Committee for Risk Assessment (RAC)

Committee for Socio-economic Analysis (SEAC)

Background document

to the Opinion on the Annex XV dossier proposing restrictions on
4.4'-isopropylidenediphenol (Bisphenol A; BPA)

ECHA/RAC/RES-O-0000001412-86-56/F
ECHA/SEAC/RES-O-0000001412-86-82/F

EC Number	CAS Number
201-245-8	80-05-7

4 December 2015

About this document

This Background Document to the opinions of RAC and SEAC is an amended version of the Annex XV restriction report submitted by France. The amendments include further information obtained during the public consultation and other relevant information resulting from the opinion making process. The assessment made by RAC and SEAC of the information presented in this document can be found in the opinions and their justification. Where relevant some additional assessment by the RAC or SEAC rapporteurs can be found in boxes in the document.

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A. Proposal

A.1 Proposed restriction(s)

A.1.1 The identity of the substance(s)

Public name: Bisphenol-A (BPA) EC name: 4,4'-isopropylidenediphenol IUPAC name: 4,4'-propane-2,2-diyldiphenol EC number: 201-245-8 CAS number: 80-05-7 Annex VI Index number: 604-030-00-0

A.1.2 Scope and conditions of restriction(s)

Based on the justifications summarised in section A.2 the following restriction is proposed as regards the use of Bisphenol-A (BPA) in thermal paper.

Entry [#]. 4,4'-isopropylidenediphenol (Bisphenol-A) CAS No 80-05-7 EC No 201-245-8	"Shall not be placed on the market in thermal paper in concentration equal to or greater than 0.02% by weight, after [entry into force + 36 months]"

Thermal paper is a paper composed of base paper which is coated with at least one chemical layer. This chemical layer is a thermal reactive coating made with binders, dyes and one developer such as BPA. BPA is a dye developer largely used in thermal paper in the EU (estimated at around 70%) and in the world (although substitution is already underway). Thermal paper is named "thermal" because it is then used in direct printing devices, placed under a heating printhead which allows the images and characters to appear. This is precisely the role of the dye developer (the BPA) contained in the thermal paper to allow this appearance. Some thermal paper can also include some additional coatings depending on the properties searched and the end-use targeted.

Thermal paper is used in many applications such as point-of-sales (POS) tickets and receipts, self-adhesive labels, lottery tickets or fax paper. In principle, all applications are likely to contain BPA although information collected during the elaboration of this proposal indicates that the POS applications mainly contain BPA. These applications stand for around 65% of the thermal tickets placed on the EU market and seem to represent the main source of BPA exposure for workers and consumers. Indeed, this type of tickets and receipts are made with relatively low quality thermal paper, namely 'ecopaper', without protective topcoating, so that the BPA contained in the thermal coating layer migrates easily to the fingers or any objects in contact with it. With respect to top coated thermal paper (or 'protected thermal papers') most often used for transportation tickets, cinema tickets and adhesive labels (food packaging, etc.), for example, BPA seems to not having been used since 2000 according to a communication from a French manufacturer of top coated thermal paper. However, this claiming is not supported by any available study. Moreover, although topcoatings might reduce the migration of BPA from the tickets, it cannot thus be excluded that BPA still migrate from them and might generate some risk. For these reasons, the restriction proposed herein aim to cover all types of thermal paper, from point-of-sales applications (namely 'ecopaper') to topcoated 'protected' thermal applications. Nevertheless, due to a higher amount of information collected for POS receipts, the exposure and risk assessments as well as the socio-economic analysis have been carried out for these specific applications. Moreover, from a control and enforcement perspective, it would be difficult to distinguish between thermal papers produced for one application or another, especially because 'thermal paper' is not explicitely defined and categorized as such in the existing classifications for products and articles (Prodcom and TARIC in particular).

As regards the measurement of BPA content in thermal paper, there is no standard analytical method to measure the content of BPA in thermal paper today in the EU but several methods still exist and could be used for that purpose. Those methods are listed and presented in section E.2.

The transitional period of 3 years (36 months) is deemed to be reasonable in terms of timing and manageability in order to give enough time for the supply chain to comply and substitute (or keep on substituting) and for the control authorities to organise and anticipate the controls.

A.2 Summary of the justification

A.2.1 Identified hazard and risk

This restriction proposal aims to address the risks for human health of pregnant workers and consumers exposed to BPA contained in thermal paper they may handle. The population at risk is more precisely their unborn children which are exposed *in utero* via their mother.

The workers targeted herein are workers who are likely to handle thermal tickets such as cashiers and the consumers at risk are any people who may receive a ticket or receipt after a purchase, an ATM withdrawal or a payment with credit card, in other words any consumer in principle. The exposure route is the dermal route.

Such as demonstrated in section B and summarized in section D.1, the risk is considered to be potentially severe and likely to concern every EU country. The evaluation of the effects arising

at low doses throughout the scientific litterature allowed to demonstrate adverse effects for the unborn children's health defined as 'at risk' on:

- The female reproductive system (increase in the occurrence of ovarian cysts, increase in the occurrence of endometriosis and disruption of ovarian cycles)
- The brain and the behaviour (alteration of spatial memory and learning functions)
- Vulnerability of the developing mammary gland (increase in the terminal end buds (TEB), terminal ducts (TD) and hyperplastic ducts (HD), considered as precursors to breast cancer with subsequent co-exposure to carcinogenic agents)
- The metabolism and obesity (increase in body weight (BW) and in cholesterol)

These adverse effects have been demonstrated at lower doses than those for fertility effects demonstrated in the recent French Harmonised Classification proposal. Indeed, it should be kept in mind that the purpose of the two dossiers (CLH and restriction), resulting from parallel exercises is different. Indeed, while the Harmonised Classification proposal was based on a comparison of the previous data that led UK to propose BPA as a Cat.2 (DSD) with recent ones, the purpose of the work carried out prior to this restriction proposal was to focus on effects arising at low doses. Moreover, three endpoints covered herein in addition to reproductive systems are effects that were mostly not investigated in older studies.

Risks for environment are not of concern herein although it is shown that the restriction could also bring some benefits for environment, avoiding in particular BPA releases in aquatic compartment from thermal paper recycling (see section F.1.2).

Such as shown in section E.2, there is no risk management measures implemented to date in any EU country restricting BPA in thermal paper. Sweden and Belgium have proposed a regulation in that purpose but they have not been adopted yet. The exposures and risks demonstrated for human health in section B are thus expected to continue to occur without this restriction. However, given its toxicity and the repetitive attacks on that ground from public opinion, medias and health and environment agencies all over the world, substitution of BPA in thermal paper is already underway. To that respect, it is shown above in section C that several 'drop in' dye developers are available, technically and economically feasible and some of them are already used in thermal paper in Europe and worldwide. This is particularly the case of BPS. Technical substitutes (alternative printing systems) and free-paper alternatives are also scrutinized in section C, showing however that they might not be economically feasible alternatives (for the former) or likely to be adopted in short-term and at large scale (for the latter). Although chemical substitution of BPA in thermal paper is ongoing, to what extent the decrease in BPA use for that application will be fast or significant without any regulatory obligation remains however uncertain. There is thus a need for regulation.

A.2.2 Justification that action is required on a Community-wide basis

As explained in section D., the main reasons for acting on a Community-wide basis are related to the risks and the internal market considerations.

The risks for human health demonstrated in section B is considered as potentially severe and extended on the whole Europe. Given that the population exposed are workers who are likely to handle thermal paper such as cashiers and consumers, in principle, all EU countries are concerned by these exposures and risks. A restriction under REACH would remove these risks and additionally some collateral environmental releases. Moreover, a restriction under REACH would ensure equal treatment among producers and importers of thermal paper and doing so it would create a level playing field on the common market.

There is thus a need for Community-wide regulation.

A.2.3 Justification that the proposed restriction is the most appropriate Community-wide measure

As shown in section E.2, the restriction proposed is referred as 'RMO 1'. It is demonstrated that it is considered as:

- *Effective in reducing the identified risks*: the concentration limit proposed is very low and at this level, the restriction is equivalent to a total ban, based on stakeholders' consultation (the thermal paper would no longer be efficient with such low BPA content). As a result, it is expected that BPA will fully phase out by the date of entry into force of the restriction and thus the exposures will totally be removed; and so will be the associated adverse effects.

- *Proportionate to the risks*:

    - The costs would mainly consist in substitution costs, namely costs associated with the replacement of BPA with another chemical dye developer and compliance control costs, related to the testing of BPA content in thermal paper. The 'direct' costs of substitution are assessed in section F.2. Indirect costs (other costs associated to substitution) are not quantified and considered as not major. The data allowing the quantitative and qualitative assessment come from one MSCAs survey carried out by ANSES in 2013, one survey by INERIS in 2013 and a review of public available data. As a whole, based on 3 scenarios (min, max and medium), and depending on the annual growth rate of the production of thermal paper in the EU and the decreasing trend of the prices of alternatives, the annual substitution cost over 2019-2030 ranges from €1 million and €25 million in 2019 value (based on the average scenario, considered as the most realistic scenario). It has to be noted that the substitution costs have been calculated based on the total production of thermal paper in the EU. However, 42% of this production is exported outside the UE. Depending on whether the manufacturers will pass on the extra costs entirely on the non-EU customers or not, the substitution costs would thus be actually only 42% of the costs calculated. A sensitivity analysis has been performed in section F.2. on several sensitive parameters. As regards the compliance control costs, borne due to the conformity tests carried our by the supply chain on the products, they are estimated between €146,255 and €254,472 per year over 2019-2030. **Overall, the costs of the restriction proposed for the thermal paper market (substitution and compliance control**

costs) are estimated to range from <u>€1.2 million and €25.3 million in 2019 value (based on the average scenario, considered as the most realistic scenario)</u>. These average costs stand for between 0.18% and 4.60% of the total production value of thermal paper manufactured for POS applications..

- As regards the health benefits associated with the restriction, they correspond to the costs avoided due to the reduction in adverse effects and diseases such as described in section B and assessed in section F.1. The health benefits have been assessed for workers as well as for consumers since both are demonstrated as at risk. The human health impact assessment is semi-quantitative. The data allowing the quantitative assessment come from the establishment of patterns and the review of economic literature. **As a whole, the total quantified potential health benefits of the proposed restriction are estimated to range from (<u>at least</u>) €3.5 million to €5.2 million, keeping in mind that all the benefits have <u>not</u> been valued and that the total benefit would be actually higher**. A sensitivity analysis has been performed in section F.1.1. on several sensitive parameters.

- The cost/benefits ratio and the proportionality of the restriction is dependent on the future substitution choice. The proportionality is analysed under two "non-use scenarios" based on whether Industry will switch to BPS (which is the cheapest and one of the alternative dye developers already largely used in thermal paper and suspected as having similar adverse effects) or to non-bisphenol alternatives. In the first situation, the proportionality might be compromised. In the second situation, under realistic assumptions and given the fact that the upper bound of the costs is probably overestimated and that not all health benefits have been valued, the restriction can be considered as proportionate.

- The transitional period of 3 years (36 months) is deemed to be reasonable in terms of timing and manageability in order to give enough time for the supply chain to comply and substitute (or keep on substituting) and for the control authorities to organise and anticipate the controls. The restriction proposed is thus expected to entry into force in 2019.

- *Practicable*: the restriction proposed is considered to be implementable, enforceable and manageable

- *Monitorable*: Given that several existing analytical methods could be used to measure BPA content in thermal paper (although no standard exists), the restriction proposed is considered to be monitorable by control authorities and customs services. As regards thermal paper imported into the EU, there might be one concern however concerning the definition of thermal paper since no specific existing TARIC code is attributed to this type of product. Several TARIC codes could in principle cover 'thermal paper'. There could be the codes 481190, 4823, 4821 and 480220 such as described in section E.2.1.3).

It has to be highlighted that, as it is shown in section C and F.2, the substitution of BPA by BPS (or other bisphenols) is expected to be likely, although it might not be a strategical choice from Industry (BPS might be also regulated sooner or later). Indeed, BPS is already used in thermal paper worldwide and appears to be the most technically and economically feasible "drop-in" alternative. However, taken into account the toxicological profile of BPS, this substitute might cause very similar adverse health effects as BPA. As a result of those expectations and hazards, it has to be pointed out that the health benefits estimated herein due to the restriction of BPA in thermal paper could be decreased and to some extent come down to zero if BPA was actually and totally replaced by BPS and if BPS was proven as much as toxic.

Another option for restriction is assessed in section E.2: a restriction under REACH limiting the migration of BPA from thermal paper. This option is named 'RMO 2' but is not considered as appropriate as regards the criteria of effectiveness (including proportionality), practicality and monitorability. It has thus been discarded.

A third option for restriction had initially been thought to be developed: a REACH restriction with a wider scope including a grouping of all bisphenols likely to be used in thermal paper. Given the fact that the other bisphenols identified and assessed in section C as possible alternatives may have the same adverse properties and effects on human health as BPA does, this option for restriction could have been of great interest and consistency. This even restriction proposal could have been scoped in that way. It would have guaranteed the non-replacement of BPA by other dye developers, such as BPS particularly, which is rather cheap. However, due to the current lack of toxicological data on some bisphenols' profile on the one hand (expected to be partially filled in by the 2014 BPS SEv by BE), and taken into account that risks from BPA in thermal paper have already been demonstrated, this option has been discarded and this proposal focuses on BPA only.

B. Information on hazard and risk

B.1 Identity of the substance(s) and physical and chemical properties

B.1.1 Name and other identifiers of the substance(s)

Public name: Bisphenol-A (BPA)

EC name: 4,4'-isopropylidenediphenol;

IUPAC name: 4,4'-propane-2.2-diyldiphenol

EC number: 201-245-8

CAS number: 80-05-7

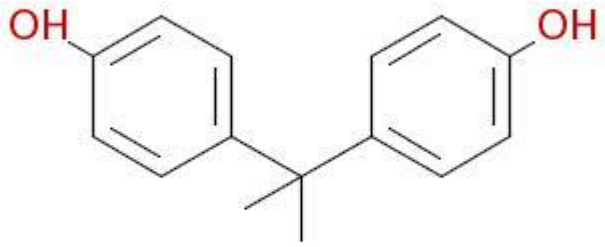
Annex VI Index number: 604-030-00-0

Deleted CAS numbers: 27360-89-0; 28106-82-3; 37808-08-5; 137885-53-1; 146479-75-6; 1429425-26-2.

Molecular formula: $C_{15}H_{16}O_2$

Molecular weight: 228,2863 ± 0,0137 g/mol

Structural formula:



B.1.2 Composition of the substance(s)

The degree of purity of BPA is superior to 80% and inferior to 100% (w/w).

Constituent	Typical concentration	Concentration range	Remarks
Bisphenol A	80 – 100%	No information	

B.1.3 Physicochemical properties

Table 1. Summary of the physico-chemical properties

Property	Value	Comment (e.g. measured or estimated)

State of the substance at 20°C and 101,3 kPa	Odourless (mild phenolic odour) solid (white crystals, flakes, prills)	Bisphenol A is a white solid at environmentally relevant temperatures
Melting/freezing point	melting point: 150-155°C	
Boiling point	at 1013hPa: 360 °C	
	At 17hPa: 250-252°C with potential decomposition	
Relative density	1.195 g/cm^3 (Air = 1) at 25° Density: 0.815 g/cm^3 at –20°C	
Vapour pressure	1.61E-09 hPa at 20°C 4.12E-09 hPa at 25°C	
Surface tension	No data	
Water solubility	Moderately soluble in water: 146-173 m g/L at 25°C	
	300 mg/L at 25°C	Tests were conducted using bi-distilled water with 24 hours mixi ng. They were performed in triplicate. HPLC was used for analytical dete rmination
Partition coefficient n-octanol/water	3.4 at 21.5°C	Experimental
	3.32 at 25°C	QSAR
Flash point	227°C @ 1013 hPa	Closed cup
Flammability	Bisphenol A is classified as not readily combustible solid	Tested according to UN Test Procedure N.1
Explosive properties	Waiving	
Self-ignition	Auto-ignition temperature:	

temperature	510°C @ 1013 hPa	
Oxidising properties	waiving	
Granulometry	Most of BPA granules are > 1mm in diameter. (1.25-2.0: 62.3-87.7%)	Experimental data
Stability in organic solvents and identity of relevant degradation products	waiving	
Dissociation constant	pKa = 10.08 at 25°C	
Viscosity	Scientifically unjustified	

Source: Registration dossier (2013)

B.1.4 Justification for grouping

Not relevant for this proposal.

B.2 Manufacture and uses

Data on manufacture and uses of BPA are documented below from different sources (registration dossiers for BPA, EC, 2003; EC, 2008, INERIS, 2010, ANSES, 2011, Danish E.P.A., 2013, UK, 2008, 2013 MSCAs consultation (see section G), 2013 Industry consultation (INERIS, 2013).

B.2.1 Manufacture, import and export of BPA

Manufacture

The market of BPA is global-oriented and supplies a high range of end markets. In 2006, the global production of BPA accounted for 3,800,000 tons (INERIS, 2010), with around 25% from the USA (1,089,000 tons in 2007) and 25% from the EU (EC, 2003; EC, 2008, UK, 2008, Danish E.P.A., 2013).

From the RAR (EC, 2003; EC, 2008), the EU BPA production is oligopolistic and divided up among four major producers. From WHO, 2010), the major world producers of BPA as of mid-2010 included Bayer (23% share in the Americas, Europe and Asia), Mitsui and Nan Ya Plastics (9% and 8% shares, respectively, in Asia) and SABIC, Dow and Hexion (16.2%, 7.3% and 5.6% shares, respectively, in the Americas and Europe). In the EU, the BPA producers operate a total of six production sites located in four EU countries (Germany, The Netherlands, Belgium and Spain) and their manufacture, based upon their submissions to CEFIC, is estimated at approximately 1,150,000 tonnes/year (taken from 2005/2006 data). However, other manufacturers exist who are not members of Cefic and so have not supplied information, so these tonnage figures may be an underestimate (EC, 2003; EC, 2008). The average amount of

BPA produced annually was estimated to be about 700,000 tonnes between 1996 and 1999, reaching 1.6 million tonnes in 2005 according to the Plastics Europe association. This figure is consistent with the abovementioned CEFIC estimate (INERIS, 2010). From DEPA 2013 survey (Danish E.P.A., 2013), Finland also produced 27 tons of BPA in 2011.

Import

They are several importers of BPA in the EU. From the different surveys carried out, there are at least 3 importers in France and 1 (Asian) importer in Poland (MSCAs survey, ANSES, 2013). However, no quantitative data has been found about the import of BPA in the EU.

Export

From the data reported in EC, 2003 ;EC, 2008 and UK (UK, 2008), net exports of BPA from the EU were in the region of 65,000 tonnes/year for 2005/2006.

Consumption

The biggest consumers of BPA worldwide are the USA (about 25%) and Japan (about 12%). Asian countries (about 35%) and Western Europe (about 25%) are also significant consumers (more than 50% of global consumption as a whole). Between 2003 and 2006, consumption of BPA grew at an average annual rate of about 10%, reaching 1,084,870 tons in 2006 (INERIS, 2010) and 1,149,870 tons in 2010 (EC, 2010), mainly due to the strong demand for polycarbonate (EC, 2010).

Table 2 summarises the available information on production, export, import and consumption of BPA. The quantity consumed is described in the next section.

Table 2. Production, import and consumption of BPA

	Production of BPA	Import of BPA	Export of BPA	Consumption of BPA
World	3,800,000 (2006) 4,300,000 tons (2012) (ETPA consultation)	-	-	4,041,000-5,500,000* (2009/2011*)
USA	1,089,000 (2007)	-	-	≈25% of global consumption

	≈25% of global production			
EU	1,150,000-1,600,000 (2005/2006) ≈25% of global production 4 producers (DE, NL, BE, ES) – 6 sites	-	> 65,000 (2005/2006)	1,084,870 (2006) 1,149,870 (EU RAR 2010)
France	0	6,480 (2005)	3,000* (2005)	2000-3,500 (2005)
Finland	27 (2011)	23-2,376 (2011)		

*estimated/prospected

B.2.2 Uses of BPA

BPA is a monomer produced and consumed for a wide range of end-uses and applications.

The following uses of BPA have been identified in the literature (ANSES, 2011).

- Use as a monomer in the synthesis of polymers:
    - Polycarbonates
    - Polyester carbonate
    - Polyarylates
    - Polysulfones
    - Polyetherimides
    - Polyols
- Use in the synthesis of resins:
    - Epoxy resins

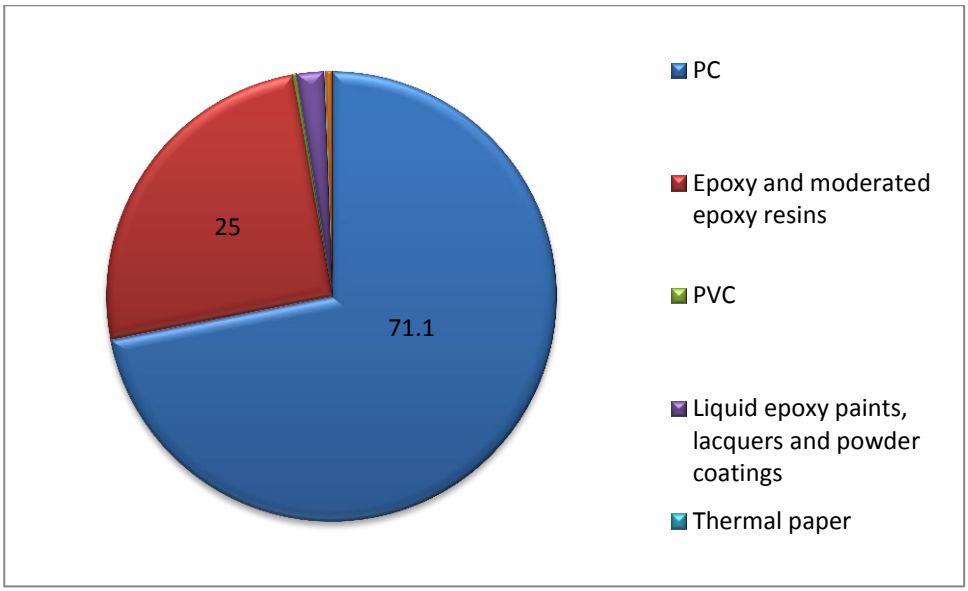
  - Vinyl ester resins
  - Phenolic plastic resins
  - Phenolic resins
  - Unsaturated polyester resins
- Use as a reagent for the synthesis of ethoxylated bisphenol A
- Use as a component of a polyamide
- Use as a reagent for the manufacture of flame retardants:
  - Tetrabromobisphenol A (TBBPA)
  - Tetrachlorobisphenol A (TCBPA)
  - Bisphenol A bis (diphenyl phosphate) (BDP)
- Use as a developer for thermal paper
- Use in the automotive industry:
  - Antioxidant in the manufacture of tyres
  - Antioxidant in brake fluids and hydraulic fluids
- Use in the paint industry
- Use in formulations for fungicides (not in the EU)

- Use of BPA-based polymers in cosmetics (lipstick, face and eye makeup, nail polish)
- Use in the composition of heat-transfer fluids and lubricants, and as a treatment for concrete resurfacing
- Use as a precursor in the synthesis of benzoxazines
- Used in dental products

In the CSR, the BPA is used for manufacturing polycarbonate, epoxy resins, coating materials, chemicals, for inclusion into or onto a matrix, for manufacture of pulp, paper and paper products, as antioxidant for processing polyvinylchloride (PVC), as epoxy resin hardeners, and as thermal paper (including paper recycling). The consumer and professional uses of thermal paper are in paper articles.

Within the wide variety of end-uses, the BPA is primarily used in the manufacture of polycarbonate (around 72% in 2005/2006 and 75% in 2009) and epoxy resins (25% in 2005/2006 and 22% in 2009). As shown in the figure below, these two largest uses stand for over 95% of the world consumption of BPA (UK, 2008 WHO, 2010).

Figure 1. Share of the main end-uses of BPA



The world consumption of *polycarbonate resins* in 2007 by end use was as follows (by decreasing quantity): electrical/electronic (TVs, monitors, PCs, telephones, Electrical kettles, mixers, switches, lamp holders, etc.), 617,000 tons; optical media (Discs, CD-ROM's, etc.), 500,500 tons; glazing and sheet (construction), 469,000 tons; transportation (automotive), 322,000 tons; and "other" (including bottles, packaging, medical and healthcare) 426,500 tons (WHO, 2010; Danish E.P.A., 2013). UK, 2008 reports that 865,000 tons of polycarbonate resins were produced in 2005/2006 in the EU.

The end-uses of BPA-based *epoxy resins* are (by decreasing produced quantity): marine and protective coatings (water ballast tanks, sea containers, Steel bridges, storage tanks and drinking water pipes of metal and concrete etc.), powder coatings (steel furniture, pipes, valves & fittings, shelves, etc.), electrical and electronics (potting/encapsulation electronic parts (trans-formers, inductors), printed circuit boards, etc.), civil engineering (flooring, fillers, crack repair, seal against water and de-icing on concrete bridges, Anti-skid coatings for park decks, etc.), can and coil coatings (food & drink cans, caps, collapsible tubes (toothpaste, cream), dishwashers, fridges, etc.), automotive coatings (Waterborne primers for cars, buses, railcars, etc.), composites (Rackets (tennis, etc.), snowboards, canoes, helmets, windmill blades, pipes, boats, aircraft, etc.), adhesives (Repair kits, adhesives for buildings, cars, boats, etc.) and photocure (Printing inks, wood coating, paper varnish, incl food packaging, coating for plastics and primed metals, etc.). UK, 2008 reports that 191,520 tons of epoxy resins were produced in 2005/2006 in the EU.

The other (minor) uses of BPA include the manufacture of liquid epoxy paints, lacquers and powder coatings (12,400 tons in 2005/2006), the manufacture of polyvinylchloride (PVC) (1,800 tons), the manufacture of thermal papers (1,890 tons) and the manufacture of tin-plating additive (2,460 tons) (UK, 2008).

Table 3 below shows the share of the main category of uses of BPA in the EU consumption.

Table 3. BPA main uses

BPA use	**EU (T/year)**	**EU % consumption**
Manufacture of polycarbonate (PC)	865,000	71.1
Manufacture of articles from polycarbonate	400*	0.05*
Manufacture of epoxy resins and moderated epoxy resins *Incl.* *Can coatings* *ethoxylated bisphenol A*	191,520 *2,755* *2,260*	25
Manufacture of polyvinylchloride (PVC) *Incl.* *Stabilizer packages* *Phthalate plasticizers* *Direct stabilization*	1,800 *450* *900* *450*	0.3
Manufacture of liquid epoxy paints, lacquers and powder coatings *Incl.* *Phenoplast* *unsaturated*	12,400 *8,800* *3,600*	1.4 0.5
Manufacture of thermal papers	**1,890**	**0.16**

	(2,400 from the registration dossier)	
Manufacture of tin-plating additive	2,460	0.4
Others	7,245	

Compilation data from the following sources: UK, 2008; Jeffs, 2011; Danish E.P.A., 2013; BPA registration dossier (04/10/2012).

BPA is thus an HTPV (high tonnage production volume) substance and a very wide range of articles or preparations are likely to contain BPA on the EU market. For illustrative purposes, Table 4 below shows the great variety range of applications likely to contain BPA on the market. It concerns polymers, resins and other products synthesised from bisphenol A.

Table 4. Items and preparations likely to contain BPA

Use	**Applications (articles or preparations) likely to contain BPA**
Polycarbonates used in the manufacture of optical media	Blank optical media
Polycarbonates used in the manufacture of optical equipment	Contact lenses; glasses made from all materials
	Prescription glasses, protective or other glasses
Polycarbonates used in the manufacture of tableware items	Crockery, other tableware and household and toiletry items, other than porcelain
Polycarbonates used in the manufacture of food containers	Food containers and packaging
	Demijohns, bottles, flasks and similar plastic items
Polycarbonates and epoxy resins used in the manufacture of domestic appliances	Domestic appliances
Polycarbonate, polyarylate resins, polysulfone resins, polyether imide resins used in the manufacture of medical equipment and dental products	Medical and dental instruments and supplies
Polycarbonate, polysulfone resins, polyether imide resins used in the manufacture of electrical equipment	Electrical installation equipment

Use	**Applications (articles or preparations) likely to contain BPA**
Polycarbonate used in the manufacture of transparent films	Plastic plates, sheets, tapes and strips, not fitted with a support or similarly combined with other materials
Polycarbonate used in the manufacture of protective equipment	Safety helmets and other safety products
Polycarbonate, epoxy resins, vinyl ester resins, unsaturated polyester resins used in the manufacture of sporting goods	Sporting goods
Polycarbonate used in the construction of roofs of sports facilities	Sports or recreational facilities
Polycarbonate, epoxy resins, vinyl ester resins, unsaturated polyester resins, polyols, polysulfone resins, polyether imide resins used in the manufacture of automobile parts Manufacture of brake fluid and tyres	Motor vehicles (tyres, safety glazing, light reflectors, headlamp inserts, bumpers, radiator and ventilation grilles, interior lighting systems, motorcycle windshields and helmets, car roof modules, etc.)
Polycarbonate, epoxy resins, modified polyamide, polysulfone resins, polyether imide resins used in electrical and electronic applications Tetrabromo Printed circuits	Computer, electronic and optical products
Epoxy resins, vinyl ester resins used in flooring (buildings)	Plastic flooring, in rolls or tiles
	Linoleum and hard flooring with non-plastic surfaces, resilient floor coverings such as vinyl, linoleum, etc.
Epoxy resins used in coatings for the insides of tins and cans	Food containers and packaging
Epoxy resins, vinyl ester resins used in surface coatings of metal containers	Metal tanks, reservoirs and containers
Epoxy resins used in coatings for tubes and pipes	Steel tubes, pipes, hollow profiles and related accessories

Use	**Applications (articles or preparations) likely to contain BPA**
Epoxy resins used in the construction of metal panels	Sandwich panels in coated steel plate
Epoxy resins, vinyl ester resins, unsaturated polyester resins used in concrete or concrete structures	Concrete parts/structures for construction
	Systems/circuits for fluids
Epoxy resins, phenolic resins used in the manufacture of glues, adhesives, etc.	Glue/adhesive/sealant/related products
Epoxy resins used in the manufacture of mastic	Mastic
Production of epoxy resins	Resins
Epoxy resins, ethoxylated bisphenol A used in the production of inks	Printing and reproduction products
Phenolic plastic resins, unsaturated polyester resins, polyols, ethoxylated bisphenol A used as binders, plasticisers, hardeners for paint, lacquers and other fillers	Paint/varnish/enamel/stain and associated products
Epoxy resins, vinyl ester resins, unsaturated polyester resins, polysulfone resins, polyetherimide resins used in aeronautical construction	Aerospace constructions
Epoxy resins, vinyl ester resins, unsaturated polyester resins used in the manufacture of boats	Ships and floating structures
	Pleasure boats
Epoxy resins, phenolic plastic resins used in the manufacture of wood panels	Veneer and wood panels
Epoxy resins used in the manufacture of tools	Tools
Epoxy resins, ethoxylated bisphenol A used in the	Varnish/lacquer for wood flooring

Use	**Applications (articles or preparations) likely to contain BPA**
manufacture of varnish	Non-water-soluble varnish/lacquer for wood flooring
Epoxy resins used in the manufacture of glass fibre	Fibreglass
Vinyl ester resins used in fibre optic media	Optical fibre cables
Vinyl ester resins used in gas cylinders	Metal containers for compressed or liquefied gas
Phenolic plastic resins used in insulation	Sealant and insulation products
Phenolic plastic resins used in abrasives	Abrasive/polishing products
Phenolic plastic resins used in friction materials	Friction linings for brakes, clutches and related products
Phenolic plastic resins used in the paper industry Manufacture of thermal paper	Paper and cardboard
Polyols used in the production of polyurethane	Polyurethane foam
Manufacture of resin-based composite materials for restoration and sealing for dental use	Adjuvants for medical prostheses (cement, glue)
Composition of lubricants	Lubricant
Composition of heat transfer fluids	Heat transfer fluids

Source: ANSES, 2011

As regards the specific scope of this proposal, and as shown above, it can be seen that the use of BPA in thermal paper is rather minor compared to other uses such as polycarbonates or epoxy resins. A detailed analysis of the thermal paper market and this particular use of concern are provided in the next sections.

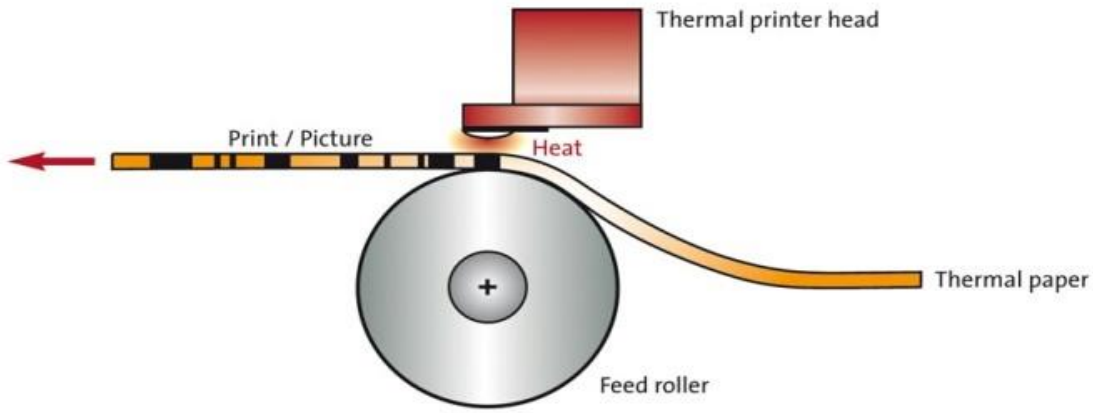
B.2.3 Manufacture, import and export of thermal paper

B.2.3.1. The thermal printing technology

Thermal printing is a commonly employed printing method typically used in point of sale receipts, such as fast food restaurants, retailers, grocery stores, gas stations, post offices and automated teller machines.

As shown in Figure 2 below, in the direct thermal printing process, a printed image or words are produced by selectively heating specific areas of the coated thermal paper as it is passed over a thermal print head. The printer's thermal head consists of numerous little heating elements. The heating elements are electronically controlled and produce thermal energy which activates a color reaction on the functional thermocoating. The numerous little dots this produces then go to make up letters, barcodes, and images.

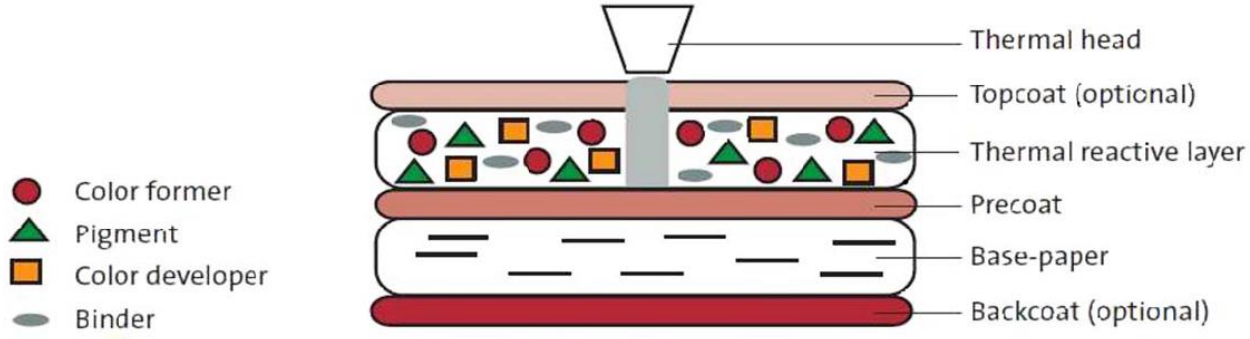
Figure 2. Thermal printing technology



Thermal printers have a relatively simple design and are easy to operate, which makes them energy-saving, quiet, fast, small, and compact – and they require no other consumable, which in turn saves money and makes them highly reliable. They keep working even under extreme conditions.

Thermal paper consists of a very smooth paper with a thin coating of a leuco dye and a dye/color developer. This leuco dye, a dye whose molecules can acquire two forms, changes colour under the application of heat, pressure or laser light and is then able to reflect light (Biedermann, 2010; Mendum, 2010).

Figure 3. Structure and function of thermal paper



As illustrated in the Figure 3 above, the thermal paper is basically composed of a base paper all over which a thermo-coating is applied in a coating machine. This thermo-coating contains the principal functional components and forms the thermal reactive layer of the thermal paper. This layer is made up of 4 components:

- a color-forming substance
- a pigment/thermochromic ink (usually spirolactones, fluoranes, spiropyranes, etc.) that passes from a colourless state to a coloured state depending on the medium's pH
- a dye/colour developer
- a binder/solvent (generally an alcohol or an ester).

When the printing stylet heats the paper at a temperature above the binder/solvent's melting point, the color developer interacts with the thermochromic ink by giving protons, which acidifies the environment and makes the system coloured. Heat applied at points by the stylet of the thermal printer to that layer produces a chemical reaction which makes the lettering or image appear. The composition of the thermal coat determines the sensitivity of the paper, the image density, the image preservation and the background density.

Thermal paper can optionally be topcoated, backcoated and/or pre-coated, depending on the quality of the paper wanted and the targeted application. An additional topcoat can be applied to the thermal coat to protect the thermal paper from mechanical abrasion (e.g. through scratches), chemical influences (e.g. through oils, fats, varnishes or organic solvents) and other environmental influences (e.g. through high humidity or water). A topcoat on the front side of the thermal paper also extends the service life of the thermal head of the printer by reducing or eliminating the transfer of residue from the thermal coating on to the thermal print heads. A top coat can also focus the heat from the thermal print head on the active coating and provide better anchorage of flexographic printing inks applied to the thermal paper (Jeffs, 2011). A coating on the back (backcoat) provides additional protection when printing, laminating, and much more. It might be essential when the reverse side of the thermal paper is exposed to migrating adhesives (e.g. adhesives which are used in the production of self-adhesive labels) or plasticizers (e.g. from plastics like PVC). Moreover, special back-coats prevent the paper from curling and enable the use of water-based solvents, inks and adhesives. Finally, pre-coating (or under-coating) may prevent heat conduction into the paper thus enabling the energy from the thermal head to concentrate in the thermal layer in order to produce high-resolution printing. This layer determines the sensitivity of the paper, the

brightness and the image density and guarantees an even and smooth surface onto which the thermal coat is applied (Jeffs, 2011).

The technological possibility of adding specific coatings also allow avoiding the traditional drawbacks of thermal paper, such as paper curling and fading of the printed image over time. These layers also allow printing to be applied to the back of the paper, such advertising.

To ensure optimal printing results, certain characteristics of the type of thermal paper and printer used have to be considered. Different grades of thermal paper have certain characteristics that render them more applicable to certain uses. One important characteristic is dynamic sensitivity, which pertains to the length of time the paper is exposed to heat. The faster a printer operates, the less time the paper is exposed to the units heating element. Thermal paper with a higher dynamic sensitivity is most appropriate for higher-speed or lower-energy printing. If thermal paper with low dynamic sensitivity is instead used, insufficient heat will be applied to the paper resulting in a reduced long-term stability of the finished product (Koehler Thermal Papers[1], US EPA, 2012). Static sensitivity is another important characteristic of thermal paper. Static sensitivity defines the temperature at which the dye and the developer begin to melt. The static sensitivity value is important for thermally-sensitive applications, such as for parking tickets or environments with high temperatures (e.g., pizza boxes, coffee cup labels). Different grades of thermal paper exhibiting varying degrees of thicknesses and sensitivities affect the lifespan of the print job. If the appropriate paper and printer combination is used, and proper storage conditions are met, an image printed on thermal paper typically lasts between five to ten years (Koehler Thermal Papers[2]; US EPA, 2012).

What are the advantages and assets of thermal printing technology?

Direct thermal printing offers significant advantages. As compiled from INERIS (INERIS, 2010), the Torraspapel website[3], Jeffs, 2011 and US EPA, 2012, they can be listed as:

- fast printing/sensitivity (up to 406 mm per second)
- high image resolution: full graphics capability, up to 400 or 600 dpi (digits per inch) outputs, image independent on the amount of data or sheets used
- very high reliability and durability/longevity (from 5 to 12 years for the best quality paper), few mobile components of the printers
- printability on two sides (depending on the process used)
- Individual printing of copies additionally to the sheets (such as credit card slip to be signed)
- small, compact, light, portable printing units ideal for handheld devices

[1] http://www.koehlerpaper.com/en/papier/thermal/

[2] http://www.koehlerpaper.com/en/papier/thermal/

[3] http://www.torraspapel.com/Conocimiento%20Tcnico/AboutPaperThermal.pdf

- no changing of peripherals
- easy handling in applications
- flexibility of paper size: large number of formats for printing, from a few centimeters to large formats
- low running costs and environmental impacts – low energy and maintenance (no additional consumables such as tapes, toners or inks)
- no particular cleaning to be performed
- low cost of ownership
- low noise due to the "non-impact" printing process
- high functionality even under extreme environmental conditions
- no fouling of the print head
- excellent ink receptivity

All of those assets stand for important competitive advantages in favour of thermal printing which is a valuable economical and fast printing system compared to alternative printing techniques available on the market.

The only shortcoming of this printing system seems to be the need for refilling the printer with paper regularly, which is actually the case for any printing devices. Nevertheless, there is another disadvantage of thermal printing (of thermal paper more precisely), being that thermal paper rolls exposed to heat may turn black, necessitating appropriate storage conditions. Thermal paper is generally very thin (especially when it is used for tickets and receipts) and may be damaged by prolonged exposure to sunlight, water, or chemicals (e.g. solvents, plasticizers) and to friction. In general, that kind of thermal paper for tickets of receipts is best suited for short-term printing needs more than longer term data storage (US EPA, 2012).

B.2.3.2. The market of thermal paper: manufacture and applications

A bit of history

The Jeffs, 2011 report includes a valuable historical development of thermal paper. A summary is provided hereunder.

The first applications of thermal papers first came to market in the 1960s, produced by NCR Corporation and 3M in the USA. The first types of thermal paper were of inferior quality, the image used to fade rapidly. Yet, this paper was attractive thanks to its relative cheapness and the market grew. The first thermal printing head was developed by Texas Instruments in 1965 and the first thermal printer, connected to a computer terminal, was launched on the market in 1969 (Jeffs, 2011). During the 1970s, Hewlett Packard began integrating thermal paper printers into its desktop computers and plotters. In the 1970s and early 1980s, Japanese producers (such as Ricoh, Jujo, and Kanzaki) using similar dye-based chemistry to that used by NCR, formed partnerships with barcode printer manufacturers (such as TEC and Sato) and

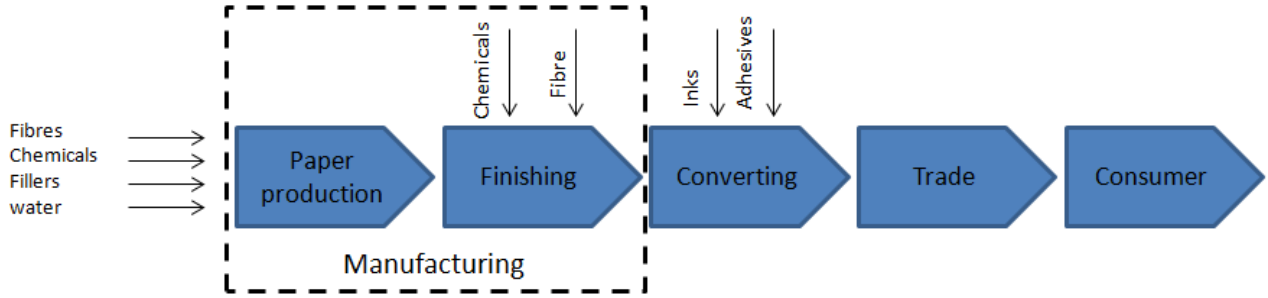
entered the emerging global bar code industry, primarily in supermarkets. U.S. producers such as Appleton (NCR's licensee), Nashua Corporation and Graphic Controls compete to gain market share. Leading pressure-sensitive label producers such as Avery Dennison became major consumers of direct thermal paper for label applications. Sales in thermal paper really took off at the end of 1980s with the launch of fax machines. This resulted in large investments in production capacity for fax papers. However, in the early 1990s the fax market had peaked and began to decrease due to the penetration of laser and inkjet fax machines which did not suffer from the fading which was common with thermal paper. Thermal transfer, laser printing, electro-photography, and to a lesser extent, ink jet printing, began to capture market share for industrial and warehouse barcode applications due to better durability. In an attempt to protect their investments in thermal paper production capacity, manufacturers were forced to seek new applications for direct thermal printing. An investment in improved performance and reliability, including image stability, printability and thermal resistance properties, has led to an increasing variety of applications. The rapid development in recent years of fast, quiet, reliable thermal printers has also allowed the speed and accuracy of the printing to improve. The result has been an overall growth in the market for thermal paper which has more than compensated for the drop in thermal fax paper.

The market penetration and growth of direct thermal printing is being maintained thanks to its inherent advantages over other alternative methods of printing. These advantages have been described in the previous section.

Market structure and value chain

As regards the value chain of the (EU or global) thermal paper market, it is structured into 5 distinct segments namely supply of raw materials, manufacturing, converting, trade and consumption.

Figure 4. Value chain of thermal paper market



(Source: Jeffs, 2011)

Supply of raw materials

The raw materials used as inputs in the manufacturing of thermal paper are fibers, chemicals, fillers and water. The suppliers of these raw materials are not specific to the thermal paper market but act on a wide range of chemicals-demanding markets. The inputs materials can be delivered to manufacturers of thermal paper either in a raw state or already pre-transformed or formulated and ready-to-use/ready-to-apply.

Manufacturing

Manufacturing is defined as both paper production (often from purchased pulp) and finishing. The 'production' *per se* consists in putting together the base (plain) paper and the different coatings (primarily thermal-coating and optionally precoat, backcoat and/or topcoat) as described in the previous section. Some manufacturers produce the plain or support paper themselves (INERIS, 2013). The coatings can be formulated on site or purchased already prepared and are only applied. Once the coatings are dry, the thermal paper generally passes through the 'finishing' operations, where it is subjected to various operations: winding, packaging and binding. Then, the thermal paper, usually conditioned in jumbo rolls (rolls with a large width, up to several thousands of meters long, so that thermal paper can be easily transformed) is warehoused before being sent to convertors or directly to traders. Those paper production and finishing operations are carried out in large, automated production factories. The manufacturing companies either own or license the patents for the different chemical formulations necessary to create the various finishes of thermal paper. Most of them operate under Japanese license[4].

Figure 5. Jumbo rolls of thermal paper

[4] http://cerig.efpg.inpg.fr/icg/Dossiers/Papier_thermique/chapitre_1.htm

The market of thermal paper manufacturing is oligopolistic. It is dominated by a handful of large business groups which are global-oriented and diversified. They usually produce a wide range of paper products in addition to thermal paper. In Europe there are in total 10 thermal paper manufacturers. The four largest are Koehler, Kanzan, Mitsubishi and Juju Thermal. Other European thermal paper manufacturers are Sihl and Torraspapel. The thermal paper manufacturers in Europe are members of the trade organisation named European Thermal Paper Association (ETPA – see section G). Between 2000 and 2006 in Europe, the thermal paper market grew from 105,000 tons to 168,000 tons which represents a 60% enhancement (Danish E.P.A., 2011). ETPA confirmed a growth of market during the past ten years (up to 10% per year). Thermal paper manufacturing continues today to be a resilient, growing, diversified industry in spite of tough competition (particularly from Asian countries whose the market grows faster) and depressed prices. The worldwide market for thermal paper in 2006 was approximately 845,000 metric tons valued at $1.5–1.6 billion at the producer level (Jeffs, 2011) and approximately 1.3 million tons in 2012 (from 2013 ETPA consultation; INERIS, 2013). ETPA estimates the European thermal paper production at 540,000 tons in 2012 (composed of 229,000 tons produced for the EU market and 311,000 tons exports[5]). Despite the specialisation in end products, margins are tight overall with profitability depending on the cost of raw materials and strong automation to achieve economies of scale in production. The high cost of plant and machinery necessitates large sales volumes for manufacturers (Jeffs, 2011).

Thermal paper is also imported into Europe from extra-EU thermal paper manufacturers mainly from Korea (e.g. Hansol), Japan and USA (e.g. Appvion, Inc. (formerly Appleton Papers Inc.)) and China (e.g. Jinan Fuzhi Paper Co.,ltd).

As regards the geographical breakdown of thermal paper production within the EU, the data collected through both the 2013 MSCA and INERIS surveys provide the following picture of the European market (INERIS, 2013).

Table 5. Production, import and distribution of thermal paper in the EU

[5] The included countries for the ETPA estimations for Europe are AT, GR, Benelux, Nordic, FR, DE, GB, IE, IT, CH, ES and PT

	Number of thermal paper producers	Number of thermal paper importers/ distributors
Bulgaria	-	10
Denmark	>1	>2
Finland	1	-
France	1	>1
Germany	4	>1
NL	-	>1
Slovenia	1	-
Spain	1	-
Sweden	1	>5
UK	-	>2
EU27	≈10 within 7 EU countries	>20+
TOTAL	540,000 tons*	N/A

*source: ETPA (INERIS, 2013): 540,000 tons include 229,000 tons produced for the EU market and 311,000 tons exported[6].

The EU countries not mentioned above either didn't answer the surveys, either didn't have the information requested, or don't count any producer or importer on their territory.

It has to be noted that up to 10% of the paper from the production process is waste (due to trimmings, etc.). This waste material is called 'broke' and is sent directly to a small number of recycling plants and so never enters actual commercial use (EC, 2008). Some recycling companies are thus linked to the manufacturing segment.

Converting

The converting of thermal paper consists in purchasing paper in jumbo rolls from the thermal paper manufacturers and then slitting them to commonly used smaller sizes for various industries, clients and applications. Converting can also include the printing of additional information on to the paper, such as advertising (often on the back side of the paper). Convertors are companies which are diversified or specialized in one specific product such as till rolls manufacturing (like Schades in Europe for example).

Trade

The trade segment corresponds to the distribution and sale of bulk-bought ready-to-use rolls purchased from the convertors to end customers. They are the very last intermediaries in the

[6] The included countries for the ETPA estimations for Europe are AT, GR, Benelux, Nordic, FR, DE, GB, IE, IT, CH, ES and PT

supply chain between the thermal paper produced and the downstream users (or final customers). The distributors operating in one particular EU country are generally companies which purchase the final products from national thermal paper manufacturers, or at least from another EU manufacturer. However, as already mentioned above, many foreign distributors (from Korea, the USA or Japan) are now well settled in several EU countries and widely marketplace their products in the whole European market.

Consumption

The downstream users or end customers of thermal paper are very numerous and can be estimated to hundreds of thousands. They go from big firms to SMEs and one-man businesses. Not exhaustively, they include large retailers, big malls, banks, shops, medical cabinets, hospitals, drugstores, parking lots, etc. as well as corner shops and stores and more generally any place where tickets, operation, payment proofs or vouchers have to be provided. The employees of those places use and handle thermal paper on an every-working-day basis; and to some lesser extent, the clients of those firms or places are also users of thermal paper when they get their receipt or proof. All of those end customers purchase the ready-to-print rolls from the traders and have to be equipped with the appropriate printing devices, corresponding to the specific thermal paper they are distributed to. There is thus a very close relationship between the thermal paper manufacturers upstream and the printing end-use technology downstream (and thus the manufacturers of those).

Recycling

After its use, some part of the thermal paper is recycled and is used in the manufacturing of recycled paper products, for example, in making napkins, toilet paper, paper towels, as well as newspapers, magazines, receipts, envelopes (Liao, 2011; UBA, 2010). Approximately 30% of thermal paper used enters the recycling circuit according to an estimate by the European Thermal Paper Association (Schreder, 2010). During the recycling operations, the basing pulp of thermal paper is treated by a de-inking treatment in order to remove chemicals contained in it and release them in the processed waters. From RPA, 2003, the recycling rate of paper was estimated to around 50% in the EU in 2000. This share is confirmed by OECD, 2009 which provides a figure slightly above 50%.

The applications of thermal paper

Thermal papers are firmly established in many areas of daily life. Today, direct thermal printing technology dominates the market for 4 printing applications.

Point-of-sale (POS) applications: this is an ever-growing market for thermal printing and is specific to bank and retail. Applications include printing of cash receipts (supermarkets, shops and stores), receipts from credit card payments (gas station, restaurants, etc.), bank statements and ATM receipts. The thermal paper used for the POS applications is usually rather thin and has to be rapidly printed.

Plotting and recording applications: they are specific to fax, medicine and office applications and include fax receipts, plotters, information terminals, medical print-outs such as ultrasound and electrocardiography. The characteristics of the thermal paper used for those applications are close to the one used for the POS applications except for some specific printings used in the healthcare sector.

Self-adhesive labels: these applications are specific to retail, industry and logistics and include barcode-labels for foods, books, clothing but also pallet labels, parcel and return stickers, luggage tags and boarding passes. As indicated in their name, an additional chemical treatment is applied to the thermal paper used for these applications in order to insure their adhesive property. One can note that, additionally to the ETPA mentioned above, this specific application also pertains to the FINAT (the international Federation of Manufacturers and Processors of Self-Adhesives Labels).

Tickets: these applications correspond to entry tickets for museums, cinemas, theatres, sport events, as well as transport tickets by air, rail, boat and bus, betting slips (such as horseracing), parking and lottery tickets. The thermal paper for ticketing is often thicker and may require security features.

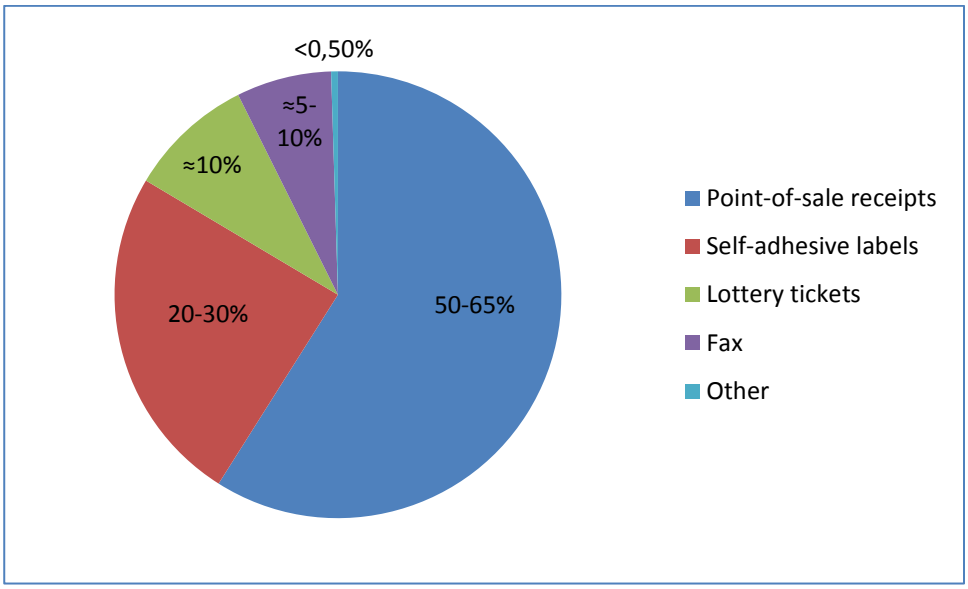
As regards the share of these different applications, the data gathered from different sources indicate the following distribution: more than the half of the thermal paper is used in the form of receipts at points-of-sale which is the dominant application, less than one-third as self-adhesive labels and the rest for lottery tickets and fax paper (EC, 2008; ANSES, 2011; ETPA consultation 2013).

Table 6. Applications of thermal paper in Europe

Application	**Share over total thermal paper (2008-2012)**
Point-of-sale receipts	50%-65%
Self-adhesive labels	20%-30%
Lottery tickets	≈10%
Fax	≈5%-10%
Other	<0.5%
TOTAL	100%

The point-of-sale receipts are the largest use of the thermal paper today in the EU and worldwide and this use keeps on growing while the fax application is declining.

Figure 6. Applications of thermal paper



On the market, two types of thermal papers may be found (EC, 2010; INERIS, 2010):

- Eco-paper or unprotected thermal paper: this is the widest used thermal paper. It is mainly used in point of sales receipts, cash register receipts and credit card slips.

- Top-coating or high-quality paper (which provides high quality images): it is used for identification tags (parcels, self-service weighing of fruit and vegetables, identification of pre-packaged fresh foods, etc.), tickets (cinema, concerts, etc.), identification badges, self-adhesive labels, lottery tickets and receipts. A range of high-quality papers also provides security measures available to reduce counterfeiting.

Whatever is the type of thermal paper or the end-use it is manufactured for, and as explained above, to be efficient, thermal paper has to be coated with particular chemicals-based formulations. Bisphenol-A is one of the chemicals used in those formulations.

B.2.4. Use of BPA in thermal paper

As shown in the section B.2.1, Bisphenol-A is used in the manufacturing of thermal paper.

B.2.4.1. BPA-containing thermal paper: tonnage and applications

As regards the tonnage of BPA used, the data gathered are shown in the following table.

Table 7. Tonnage of BPA used in thermal paper in Europe

	Tons/year	**Percentage of EU consumption**
Source 1 : EC, 2008	1,890	0.16 (2008)
Source 2: Registration dossier, 2012	2,400	0.2 (2013)

Total ('broke' waste excluded)*	**1,700 (2008)** **2,160 (2013)**	**0.14 (2008)**

*calculated figures

The use of BPA in thermal paper is very minor, especially compared to its use on polycarbonate or epoxy resins. It is estimated that 1,890 tons of BPA was used in thermal paper in the EU during the period 2005/2006, which is 0.16% of total BPA use in Europe (EC, 2008)[7]. The data extracted from the registration dossier for BPA provides the figure of 2,400 tons, which is consistent with the RAR figure and shows a rather steady growth of the market. Moreover, as explained in the previous section, 10% of thermal paper (the 'broke') is wasted during the manufacturing process and is sent out for recycling. As a result, of the 1,890 tons of BPA used as inputs in the supply chain, around 190 tons are wasted every year. The amount of BPA actually used in thermal paper in the EU can be estimated therefore at 1,700 tons (EC, 2008).

This quantity of BPA was used in 2006 to make $2.4x10^9$ m² of thermal paper, equivalent to approximately 168,000 tons of paper (EC, 2008). As already mentioned above, ETPA estimates the European thermal paper production at 540,000 tons in 2012 (including 229,000 tons produced for the EU market and 311,000 tons exported). Referring to the distribution of thermal paper among the different applications presented in Table 8, the tonnage used by application can be calculated. The following table shows this tonnage by application (third column).

Table 8. Tonnage of thermal paper by application produced in Europe (2008-2012)

Application	**Share for each application (2008-2012)**	**Corresponding tonnage of thermal paper for each application (2008)**	**Corresponding tonnage of thermal paper for each application (2012)**
Point-of-sale receipts	50%-65%	84,000t	351,000t
Self-adhesive labels	30%-20%	50,400	108,000t
Lottery tickets	≈10%	≈16,800t	54,000t
Fax	≈10%-5%	≈16,800t	27,000t
Other	<0.5%	<840t	-
TOTAL	100%	168,000t	540,000t*

*Source: ETPA (INERIS, 2013); 540,000 tons include 229,000 tons produced for the EU market and 311,000 tons exported.

[7] For comparison purposes, the worldwide use of BPA in thermal paper is estimated at 4,300 tons in 2012 (ETPA consultation 2013).

The figure for every EU country has not been possibly got, except that 35,000 tons per year BPA-containing thermal paper are placed on the DE market.

As shown in Table 9 below, the use of BPA in thermal paper has increased since the 1990s at a rather steady rate.

Table 9. Use of BPA for thermal paper manufacturing

Use	**BPA Tonnes/ year, 1996-1999**	**% EU consumption, 1996-1999**	**Tonnes/ year, 2005/2006**	**% EU consumption change since 1996-1999**	**Tonnes/ year, 2013**	**% EU consumption change since 2005-2006**
Thermal paper manufacture	1,400 (corresponding to 105,000 tons of thermal paper produced)	0.2	1,890 (corresponding to 168,000 tons of thermal paper produced)	+35	2,400 (registration dossier – 24.10.12)	+27

The fourth first columns are extracted from Danish E.P.A., 2013

Used in the manufacturing of thermal paper, BPA acts as a dye or color developer as a component of the thermal reactive layer (see figure above). As a developing agent, it causes a chemical reaction when the paper is heated, resulting in colour, images, figures, codes or words being produced on the paper EC, 2010; INSERM, 2010; INERIS, 2010.

Estimate of the share of BPA-containing thermal paper compared to total thermal paper on the common market

According to the data gathered on the use of BPA in thermal paper and to the different consultations carried out during the elaboration of this proposal (INERIS, 2013, see section G), BPA remains today the largest used dye developer in the thermal paper in Europe. The data gathered to that respect from the MSCA survey carried out by ANSES in 2013 indicate **an estimated share of BPA-containing thermal paper compared to the total thermal paper placed on the EU market ranging from 75% (1 claim) to 100% (1 claim) with a central estimate between 90% and 99% (3 claims). ETPA indicates that around 70-80% of thermal paper produced in Europe contains BPA (ETPA 2013 consultation).**

BPA has been used in the thermal printing of receipts for the last fifty years (Mendum, 2010; Schreder, 2010) and is widely used in eco-paper particularly. In that type of thermal paper, used mainly for cash receipts and credit card receipts, BPA is present in the free monomer form and offers no significant resistance to abrasion (Mendum, 2010). It may then be

transferable by contact with objects such as banknotes (EWG, 2010; Liao, 2011) and skin (Biedermann, 2010; Zalko, 2011).

BPA still dominates the market of dye developers in thermal paper due to its efficacy, availability, and low cost. Indeed, BPA is considered as efficient, available and cheap.

BPA is deemed very performing in particular for thermal eco-paper used for points-of-sales tickets and receipts which have to be printed fast and do not require any particular security features or longevity characteristic. BPA is less stable than other dye developers but is regarded as stable enough under standard conditions (Danish E.P.A., 2013).

As regards its availability, it can be considered as significant since it has been explained above that BPA is an HPVC with a worldwide production of 3,800,000 tons in 2006 (considered as growing steadily) and a EU production between 1,150,000-1,600,000 (see Table 9 above).

BPA is a rather cheap dye developer. As regards its price, the data gathered are shown in Table 10 below and indicates a EU market price ranging from 1,100€/ton and 1,800€/ton on average, that is an average price of about 1,500€/ton. A comparison of BPA price with the price of its potential substitutes is made in section F.2.

Table 10. Price of BPA in the EU per ton

EU BPA price	**Source**
1,200-1,800 €/t	MSCA Survey (ANSES, 2013)
1,535-2,800 €/t	INERIS, 2013
1,055-1,120 €/t	ICIS, 2009 (quoted in INERIS, 2010)
Average (min; max) range= [1,263; 1,906]€/ton	

However, given its toxicological and ecotoxicological profiles, the growing consumers demand for substitution solutions, the availability of alternatives dye developers on the market and the increasing regulatory actions BPA is being subjected to, its use in thermal paper is undoubtedly declining. The French Pulp and Paper Research & Technical Centre (CTP) confirms that trend as well as major manufacturers of thermal paper who claim to having started to substitute BPA to some other dye developers. One manufacturer in Slovenia states not using BPA anymore and according to a communication from Ricoh, the French manufacturer of top coating paper, BPA has not been used in top coating thermal paper since 2000, as it is not a rapid enough developer for this type of paper.

B.2.4.2. Concentration of BPA in thermal paper

As regards the concentration of BPA in thermal paper, several investigations have been carried out, leading to an average concentration between 1% and 2% (% weight). Thermal paper is

provided in different grades with differences in concentrations of BPA depending on specific needs. The reason for different grades is that different printers need different quality both in terms of run-ability (i.e. paper thickness) and print head temperature (Danish E.P.A., 2013). The investigations consisted, on the one hand, in a review of scientific literature and on the other hand, in the acquisition of own data by ANSES.

A review of the scientific literature

The literature review has identified 8 studies that document levels of BPA in thermal receipts. They are the studies by Biedermann, 2010, EWG, 2010, Mendum, 2010, Schreder, 2010, Liao, 2011, Danish E.P.A., 2011, Östberg, 2010 and Geens, 2012. They are summarised in ANSES, 2013 and below.

Biedermann, 2010 conducted a study in order to produce data on BPA concentration in a few thermal papers collected in Switzerland and evaluated the transfer of BPA from thermal papers to the skin of fingers under several conditions that were as realistic as (Biedermann, 2010). BPA transferred to fingers was recovered by moving fingers in 10 ml ethanol for 30 s. Ethanol extracts were diluted 1:10 with deionized water. For analysis, 500 µl diluted extract was injected into a 250×4.6-mm-i.d. column packed with Spherisorb ODS-2, 5 µm. BPA was eluted with a gradient of 50 to 100% acetonitrile (1 ml/min). Fluorescence was detected at 226/296 nm. Thirteen thermal papers were tested: 2 papers from chromatography recorders, 8 from various stores, 1 tram ticket, 1 train ticket and 1 cafeteria receipt. The papers were tested using HPLC with a quantification limit of 0.05%. Biedermann (2010) also studied the influence of the period between the transfer of BPA on the skin and its extraction, the nature of the vector and the washing of the hands.

Two papers out of the 13 did not contain any BPA (< DL). For the others, the mean concentration was 1.3% of the mass of the paper. Values were quite consistent and ranged from 0.8% to 1.7%. The mean amount of BPA deposited on normal skin was 1,13 µg/finger, values ranging from 0,1 to 3 µg/finger after handling the thermal paper for 5 sec. BPA amount transferred to skin was about ten times more if these fingers were wet or very greasy. Biedermann *et al.* (2010) show an almost constant BPA amount on skin whatever the contact duration (from 5 to 60 sec) and the contact repetition (from 3 to 10 contacts) with thermal paper. The contact with a BPA-containing thermal paper, followed by 3 contacts with a BPA-free paper, did not show any significant decrease of BPA amount on skin. Biedermann estimated a 27% dermal absorption rate calculated from the amount of BPA transferred onto the skin of the finger after a single contact of 5 seconds with a receipt, and the amount of BPA which was no longer removable from the skin by soap and water 2 hours after this contact. Similarly, a 60% maximal absorption rate is estimated by Biedermann, 2010 2 hours after immersion of the finger in a BPA/ethanol solution.

The North American organization "Environmental Working Group" determined the BPA content in sales receipts collected on the North American continent and in Japan, and evaluated transfer of BPA from one receipt to another (EWG, 2010). Thirty-six receipts were collected in retail shops in 7 States and in the district of Columbia: 10 private national chains (supermarkets, hypermarkets, service stations, pharmacies, fast food outlets, banks and ATMs); 3 public institutions: the U.S. Postal Service, and the cafeterias of the Chamber of Deputies and the Senate; 1 local supermarket in Colorado.

For each establishment chain, one receipt was sampled in 2 or 3 different cities. Three receipts were also collected in Japan in chains present in the United States (McDonalds, KFC, etc.). The receipts were collected at tills and immediately placed in a polyurethane tube. The date, place, the humidity and temperature were recorded. The receipts were weighed, measured and photographed. A 200 mg portion of the receipt was cut and placed in a glass tube. The samples were incubated in methanol for 3 hours at room temperature and stirred sporadically. Methanol was then transferred to clean and diluted tubes. BPA was extracted and determined by HPLC-CoulArray. The DL and QL were not specified in the study. To quantify the BPA transferable from one paper to another, 4 receipts containing BPA were rubbed with a previously moistened receipt without BPA**. BPA was detected in 16 of the 36 receipts of the sample, with a mean concentration of 1.9%.** The values ranged between 0.8 and 2.8%. None of the 3 receipts collected in Japan contained BPA in detectable amounts. With respect to the extent of the transfer of BPA by friction of a receipt containing it to a moistened receipt not containing it, the 4 receipts tested all showed BPA transfer. The transferred share ranged from 0.7 to 3.8% of the BPA contained on the receipt, with an average of 2.4%.

Mendum, 2010 collected unprinted receipts from 10 local, North American retail stores (1 receipt per business) to determine the BPA content of receipts collected (Mendum, 2010). The objective of the study was not to tend towards a representativeness of either the typology of the shops or the geographical distribution. To carry out the BPA extraction, a 200 mg portion of each receipt was cut, weighed precisely and placed in a capped Teflon tube. BPA was extracted and determined by GC/FID. The DL was 3.1µg/mL and the QL was 9.4 µg/ml. **Eight of the 10 receipts tested revealed quantifiable levels of BPA ranging from 0.3 to 1.7%.**

Schreder investigated the extent of thermal papers containing BPA in the North American market and the migration of BPA from the paper to the skin (Schreder, 2010). BPA concentration was measured in receipts received and bank notes, as well as the amount of BPA transferred to the skin after the normal handling of receipts. Twenty-two receipts were collected in 10 States, as well as in Washington DC. The receipts were minimally handled and placed in aluminium foil for transport to the laboratory. To achieve the extraction of BPA, the receipts were weighed and calibrated at 0.1 g of paper. BPA was extracted and determined by GC/MS. The DL was 50 ppm. Half of the paper tested contained BPA, indicating that this type of paper is very widespread but there are alternatives**. The BPA represented up to 2.2% of the total weight of a receipt (0.9% to 2.2%; average about 1.7%)**.

Liao, 2011 measured the concentration of BPA in various types of paper and related products present in the North American market as well as in Japan, South Korea and Vietnam, and estimated the potential exposure to BPA after the handling of these papers (Liao, 2011). Receipts (n = 103) were collected in various American States, as well as in Japan, South Korea and the Vietnam. A sample of approximately 19 mm in diameter was taken at the receipt centre and was weighed (about 0.0172 g). The BPA was extracted and tested using LC-MS/Ms. The QL was 0.1 $ng.g^{-1}$. **The BPA was detected in 94% of the receipts tested with concentrations ranging from a QL of 13.9 $mg.g^{-1}$ (geometric mean = 0.211 $mg.g^{-1}$), or 1.10^{-7} to 1.4% (geometric mean = 0.0211%)**. Liao, 2011 also measured the concentration of BPA in various parts of 6 thermal paper receipts. There was no difference in BPA concentration dependant on the location of the receipt analysed. BPA was also found in an unused roll of thermal paper (average: 3.06 ± 3.65 $mg.g^{-1}$).

In 2010, the Danish EPA wanted to illustrate to what extent exposure to BPA through receipts may be a health problem for Danish consumers (Danish E.P.A., 2011). Twelve receipts were collected in several types of stores: Grocery stores, stores using thermal paper with a long shelf life (furniture stores, etc.), stores using resistant thermal paper ("top-coated") and a library. The receipts were measured and weighed. The samples were analysed by GC/MS in order to search for the presence of BPA and BPS. BPA was extracted and assayed by HPLC in reversed phase. The detection limit was 0.1 $mg.kg^{-1}$.

Nine of the 12 receipts contained BPA but 2 of these receipts contained very low concentrations suggesting that they were contaminated by other receipts. For the other 7 receipts containing BPA, the concentration varies between 8,700 and 17,000 mg BPA/kg (46-77 µg/cm²; mean = 11 400 mg BPA/kg, equivalent to 57 µg/cm²), or between 0.87 and 1.7% (average = 1.14%). The immersion of the receipts in artificial sweat for 5 seconds showed a migration from the receipts of 7-21 µg of BPA/cm² (10-37% of the concentration of BPA in the receipt). Four receipts were analysed to simulate a realistic situation of receipt handling. The average amounts of BPA found on dry, wet fingers, and with a cream were significantly different (11,103 and 28 µg BPA respectively).

A Swedish study by Östberg, 2010, quoted in the Danish EPA report, reported concentrations of BPA in receipts (Danish E.P.A., 2011). This publication is available only in Swedish - the data cited was extracted from the Danish EPA report. Four Swedish families collected receipts for a certain period. **BPA was detected in 100% of samples at concentrations ranging from 5,000 mg.kg-1 to 32,000 mg.kg-1 with an average of 15,800 mg.kg-1, or from 0.5 to 3.2% with an average of 1.58%**. The study also assessed BPA's migration ability between the receipt and the lining of the wallet. More than 2,000 $mg.kg^{-1}$ were found in the wallet lining and more than 86 mg/kg on 20 Krona coins. Therefore, the authors concluded that there is a secondary exposure to BPA from the same source, although it may seem negligible compared to the primary exposure.

Geens, 2012 collected 44 receipts in Belgium between the months of September and October 2011 (Geens, 2012). The receipts included ATM receipts, receipts from various businesses (book shops, service stations, clothes shops, cosmetics shops, food shops, supply store, gift shops and multimedia stores, etc.), restaurants and parking. **BPA was measured in all of the receipts collected. 73% of the samples had concentrations ranging between 0.9% and 2.1% (average = 1.46%)** which corresponded to 2.4 and 22.7 mg of BPA per receipt, taking into account the weight of the receipt. The 27% of the remaining samples had extremely low concentrations, between 0.0000044% and 0.1%, which could be due to contamination by other papers or traces due to recycling.

Acquisition of French data

Anses has commissioned a specific study of the SCL (*Service Commun des Laboratoires* or Joint Laboratories Services) (DGCCRF, 2011). The objective of this study was to measure the frequency and concentration of BPA in receipts collected in various retail stores, and a few receipts collected from various ATMs.

Between September 26 and October 5 2011, 50 printed receipts were randomly collected in different shops and ATMs of greater Lyon:

- 40 till receipts: 28 (70%) from supermarkets (large and medium supermarkets, hard discount, fast food chains, etc.), 10 (25%) from local shops (small supermarket, bakery, news shop, market, etc.) and 2 (5%) of fuel distribution service stations.

- 10 receipts from ATMs

The receipts were received, immediately after printing, from the hands of a cashier or from an ATM, and inserted (holding at the edges) between two sheets of aluminium foil placed in a paper envelope. In the laboratory, they were stored for 6 to 15 days in a dry area, away from sources of heat and light while awaiting analysis. Every receipt was measured and weighed. A sample measuring approximately 10 cm^2 was collected from the centre of the receipt. The latter was weighed (approximately 50 mg) and then subjected to extraction at room temperature in 10 ml of absolute ethanol in an ultrasonic bath for 10 minutes. The extract obtained was diluted 100 times in a 90: 10 acetonitrile/water mixture in a volumetric flask in which a known amount of internal standard (bisphenol A d-16) used for BPA was introduced. The resulting solution was injected twice in LC-MS/MS. The validity of the BPA calibration was between 0.02% and 10% of the weight of the receipt (for a 50 mg sample). The quantification and theoretical detection thresholds were 0.02% and 0.01% for BPA.

The results of that study are the following: of the 50 receipts tested, 72% (36 receipts) contained between 0.80 and 1.93% of BPA (median: 1.33%) or between 44 and 88 $\mu g/cm^2$ (median: 69 $\mu g/cm^2$).

An analysis of the study data showed a lack of correlation between the number of days separating the collection date and the analysis date on the one hand, and the BPA concentration in the receipts on the other. This analysis suggests the absence of influence of the period of sample storage on BPA concentration in the study's conservation conditions.

Summary of the data related to the concentration of BPA in thermal paper

The following table summarises the various studies described above.

Table 11. Summary of the 9 studies documenting the measurement of BPA in thermal receipts

	DGCCRF, 2011	Biedermann, 2010	EWG, 2010	Danish E.P.A., 2011	Östberg, 2010 quoted in Danish E.P.A., 2011	Mendum, 2010	Schreder, 2010	Liao, 2011	Geens, 2012
Country and year of sampling of the receipts	France 2011	Switzerland nd (2009-2010 ?)	USA and Japan nd (2009-2010 ?)	Denmark 2010	Sweden 2010	USA nd (2009-2010 ?)	USA nd (2010 ?)	USA, Japan, Korea and Vietnam 2010-2011.	Belgium 2011
Points of receipt collection	Large retail food or not, nearby businesses, service stations, ATMs	Chromatography, shop, public transport and cinema receipts	National stores, public establishments and local stores	Supermarkets, grocery stores, toy stores, bookshops, service stations, ATMs	Collection by 4 Swedish families	Not specified	Stores and restaurants	Supermarkets, grocery stores, banks, bookshops, service stations, restaurants, fast food	Bank, bookshops, shops (clothing, cosmetics, multimedia, etc.), restaurant, parking receipt, food business
Number of receipts tested	50	13	36	12	16	10 (unprinted	22	103 (different types of	44

						receipts)		papers and related products)	
Number of receipts containing BPA	36 (or 72%)	11 (or 85 %)	16 (or 44 %)	9 (or 75 %)	100 %	8 (or 80 %)	11 (or 50 %)	97 (or 94 %)	32 (or 73 %)
Concentration of BPA in the receipts (% m/m) **Average** **Min – Max**	**1.33 %[1]** **0.8–1.9 %**	**1.33 %** **0.8-1.7 %**	**1.9 %** **0.8-2.8 %**	**1.14 %** **0.9-1.7 %**	**1.58 %** **0.5-3.2 %**	**1.24 %** **0.3-1.5 %**	**1.70 %** **0.9-2.2 %**	**0.0211 %[2]** **<10-7–1.4 %**	**1.46 %** **0.9-2.1 %**
DL/QL	DL: 0.01% QL: 0.02%	QL: 0.00005%	Not specified	DL = 0.00005%	DL = 0.00005%	DL: 0.09% QL: 0.26%	DL: 0.005%	QL: 10-7%	QL = 0.000001%
Dosage method	LC-MS/MS	HPLC/fluo	HPLC/ CoulArray	HPLC/GC/MS	-	GC/FID	GC/MS	LC/MS-MS	GC-ECNI/MS
Search for other bisphenols	BPS	-	BPB, BPS, BPF	BPS	-	-	-	-	-

[1] Median ; [2] Geometric mean, includes receipts containing traces of BPA contrary to other studies

Overall of these studies, the BPA concentrations range from 0.3% to 3.2% of the weight of the receipt with an average at 1.46% (receipts containing trace amounts of BPA not taken into account and median and geometric mean excluded).

These concentrations are consistent with the data collected from the INERIS, 2013 survey and the US EPA, 2012 report which indicate a concentration between 1% and 2% (% weight) (INERIS, 2013; US EPA, 2012). The Danish EPA figures, concerning evolution of BPA quantity used in thermal papers between 2000 and 2006, indicate that the average concentration of BPA in thermal papers have decreased from 1.3% to 1.1% between 2000 and 2006 (percentage in weight of paper) (Danish E.P.A., 2011).

INERIS, 2013 also indicated that recycled paper may contain traces of BPA because of the frequent presence of POS receipts in the paper recycling chain. However, the four thermal paper manufacturers consulted declared not using recycled paper to produce thermal paper (ETPA also confirmed this information, personal communication). Therefore, logically, thermal paper should not contain BPA if this substance is not intentionally added.

Moreover, as regards this concentration of BPA in thermal paper, it has been raised from the consultation of stakeholders that this concentration is optimized and fully adjusted to the functional characteristics targeted for each specific end-use (printing durability, speed, printing device, etc.). As a result, the BPA content currently present in thermal paper can be considered as the content which guarantees the technical efficiency of the thermal paper. In other words, any significant decrease of this content would degrade the thermal paper properties (INERIS, 2013). For example, a reduction in the concentration of BPA in thermal paper could impact on the thickness of the thermal coating and subsequently its performance: the density of image on thermal paper relates directly to the concentration of BPA in the coating layer and the thickness of the layer is often dictated by the requirements of the client (RPA, 2003).

Finally, it has to be noted that the 'concentration' of BPA is a term to be handle with precaution. Indeed, only the reactive layer of the thermal paper actually contains BPA and the data collected from the studies mentioned above correspond to the analysis of the entire ticket thickness. It might thus be rather easy to make the BPA content of a thermal ticket artificially fallen down by increasing its thickness (its so-called 'grammage').

Discussion about the studies measuring the concentration of BPA in thermal paper

Five of these studies collected receipts in order for the sample to tend towards a representation of receipts circulating over the territory, in terms of geographical distribution or type of trade from which they originate (Schreder, 2010; EWG, 2010; Liao, 2011; Geens, 2012,; DGCCRF, 2011). In fact, in the Schreder study, receipts were collected in 10 Northern States, as well as in Washington, DC. Although the receipt sample may not be representative in terms of nature and size of store investigated, the receipt collection method seems to show willingness for geographical representativeness across the United States. In EWG, 2010, the receipts collected came from different types of distributors and were collected in 7 different States and in the District of Columbia. Of the 36 receipts collected, 10 receipts came from service or national retail chains, 3 public institutions and a local supermarket in Colorado. Similarly, in the Liao and Kannan study (Liao, 2011), of the 103 receipts collected, 83 came from 7 different cities in the United States, and the other receipts from other countries.

The nature of the business from which the receipts were collected varies: supermarkets, grocery stores, banks, bookshops, service stations, restaurants and fast food restaurants. As well, in the Smith *et al.* study, 44 receipts were collected in Belgium in a variety of businesses (grocers and other shops, bank, book shop, service station and parking lot), randomly, but without information on their geographical origin. In the French study, all of the receipts were collected from the Lyon metropolitan area, the objective is therefore not representativeness in terms of geographical distribution, although it can be assumed that these results can be extrapolated to other similar towns in France. However, the nature of the shops investigated, following the example of the EWG, 2010, Liao, 2011 and Smith *et al*. studies, was voluntarily varied: Retail food or not, local businesses, service stations, ATMs.

On the other hand, Mendum (2010) indicated that the sample was not representative of paper used in the United States, either in terms of size and nature of the store, or according to their geographical distribution. Similarly, the variability between different stores of the same chain was not taken into account. In the Biedermann (2010) study, of the 13 thermal papers collected, 2 are derived from chromatography registers, 8 come from various stores, 1 is a tram ticket, 1 is a train ticket and 1 is a cafeteria receipt. The choice of shops, as well as the proportion of the number of receipts from stores in relation to the rest have not been justified, but reveal a willingness to mix the sources of the paper, although that is not representative of the receipts available to the general population. Furthermore, no information as to the location of the collection of the receipt was given.

As a result, only the Schreder, EWG and Liao and Kannan studies can be considered as a first estimate of the diversity of receipts containing BPA accessible to the American population, as well as the study by Geens, 2012 for the Belgian population and the SCL study for the population of Lyon (Schreder, 2010; EWG, 2010; Liao, 2011; Geens, 2012; DGCCRF, 2011). The first two consider that between 44% and 50% of retail receipts contain BPA (excluding receipts containing trace amounts of BPA). These values are comparable to the SCL study, which reported a proportion of 57 percent originating from retail receipts. More remarkable is the coherence between this SCL study and the Geens study that reports, respectively, a proportion of 72% and 73% of receipts containing BPA, regardless of the nature of the business where they originated. In the Liao and Kannan study (Liao, 2011), almost all receipts collected in the United States, Korea and Vietnam contained BPA in detectable amounts, however, this result includes receipts containing very low traces of BPA, unlike the proportions cited in other studies. With respect to the receipts collected in Japan, none contained BPA, probably in connection with the prohibition of its use in 2003 and initiated in 1998 by the Japan Paper Association (EWG, 2010).

Although the analytical methods were not always similar in these studies, the BPA concentrations in the receipts are consistent. The concentrations measured on the BPA-based receipts are all largely quasi-systematically higher than the analytical detection and quantification limits, strengthening confidence in the results obtained from this test. The average surface of a receipt varies according to the studies: Mendum, 2010 evaluate it to about 240 cm², whereas it reaches between 382 and 2294 cm² according to EWG, 2010. In DGCCRF, 2011, the average surface of 136 cm2 was lower. In fact, the length of the receipt is variable depending on the number of items purchased and if the store prints promotional receipts on thermo-printed receipt in addition to the cash receipt. According to American scientists, the total mass of BPA on the thermal receipts would be 250 to 1000 times greater

than the amount of BPA typically found in a food can or the quantity of BPA leaching from a BPA-laden plastic baby bottle to its content (EWG, 2010).

Finally, French data confirm that BPA is mainly used in the thermal "eco-paper"-type paper (receipts, cash receipts, credit card slips, debit card slips). With respect to top coated thermal paper (or "protected thermal papers") most often used for the transportation tickets, cinema tickets and adhesive labels (food packaging, etc.), for example, BPA seems to not having been used since 2000 according to a communication from a French manufacturer of top coated thermal paper (ANSES, 2011).

Although the use of BPA in thermal paper is decreasing according to the Technique Paper Center (CTP), all of these data show that BPA seems to be still widely used as a component of thermal paper which is placed on the market. Together with the MSCAs and INERIS surveys carried out in 2013 (INERIS, 2013), the market data show that BPA is at least undoubtedly present in 13 EU countries (France, Belgium, Denmark, Germany, Bulgaria, Ireland, Poland, the Netherlands, Portugal, Slovenia, Spain, Sweden and the UK). There is no particular reason that this would not be also the case in the other EU countries. Therefore, one can realistically expect that BPA is still largely present in the market of dye developers in thermal paper in the whole EU (at least in eco-paper receipts – the most common type).

B.2.5 Uses advised against by the registrants

There are no uses advised against in the CSR of the lead registrant dated from the 12 March 2012.

B.2.6 Description of targeting

As already mentioned above, the targeted population for that restriction proposal is pregnant women in terms of potential risks to the unborn child, due to their exposure (as workers and consumers) to BPA contained in the thermal paper they might handle.

B.3 Classification and labelling

B.3.1 Classification and labelling in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation)

The classification of BPA is harmonised in Annex VI of CLP under the index number 604-030-00-0 as follows as a direct translation of TC C&L:

Table 12. Classification of BPA under CLP and 67/548/CEE regulations

According to the CLP (regulation 1272/2008)	According to the 67/548/CEE directive / DSD (Dangerous Substance Directive)
Repr. 2 – H361f; STOT SE 3 – H335; Eye Dam. 1 – H318; Skin Sens. 1- H317;	Repr. Cat. 3; R62; Xi; R37-41; R43; R52;

Classification of BPA was inserted in the 29th ATP (directive 2004/73) of Annexe I of Directive 67/548/EEC for the human health effects and in the 30th ATP (directive 2008/58) of Annexe I of Directive 67/548/EEC for the N; R52 classification.

A classification proposal for BPA was submitted by the UK CA at the TC C&L during its work for the Risk Assessment Report (RAR) on this substance (UK, 2008). In 2002, BPA was classified as reprotoxic cat. 3. The initial proposal of the UK was to classify the BPA as Repr. Cat. 2; R60. Nevertheless as some member states stressed the fact that classifying the BPA as Repr. Cat. 2 is a borderline case and could create precedence, the member states chose (after a divided vote) to rather classify the BPA as Repr. Cat. 3 for fertility and to discuss concerning the effects on development when more studies would be available. However, it seems that these new data have never been provided and then the classification was not discussed again later.

In 2011, ANSES (French Agency for food, environmental and occupational health and safety) published a report on the hazards of BPA demonstrating its effect on fertility. Therefore, the French Competent Authority considered that the classification for sexual function and fertility needs to be revised on the basis of the report of ANSES (ANSES, 2011). The French classification proposal is Repro 1B H360F. Currently, the classification dossier is in progress. The public consultation on the BPA classification dossier took place during summer 2013.

B.3.2 Classification and labelling in classification and labelling inventory/Industry's self classification(s) and labeling

At present, 41 aggregated notifications exist in the inventory of industry's self classification and labeling. The endpoints proposed for self classification are the following: Skin Sens. 1 H317; Eye Dam. 1 H318; STOT SE 3 H335; STOT SE 3 H370; Repr. 2 H361; Aquatic chronic 2 H411; Asp. Tox. 1 H304; Muta. 1B H340; Carc. 1B H350; Ox. Sol. 3 H272; Acute Tox.4 H302; Eye irrit. 2 H315, H319; Acute Tox 4 H332;

Some of industry's self classification proposes not to classify BPA.

B.4 Environmental fate properties

Not relevant for this proposal.

B.5 Human health hazard assessment

The request of the French authorities was to evaluate if effects through reproductive and reliable studies could be identified at lower doses than the NOAEL of 5 mg/kg bw/day of BPA which was used to establish the current TDI (Tolerable Daily Intake) by EFSA.

B.5.0 The choice of the studies for BPA risk assessment

B.5.0.1. General considerations

B.5.0.1a Mechanisms of action

Knowledge of BPA's mechanisms of action is an important element to consider in order to be able to transpose to humans the effects observed in animals. BPA is known as a weak agonist of oestrogen receptors α and β (ERα and ERβ). It can be affirmed that not all BPA's

mechanisms of action are yet known. However, a growing number of *in vitro* or molecular studies suggest that interpretation of BPA's toxicological effects cannot be limited to a classical oestrogenic mechanism (NTP-CERHR, 2008). BPA may also interact with other cellular receptors such as the androgen receptor AR and cause a moderate anti-androgenic effect, and the aromatic hydrocarbon receptor (AhR), the transmembrane oestrogen receptor, the thyroid hormone (TH) receptors, as well as the transmembrane receptor GPR30 which is involved in cell proliferation (Bonaccorsi *et al.*, 2008; INSERM, 2011; Iordanidou *et al.*, 2010). In addition, BPA diglycidyl ether (BADGE) and BPA are capable of inducing expression of the nuclear receptor involved in the proliferation of PPAR γ (Bishop-Bailey *et al.*, 2000; Kwintkiewicz *et al.*, 2010). Most recently, BPA was also identified as an oestrogen-related receptor □ (ERR γ) ligand, whose natural ligands and specific physiological functions are unknown. Consequently, any interpretation of BPA's effects only in terms of an oestrogen-mimicking effect would be simplistic. The involvement of several of these systems during exposure to BPA could explain some effects observed at low doses, due to a possible synergy of action, but the non-monotonic dose-response relationships reported in some studies could explain them as well. Indeed, it is easy to imagine that strong responses to low doses on a given hormonal pathway may trigger feedback phenomena, well known for some hormones, and that at higher doses of BPA, the effects observed are lower. Finally, mechanisms of action other than those via links with hormone receptors are also mentioned, such as the activation of expression of certain genes at embryo level, or the modulation of second messenger systems.

B.5.0.1b Study models

(a) Epidemiological studies

Epidemiological studies provide very valuable data for highlighting associations between exposure to a substance and the presence of health effects, as they avoid interspecies transpositions. However, the epidemiological data identified for this risk assessment have many limitations, rendering difficult their use to determine an association between health effects and exposure to BPA.

First, many studies are hampered by classic methodological biases (sample size too small, selection of exposed population and controls, method of data collection, etc.). Secondly, many epidemiological studies are cross-sectional studies that include a single sample as a measure of exposure. In general, cross-sectional studies are rarely suitable for studying effects requiring a long latent period: extrapolating from a single exposure measurement taken at a period contemporary with the study may not be representative of the exposure that led to the initiation of the disease, mainly because of the changing uses of the substance and exposure to it. However, in this specific case, BPA is a ubiquitous substance with recurrent exposure and a short half-life compared to other environmental contaminants. Obtaining a single sample to assess the mean internal exposure levels in a population may therefore in some cases (e.g. adequate sample size and random sampling throughout the day) be legitimate (Ye *et al.*, 2011). Moreover, cross-sectional studies may be appropriate in the following two cases:

1. The study of a link between exposure at time *t* and a short-term effect (for example, the association between a single measurement of BPA in plasma and plasma levels of a hormone, in the case in which BPA induces a change in hormone secretion, synthesis, transport and metabolism).

2. The study of an effect resulting from exposure during a known and well-defined window of susceptibility, in order to characterise exposure at the most relevant period with respect to the expected effect.

The limitations of epidemiological studies should therefore be analysed on a case-by-case basis, taking into account the exposure period with regard to the critical phases of development, and the dosing period for BPA, in particular for assessing deferred effects due to exposure during development.

Finally, some studies were excluded because of known bias in selection of the study population, bias related to the exposure assessment (blood samples stored in plastic tubes containing BPA) or inaccuracies in measuring the health effect (self-questionnaire).

For this risk assessment, 29 epidemiological studies were identified and assessed. Eleven studies were not selected for the characterisation of health effects because they were considered as having major methodological limitations. The analysis of these studies is described here below.

(b) Technical limitations in experimental studies

Many parameters can influence the results of experimental studies. Taking them into consideration is particularly important since the doses administered in studies are low, in some cases close to the contamination levels making up the environmental background, and the observed effects are also sensitive and subject to wide variability. This is largely true in the toxicity studies on endocrine disruptors in general and BPA in particular. Failing to consider these parameters in the study protocol can, in some cases, lead to bias in the results observed. The main parameters are therefore detailed in the Annex to AFSSA's Opinion of 29 January 2010 (AFSSA, 2010a).

Most of the expert appraisals conducted by other national or international bodies also address this issue. Thus, Health Canada in its 2008 report considers that the divergent results on exposure to BPA at low doses could be explained by a number of experimental variables (Health Canada, 2008). For example, those parameters include the choice of animal species, the strains used, the variability related to tissues, food (especially the level of oestrogenic contaminants), the inappropriate use or lack of positive controls and the consideration of exposure-related effects that present a non-monotonic dose-response curve (Richter *et al.*, 2007; vom Saal and Hughes, 2005) (vom Saal *et al.*, 2005). In addition, the period of exposure to BPA with regard to the critical phases of development is an important consideration, especially for the assessment of delayed effects resulting from exposure during development. Moreover, the nature of the effects is such that it is difficult to characterise the degree of potential 'harmfulness' and, therefore, to determine their importance in a human health risk assessment.

As part of ANSES' expert appraisal, depending on whether or not some of these biases were taken into consideration when analysing the studies, they may or may not have been used to assess the toxicity of BPA. Thus, the Experts Working Group considered the following points:

- Choice of animal species and strain,
- Sample size,
- Presence or absence of one or more positive control groups,

- Nature of cages and containers (feeding bottles, etc.),
- Composition of the litter, diet and water quality,
- Route of exposure and method of administration.

The assessment of some parameters such as route of exposure, method of BPA administration (gavage, infusion, injection, etc.), period of exposure *(in utero,* during lactation, in adulthood, etc.), or co-exposure to oestrogen-mimicking substances is important for interpreting the study results. They are not, however, strictly speaking, major methodological limitations that could jeopardise the quality of the study. Studies whose methods have non-major methodological limitations have therefore not been excluded *a priori.* The working group considered that treated and untreated batches were subject to the same co-exposures. The risk here is not to demonstrate an effect that does not exist, but rather a loss of potency for the study. Although weighted, these studies with "non-major methodological limitations" add to the weight of evidence formed by all the studies, which is constructed by using a method detailed here below based on the methodology used for the hazard assessment. However, studies conducted without a negative control were considered as having major methodological limitations and were not selected for the health effect assessment.

In addition, in most experimental studies, internal exposure data appear only rarely, which is considered as a major limitation to judge about the relevance of experimental exposure schemes with regard to the level of contamination of human populations.

(i) Choice of laboratory animals: species, strain and origin

It has been proven that different animal species have varying sensitivity to hormone-mimetic compounds. In addition, the sensitivity of strains can vary within the same species; for this reason, the NTP stated in 2001 that due to clearly demonstrated differences in sensitivity between species and strains, selection of the animal model should be based on the ability to respond to compounds with an endocrine activity (i.e. the response to positive controls) and not on convenience and habit (NTP, 2001).

As with many chemicals, most experimental studies are performed in rodents. According to Chapel Hill, the Sprague-Dawley (SD) rat marketed by Charles River Laboratories (CD-SD) may have lost susceptibility to exogenous oestrogens (Richter *et al.*, 2007). However, this observation should be modulated depending on the parameter analysed (EDMVS, 2003). Moreover other authors have shown effects at low oestrogen doses using the SD rat strain: for example, in a four-generation study associating exposures with different windows of development and a chronic toxicity study, mammary hyperplasia in males was induced at 0.2 mg/kg bw/day of EE2 (Latendresse *et al.*, 2009).

According to Richter *et al.* the CR-SD strain was developed from the Sprague Dawley strain by the Charles River laboratory in 1950 (Richter *et al.*, 2007). This colony continuously underwent selective breeding based on rapidity of postnatal growth and large litter size. Then in 1991 and 1997, new colonies were established from selected animals (vom Saal and Hughes, 2005). Spearow *et al.* observed that rodents selected for their high fertility and high growth rate, such as the CD-1 mouse, were more oestrogen-resistant (Spearow *et al.*, 1999) and this observation is consistent with the loss of oestrogen sensitivity in CR-SD rats reported by Chapel Hill. Similarly, according to the NTP in 2008, *"it is evident that the SD rat and other rat strains are less sensitive to the effects of estrogens than the F344 rat. However for some traits, the reverse is true"*.

(ii) Sample size

Sample size is a criteria taken into account in the classification of studies. Low numbers of animals may lead to a reduction in the potency of the study, and thus a failure to demonstrate existing effects. However, there is no required minimum sample size for studies, because it depends on the incidence of the effect sought in the control group and its variability. According to the NTP-CERHR, a sample size of 6 animals per experiment and per dose seems reasonable for effects with a low degree of variability (e.g. body weight), but is not sufficient for effects with high inter-individual variability (rate of circulating hormones, etc.) (NTP-CERHR, 2008).

(iii) Positive control

The opinion of the NTP-CERHR is consistent with the view of several panels of scientists on the fact that the use of a positive control group can be very useful in evaluating the sensitivity and performance of an experimental model (NTP-CERHR, 2008). According to the experts panel that met at Chapel Hill, a study without a positive control should be considered uninterpretable (Richter *et al.*, 2007), while the NTP considers that the positive control is not essential in animal studies, especially when using animal models that are well known for the characterisation of certain effects (NTP-CERHR, 2008). In contrast, both panels agree that a study showing no effect in the treated groups and no significant effect in the positive control is not admissible.

According to the NTP, the substances most commonly used as positive controls are diethylstilbestrol (DES), ethinyloestradiol (EE2), 17 β-oestradiol and oestradiol benzoate (NTP-CERHR, 2008). These are the substances that initially led to BPA being considered as an oestrogen-mimicking substance. However, 17 β-oestradiol cannot be used as a positive control for studies using the oral route because only 3% of the dose is absorbed (vom Saal and Welshons, 2006).

In previous assessments of the effects of BPA, studies which resulted in no response being observed for the positive control generally mattered less for evaluating the effects of bisphenol A. In addition, although natural or synthetic oestrogens are used as positive controls for BPA, a growing number of *in vitro* or molecular studies suggest that interpretation of BPA's toxicological effects cannot be limited to a classical oestrogenic mechanism (NTP-CERHR, 2008). INSERM states that BPA is a weak agonist of oestrogen that can bind to the nuclear receptors ERα and ERβ, but that it is also capable of binding to other nuclear receptors such as the androgen receptor AR and causing a moderate anti-androgenic effect (INSERM, 2011). Furthermore, BPA diglycidyl ether (BADGE) and BPA are capable of inducing expression of the nuclear receptor involved in the proliferation of PPAR γ (Bishop-Bailey *et al.*, 2000; Kwintkiewicz *et al.*, 2010). Most recently, BPA was also identified as an oestrogen-related receptor □ (ERR γ) ligand, whose specific physiological functions are unknown. Finally, BPA also has the property of binding to membrane forms of oestrogen, androgen or thyroid hormone receptors (Bonaccorsi *et al.*, 2008; Iordanidou *et al.*, 2010) as well as the transmembrane receptor GPR30 which is involved in cell proliferation (INSERM, 2011). Under these conditions, the positive controls selected do not necessarily cover all these binding possibilities and, therefore, all the effects that may arise.

In view of this, the working group chose not to immediately rule out studies that did not use a positive control: firstly, because BPA's mechanism of action has not been clearly identified, and therefore the relevance of a solely oestrogen-mimicking positive control (and similar to

oestradiol, DES, EE2 or oestradiol benzoate) is questionable; secondly, if an effect is observed with BPA in the absence of a positive control, it can be considered. However, if no effect is observed and there is no positive control, the study may be excluded. The choice of a positive control implies that strong assumptions are made *a priori* concerning BPA's potential mechanism of action. Therefore, a study showing a lack of response with a positive control was considered as acceptable when the mechanism of action is unknown.

(iv) Uncontrolled exposure

The AFSSA report summarises the consequences of "accidental" co-exposure in experimental studies that can lead to bias (AFSSA, 2010a). Indeed, cages, litter, food and water can cause uncontrolled exposure to BPA and other endocrine disruptors and thus modulate oestrogenic activity. In addition, studies use exposure to BPA at increasingly low doses which are thus ever closer to the background levels.

The study by Howdeshell *et al.* demonstrated BPA's transfer potential from the wall of the polycarbonate or polysulphone cage (Howdeshell *et al.*, 2003). The authors concluded that the animals are subjected to chronic exposure to bisphenol A, which may occur by contact or by licking the walls. Oestrogenic activity was measured *in vitro* by an "E-screen" assay, *in vivo* by a uterotrophic assay and the concentrations of BPA released in the cage were quantified by GC/MS.

Similarly, AFSSA mentions the possibility of oestrogenic contamination depending on the nature and quality of litter, but to date, very few studies have taken into account the contribution of litter to the total oestrogenic load the animals are subjected to (AFSSA, 2010a).

Finally, AFSSA summarises several articles indicating that the presence of phyto-oestrogens in the diet has an impact on the oestrogenic response (AFSSA, 2010a). Owens *et al.* show that the use of foods with a phyto-oestrogen content lower than 325-375 mg/kg bw/day does not affect the response to BPA of the OECD uterotrophic assay (Owens *et al.*, 2003). However, a uterotrophic effect was measured for phyto-oestrogen concentrations in excess of 600 mg/kg bw/day. In a review of the literature, Jensen *et al.* qualify these findings by stating that the sensitivity to the presence of phyto-oestrogens depends on the toxicological targets (Jensen and Ritskes-Hoitinga, 2007). While in many studies, the thresholds above which responses are influenced by phyto-oestrogens are between 300 and 400 mg/kg of food, some studies show that certain toxicological targets such as behaviour or development of hormone-dependent cancers can be affected by significantly lower levels. The presence of phyto-oestrogens may have significant effects on the reproductive system (daily weight gain, anogenital distance and vaginal opening) (Thigpen *et al.*, 2007), age of puberty (Thigpen *et al.*, 2003), feeding behaviour, body fat, serum parameters associated with metabolism (Lephart *et al.*, 2004) and social behaviour of adult male rats (Hartley *et al.*, 2003). The effects on reproduction and development may instead be exacerbated by a diet devoid of oestrogen, in laboratory animals subjected for several generations to diets rich in phyto-oestrogens. Ruhlen *et al.* explain that these laboratory animals develop an adaptive process that results in an oestrogenisation syndrome, when a diet rich in phyto-oestrogens is stopped (Ruhlen *et al.*, 2008).

According to the report of the panel of experts that met at Chapel Hill, even soy-free diets may contain phyto-oestrogens, so it is recommended to use the same batch of food throughout the study (Richter *et al.*, 2007). Vom Saal and Hughes therefore recommend developing a

standard diet appropriate for studies involving toxicological targets that are sensitive to oestrogenic substances (vom Saal et Hughes, 2005).

Concerning the drinking water provided to laboratory animals, this is most frequently tap water. However, it may contain chemical contaminants at trace levels, some of which may have a hormone-like activity. Nevertheless, all the data in the literature, when referring to BPA that may be present in drinking water intended for human consumption, mention concentrations of the order of a nanogram per litre. Furthermore, it is important to check whether the studies indicate the nature of the container that was used to dispense the drinking water.

Some studies have assessed the oestrogenicity of the cage, litter and food after successive extractions with organic solvents and optional purification on a Sep-Pak C18 cartridge, according to a previously published method. The extracts are ultimately suspended in the culture medium and their oestrogenicity measured by the E-Screen assay based on the MCF-7 breast cancer cell line's ability to proliferate in the presence of oestrogen (Soto *et al.*, 1992). Under these conditions, the oestrogenicity of the animal feed was estimated at less than 20 femtomoles of oestradiol equivalent per gram.

It should be noted that the E-Screen test, which is based on cell proliferation, is not recommended by the ICCVAM (Interagency Coordinating Committee for the Validation of Alternative Methods) since this proliferation may be due to mechanisms other than those strictly associated with the transcription of oestrogen response genes (ICCVAM, 2003). In addition to the E-Screen test, other bioassays, such as those based on the ability of genetically modified cell lines or yeasts to express one or other of the oestrogen receptors in response to oestrogens, are commonly used to measure the oestrogenic activity of materials, of feed matrices or of water (Ankley *et al.*, 1998; ICCVAM, 2003; Mueller 2004; OECD, 2009).

(v) Administration route, method and vector

- **Oral administration**

According to AFSSA, studies in relation to food contamination favour exposures *per os*, either by using gastric tubes for gavage, or by directly depositing the test compounds in the oral cavity using a micropipette (AFSSA, 2010a) (Palanza *et al.*, 2002).

The oral administration routes most widely used are **gavage** and **dispersion in feed** or **drinking water**. Administration by gavage offers greater accuracy of the administered doses than administration in feed and drinking water. On the other hand, it causes stress to the animal and does not offer the same kinetics as the other two methods of administration. Indeed, the dose of BPA is administered in one go, thereby inducing a plasma concentration peak of the substance. Administration in the drinking water and feed gives more linear kinetics, since the animal has the feed and water at will throughout the day, but the doses given are not as accurate. The feed is weighed before each administration, and the water bottles are graduated in order to evaluate the amount consumed. However, the feed can be spilled in the cage and the feed distribution is collective for all the animals in a single cage, which gives only an average consumption per animal.

Moreover, according to AFSSA, the vehicle used to solubilise and administer the test substances can modify the absorption or introduce compounds which are themselves active on the targets studied (AFSSA, 2010a). Thus, protocols using olive oil, which is rich in

polyphenols, introduce a possible risk of interaction between these polyphenols and endocrine disruptors tested at low dose.

- **Subcutaneous administration**

Numerous studies use the subcutaneous route to administer BPA, often diluted in DMSO (dimethyl sulphoxide). This may involve subcutaneous injection or a slow diffusion system such as implanted miniature pumps or a capillary system (permeable or with small pores throughout).

When BPA is administered by subcutaneous injection the daily dose can be controlled with greater accuracy. The dose administered can be corrected according to the modification of body weight during the study for long-term studies or exposures during gestation. The use of an osmotic pump or of a diffusion system facilitates repeated exposure studies and limits the stress on the animal subjected to repeated and invasive administrations, as well as making it possible to reproduce a linear exposure scheme, i.e. a scheme without an absorption peak. However, this method of administration has certain limits. Adapting doses according to changes in body weight during long-term exposures or during gestation is incompatible with the use of diffusion pumps. However, the age of the animal and its growth curve are important factors.

Subcutaneous administration bypasses the digestive barrier, intestinal and/or skin metabolism and the hepatic first-pass effect. In addition, transfer from the subcutaneous compartment to the bloodstream can also be influenced by the vector in which the substance tested was administered. According to the NTP, DMSO alone can cause a biological activity and is known to facilitate cell diffusion through the formation of channels (Zafar *et al.*, 2010). However, the NTP concludes that the impact of the use of high concentrations of DMSO is uncertain, and that this effect is probably weak at the amounts described in subcutaneous studies. Certain studies replace the use of DMSO with a 10:90 ethanol/sesame oil mixture in order to cause less skin irritation (Adewale *et al.*, 2009; Patisaul *et al.*, 2006). Moreover, when exposure is via the implantation of subcutaneous minipumps, some authors use pure DMSO as solvent. This practice is strongly advised against by the manufacturer of these pumps, which recommends a maximum concentration of 50% DMSO, otherwise the implant may dissolve, leading to tissue inflammation and oedema (NTP-CERHR, 2008).

Pottenger *et al.* used a kinetic approach to study exposure routes such as the oral, peritoneal or subcutaneous route (Pottenger *et al.*, 2000). The authors report a substantial difference in the pharmacokinetic parameters (bioavailability and metabolism) according to the exposure routes used. They warn against transposing the effects observed during subcutaneous exposure in particular, and recommend making this comparison with great care. Tominaga *et al.* also studied the impact of exposure routes (oral and subcutaneous) on BPA toxicokinetic parameters after administration at doses of 10 mg/kg and 100 mg/kg, in rats, chimpanzees and *Cynomolgus* monkeys (Tominaga *et al.*, 2006). Notable differences in kinetics were observed depending on the species and the routes of administration used. Thus, according to the NTP-CERHR in 2008, the main difference between oral and subcutaneous administration lies in the absence of a hepatic first-pass effect with subcutaneous administration (NTP-CERHR, 2008). BPA is known to undergo a strong hepatic first-pass effect. However, in rodents as in humans, hepatic metabolism in newborns is limited, consequently reducing the hepatic first-pass effect. The higher the doses, the greater this difference. Consequently, according to this report, the effects obtained with the subcutaneous studies are relevant only if the

exposure took place during the neonatal or juvenile period. Studies with subcutaneous administration in which the exposure took place in adults were only considered to be informative during the identification of the biological effects due to BPA.

To date, studies using subcutaneous exposure routes have not been taken into account in assessing the health risks arising from exposure through food, owing to the pharmacokinetic differences between the two routes of administration. Human exposures to BPA via routes other than the oral route, such as the cutaneous route (thermal papers, etc.), and quantitative studies of BPA penetration through the skin, have also recently been reported. Moreover, biomonitoring studies indicate urinary concentrations that are very much higher than those anticipated on the basis of the current food contamination data. One of the hypotheses put forward to explain this difference is that it could be due to the underestimation of an exposure route such as the cutaneous route.

Dose bioequivalences could be established on the basis of robust toxicokinetic data, in order to be able to use the results of subcutaneous studies as part of a health risk assessment.

The working group identified and examined 17 studies performed via subcutaneous exposure. These studies are recent since they were published between 2002 and 2010, eight of them published between 2009 and 2010. These studies cover prenatal or perinatal exposures and correspond to administered doses ranging between 0.1 and 97 000 μg of BPA/kg bw. Effects were observed at administered doses of less than 5000 μg of BPA/kg bw/d. The species used in the studies are predominantly rodents (nine studies carried out in rats, in particular on the Holtzman, Wistar and Sprague-Dawley strains) and seven in mice (CD-1, ICR/Jcl, BALBC). One study was carried out in Suffolk sheep. Ten studies relate to reprotoxicity and development. Given the types of effects observed and the low doses administered, the endocrine disruptor working group decided to examine closely the studies based on subcutaneous administration and to assess to what extent the results obtained could be extrapolated to other routes, in particular exposure via the oral route.

- **Other routes of administration**

The other routes of administration used are anecdotal. One study uses the intracerebral route (Matsuda *et al.*, 2010). This route is not representative of a human route of exposure, but is part of experimental protocols aimed at demonstrating mechanisms of action. In the context of risk assessment, it cannot be included in a characterisation of the effects of BPA.

Finally, some studies use the intraperitoneal route (Pottenger *et al.*, 2000), intravenous route (Kurebayashi *et al.,* 2002) or respiratory route, but these concern pharmacokinetic studies aimed at comparing the bioavailability of various routes. One study in ewes via the intravenous route is reported, aimed at determining the effectiveness of BPA as an oestrogen-mimicking substance that inhibits pulsed secretion of LH (Collet *et al.*, 2010).

(vi) Exposure doses

The recent data of Taylor *et al.* on animal models suggests that an external dose of 400 μg/kg bw/d (eight times the current TDI) given orally would be required to reproduce the plasma concentrations commonly described in humans (of about 1 ng/mL) (Taylor *et al.*, 2011). Most of this report is based on studies in which the administered doses are of this order of magnitude, or lower and/or below the NOAEL (5 mg/kg bw/d; orally).

It should also be noted that the purity of the BPA tested has not been taken into account because the level of details (most of the time the degree of purity is specified but the impurities are not specified) on that point vary a lot in between different studies.

B.5.0.2 Methodology used for the studies selection

At first, the methodology on the choice of the most reliable studies to carry out the risk assessment is explained in this part.

- **Bibliographical analysis**

The health risk assessment is based on prior work undertaken by expert assessment authorities, and particularly the European Risk Assessment Report prepared in 2008 by the United Kingdom (UK, 2008), the preliminary INSERM collective expert assessment report on BPA (INSERM, 2010), and the expert appraisal work undertaken by AFSSA in 2010 (Afssa, 2010). Moreover, the EFSA expert assessment report published in September 2010 (EFSA, 2010) and the report written by the expert panel which met under the leadership of the FAO/WHO that was published in November 2010 (FAO/WHO, 2010) were also taken into account. Past expert assessments have mainly considered recognised effects by the oral route of exposure, which have been deemed more representative of dietary exposure.

In addition to these expert assessment reports on BPA which have recently been published, original papers of studies considered as key studies for certain types of effects linked to a BPA exposure were also analyzed. Furthermore, particular attention was paid to **epidemiological studies** likely to contain information that could be interpreted in terms of human effects and experimental studies using the **subcutaneous** route of exposure. In fact, the latter type of study has not undergone systematic analysis in past expert assessments, generally effects observed following oral route are considered as relevant, since they have been deemed more representative of dietary exposure. Nevertheless, given the fact that questions have recently been raised regarding non-dietary BPA exposure, including **dermal exposure** and that subcutaneous route can highlight effects at doses much lower than those administered orally, it was indeed considered as relevant to take these studies into account.

Lastly, new scientific paper published from 2010 (when the preliminary INSERM report was published) to January 2011 (bibliography end date) were listed by ANSES and analysed (INSERM, 2010). In addition, some publications dated after January 2011 were included when they were relevant.

- **Criteria for the selection of the key studies for the previous HRA (EFSA TDI, Tyl *et al.,* 2002, 2008)**

There are two multi-generation reproductive studies investigating a wide range of BPA doses and carried out according to standardized test guidelines available (Tyl et al., 2002; 2008). These two studies currently serve as the basis for regulatory risk assessment of BPA in the EU (ECB, 2008; EFSA, 2006) and the USA (US FDA, 2008). These studies and their limitations are described in the relevant section.

A Tolerable Daily Intake (TDI) of 0.05 mg/kg bw/day has been defined in 2006 by the EFSA CEF panel, based on the multi-generation study of Tyl et al., 2002. This study, described in more details in the section B.5.9 provides a NOAEL of 5 mg/kg bw based on reduced body weights and body weight gains at the LOAEL corresponding to 50 mg/kg bw/day. Reproductive effects are observed only at the top dose level corresponding to 500 mg/kg bw/day. A standard safety factor of 100 was applied, without any additional uncertainty factor. In addition, the Panel considers, however, that its derived health-based guidance value should be a temporary Tolerable Daily Intake (t-TDI) rather than a full TDI, pending the outcome of the

long-term study in rats involving prenatal as well as postnatal exposure to BPA, currently being undertaken by NTP. This study will clarify whether the changes in the mammary gland seen in rats (as well as other species) will result in an increased incidence of tumours in this species. In 2010, a second opinion on BPA has been published by EFSA CEF panel, taking into account the new studies appeared since 2006 on BPA. The new studies pointed out some effects deserving further investigation but were not considered robust enough to modify the TDI. In January 2014, EFSA identified likely adverse effects on the liver and kidney and effects on the mammary gland as being linked to BPA exposure. Therefore, EFSA recommended that the current TDI be lowered from its current level of 0.05 mg/kg bw/d (equivalent to 50 µg/kg bw/d) to 5 µg/kg bw/d.

- **Methodology of the ANSES HRA: Classification of effects by organ and system**

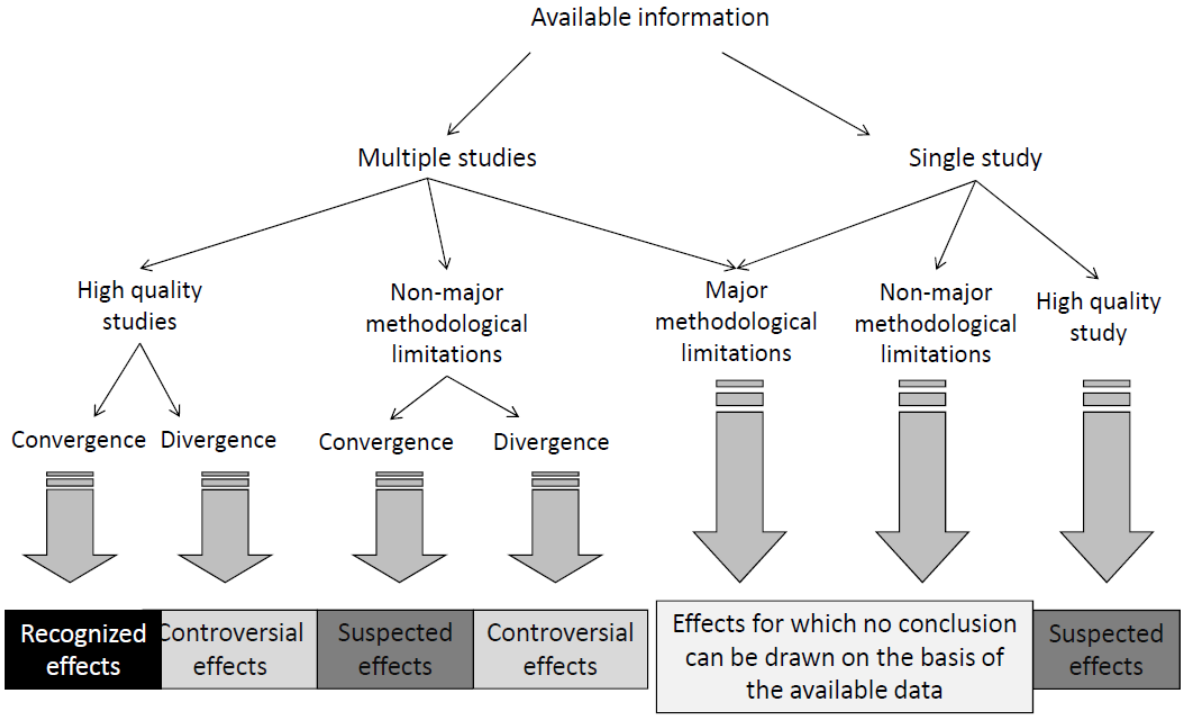
At first, the health effects associated with BPA (Anses, 2011) were classified by organ and system and were qualified using a decision tree by periods of exposure and by distinguishing the effects as: recognised, suspected, controversial, and effects for which the available data was not conclusive.

For each type of effect, the available data were presented by windows of exposure: gestational or *in utero*, prenatal, perinatal, neonatal, postnatal exposure or exposure during puberty or adulthood. The term 'exposure' does not provide information on the number of administrations (e.g. single or repeated).

For those references considered as significant in providing information about the health effects of BPA, particularly at low doses for which there is currently no consensus in the international scientific community, a publication analysis chart was used. The items on this chart list the important points to be specified when analysing articles, considering the limiting factors likely to interfere with the interpretation of results.

In order to qualify the health effects of BPA, the following decision tree was used.

Figure 7. Decision tree to qualify the effects of BPA



All the available information regarding a health effect was assessed using the decision tree, which can be interpreted as follows:

- When the available information was obtained from one or more studies, each study was analysed and considered either to be of 'good-quality', having 'non-major methodological limitations' or having 'major methodological limitations'.

A 'good-quality' study was defined as containing an appropriate methodology (coherence of the exposure model, confounding factors taken into account, etc.) and a sufficient number of observations.

A study was considered to have 'non-major methodological limitations' when one of the above aspects was not considered to be fully fullfiled. Nevertheless, the study could be taken into account in light of its contribution to the expert appraisal. Moreover, co-exposure had to be controlled (composition of feed for laboratory animals, type of cage, type of drinking container, etc.). If not controlled, the way the co-exposure was managed had to be mentioned. When a study had unacceptable shortcomings, it was considered as having 'major methodological limitations'.

- When the results of multiple 'good-quality' studies undertaken by different scientific teams:
    - converged: the effect was considered to be 'recognised',
    - diverged: the effect was considered to be 'controversial'.
- When studies having 'non-major methodological limitations':
    - converged: the effect was considered to be 'suspected',
    - diverged: the effect was considered to be 'controversial'.

- Studies having 'major methodological limitations' were excluded as they could not be used to draw conclusions.
- Lastly, when information was reported in only one study, the methodology was assessed:
    - when it was 'good-quality', the effect was considered to be 'suspected',
    - when it had 'major or non-major methodological limitations', the study was considered to be excluded and could not be used to draw conclusions regarding the effect under consideration.

Lastly, once the various types of effects had been characterised according to their level of evidence, the significance of the observed biological effects was thus discussed in order to estimate their relevance in terms of transposition to humans for the Health Risk Assessment (HRA).

Further to the hazard analysis, critical effects and key studies for the various exposure routes will be determined in order to define human toxicity values so as to undertake a quantitative HRA.

All of the studies analysed in this report were the subject of NOAEL or LOAEL determination. The NOAEL/LOAEL arising from these publications have been identified and classified by type of effect on a diagram to show the position of the various studies in relation to each other. For each organ or function considered, the most relevant critical effect(s) were selected, both in terms of levels of doses and transferability of this type of effect to humans. Then, for each effect, a key study was selected. The studies considered to be of good quality that reported these effects were attributed greater weight. Lastly, the DNELs derived from these studies (NOAEL and/or LOAEL) were proposed for use in the HRA.

As no proven effect was identified in humans, only the effects considered as "recognised" in animals or "suspected" in humans has been used as the main focus of the risk assessment.

- **The Study of BPA dose-response relationships: Non-monotonic relationships**

By definition, a dose-response relationship (or curve) is referred to as non-monotonic when the slope of the tangent (to the curve) changes in the range of the doses studied. Non-monotonic dose-effect relationships give rise to many debates. They are regularly described in numerous *in vitro* and *in vivo* toxicology studies on substances acting on the hormonal system, whether concerning endogenous hormones or ED. Several publications on BPA thus report greater effects at lower doses than those induced by higher doses and therefore describe non-monotonic dose-response relationships.

A survey of the publications describing non-monotonic dose-effect relationships concerning ED and more specifically BPA was conducted as part of a thesis (Lagarde F, 2012). However, before considering how to take them into account or not for the HRA, it is necessary to assess the plausibility of their existence. In January 2012, the bibliographical research on PubMed® enabled us to identify 17 BPA-related publications (8 relating to *in vitro* studies and 9 studies on animals). In total in these publications, 59 non-monotonic dose-effect relationships were identified for different types of effects: 11 *in vitro* and 48 *in vivo*. The effects associated with this type of relationship have also been reported.

In vitro, the non-monotonic relationships observed concern effects on the pituitary gland (prolactin release, phosphorylation of protein kinases), the heart (cardiomyocyte contractility), lipid metabolism (expression and release of adiponectin), prostate, and testes (cell proliferation).

In vivo, non-monotonic relationships identified in the literature concern effects on development (age of puberty, total weight), sexual behaviour, the activity of numerous genes involved in gluco-lipid metabolism, the mammary glands (structure and number of breast buds), the female reproductive system (ovarian transcriptional activity, alteration in the expression of hormonal receptors on uterine epithelial cells) and the male reproductive system (weight of the epididymis, seminal vesicles and preputial glands).

Then, for each non-monotonic dose-response relationship identified, the statistical and biological plausibility of its monotony was studied. The statistical plausibility rests on both the experimental conditions and the results of the statistical analysis of the observed data. As for the biological plausibility, it is based on the mechanisms of action that can explain the phenomena observed during the study (i.e. the interactions with receptors, activation of metabolic pathways, etc.). These two aspects were assessed but are not detailed here.

The results of the application of the analysis criteria of statistical plausibility to BPA are as follows (**Table 13**).

Table 13: Results of the statistical plausibility of the non-monotonic dose relationship applied to BPA in *in vitro* and *in vivo studies*

Statistical plausibility of the non-monotonic dose-effect relationship	***n* (*in vitro*)**	***n* (*in vivo*)**	***n* (total)**
null	0	0	0
Very Low	2	12	14
Low	2	10	12
Average	2	5	7
High	2	8	10
Very high	2	7	9

Half of the non-monotonic relationships observed concerning BPA have an average, high or very high statistical plausibility.

With respect to the assessment of biological plausibility, if one eliminates the dose-effect relationships where no return to the base effect is observed and those of "null", "very low" and "low" plausibility, 23 relationships out of 59 remain, which can be considered to have a medium, high, or very high plausibility. Of these 23 remaining dose-effect relationships, only one is not the subject of mechanistic explanation on the part of the authors. The other 22 envisage two valid mechanistic assumptions: the plurality of molecular targets and/or a negative retro-control.

In conclusion, before affirming the existence of a non-monotonic dose-response relationship, the statistical and biological plausibility of this non-monotonic relationship must be evaluated, for example, using the criteria proposed above.

Therefore, when a non-monotonic dose-effect relationship is present in a study (whether mentioned by the authors or not), it is necessary to apply the following decision tree (Figure 8).

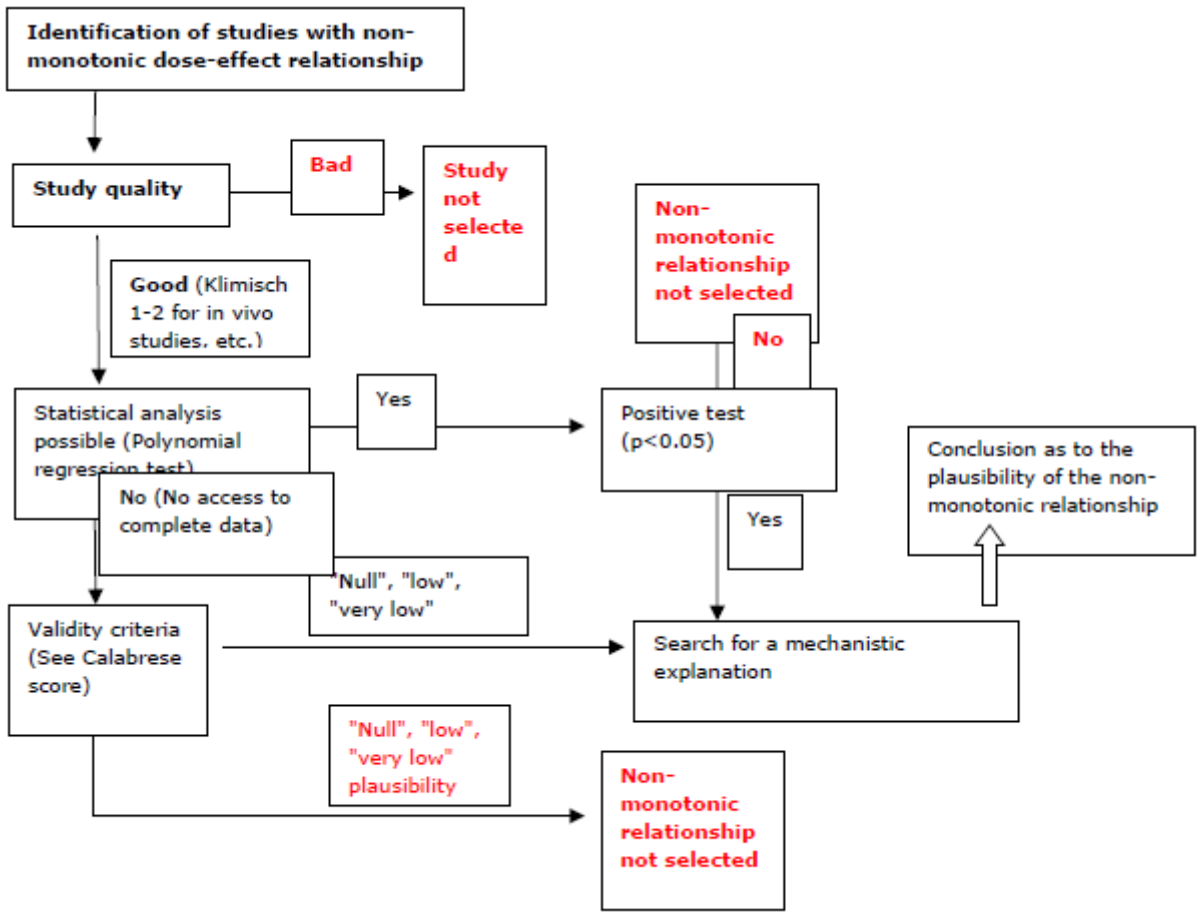


Figure 8 Diagram to aid in the analysis of studies showing a non-monotonic dose-effect relationship

However, more suitable analysis criteria remain to be defined. In fact, those used within the framework of this study relate only to the phenomena of hormesis. In order to assess any type of non-monotonic relationship, new criteria should be developed.

Taking into account non-monotonic dose-effect relationships in the quantitative assessment of risks associated with BPA was not possible due to methodological difficulties. The "standard" approach based on the choice of a starting point associated with a critical effect (NOAEL/LOAEL/BMD) was used by the experts at this stage. Discussions are currently underway at European (EU, EFSA) and

international levels (NIEHS) to define the best way to take into account these data showing a non-monotonic dose-response relationship. Depending on the conclusions drawn from these discussions, the BPA HRA process may be subsequently adapted and reviewed.

B.5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

This section included articles published until March 2012 only.

The analysis presented in this part has the objective of determining the absorption factors to be used for the health risk assessment (HRA) of BPA for both oral and cutaneous absorption. Particular interest is also given to the possibility of determining an absolute or relative bioavailability factor for the subcutaneous route, since this route of administration have been widely used during the experimental protocols put in place to investigate the toxicological properties of BPA. In addition, the option of using the kinetic models available in the scientific community has also been investigated. The main points of this analysis are given hereafter.

1. Absorption

    - Oral

In humans and other primates, BPA is rapidly absorbed by the gastrointestinal tract, consistent with its substantial aqueous solubility and lipophilicity. Analysis of the areas under the plasma concentration time curve (AUC) shows that gastrointestinal absorption is greater than 85% in rats and monkeys. The experiments carried out in adult human at relatively low doses (0.025 to 5 mg in total) show that BPA is rapidly and completely absorbed by the gastrointestinal tract (Tsukioka T, 2004; Volkel W, 2002; Volkel, 2005). After a single dose, the plasma peak is reached approximately 80 minutes after ingestion.

Bioavailability via the oral route:

Method of analysis of Bisphenol A in biological samples- a critical analysis

In addition to the human health effect assessment of BPA, an additional analysis was carried out in order to identify the critical points of the toxicokinetic studies, from a methodological point of view as well as from the point of view of interpreting the results for humans. This analysis also had the objective to determine the absorption factors to be used during the HRA of BPA for both oral and cutaneous absorption. Particular interest was also given to the possibility of determining an absolute or relative bioavailability factor for the subcutaneous route, this route of administration having in effect been widely used during the experimental protocols put in place to investigate the toxicological properties of BPA. In addition, the option of using the kinetic models available in the scientific community has also been investigated.

The pharmacokinetic and toxicokinetic studies conducted on rats, mice, monkeys, dogs, ewes, pigs, the publications on monitoring in humans, a study on the hepatic metabolism, two studies describing a PBPK model and publications on analytic methods have been analysed - in total 35 publications. Analysis of these data has been focused on the critical points of the experiments (animal phase or experimental phase, analytical method of the samples, PK or TK analysis of the data) likely to cause methodological biases

Overall, the analysis methods do not precisely meet the assessment criteria, at times using a value calculated between the DL and the QL. The estimate of the QL, based on the ratio DL/√2, influences, in particular, estimates of the time of elimination half-life. Asimakopoulos *et al.*, (2012) highlights this problem and its consequences in terms of predicting data between the DL and the QL.

Some recent articles (Yi *et al.*, 2010 ; Cunha *et al.*, 2010 ; Markkam *et al.* 2012) take care to validate the methods used. These studies describe the developments of new methods using detectors other than mass spectrometry (fluorometric detection) or optimised purification techniques (micro-extraction by liquid-liquid dispersion or DDLME). Furthermore, the simultaneous assay of BPA and BPA-G has also been the subject of specific developments (Lacroix *et al.*, 2011). These methods are generally developed in order to analyse biological matrices (plasmatic or urinary in animals and humans).

In terms of constructing a PBPK model, knowledge of the processes of metabolisation or of the enterohepatic cycle is essential. In fact, these processes have a direct influence on the level of circulating concentrations and thereby, on internal exposure to the investigated component. The necessity of a foetal compartment is also to be taken into consideration, as well as taking into account the passage into breast milk.

Relevance of the results

It is important to note that certain publications present calculations of unadapted parameters (e.g. time of elimination half-life, total clearance). In fact, the half-life times estimated on the basis of arithmetical averages are erroneous because the latter should be calculated on the basis of harmonic averages of the individual values (see Lam *et al*., 1985).

The choice of species which is the most representative of human toxicokinetics is particularly crucial. Whichever species is considered, there does not seem to be an accumulation of BPA over time. For a rapidly eliminated component (elimination half-life time of around 4 to 6 h in mice or monkeys), it is not surprising to observe an absence of accumulation during administration by tube feeding carried out every 24 hours. However, major differences are found from one species to another, even depending on the strains tested (see Prins *et al.*, 2011) and the gender (see Kurebayashi et al., 2005 - Table xxx). An enterohepatic cycle has been demonstrated in rats and dogs but has not been found in species such as monkeys, sheep, pigs and humans. The elimination routes also differ. The faecal route prevails in rodents and dogs, the urinary route is predominant in humans. The principal metabolite, the conjugated glucuronide of BPA, is found in every species with, however, major differences between rats, monkeys and humans. A sulphate form is also observed in a lesser quantity. It should be noted that the results appear similar between mice and monkeys for parameters such as total clearance. Recent results Collet, 2012 (Collet, 2012) demonstrate similarities in ewes, dogs and pigs in terms of kinetics for BPA and BPA-G: high metabolism, low bioavailability, high clearance, urinary excretion, and no accumulation. Moreover, Collet, 2012 has shown that the presence of BPA-G in the bile of dogs cannot be attributed to a phenomenon of coprophagia, a phenomenon commonly encountered in certain animal species.

Several authors have proposed models using either conventional approaches or using a PBPK model (Mielke and Gundert-Remy, 2009)[8]. The latter model presents a good departure point. However, for the time being validation of this model has only been done with a single point, and a sensitivity study remains to be conducted. The working hypotheses must also be refined with, for example, the influence of food (Sieli *et al.*, 2011) and the use of data obtained on

[8] Mielke H. and Gundert-Remy U., Tox letters, 2009

mice and monkeys, rather than on rats. Fisher *et al.*, 2011[9] have also developed a model specific to BPA, which seems ill-adapted to the prediction of data pertaining to oral administration. However, this model takes into account the intestinal and hepatic metabolisms. A comparative analysis of the different existing models is presented in appendix 6. Prospects for improvement are proposed; they are currently implemented as part of the development of the PBPK model of BPA sponsored by Anses.

On the basis of the available data, only the studies by Doerge *et al.* (2010) carried out on the Sprague Dawley rat and the Rhesus monkey for an administered dose of BPA of 100 µg/kg and Collet (2012) carried out on several species (ewe, pig, dog, Wistar rat, CD1 mouse) for an administered dose of BPA of 100 mg/kg **enable the determination of an absolute bioavailability of unconjugated BPA for the oral route**. These two studies report an absolute bioavailability in unconjugated BPA in rats of the same size, specifically: 2.8% ± 3.1% (Doerge *et al.* (2010)) and 3.03% (Collet, 2012) whereas the other values of absolute bioavailability in unconjugated BPA obtained on other species (e.g. Rhesus monkey, mouse, pig, etc.) are not supported by other experimental studies.

Consequently, it is recommended to retain an absolute bioavailability in unconjugated BPA of 3%. The value of absolute bioavailability of 0.2% is not retained due to the limited number of animals (4) and the high variability observed (of around 100%) in the study by Doerge (2010). The physiological model of Mielke and Gundert-Remy, 2009 constitutes a good departure point for modelling the kinetics of BPA. However, this model could be combined with that of Fisher in order to incorporate the intestinal metabolism. However, it should be observed that these models are developed for adults and do not take into account the foetus and the placentary passage. Biomonitoring studies are discussed in section B.9.3.2.2.2.

The absolute bioavailability factors determined by the oral route are recorded in the following table.

Table 14. Absolute bioavailability factors after administration of unconjugated BPA via the oral route depending on the animal species

Author	**Species**	**Dose**	**$F_{absolute}$ (bioavailability)**	**Measured component**	**Comments / Limits**
Doerge, 2010	**Rhesus Monkey**	100 µg/kg	0.19% ± 0.18%	**unconjugated BPA**	Number of animals: 4 females
Doerge, 2010	**Sprague Dawley Rat**	100 µg/kg	2.8% ± 3.1%	**unconjugated BPA**	Number of animals: 4 females

[9] Fisher *et al.*., Toxicology and Applied Pharmacology, 2011

Collet, 2012	**Ewe**	100 mg/kg	1.2% ± 1.1%	**unconjugated BPA**	Number of animals: 8 females – cross-over
	Pig	100 mg/kg	1.1% ± 0.7%	**unconjugated BPA**	8 males – cross-over
	Dog	100 mg/kg	1.9% ± 0.4%	**unconjugated BPA**	6 females – cross-over
	Wistar Rat	100 mg/kg	3.03%	**unconjugated BPA**	12 males (6 VO? and 6 IV?)
	CD1 Mouse	100 mg/kg	6.03%	**unconjugated BPA**	99 females divided according to OV, IV groups, blood or urine sample.

Table 15. Absolute bioavailability factors after administration of total BPA via the oral route depending on the animal species

Author	**Species**	**Dose**	**$F_{absolute}$ (bioavailability)**	**Measured component**	**Comments / Limits**
Doerge, 2010	**Rhesus Monkey**	100 µg/kg	79% ± 23%	**Total BPA**	Number of animals: 4 females
Doerge, 2010	**Sprague Dawley Rat**	100 µg/kg	77% ± 47%	**Total BPA**	Number of animals: 4 females
Kurebayashi *et al.*, 2002	**Cynomolgus Monkey**	100 µg/kg	70% (males) ±16% 66% (females) ±13%	**Total BPA**	3 animals / dose/gender radioactivity
Kurebayashi *et al.*, 2003	**Fischer Rat 344 M**	100 µg/kg	97% (males)	**Total BPA**	Number of animals: 3 males radioactivity

Kurebayashi *et al.*, 2005	**Fischer Rat 344 M**	20 µg/kg	82% (males) 35% (females)	**Total BPA**	Number of animals: 3 males 3 females radioactivity
		100 µg/kg	81% (males); F: 50% (females),	**Total BPA**	
		500 µg/kg	60% (males); 50% (females)	**Total BPA**	

The two reliable studies report an absolute bioavailability in unconjugated BPA in rats in the same range, specifically: **2.8% ± 3.1%** (Doerge, 2010) and **3.03%** (Collet, 2012 – see detailed description below).

Consequently, the value of absolute bioavailability in unconjugated BPA chosen for the risk assessment, that will be conducted for humans, is **3%** based on the studies performed in Sprague Dawley (Doerge, 2010) and Wistar rats (Collet, 2012).

- Sublingual

A recent study (Gayrard, 2013) using the sublingual route of exposure, was published after the Health Risk Assessment report (ANSES, 2013) and has not formally been taken into account in the HRA herebelow. However, it can be considered that this sublingual route of exposure has been taken into consideration by default by the oral studies through the diet or the water, which were used to derive DNELs.

The study of Gayrard performed on six dogs shows that the systemic bioavailability of BPA deposited sublingually is high (70-90%) and that BPA transmucosal absorption from the oral cavity led to much higher BPA internal exposure than obtained for BPA absorption in the gastro-intestinal tract after oral administration. This efficient systemic entry route of BPA may lead to far higher BPA internal exposures than known for BPA absorption by the gastro-intestinal tract. The main difference between both exposures ways is that the conjugated [BPA-Glucuronide: free BPA] ratio is 100 times lower by sublingual route than the one obtained after absorption by the gastro intestinal tract following oral absorption. The sublingual route of exposure bypasses the first-pass hepatic metabolism and this may explain the much higher internal exposure to unconjugated form of BPA entry into the systemic circulation. Indeed, BPA human clearance of 30 mL/kg/min has been predicted from animals' clearance. This value reveals a major inconsistency between BPA concentrations reported in biomonitoring studies, and a BPA daily intake of 13 µg/kg reported by EFSA combined to its clearance (Collet, 2012).

- Dermal

Bioavailability via the subcutaneous route:

Comparison of the different routes of administration, meaning oral route *versus* the subcutaneous (SC) route was discussed. The following table summarises all of the reports available for different species and at various doses.

Table 16. Comparison of areas under the curve (AUC) obtained after exposure to BPA by the oral route and by the subcutaneous route

Author	**Component**	**Species**	**Dose**	**AUC_{SC}/ AUC_{OR} Ratio**	**Comments / Limits** **Number of animals used per experimental point**
Taylor, 2008	BPA	PND3 Mouse	35 µg/kg	1.11	
			395 µg/kg	0.97	
Prins (2011)	**Unconjugated** BPA	PND3 Sprague Dawley Rats	10 µg/kg	4.35	
	Total BPA			1.80	
Doerge, 2010	**Unconjugated** BPA	PND3 Sprague Dawley Rat	100 µg/kg	16.6	Number of animals: 4 females
	Total BPA			5.90	Number of animals: 4 females
	Unconjugated BPA	PND10 Sprague Dawley Rat	100µg/kg	36.23	Number of animals: 4 females
	Total BPA			11.82	Number of animals: 4 females
	Unconjugated BPA	PND21 Sprague Dawley Rat	100µg/kg	11.44	Number of animals: 4 females
	Total BPA			11.80	Number of animals: 4 females
Tominaga (2006)	BPA	F344/N Rat	10 mg/kg	274.6	Number of animals: 3 females per sampling point
	BPA	F344/N Rat	100 mg/kg	44.5	Number of animals: 3 females per sampling point
	BPA	Chimpanzee	10 mg/kg	181	Number of animals: 2 females
	BPA	Cynomolgus	10	443.6	Number of animals:

		Monkey	mg/kg		3 females
	BPA	Cynomolgus Monkey	100 mg/kg	227.9	Number of animals: 3 females
Pottenger, 2000	Total BPA	F344/N Rat	10 mg/kg	NC	Number of animals: 5 males
	Total BPA	F344/N Rat	10 mg/kg	7.38	Number of animals: 5 females
	Total BPA	F344/N Rat	100 mg/kg	245	Number of animals: 5 males
	Total BPA	F344/N Rat	100 mg/kg	7.16	Number of animals: 5 females

NC: non calculable

N: Number of animals used per experimental point

F: Female

M: Male

It appears from this analysis that the levels of circulating BPA observed are higher following an administration via the subcutaneous route compared to the oral route. The ratio of AUC_{SC}/ AUC_{OR} ranges from 0.97 to 443.6. Overall this ratio is close to 1.0 in the young mice (3 day-old), but this is different from the value obtained for young rats (3 day-old) for which the ratio varies from 16.6 to 5.90 respectively for the free form and the total BPA. A metabolism which is not totally mature in the newborn (Doerge, 2011) is probably involved, which would explain the comparable AUC values regardless of the route of administration used - oral or subcutaneous. With the capacity to metabolise BPA being lower in newborns, the bioavailability will be higher in the latter than in adults in whom a strong coefficient of hepatic extraction has been demonstrated in animal species (He > 0.94). In adult animals, the studies available showed fluctuating ratios of 7.2 to 274 in adult rats and from 181 to 444 in monkeys. The strain of rats used (Sprague Dawley *versus* Fisher) is also be a significant parameter.

Due to the high variability of the AUC_{sc}/AUC_{oR} ratios reported in the literature, whether between different animal species or within the same species, and in the absence of an adapted PBPK model, no estimation of the relative bioavailability for the subcutaneous route has been carried out. Moreover, no study to date provides information on the absolute bioavailability for this route.

<u>Bioavailability via the cutaneous route:</u>

None of the experimental study available enables a **factor of absolute bioavailability** to be determined **for the cutaneous route**. In the absence of specific study on the cutaneous absorption, the **bioavailability value via cutaneous route** used by default **in the HRA** will be **100% of bioavailability after absorption,** for professionals and consumers.

Differences between professionals and consumers for the exposure modelling by cutaneous contact in the HRA:

Professionals and consumers are differently exposed to BPA in term of duration and frequency of exposure. Indeed, the professionals are exposed to a constant quantity of BPA transferred to the surface of the skin of the finger whatever the duration (between 5 and 60 seconds) or the number (between 3 and 10) of contact with the receipts, based on the study of Biedermann, 2010. Thus, the **percutaneous absorption flow** has been used to model the **professionals' exposure**. At the contrary, the consumer will touch relatively few receipts over the course of a day and it is likely that the quantity of bisphenol A on the fingers is not constant through time. It appeared therefore justified to use an approach based on the **rate of absorption** combined with contact with a thermal receipt with BPA for the **consumers**. The absorption rate is expressed in percentage absorbed of the quantity of BPA transferred onto the skin.

Regarding **absorption via the cutaneous route**, **the European Commission** (European, 2010) considers that **only 10%** of the dose in contact with the skin is absorbed (European, 2004). This estimation is confirmed by a study using a pig skin model (Kaddar *et al.*, 2008). However, new studies may suggest that the cutaneous absorption of BPA could be **greater** (Morck, 2010; Marquet, 2011; Demierre, 2012). The recent study by Demierre *et al*., 2012 stated that the dermal penetration contributes only in a negligible way to the total exposure (**8.6%** of penetration in human skin *in vitro*). Other studies contradict this statement: Zalko, 2011 studied the distribution and metabolism of BPA in cultures of pig ear skin and human skin explants. This study, (not a study of penetration as recommended by the OECD guideline 428 and with limitations (the incubation time of 72 hours is well beyond the recommended 24 hours to preserve the integrity of the explants), shows that the BPA in the conditions of the experiment and after 72 h incubation diffuses significantly through the two skin models: absorption of about **65%** for pig ear skin and **46%** for human skin explants. On the oterh hand, this study shows that at low concentrations applied to human skin, approximately 40% of the dose which diffuses into the liquid receiver is as glucuronide and sulfate.

Another study determined the percutaneous absorption of BPA *in vivo* in rats and *ex vivo* in rats and humans after an exposure of 24 hours (Marquet, 2011). The permeability was found to be 12 times higher in rats than in humans. The *ex vivo* percutaneous absorption in rat and human skin was measured on a static Franz cell. 15 samples of fresh human skin were collected from abdominal explants of 6 patients who undergo plastic surgery. Skin was dermatomed to approximately 400 microns thickness, was cut into circular sections of 1.76 cm² and was placed on the diffusion cell. The diffusion cell was maintained at a temperature of 36 ° C, which corresponds to a temperature 32 ± 1 ° C at the surface of the skin. [14C]-BPA in acetone (4 mg BPA/ml) was applied to the skin (50 ll/cm2 and 200 lg/cm2) for 24 h. An aliquot of receptor fluid was collected regularly over the 24-h period with an automatic fraction collector (Gilson FC 204, Middleton, WI, USA). The skin was digested in an 80% ethanol solution of KOH (1.5 M). Total radioactivity in the various fractions was determined by direct liquid scintillation counting. The integrity of each skin sample was assessed before performing permeation experiments by the measurement of trans-epidermal water loss (TEWL), one of the three methods recommended by the OECD (guidance 28, 2004). Samples with a TEWL value higher than 13 $g/m^2/h$ were discarded. The rate of metabolism of water-soluble tetrazolium salt (WST) into formazan was used to evaluate cellular viability. A non-viable skin control was obtained by freezing samples for 1 h at -20°C.

Fom the 15 human skin samples exposed to 200 mg BPA.cm-2, the mean value of percutaneous absorption flow was 0.120 µg.cm^{-2}.h^{-1}, ranging from 0.026 to 0.331 µg.cm^{-2}.h^{-1}. Inter-individual variability was found in humans. Finally, contrary to the study by Zalko, 2011, the authors found most of the BPA in unchanged form in the receptor fluid, which can be explained by the significantly much higher dose of BPA applied to the skin samples in the study by Marquet (Marquet, 2011).

Athough varying in terms of permeability, the overall data show that dermal absorption occurs and the minimal dermal absorption value estimated in the European Risk Assessment Report is 10% (EC, 2010).

The studies conducted by the Marquet *et al.* (Marquet, 2011) and Demierre *et al.*'s teams aimed to determine a value for the **transcutaneous absorption flow** of BPA while the initial aim of the study by Zalko, 2011 was to analyse the metabolites of BPA and compare the models of the skin from the ear of a pig with models of human skin. As the objectives of the studies were not identical, the protocols used were markedly different. It is difficult to compare the results (Demierre, 2012).

Table 17. Comparative table of the studies which evaluated the cutaneous penetration of *in vitro* BPA on human explants

	Zalko, 2011	**Marquet, 2011**	**Demierre, 2012**	**Morck, 2010**
Number of specimens	NC	15	7	11
Number of donors	NC	6	2	NC
Nature of the skin	Cold	Cold	Defrosted	NC
Thickness of the skin	500 µm	400 µm	200 µm	800 - 1000 µm
Anatomical region of the skin	Abdomen	Abdomen	Thigh	NC
Dose	2.75 µg.cm^{-2}	200 µg.cm^{-2}	1.82 µg.cm^{-2}	422 µg
Solvent	Ethanol/phosphate buffer	Acetone	Physiological serum	NC
Number of points to evaluate the flow	-	NC	4	-
Flow ± standard	-	0.12 ± 0.09	0.022 ± 0.011	-

deviation		$\mu g.cm^{-2}.h^{-1}$	$\mu g.cm^{-2}.h^{-1}$	
% of absorption	45.6 ± 6.2 % in 72 h	-	-	17.2 ± 6.45 % in 48 h

NC = not communicated

With regard to the HRA on **workers** exposed by skin contact with thermal papers containing BPA, percutaneous absorption of BPA is considered to be continuous during the period of work. This hypothesis is based on the observations of Biedermann, 2010 which shows a constant quantity of BPA transferred to the surface of the skin on the finger, regardless of the duration (between 5 and 60 seconds) and number (between 3 and 10 contacts) of contact with the receipts. The quantification of this absorption corresponds to the interval of the percutaneous absorption flow values measured in an experimental *in vitro* study (Marquet, 2011). Although the *in vitro* model on the Franz static cell used in this study cannot replace an *in vivo* model, it enables the mechanisms of interaction during absorption to be investigated, the tests to be multiplied, and work to be carried out on human skin. Moreover, the validity of the experimental protocol used on human skin explants is supported data on rats with an absorption flow value measured *in vitro* (1.5 $\mu g.cm^{-2}.h^{-1}$) of the same size as that measured *in vivo* (2.5 $\mu g.cm^{-2}.h^{-1}$).

With regard to the HRA for the **general population** relating to cutaneous contact with thermal papers containing BPA, the estimate of the percutaneous absorption flow (expressed in % absorbed by the dose transferred onto the skin, and not in the quantity absorbed by surface unit of skin and time) corresponds to values of the least probable rate of a minimum of 10 % and a maximum of 60 %, encompassing a most probable value of 27 %. The rate of 27 % was used in an experimental study (Biedermann, 2010). The data from this study cannot be considered as representative on a population scale. However, the experimental protocol is considered to be similar to the conditions of exposure for a person handling cashier's tickets on an occasional basis during the day, different to cashiers. This rate was estimated from the quantity of BPA transferred to the skin of the finger after a single contact of 5 seconds with a ticket, and the quantity of BPA which was no longer removable from the skin by soap and water 2 hours after this contact. The maximum rate of 60 % corresponds to the rate estimated by Biedermann, 2010 2 hours after immersion of the finger in a solution of BPA in ethanol; while the minimum level of 10 % corresponds to a (default) recommended value by the European Commission when a substance has a molecular weight over to 500 $g.mol^{-1}$ and an octanol-water distribution coefficient lower than -1 or higher than 4 (European, 2004). Therefore, with the absorption rates being estimated by Biedermann, 2010 for a period of exposure to the skin to BPA of 2 hours, they must be weighted by an adapted period of exposure for the general population in the handling of BPA-based thermic papers.

- Inhalation

There is no experimental data on BPA toxicokinetics after exposure by inhalation. However, the changes in absolute organ weights highlighted in a study of repeated inhalation toxicity in rats exposed during 13 weeks, show that absorption through the lungs occurs (EC, 2010). Decrease in absolute liver weight in males exposed to 10 or 150 mg/m^3, decreased absolute liver and kidney weights in females at 150 mg/m^3, increased relative brain weights in females at 50 or 150 mg/m^3, and increased relative lung weight in females at 150 mg/m^3 were

observed in this study. In rats sacrificed 4 weeks after exposure, males exposed to 150 mg/m^3 BPA had an increased relative brain weight. In rats sacrificed 12 weeks after exposure, decreased absolute kidney and lung weights were observed in males at 150 mg/m^3 and decreased absolute and relative kidney weights were observed in females at 150 mg/m^3. This is in line with its octanol / water favourable partition coefficient (3.2) indicating that absorption through the lungs can occur.

However, in the absence of data, absorption by inhalation cannot be quantified (EC, 2010). For the characterization of the risk behavior in European 2008 report, the oral and inhalation absorption values were set at 100% (EC, 2008).

Conclusion on the absorption via the oral, subcutaneous and cutaneous route and by inhalation:

BPA is rapidly absorbed after administration via the oral, subcutaneous or cutaneous route and totally by inhalation.

- Distribution

Once absorbed, the BPA is rapidly distributed in all tissues without real affinity of BPA for one particular organ. However, in rodents, a few hours after oral administration of radiolabelled BPA, the highest concentrations are found in the liver and kidneys.

Following an intravenous bolus administration in adult mice, unconjugated d6-BPA is rapidly taken up into adipose tissues but does not exceed the initial measured serum level (Doerge, 2012).

Krotz *et al*., (Krotz S.P., 2012) show that BPA does not accumulate (no BPA detected) in ovarian follicular fluid after a brief exposure to medical plastics during an IVF (in vitro fertilization) cycle in five women (small sample size). However, two previous studies contradict these results. In 2002, Ikezuki *et al*. (Ikezuki, 2002) measured BPA in non pregnant Japanese patients yielding follicular fluid levels averaged 2.4 ng/ml (n=32) and in 2005, Tsutsumi measured a follicular fluid levels ranged 1-2 ng/ml.

- Metabolism

After oral absorption, BPA is conjugated into his inactive form (also called aglycone BPA or unconjugated form). Thus, the percentage of active BPA released after oral absorption (the absolute bioavailability of active BPA) has been estimated to be at 3% based on Doerge *et al.* and Collet studies (Doerge, 2010; Collet, 2012). This approach allows refining for the health risk assessment. Doerge and Collet (Doerge, 2010; Collet, 2012) studies results are similar concerning the value of absolute bioavailability of active BPA (3%): that is why the health risk assessment is based on this value of 3%.

Toxicokinetic data obtained in rats and humans show a first-pass effect and indicate that the plasma residues are mainly (92-99%) as glucuronide. Several reports highlight the existence of an enterohepatic circulation in rats after the glucuronide hydrolysis in the intestine, which results in a relatively slow elimination of BPA in rats compared to humans (EC, 2010; INERIS, 2010; INFOSAN, 2009). This major difference in toxicokinetics between these 2 species has often been put forward to highlight the limitations of the rodent model in the risk assessment of BPA for humans (Mielke, 2009; Ginsberg G, 2009). Recent studies combining the use of

tritium/deuterium-labeled BPA and specific and sensitive detection techniques (LC-MS/MS) confirm the existence of an enterohepatic cycle in rodents unlike primates. However, they indicate that this cycle has very limited consequences on the clearance of BPA (Doerge, 2010; Doerge, 2010; Taylor, 2011) arguing for the relevance of the rodent model to humans for oral exposure to BPA.

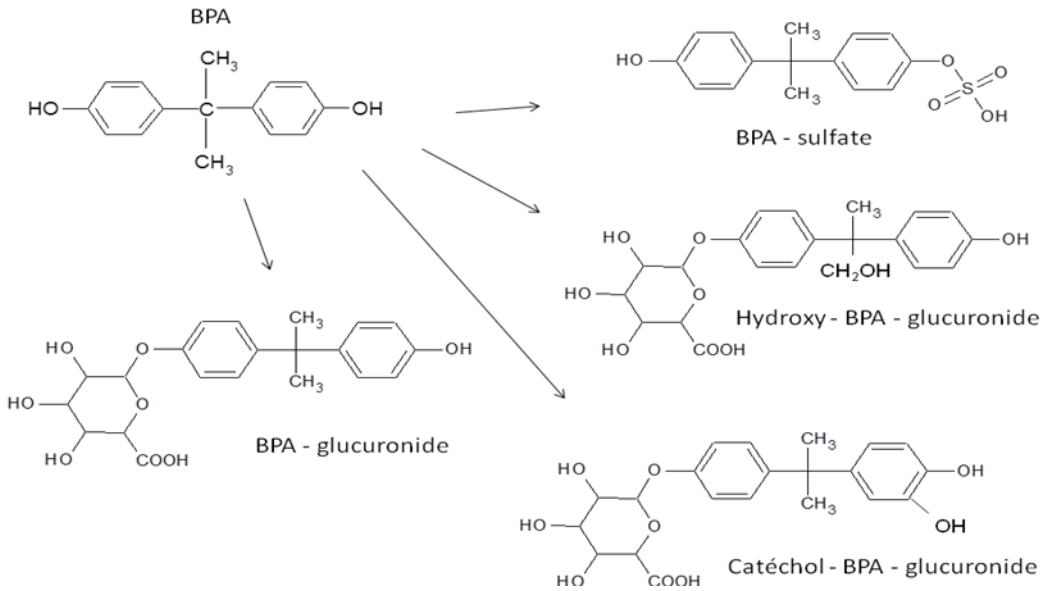
In all species studied, the majority metabolic pathway is the combination of BPA with glucuronic acid to form the BPA-glucuronide (BPA-G) (figure below). This combination takes place mainly in the liver and to a lesser extent in the intestine. It is catalyzed by UGT2B1 in rats, whereas in humans the UGT2B15 and UGT2B7 isoforms are responsible for the glucuronidation (Mazur CS, 2010). The genetic polymorphism of the UGT2B15 could result in individual differences in the ability to detoxify BPA (Hanioka N, 2008; INSERM, 2010).

Human pharmacokinetic studies show that the urinary metabolites profile is composed of almost BPA-G. Monitoring studies conducted using urine samples collected from adults (Ye, 2005) indicate different proportions (9.5% BPA free, 69.5% BPA-glucuronide and 21% BPA-sulfate). Kim *et al*. analyzed the proportion of BPA and its metabolites in urine collected from 15 women and 15 men (Kim, 2003). The average urinary composition observed in men was 29.1% of free BPA, 66.2% of BPA-glucuronide and 4.78% of BPA-sulfate, whereas in women the proportions were 33.4% of free BPA, 33.1% of BPA-glucuronide and 33.5% of BPA sulfate. The authors conclude that women have a better sulfation capacity than men (NTP, 2007).

Other metabolites have also been identified using urine or bile samples in rodents or during incubations with hepatocytes in primary culture. These include BPA and BPA-hydroxy sulfate (figure below). In total, these metabolites only rarely exceed 5 to 10% of the total metabolites in the urine of rodents. Other minor metabolites such as double conjugates or Metanephrine metabolites were also identified in rodents.

Several other metabolites formed by oxidation, have been identified *in vitro* using subcellular fractions (4-methyl-2, 4-bis (p-hydroxyphenyl) pent-1-ene, isopropyl-hydroxyphenyl, glutathionyl-phenol, glutathionyl 4 isopropylphenol-and bisphenol A dimers), but to date they have not been described *in vivo* (INRS, 2010).

Figure 9. BPA metabolites formed by oxidation



BPA-G and BPA sulfate forms represent BPA detoxification pathways insofar as they are not active on the estrogen receptor. Ginsberg *et al.* suggested that deconjugation by the β glucuronidase and arylsufatase C enzymes at specific tissue sites could convert conjugated and sulfated metabolites into "free"-BPA active on the estrogen receptor (Ginsberg G, 2009). The β-glucuronidase is present in the intestines but also in the placenta and fetal liver, which could result in exposure of the fetus to "free" BPA (Aschberger K, 2010).

The route of administration affects the forms and circulating levels of free BPA and conjugates (Doerge, 2010; Pottenger, 2000; Taylor, 2008). The data collected in rodents show significantly higher proportions of free BPA after subcutaneous and intraperitoneal administrations than in the case of an oral administration.

It is well established that ATP-Binding-Cassette (ABC) transporters play a fundamental role in the absorption, distribution, metabolism and excretion of endogenous and exogenous chemicals, and transporter membrane localization can directly influence these processes (Glavinas *et al*., 2004).

Mazur (Mazur CS, 2012) show that in rat, possible transport preferences of BPA and BPA-G is into intestinal lumen and hepatobiliary excretion whereas in humans, BPA-G is preferably transported into the blood supply of the liver or portal blood supply of the small intestine.

- Elimination

Orally administered BPA undergoes complete first-pass metabolism in the liver to BPA-glucuronide as major metabolite, which is rapidly excreted in the urine, with a half-life of less than 6 hours (Volkel W, 2002; Volkel, 2008). BPA-sulphate has been reported as a minor urinary metabolite of BPA in humans (Ye, 2005; Ye, 2006). Because this first-pass metabolism is effective, there is low systemic availability of free BPA (estimated at 3% in the HRA) in humans after oral exposure. The conjugated forms of BPA have no endocrine activity (Snyder RW, 2000; Shimizu M, 2002; Willhite, 2008). Therefore, these conjugation reactions represent detoxication pathways.

In rats, BPA is also predominantly glucuronidated, with sulphation representing a minor pathway (Pottenger, 2000), but the BPA-glucuronide formed is excreted from the liver via bile into the gastrointestinal tract, cleaved back to BPA and reabsorbed into the blood. Thus it undergoes enterohepatic recirculation resulting in slower elimination of BPA including its conjugate in rodents compared with humans (EFSA, 2006); this results in slow elimination (half-lives between 20 and 80 hours). The enterohepatic cycling and decreased first pass metabolism of BPA in rats results in higher plasma levels of unconjugated BPA in rats compared to humans given the same dose. There are differences in the molecular mass threshold for biliary elimination in rats and humans. The molecular mass of the BPA-glucuronide (484 D) is well above the threshold for rats (300 – 400 D) but below that of humans (500 - 600 D) (Hirom, 2014; Walton, 2001; Ghibellini, 2006).

Teeguarden (Teeguarden, 2011) show that after a 24 h period of dietary exposure to high dose of BPA in 20 humans, the total BPA concentrations serum are undetectable in 83% of the samples and when it is less than or equal to limit detection, BPA concentrations in serum is on average 42 times lower than urine concentrations. The rapid absorption and elimination kinetics of BPA observed in this study clearly demonstrate that spot urine sample reflect exposure in the prior meal or prior 4 h to 6 h period but not the full day's exposure.

Although the main pharmacokinetic differences between 5 species (Collet, 2012) concern the elimination of BPA and BPA-G, they have not impact on the BPA plasmatic concentrations which are the reflection of the internal exposure of BPA.

Toxicokinetic of BPA during gestation and in foetuses

Maternal exposure to BPA results in embryos and newborns exposed to BPA via placental transfer and milk (Doerge, 2010). Concerning the toxicokinetics of BPA *in utero*, the presence of BPA in human fetal tissues at almost the same concentrations than in maternal blood, demonstrates that BPA crosses the placenta barrier. This is confirmed by a study by Balakrishnan *et al.* (Balakrishnan, 2010) on seven human placentae perfused *ex vivo* with 10 ng/mL (environmentally relevant concentration) of BPA for 180 minutes: the transfer percentage of BPA is 27.0% ± 1.88% and only 3.2% ± 1.6% of BPA in the fetal compartment is in the conjugated form. Thus BPA can transfer across the human placenta, mainly in active unconjugated form. Moreover, BPA have a high transplacental transfer rate much similar to passive diffusion according to the meta-analysis of data from human ex vivo placental perfusion studies which confirm that the placental barrier is not protective against exposures to BPA (Mose T., 2012).

Nevertheless, the fetus has the capacity to deconjugate BPA into its "free" active form with the placental enzyme β-glucuronidase (Edlow, 2012). Free and total BPA were identified in both second and third trimester amniotic fluid. Thus, deconjugation of BPA by the placenta and limited capacity of the fetal liver to conjugate BPA, may increase fetal exposure to the active, endocrine-disrupting form (Edlow, 2012).

However, the study of Patterson (Patterson, 2013) done with 5 pregnant monkeys argues against the hypothesis that BPA conjugates are selectively deconjugateg by either the placenta or fetus. Indeed, it is explained by the monotonic elimination of aglycone BPA from the fetal compartment accompanied by persistent conjugate levels. This study measures concurrently the pharmacokinetics of aglycone (active) and conjugated (inactive) deuterated BPA (d6) in 5 maternal and fetal rhesus monkey serum, amniotic fluid and placenta following intravenous

injection in the dam. Internal exposures of the fetus to aglycone BPA is attenuated by maternal, placental, and fetal phase II metabolism to less than half that in the dam.

Exposure of human infants to BPA directly, in the absence of maternal transfer or excretion, also occurs through polycarbonate bottle feeding and/or infant formula feeding (Afssa, 2010). The fetus and neonate may therefore be a sensitive and more highly exposed subpopulation deserving special attention.

Toxicokinetic of BPA for newborns

In human neonates, several metabolic pathways, such as glucuronidation (2-5 times lower in preterm infants), and several excretory functions such as glomerular filtration rate (1.7 times lower) have a lower efficiency compared to adults; these functions reach their full capacity 1 month and 7 months after birth, respectively (EFSA, 2008). In 2008, EFSA was asked to review the toxicokinetics of BPA based on age and involvement in risk assessment and thus in the construction of the TDI. EFSA concluded that immaturity in glucuronidation capacity in newborns could be compensated by the presence of sulfo-transferases, which would result in an effective detoxification EPS (Aschberger K, 2010; EFSA, 2008). Contrary to the CGU, sulfotransferases (SULT), for which the substrates of UGT have high affinity, are active in the developing fetus and are functional at birth. These enzymes efficiently catalyze the formation of BPA-sulfate in vitro in humans. Finally, EFSA concluded that the ability of BPA biotransformation to inactive metabolites was sufficient in human neonates.

Studies in rats have shown that in infants, the glucuronidation pathway was more saturable than adults, which could lead to a greater concentration of BPA "free" in the target tissues. The ability of glucuronidation through the activity of UGT is also low after birth and remains low after weaning (Aschberger K, 2010).

Studies in newborn rat and rhesus monkey confirm that most of their toxicokinetic parameters are significantly different from those determined in adults, particularly with regard to the total BPA (Doerge, 2010; Doerge, 2010). However, with regard to BPA free maximum serum concentrations (Cmax), if it is significantly higher in the newborn rat (to NDP3 or PND10) than in adults rats, it does not seem to be the same in monkeys.

These same authors also showed in adult rats and newborn that subcutaneous administration of BPA significantly altered the toxicokinetic parameters, but also the free BPA/ conjugated BPA ratio in serum in the neonatal rat (Doerge, 2010).

BPA in breast milk

There is contradicting results regarding the presence of BPA in milk. Indeed, Vandenberg has shown limited excretion of BPA in breast milk (Vandenberg, 2007).

In a recent study, the level of BPA in milk was determined from experiments in rats exposed orally (Doerge, 2010). Lactating rats (n = 5) were force-fed daily for a week with deuterated BPA (100 mg / kg bw) from the day of birth of newborns. A control group (n = 3) was treated with the vehicle only (ethanol / water, 1:9 v / v). The milk samples were performed after injection of oxytocin and take place exactly one hour after administration of BPA. Analyses of milk and serum were performed in LC / MS-MS. They were made to PND7 for milk and PND10 for serum (for mothers and their young). Serum analyzes confirm the low percentage of aglycone (0.5%). The assays performed on milk indicate average concentrations of free and

total BPA corresponding to 0.87 and 7.6 nM, a milk / serum 1.3 for BPA free and 0.062 for total BPA report.

This article clearly shows that exposure of newborns to free BPA via lactation, following exposure of the mother, is very low. The serum concentrations of total BPA are 300 times lower than in young mothers, the BPA free being undetectable in the offspring. The results, compared with previous data obtained by the same authors in rat pups at PND10 indicate that serum concentrations here are 500 times lower than those obtained when administered by gavage at a dose of 100 mg /kg bw this is to say that administered to mothers in this study.

Several studies have demonstrated the presence of BPA (unconjugated and conjugated) in breast milk or in human colostrum. The studies which analysed breast milk involved a limited number of subjects (n=3 to 23). In these studies unconjugated BPA was detectable in the majority of cases (60% or more) (Otaka *et al*., 2003; Sun *et al*., 2004; Ye *et al,* 2006; Ye *et al.,* 2008), with average concentrations ranging from 0.61 to 1.3 ng/ml, but which could reach up to 6.3 ng/ml (Ye *et al*., 2006). The total BPA was detectable in nearly all of the studies, with the average concentrations comprised between 1.0 and 1.9 ng/ml, but which could reach up to 7.3 ng/ml (Ye *et al*., 2006). It should be noted that the methods of assay used in the studies mentioned previously (specifically those by Ye *et al*., 2006 and 2008) did not present satisfactory validation criteria (*e.g.* no quantification limits), which could have resulted in an overestimation of the declared sensitivity and therefore an under-estimation of the actual number of samples, the concentration of which was higher than the value used as a detection limit.

Kuruto-Niwa *et al.,* 2007 (Kuruto-Niwa, 2007) analysed the BPA in colostrum collected within the 3 days after birth (n=101). They report concentrations of total BPA of 3.4 ng/ml on average, which could reach up to 7 ng/ml. The method used (immuno-assays), although presented as detecting both unconjugated and conjugated BPA, could however have under-estimated one (or more) of the different forms present.

More recently, using the LC-MS/MS method, Cariot *et al*., 2012, analysed three samples of milk taken several days after birth (without further clarification). The data obtained showed concentrations of free BPA of 0.80; 3.29 and 3.07 ng/ml.

These studies indicate that the concentrations of BPA in colostrum (collected within 3 days after birth) and breast milk are of the same size.

A daily exposure dose, calculated on the basis of a volume of 600 ml breast milk consumed for an infant of 3.5 kg, would result in the ingestion of 171 ng/kg for milk containing 1 ng/ml of BPA, and 1200 ng/kg for milk containing 7 ng/ml of BPA. These values place the exposure of infants to higher levels than those shown on average for adults. In addition it should be noted that the majority of the BPA detected in breast milk is in the unconjugated form (up to 80%) and that it is likely that a significant proportion of the conjugated BPA absorbed is deconjugated by acid hydrolysis during passage into the stomach and/or by the intestinal flora.

Conclusion regarding the toxicokinetics of BPA

Based on the studies available, ANSES concludes that:

- BPA is rapidly absorbed after administration via the oral, subcutaneous or cutaneous route,

- BPA is rapidly eliminated in the form of glucuronide in all species, however with major differences between rats, monkeys and humans. The sulphate form is also observed but in a lower quantity. BPA and its metabolites are preferentially eliminated via the urinary route in humans and by the faecal route in rats. Comparison of the AUC ratios via the subcutaneous and oral routes shows a great disparity in these ratios in adult rats while these values are quite similar in newborns. **Due to these discrepancies, the absence of studies of absolute bioavailability for the subcutaneous route, and the absence of an adapted PBPK model, an estimate of the relative bioavailability for the subcutaneous route cannot be proposed at present,**
- The absolute bioavailability value retained in unconjugated BPA via the oral route is **3%** (Doerge, 2010, Collet, 2012), based on the similar results found between these two studies,
- The cutaneous absorption flow value measured by Marquet (Marquet, 2011) may be retained with, as interval of variation of the distribution, the minimum (0.026 $\mu g.cm^{-2}.h^{-1}$) and maximum (0.331 $\mu g.cm^{-2}.h^{-1}$) values measured,
- Estimation of the percutaneous absorption rates of BPA (expressed in % of BPA transferred on the skin that is absorbed into the skin) is estimated between 10% and 60%, the most probable value being 27% Biedermann, 2010,
- The physiological model of Mielke and Gundert-Remy, 2009 constitutes a good departure point for modelling the kinetics of BPA. However, this model could be combined with that of Fisher in order to incorporate the intestinal metabolism. However, it should be observed that these models are developed for adults and do not take into account the foetus and the placentary passage.

B 5.2 Acute toxicity

Not relevant for this proposal.

B 5.3 Irritation

Not relevant for this proposal.

B 5.4 Corrosivity

Not relevant for this proposal.

B 5.5 Sensitisation

Not relevant for this proposal.

B 5.6 Repeated dose toxicity

2014 studies showing immunotoxicity of BPA

It has to be noted that these studies were not available at the time of the submission of this proposal. They have been quoted during the public consultation on this dossier.

In the study from MENARD et al. (2014) titled "Perinatal Exposure to a Low Dose of Bisphenol A Impaired Systemic Cellular Immune Response and Predisposes Young Rats to Intestinal Parasitic Infection", the authors investigated the consequences of developmental exposure to a low dose of BPA on immune functions in juvenile rat. **For the oral tolerance and immunization protocol**, Wistar female rats were orally exposed to 5 µg/kg bw/d BPA from GD 15 to PND21 and then F1 female were tested with ovalbumine (OVA) for oral tolerance induction and immunization. The humoral response was then tested by measuring plasmatic anti OVA-IgG/IgE (73 days) and the cellular response (53 days- splenic and Lymph Nodes/MLN cells).

For the infection protocol, females rats aged of 25 days post-partum and prenatally exposed to BPA 5 µg/kg bw/day were infected subcutaneously with 1000 infective-stage larvae of *Nippostrongylus brasiliensis*. The living larvaes were then counting after feces culture and the myeloperoxidase (MPO), jejunal activity (32d jejunum cells) and cytokines were measured. Cytokine measurements were also done in supernatants of primary cell cultures of spleen, MLN and jejunal tissue segments: IL13, IL4, Growth-Regulated Oncogene/Keratinocyte Chemoattractant (GRO/KC) in the jejunal tissue cells and IFNγ, IL10 in all cells. The following results were observed:

- **Oral tolerance and immunization to OVA protocol**: concerning the humoral response, no difference in Ig titers was observed. Using this protocol, no anti-OVA IgE response occurred excluding any allergic response after exposure to BPA. For the cellular response, it was shown that perinatal exposure to BPA did not affect basal IFNγ concentration (ie without OVA restimulation) in splenocytes or MLN supernatants from either OVA-tolerized or OVA-immunized rats compared to their respective controls non-exposed to BPA. However, perinatal exposure to BPA led to a sharp decrease in the *in vitro* OVA-induced IFNγ production by splenocytes compared to non-exposed controls with OVA-tolerization or –immunization. In MLN cells, *in vitro* OVA restimulation induced a significant increase of IFNγ production in control OVA-

immunized rats only, while BPA exposure significantly decreased by 3-fold the OVA-induced IFNγ secretion in these animals. No significant change in IL10 secretion was observed in rats perinatally exposed to BPA compared to their controls.

Perinatal exposure to BPA decreased TReg and Thelper lymphocyte and dendritic cell populations in the spleen and MLN.

- **Concerning the susceptibility to N. brasiliensis infection**, rats perinatally exposed to BPA developed a susceptibility to intestinal infection: for the BPA-treated rats, an increase of living larvae and a decrease of jejunal MPO activity were observed but no changes in IgE were observed after BPA exposure. An increase of Th2 cytokines (IL13, IL4) and anti-inflammatory (IL10) and pro-inflammatory cytokines (GRO, IFNγ) were also reported in infected BPA-treated rats.

In conclusion, juvenile rats perinatally exposed to BPA failed to induce a proper cellular immune response after systemic immunization. Perinatal exposure to low dose of BPA (5µg/kg bw/d) increases susceptibility to N. brasiliensis parasitic infection by deregulating TH1/Th2 cytokines profile in infected intestinal mucosa.

In the study from MENARD et al. (2014) titled "Food intolerance at adulthood after perinatal exposure to the endocrine disruptor bisphenol A", the authors investigated the consequences of low-dose exposure to BPA during the perinatal periode on mucosal and systemic immune responses to the food antigen ovalbumin in rats at adulthood. In this study, pregnant Wistar female rats were orally exposed (by feeding) to 0.5, 5 or 50 µg/kg BW/d BPA (nominal value) or vehicule alone and offspring females rats aged of 45 days were assessed for oral tolerance induction and immunization.

Offspring female rats (all groups) at 45 day of ages were tested for oral tolerance induction (OVA-tolerized) and immunization (OVA-immunized). In groups of offspring's rats perinatally exposed to either BPA at 5 µg/kg bw/d or vehicle (control), a chronic oral challenge with OVA was performed to determine the physiological consequences of the lack of immune tolerance at humoral and cellular levels after OVA-tolerization and immunization in rats perinatally exposed to BPA. The following measurements were performed:

<u>For the oral tolerance and immunization to ovalbumin (OVA) protocol</u> :

- Humoral response : measurement of plasmatic anti OVA-IgG/IgE (73d)
- Cellular response (73d splenic cells):
  - Percentages of T lymphocytes
  - Splenocytes proliferation measurement
  - Cytokine measurement in supernatants of spleenocyte cultures

<u>For the oral challenge protocol:</u>

- Colonic MPO activity (67d colonic cells)
- Cytokine measurement in colonic tissue segments (73d): TGFβ, IFNγ, IL10

The results have shown that, **concerning the humoral response**, after perinatal exposure to BPA, whatever the dose, a sharp decreased in anti-OVA IgG titers was observed in OVA-tolerized rats compared to immunized rats, as expected. BPA exposure increased anti-OVA

IgG (significantly higher in all BPA-exposed rats than in non exposed controls but a non linear dose-response relationship is reported response with 5 µg/kg BW/d BPA exposure showing the highest effect). No IgE response was observed.

Concerning the **cellular response in spleen**, perinatal exposure to 5 µg/kg bw/day of BPA increased the percentage of activated T lymphocyte subpopulation with no effect on regulatory T lymphocytes. Basal cell proliferation in the spleen (ie without OVA stimulation *ex vivo*) was not affected by perinatal exposure to BPA in OVA-tolerized rats or OVA-immunized rats. Splenocytes from BPA-exposed rats showed a 2-fold increase of cell proliferation for OVA-tolerized group after OVA restimulation. In BPA-treated rats, OVA stimulation *in vitro* induce a sharp increase of INFγ concentration release by splenocytes in both OVA-tolerized and OVA-immunized rats compared to the corresponding unstimulated conditions. No modification for IL10 secretion whatever the treatment group.

Concerning **the tolerogenic GALT function on OVA-tolerized rats**, perinatal exposure to 5 µg/kg bw/day of BPA increased neutrophilic infiltration (MPO activity) in colonic tissues compared to control. In addition, in the same groups of rats, significantly higher concentrations of IFNγ and IL10 than in tolerized control rats were observed; this was accompanied by a significant decrease in TGFβ production.

Based on these results, the authors concluded that perinatal BPA exposure impaired oral tolerance and sensitization to dietary antigens in adulthood. BPA not only affected local GALT function but also systematically activated the T-cell population and increased immune response to immunization.

B 5.7 Mutagenicity

Not relevant for this proposal.

B 5.8 Carcinogenicity

Not relevant for this proposal.

B 5.9 Toxicity for reproduction

B.5.9.1. Effects on the female reproductive system

B.5.9.1.1. Effects in human

B.5.9.1.1.1. Previous expert assessment of data in human

Epidemiological studies have been evaluated by organisations such as EFSA, NTP-CERHR, OEHHA, FAO/WHO, AFSSA, INSERM, etc.

According to reports from Health Canada (2008) and the OEHHA (2009), epidemiological studies report a link between BPA exposure and endometrial hyperplasia, recurrent miscarriage, polycystic ovary syndrome and elevated levels of androgens. However, these studies have significant methodological flaws that prevent consideration of these effects as recognized (Health Canada, 2008), (OEHHA, 2009).

According to the FAO/WHO (FAO/WHO, 2010), in women, only one study with a small number (n=84) examined the link between BPA and oocyte production and peak serum oestradiol (Mok-Lin, 2010). According to an expert panel, conclusions cannot be drawn from this sole study. Two studies have examined the link between BPA and the advancement of the age of puberty, but are of limited quality and not convergent (Wolff MS, 2010; Wolff, 2008). The expert panel recommended a prospective study to investigate the association between BPA and the effects of BPA on the age of puberty. In addition, experts on the panel underlined the lack of any study undertaken in boys (FAO/WHO, 2010).

According to INSERM (2011): "In conclusion, overall, the epidemiological studies are too few to determine the probability in humans of the effects observed in animal experiments. At present, studies conducted in women concerning the risk of breast cancer or endometriosis are all based on a retrospective approach (particularly limited for a non-persistent compound like bisphenol A), and convenient clinical populations, without a specific sampling plan.

A summary table of the epidemiological studies analysed in this report is available here below. This table includes a reference to the quality of these studies.

B.5.9.1.1.1.1 Studies of high quality, or not presenting major methodological limitations

The cross-sectional study of Itoh *et al,* did not find any association between urinary BPA and **endometriosis** in 140 Japanese patients from an infertility clinic (Itoh *et al.*, 2007). Women who had a pregnancy with childbirth were excluded. For unknown reasons, the authors recruited nine women with no fertility problems "to increase the statistical power." BPA was analysed in urine collected just before laparoscopy. The diagnosis was made according to the laparoscopic criteria of the American Fertility Society Association, stage 0 to IV. The median level of the group 0-I was 0.80 µg/g creatinine versus 0.93 µg/g creatinine in the group II-IV.

The study by Wolff *et al,* (**growth parameters at birth**, 367 subjects) demonstrated no correlation with the BPA analysed once during the third trimester of pregnancy (Wolff *et al.*, 2008b). The authors analysed five phenols including BPA and ten phthalate metabolites. In a recruited population of 479 women, 75 (16%) were excluded from the analysis, mainly because of complications of pregnancy or loss to follow-up (n=19). Concentrations of BPA ranged from the limit of detection to 35.2 µg/L (median 1.3 µg/L).

The study by Wolff *et al,* (**puberty in girls, breast development**, 192 subjects) demonstrated no relationship between BPA and the stage of puberty (Wolff *et al.*, 2008a). In addition to BPA, phytoestrogens, lead, DDE and PCBs were analysed. This result was confirmed in a cohort of 1151 girls (Wolff *et al.*, 2010). The latest study included the analyses of phytoestrogens, phthalates, triclosan, and phenols other than BPA. In both cases, the authors do not provide the range of concentrations across the population; they are presented by study groups. The geometric means ranged from 1.6 to 2.4 µg/L.

Two studies (Lang 2008, Melzer 2010) **analysing cardiovascular disease, diabetes and biochemical blood parameters** in adults deserve special attention, because they concern an NHANES population representative of the general US population (Lang *et al.*, 2008) (Melzer *et al.*, 2010). In a sample of 1455 persons in 2003-2004, a positive association was observed between urinary BPA, certain liver enzymes, the risk of diabetes, and cardiovascular disease. In 2005-2006, the levels of BPA were significantly lower. Only an association with the risk of cardiovascular disease was observed. No animal studies have been done to support these observations. The authors have reservations about causality in these associations, and indicate that further studies are needed.

Two studies have examined the relationship between BPA and **psychomotor development**. That of Braun *et al.* has been quite criticised (Braun *et al.*, 2009), especially by AFSSA, which in 2010 did not consider it to be of acceptable quality. It is highly probable that the positive association found for the externalising behaviour found only in girls is related to chance. However, it should be noted that FAO/WHO experts consider it a priority to replicate this study in a large cohort, combined with several urinary measurements, particularly in early pregnancy (FAO/WHO, 2010). A second study by Miodovnik *et al.* sought to correlate the level of urinary BPA and phthalates analysed during pregnancy with the sociability of multi-ethnic urban children aged 7 and 9 years in 137 children. No significant association was found between urinary and social problems for BPA. BPA was positively correlated with the severity of social problems ('Social Responsiveness Scale'), but this relationship was not statistically significant (Miodovnik *et al.*, 2011).

The study by Hong *et al.* (**markers of oxidative stress and insulin resistance**, 960 subjects) demonstrated no correlation with markers of oxidative stress (Hong *et al.*, 2009). However, subjects with high fasting insulin levels had more urinary BPA. The HOMA index (Homeostatic Model Assessment) was not linked to BPA. BPA was analysed in 516 samples. In 24% of subjects, BPA was not detectable, and the median was 0.63 ng/mL.

The study of Mok-Lin *et al,* conducted in women treated by *in vitro* fertilisation (IVF), demonstrated a negative association between BPA, oestradiol, the number and stage of oocyte maturation (Mok-Lin *et al.*, 2010). Urinary concentrations of BPA ranged from <0.4 to 25.5

µg/L. The geometric mean was 2.5 µg/L. The study was conducted on 112 cycles (total of 84 women), and 203 urine samples (2 samples for 91 cycles and one sample for 21 cycles).

The study by Fujimoto *et al.* examined the relationship between serum BPA and maturity of oocytes and fertilisation rate in 58 women treated with IVF (Fujimoto *et al.*, 2011). Urinary BPA was analysed in women and in 26 male partners. The median concentration of BPA was 2.53 ng/mL, with maximal concentrations of 67.4 ng/mL in women and 0.34 ng/mL in men (with maximal concentrations of 22.7 ng/mL). Of 59 cycles, 13 oocytes on average were collected per cycle. The authors report a significant association between serum BPA in women and decreased fertilisation rates. However, patients who used two procedures for *in vitro* fertilisation (with and without sperm microinjection) were considered as one group, despite the fact that the quality of male gametes was different in these two groups.

The analysis of all of these studies allows some doubt to remain about the impact on the quality of gametes in sterile females followed for medically-assisted procreation (MAP).

The epidemiological studies available at present have failed to demonstrate an association between urinary BPA concentrations measured postnatally and the development of puberty in girls.

In addition, as noted in the introduction to this section, all these studies will be discussed in the different sections of this dossier.

Miscarriages / spontaneous abortions								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Sugiura-Ogasawara *et al.*, 2005) Exposure to bisphenol A is associated with recurrent miscarriage	Case-control study	Study population: general population: women having had at least 3 first-trimester miscarriages N=45 cases vs 32 controls (doctors, nurses, secretaries at the school of medicine) → Small population size	Serum	ELISA	Age: no Sex: no Medication: no Tobacco: no BMI: no Other contaminants: No	Results: - positive association with antinuclear antibodies but not with the other parameters - serum BPA levels higher in women having had at least 3 miscarriages.	Studies not taken into consideration since they have major methodological limitations This study was excluded due to the following methodological weaknesses: - small population size, - questionable choice of control group (no proof of attempted pregnancy in this group), - limited list of	Information from epidemiological studies Effects on the female reproductive system

							confounding factors to be considered, - an analytical method (ELISA) that does not distinguish between the various forms of BPA, - other confounding factors for miscarriage, - an inadequate analysis of results (identical median serum levels in the two groups) - inadequate choice of statistical tools	

Puberty and breast development

Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Wolff *et al.*, 2008a) Environmental exposures and puberty in inner-city girls	Cross-sectional study	Study population: 9-year-old girls N=192 => 186 in the end → OK population size	Urinary	Not specified	Age: yes Sex: NA Medication: yes Tobacco: yes BMI: yes Other contaminants: yes Other: race, ethnic group, urinary creatinine, height, combined with a set of predictors identified through significant comparisons with a 20% threshold.	Results: No change in the age of puberty onset in the girls. Comments: the study's power is not known and the study size is not so large	Studies of high quality or having no major methodological limitations	Information from epidemiological studies Effects on the female reproductive system
(Wolff *et al.*,	Prospecti	Study	Urinary	Not	Age: yes	Results:	Studies of	Information

2010) Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls	ve cohort study	<u>population</u>: girls between the ages of 6 and 8 years N=1151 → Excellent population size		specified	<u>Sex</u>: NA <u>Medication</u>: yes (in particular, "endocrine medical conditions excluded") <u>Tobacco</u>: no <u>BMI</u>: yes <u>Other contaminants</u>: yes <u>Other</u>: race/ethnic group (for patients from Mount Sinai School of Medicine)	No change in the age of puberty onset in the girls.	high quality or having no major methodological limitations	from epidemiological studies Effects on the female reproductive system
Effects on prematurity								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**

(Cantonwine *et al.*, 2010) Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study	Mexican, retrospective case-control study nested in a cohort study	Study population: pregnant women N=30 cases (delivery < 37 weeks of pregnancy) vs 30 controls (delivery > 38 weeks of pregnancy) → limited population size	Urinary	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: NA (non-smoking women but passive smoking not taken into account) BMI: yes Other contaminants: yes (urinary phthalate metabolites) Other: maternal education, marital status, gender of children	Results: the 'premature' group (delivery < 37 weeks of pregnancy, n=12) had about twice as much BPA as the controls Comments: - Prematurity based solely on gestational age at delivery, no sonogram measurements. In light of the heterogeneity of this group (elective caesareans, spontaneous delivery, pre-eclampsia,	Study having major methodological limitations This study was not taken into consideration due to the following limitations: - passive smoking not taken into account, - other risk factors for prematurity not taken into account (obstetrical history) - Mode of delivery not specified (caesarean? spontaneous	Information from epidemiological studies Effects on the female reproductive system

						etc.), it is difficult to pinpoint the hypothetical effect; - No measurements of lead or other contaminants; - Only one BPA measurement (one single spot urine sample), no repeated measurements, - No information about passive smoking or other risk factors for prematurity (obstetrical	births?) - Population size too small to have sufficient statistical power to determine the effect of low-dose environmental exposure. - In fact, this population size is barely sufficient for the application of parametric statistical tests as undertaken by the authors.	

						history)		
Ovarian effects								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Mok-Lin *et al.*, 2010) Urinary bisphenol A concentrations and ovarian response among women undergoing IVF	Prospective cohort study	Study population: women undergoing an ovarian stimulation protocol in the framework of IVF (21-44 years) N=84 women (112 IVF cycles) → Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS/MS	Age: yes Sex: NA Medication: no Tobacco: no BMI: yes Other contaminants: no Other: specific gravity, day-3 FSH	Results: urinary concentrations of BPA were associated with: - a decrease in the number of oocytes retrieved after stimulation - a decrease in peak serum oestradiol levels BPA was detected in the majority of women undergoing IVF Comments:	Study of high quality or having no major methodological limitations	Information from epidemiological studies Effects on the female reproductive system

						- urine was sampled twice for BPA, a geometric mean was calculated for each subject - The urinary concentrations of BPA reflected exposure at the time of sampling and not during the period of follicular maturation, several months prior. - It is difficult to extrapolate results observed in sample of infertile women consulting for IVF to the general population.		
(Cobellis *et al.*, 2009) Measurement of Bisphenol A and Bisphenol B Levels in	Study in humans	Study population: fertile women consulting a gynaecological-obstetric service for	Serum Note: Bisphenol B was also	HPLC/ fluorescence	Age: no Sex: NA Medication: no Tobacco: no	Results: Absence of bisphenols in the control group BPA found in 30	Studies not taken into consideration since they have major methodologic	Information from epidemiological studies Effects on the

Human Blood Sera From Healthy and Endometriotic Women		chronic pelvic pain, dysmenorrhea or ovarian cysts N=58 cases (endometriosis) vs 11 controls (same population but without endometriosis) → Small control group	measured		BMI: no Other contaminants: no	sera (51.7%) Presence of at least one of the two bisphenols verified in endometriotic women (63.8%) Comments: This study mainly focused on analytical aspects, and particularly the assay techniques used to analyse serum BPA.	al limitations This study was excluded due to: - the small population size (only 11 controls), - the very limited description of results - simple descriptive statistical analysis without adjustment	female reproductive system
(Fujimoto *et al.*, 2011) Serum unconjugated bisphenol A concentrations in women	Cohort study	Study population: couples undergoing IVF (infertile women undergoing	Serum (un-conjugated BPA)	HPLC/ESA coularray 5600 detector	Age: yes Sex: no Medication: no Tobacco: yes	Results: Significant association between the serum BPA concentrations of the women and decreased oocyte	Studies of high quality or having no major methodological limitations	Information from epidemiological studies Effects on the

may adversely influence oocyte quality during *in vitro* fertilization		ovarian stimulation and their male partners) N=58 women and 37 men			BMI: no Other contaminants: no Other: ethnic group	fertilisation Comments: Patients who underwent both *in vitro* fertilisation procedures (with and without sperm microinjection) were considered as one single group. And yet male gamete quality was different in these two groups.		female reproductive system
(Hiroi *et al.*, 2004) Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia	Cross-sectional study	Study population: women N=19 female patients with endometrial hyperplasia (2 groups according to complexity: 10 with 'simple' hyperplasia and 9 with	Serum	ELISA	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: The correlation was the opposite of what was expected: the controls had more BPA than the cases (non-significant). Serum BPA concentration=2.9 ng/mL in women with simple hyperplasia vs 1.4 ng/mL in women	Studies not taken into consideration since they have major methodological limitations This study was not taken into consideration due to the following	Information from epidemiological studies Effects on the female reproductive system

		'complex' hyperplasia) and 7 with an endometrial carcinoma vs 11 controls →Limited population size				with complex hyperplasia. Same inverse relationship observed in women with an endometrial carcinoma	limitations: - limited population size, - confounding factors not taken into account.	
(Itoh *et al.*, 2007) Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study	Cross-sectional study	<u>Study population</u>: Female patients primarily complaining of infertility (endometriosis, 24-43 years) N=140 -> Sufficient population size	Urinary (total BPA)	HPLC/MS	<u>Age</u>: no <u>Sex</u>: NA <u>Medication</u>: no <u>Tobacco</u>: no <u>BMI</u>: no <u>Other contaminants</u>: no <u>Other</u>: creatinine	<u>Results</u>: No significant association between urinary BPA levels (not adjusted and adjusted for creatinine) and the stage of endometriosis <u>Comments</u>: - urine testing for BPA reflects recent exposure and not long-term contamination. - no control group truly free from	Study of high quality or having no major methodological limitations	Information from epidemiological studies Effects on the female reproductive system

						disease, - urinary samples stored in plastic tubes in a freezer for 5 years		
(Kandaraki *et al.*, 2011) Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Serum Levels of Bisphenol A in Women with PCOS	Age- and BMI-matched cross-sectional study	Study population: women N=71 cases (women with PCOS) vs 100 controls → Sufficient population size	Serum	ELISA	Age: yes (via matching) Sex: NA Medication: NA Tobacco: NA BMI: yes (via matching) Other contaminants: no Other: via a multivariate analysis (anthropometric, hormonal and metabolic parameters)	Results: - Serum BPA concentrations significantly higher in women with PCOS (obese or not) compared to normal control women. - In women with PCOS (obese or not): significant increase in testosterone levels and the LH/FSH ratio while SHBG levels were lower than in the controls. - BPA concentrations were significantly correlated with	Studies not taken into consideration since they have major methodological limitations This study was excluded due to: - an analytical method (ELISA) that does not distinguish between the various forms of BPA.	Information from epidemiological studies Effects on the female reproductive system

						testosterone and androstenedione concentrations and insulin resistance. - BPA concentrations were significantly correlated with the existence of PCOS.		
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and BMI secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments:	Studies not taken into consideration since they have major methodological limitations This study was excluded in light of the following methodological weaknesses: - small population size, - statistical analysis	Information from epidemiological studies Effects on the female reproductive system

		size				The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	lacking detail, - the final comparison was made in relation to non-obese women, with normal cycles (considered as controls) - no adjustment for confounding factors, - plasma BPA measured using the ELISA technique (lower limit).	

B.5.9.1.1.1.2. Studies not selected due to major methodological limitations

When analysing the quality of epidemiological studies, it appeared that some of these studies had major methodological limitations, such as low population numbers investigated, not taking into account relevant confounders, determination of BPA using an unsuitable technique, an unsuitable method of sample storage, etc. The studies below have therefore not been taken into account when assessing the health effects of BPA:

- (Cantonwine *et al.*, 2010),
- (Cha *et al.,* 2008),
- (Cobellis *et al.*, 2009),
- (Hanaoka *et al.,* 2002),
- (Hiroi *et al.*, 2004),
- (Kandaraki *et al.*, 2011),
- (Meeker *et al.,* 2010a),
- (Padmanabhan *et al.*, 2008),
- (Sugiura-Ogasawara *et al.*, 2005),
- (Takeuchi *et al.*, 2004),
- (Yang *et al.*, 2009).

In humans, two studies on the effect of BPA on **ovocyte maturation** in women appear to be of interest: the **good quality study** by Mok-Lin *et al*., 2010 (Mok-Lin, 2010) and the study by Fujimoto *et al*., 2010 **with no major methodological limitations**. There are few other epidemiological studies but they have **methodological limitations** (size of the study population, selection of participants, statistical analyses, confounding factors, etc.). All these studies are presented below by type of effects.

B.5.9.1.1.2. Uterine effects

Endometriosis

In an Italian study, BPA was more commonly detected in the plasma of women with endometriosis (n=58) than in women without endometriosis (n=11). BPA was not found in the control group. In 51.7% of endometriosis cases, BPA was above the detection limit. Only 25.9% of cases had levels of BPA greater than the limit of quantitation (LOQ) (Cobellis, 2009). The methodology is questionable in terms of the constitution of the groups (inclusion criteria, study dates, very small number of subjects in the control group, and diseases existing in the control group). The analytical technique used is adapted (HPLC-fluorescence and MS detection). However the impact of deconjugation during the extraction was not evaluated. It should be noted that free BPA was never detected in the plasma of the control population.

A second study evaluated the association between endometriosis and urinary levels of BPA in 140 Japanese women seen for primary infertility between January 2000 and December 2001, divided into two groups: endometriosis stage 0-I, n=81; and stage II-IV, n=59 (Itoh H, 2007). A cross-sectional analysis was performed between the urinary level of conjugated BPA (unadjusted and adjusted for creatinine) and stage of endometriosis. The authors found no significant association. The urinary levels of conjugated BPA found appear consistent with rates found in Japan in several studies of the general population. Two main limitations weigh in the interpretation of this study. First, the determination of urinary BPA reflects not long-term, but recent exposure. Second, there is no true control group devoid of pathology.

Endometrial hyperplasia

An *a priori* prospective study (inclusion criteria and dates not specified) suggests that circulating levels of BPA would be lower in women with complex uterine hyperplasia (n=9) and/or uterine adenocarcinoma (n=7) than in women with normal endometrial histology (n=11) or moderate endometrial hyperplasia (n=10) (Hiroi, 2004). The analytical method (ELISA) is questionable and BPA was measured in a single plasma sample in uncontrolled conditions (it is present in all subjects). In addition, the number of patients in each subgroup is very limited. This study was excluded.

B.5.9.1.1.3. Pregnancy

A case-control study evaluated the association between BPA exposure and the incidence of spontaneous miscarriages (Sugiura-Ogasawara, 2005). The authors report a higher serum level of BPA in women with a history of three miscarriages. However, this study remains very controversial, especially in terms of the protocol for measuring BPA (ELISA method), the comparability of groups, because of other confounding miscarriage factors, in terms of analysing the results (median serum levels identical in both groups), and statistical tools chosen (Berkowitz, 2006).

Cantonwine *et al,* studied the relationship between the rate of premature births and total urinary BPA on a single sample taken between 30 and 37 weeks of pregnancy in a Mexican population (Cantonwine, 2010). The most conclusive result for the authors was a higher concentration among women delivering before 37 weeks, and that an increase of 1 log in BPA concentration was associated with an advance of the delivery date by 4.5 days ("odds ratio" method). Analysis of these data is problematic: only 12 of 60 women gave birth before 37 weeks. In addition, the difference compared to women who delivered at term is no longer significant if one normalises the concentrations of BPA in relation to urine specific gravity and/or creatinine concentration. Finally, the absence of certain information further limits the scope of the study (time of urine collection relative to the stage of pregnancy and in relation to food intake, etc.).

B.5.9.1.1.4. Ovarian effects

One prospective study that included women (n=) following an ovarian stimulation protocol as part of an *in vitro* fertilisation indicated that there is a negative correlation between urinary levels of BPA (n=203 urine samples in 112 cycles of IVF) and ovarian response (number of oocytes collected and amplitude of the preovulatory oestradiol peak). A mean decrease of 12% in the number of oocytes recovered per cycle and of 213 pg/mL from the oestradiol peak for each log unit increase of urinary SG-BPA (BPA specific gravity, i.e., the BPA concentration corrected by the urine specific gravity) was observed (Mok-Lin, 2010). The study compared

urinary BPA concentrations to those observed in the general population in the NHANES 2003-2008 cohort. The concentration of urinary BPA found reflects exposure at the time of collection, and not during the period of follicular maturation several months earlier. In addition, it is difficult to extrapolate the results observed in a sample of infertile women seen for *in vitro* fertilisation to the general population. These results are nonetheless consistent with those of a recent study showing that exposure to BPA is associated with a decreased likelihood of success of IVF (fertilisation rate), attributed to impaired oocyte quality (Fujimoto *et al.*, 2011). Although this is a fairly limited group of patients, the authors indicate that the units of study were oocytes whose quantity was on average 13 per cycle and per woman.

A cross-sectional study was conducted in Japan in women with polycystic ovary syndrome (PCOS) (Takeuchi T, 2004). The women with PCOS were obese (n=6) or not (n=13), and the women without PCOS were divided into several categories: no disruption of the menstrual cycle and normal body weight (n=19), no cycle disorders and obesity (n=7), cycle disorders associated with hyperprolactinaemia (n=7), and cycle disorders associated with hypothalamic amenorrhea (n=21). BPA was measured in fasting plasma by a non-validated immunoassay method. BPA was present in all subjects. The statistical analysis was poorly detailed, the numbers were low; the final comparison was made with respect to non-obese women without cycle disorders (considered as controls). For the entire group, the study demonstrated a correlation between plasma concentrations of testosterone (free and total) and BPA on the one hand, and the concentration of BPA and body mass index on the other hand: the levels were significantly increased in women with PCOS (obese or not) and in the obese without ovulation disorders. The results remain difficult to interpret as they are, because of the imprecision of the sampling, the lack of information on inclusion criteria, and the lack of accounting in the results of disorders in the controls.

However, this study is in line with that described by Kandaraki *et al.* who found serum concentrations of BPA significantly higher in women with PCOS (n=71) (obese or not) compared to normal control women (n=100) (Kandaraki, 2010). In addition, BPA concentrations were significantly correlated with testosterone concentrations and insulin resistance. Women with PCOS were divided into obese (n=33) or non-obese (n=38) and were compared to women with normal ovarian cyclicity (obese: n=49 and non-obese: n=51). The main limitation of this study is the analytical method (ELISA) which does not discriminate between different forms of BPA. However, the concentrations obtained can be considered as a global indicator of exposure to BPA.

B.5.9.1.1.5. Conclusion on effects of BPA on humans reproductive data

There are relatively few epidemiological studies examining a link between exposure to BPA and effects on reproduction in women. These studies have methodological limits (size of the population studied, selection of participants, statistical analysis, etc.) which make them difficult to interpret. Correlations in populations (with many possible confounding factors) can only be convincing on the basis of a very large number of individuals, regardless of the statistical approach used to analyse these data. The human data are therefore to be considered with a great deal of circumspection and are in no way conclusive of an effect of BPA on the parameters studied. Based on current knowledge, human data relative to the effects of BPA on the **endometrium with endometriosis** (Cobellis, 2009; Itoh H, 2007) and **hyperplasia (**Hiroi, 2004), on the **ovaries (with polycystic ovary syndrome,** Takeuchi T, 2004, Kandaraki, 2010) and the **outcomes of pregnancy (miscarriage and premature**

births, Sugiura-Ogasawara, 2005; Cantonwine, 2010) in women are not conclusive and will not be used for the HRA.

The effects of BPA on **oocyte maturation** in women (decrease in the number of oocytes after ovarian stimulation and alteration in the quality of the collected oocytes), **in a context of ART (Assisted Reproductive Technology),** are suspected on the basis of a study of **high quality (Mok-Lin, 2010)** and of another which has **non-major methodological limitations (Fujimoto et al., 2011)**. Besides, they are comforted by the recent study from Machtinger (Machtinger, 2013) (not presented in this dossier because the date of the bibliographical search was stopped in 2012) on the effects of BPA on the **maturation of human oocytes** *in vitro*.

B.5.9.1.2. Animal data

B.5.9.1.2.1 Summary of previous expert assessments

According to the NTP-CERHR, the results obtained in different animal models are conflicting, and some studies have methodological flaws that make it difficult to interpret them (NTP, 2008). The results concerning early puberty are considered controversial.

However, according to the FDA, studies on delayed vaginal opening at high doses in animals are convincing, even if this parameter is not a direct measure of puberty, but an indicator of sexual maturation (FDA, 2008).

According to the FAO/WHO, many studies have been conducted in rodents and in other pets. Developmental or reproductive toxic effects were observed only in rodents, and their extrapolation to humans is still subject to discussion, and they believe it is important to consider species differences in terms of critical periods of development and sexual differentiation (FAO/WHO, 2010).

The experts on this panel believe that the evidence is controversial for the following effects:

- alteration of the age of puberty in F1 females,

- change in weight and histology of female reproductive organs of exposed adults,

- changes in hormone levels in exposed adults and F1 females.

According to INSERM, low doses of BPA during critical periods of development have an impact on the advance in the age of puberty, lead to changes in the oestrus cycle, sexual and maternal behaviour and benign, pre-malignant, and neoplastic effects on the female genital tract (histological alteration of the uterus and vagina, endometrial cystic hyperplasia, ovarian cysts) INSERM, 2010.

B.5.9.1.2.2 Existing multi-generation animal data and their limitations

<u>These studies are reported herein in the reproductive system-related section as they did not investigate neither the brain development nor the metabolism or the mammary glands.</u>

Continuous breeding study (Copy of the RAR-UK, Final report 2003, study considered as key study)

The effects of bisphenol-A on fertility and reproductive performance have been extensively studied in CD-1 mice (n= 20/ treated group/ sex (F0 generation), n= 40/ control group/ sex) using the test system known as the "Fertility Assessment by Continuous Breeding" (NTP, 1985b). This system involves four successive tasks (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.). Bisphenol-A was administered in the diet at concentrations of 0, 0.25, 0.5 or 1.0% (daily intakes of BPA 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg in males or females repspectively) during a one-week premating period and a 14-week mating trial (Task 2). After the premating period, males and females from each group were randomly paired and allowed to cohabit for 14 weeks. During the cohabiting period the reproductive performance was monitored by counting the number of F1 generation litters produced by each breeding pair and recording on the day of birth the litter size, proportion of live pups, litter size and sex ratio of the pups; all pups were then immediately removed and discarded. All litters produced after the cohabiting period remained with their mothers until weaning on day 21 post partum. The twenty F0 males and twenty F0 females from the top dose group (1.0% bisphenol-A) were then mated with twenty control females and twenty control males, respectively. Bisphenol-A was discontinued in the diet during this 7-day cohabitation period and then reinstated for 21 days upon separation of the breeding pairs. A control group of twenty untreated breeding pairs was also included (Task 3). The same reproductive assessment as described for the continuous breeding phase was conducted. Parental animals were sacrificed within 1 week of delivery. A maximum of twenty male and twenty female F1 generation offspring (from the final litters of the control and highdose groups in the continuous breeding phase) were retained after weaning for assessment of their reproductive capacity (Task 4). After rearing to sexual maturity, each F1 female was paired with a F1 male from the same dose group for 7 days. The resulting litters were evaluated and discarded on the day of birth as described for the litters produced during the F0 generation cohabitation phase. For all control and high-dose F0 and all reared F1 animals, liver, kidneys, adrenals and reproductive organs were weighed and subjected to histopathological examination. In males, sperm analysis (concentration, motility and morphology) was undertaken, and effects on the oestrous cycle assessed in females. There were no clinical signs of toxicity among F0 generation animals. In the continuous breeding phase, a statistically significant decrease in maternal body weight was observed after each litter (between 6 and 9%), at the top dose, on postnatal day 0 compared to controls. No effect was observed on maternal postnatal (day 0) body weight following the cross-over mating phase. However, at study termination, a small but statistically significant decrease in body weight (4%) was observed in treated females compared to controls. No adverse effects on body weight gain were observed in treated males. An adverse effect on fertility was observed in the continuous breeding experiment and cross-over mating experiment. In the continuous breeding phase, a statistically significant decrease compared to controls was observed in the number of litters produced per pair (4.5 and 4.7 compared to 5.0 for controls), litter size (6.5 and 9.8 compared to 12.2 for controls) and the number of live pups per litter (6.3 and 9.7 compared to 12.1 for controls) in the high and mid-dose group. The litter size reductions occurred across all matings and the magnitude of all these decreases were dose-related. No effects on fertility were observed in the low-dose group. A statistically significant decrease in litter size (controls: 11.4, treated males: 9.1, treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5) were observed in the cross-over mating. In the continuous breeding phase, a statistically significant decrease in live pup weight (6%) on postnatal day 0 was observed in females at the top dose after adjustment for litter size, including live and still births. In the continuous breeding phase a small but statistically significant decrease in body weight gain (4%) was only observed in

treated females at study termination. No effect was observed on the sex ratio in the F1 generation. In the F1 litters used in the cross-over breeding experiment, post natal (day 0) pup weights were significantly increased in males (9-11%) and in females (8-10%) in the mid- and high-dose groups compared to controls. These increases were no longer evident in either sex at 21 or 74 days of age. Deaths among F1 generation were observed during lactation (day 0-21) and post weaning (day 21-74). At the top dose there were only 8 litters that had at least one male and one female for the mating phase, and therefore only 11 breeding pairs at the top dose compared to 19-20 breeding pairs in the control, low-dose and mid-dose groups. In those litters selected for mating deaths had been observed in 6%, 4%, 14% and 38% animals up to day 74 in the control, low-dose, mid-dose and high-dose groups, respectively. It is not known how many animals of this total died during lactation. However, it does raise the possibility that there may be potential effects on pups due to exposure to bisphenol-A via the milk. In the F1 generation, bisphenol-A treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or mean pup weights at birth. At necropsy of the F0 generation (controls and top dose group only), treatment-related effects were seen at the highest dose level; for both sexes relative liver weight was increased about 28% and relative combined kidney/adrenal weight increased 10-16% compared to controls. No histological changes were observed in female reproductive organs and no effect was observed on the oestrous cycle. Overall, the signs of general systemic toxicity were not marked in this study and therefore the effects on fertility are not considered to be a consequence of parental toxicity. At necropsy of the F1 generation, treatment-related effects of similar magnitude were generally observed in males and females; compared to controls, increased relative liver weights (6-29%) and kidney/adrenal weights (13-20%) were observed in all treated groups. No histological changes were observed in the female reproductive organs. In this study, adverse effects on fertility, namely a reduction in litter size and number of live pups per litter, were observed in each litter from the F0 generation in the continuous breeding experiment at approximately 600 mg/kg and above, and at the only dose level tested in the crossover breeding experiment, approximately 1,200 mg/kg. A treatment-related decrease in the number of litters produced was also observed at 1,200 mg/kg during the continuous breeding phase. These effects were observed in the absence of significant parental toxicity. No effect on fertility was observed at 300 mg/kg, though no histopathology was conducted on these animals. Histological examination was conducted on all F animals, and the only effects observed were toxicity to the liver and kidney at all doses. No adverse effect on fertility was observed in the F generation up to approximately 1,200 mg/kg, which might have been expected in view of the observed effects on fertility in the F generation. Nevertheless, the absence of effects following the single F mating does not detract from the reproducible results across the 4-5 litters produced by each F generation pair. Therefore, overall, an adverse effect on fertility has been observed with BPA at approximately 600mg/kg and above.

Tyl et al. performed two multigeneration studies in 2002 and 2008. The studies design and the systemic toxicy findings are reported here with the results on females' reproductive toxicity. The results for the males are described below in the paragraph 4.11.2.1.6. The latest study is presented at first because a lot of supplementary data were available allowing an in depth evaluation.

In 2008, a 2-generation study was performed according to the OECD guideline 416, in mice (Tyl et al., 2008). Mice were exposed by gavage to 0, 0.018, 0.18, 1.8, 30, 300 and 3500 ppm (equivalent to approximately 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg bw/day) of BPA (purity

99.7%). The positive control group was exposed to the 17β-estradiol, and the negative control group received vehicle only. Each of the 8 groups was composed of 28 male and 28 female CD-1 mice (F0). Mice were exposed 8 weeks prior to mating, and then from conception to adulthood (chronic exposure). No toxicity was observed in the F0 or F1 generations and effects on the fertility were only observed at the highest dose (3500 ppm: 600 mg/kg bw/d). Although it has been described in the paper that systemic toxicity can be observed at this dose, a thorough observation of the data provided as supplementary tables with the paper did not allow validating this statement. Indeed, F0 male body weights (BW) were comparable all along the study between the various treatment groups and control. As shown in figure 1, F1 male (parental and retained (not presented here)) have the same growth curve as controls during the treatment period whatever the treatment group. The difference observed comes from PND14's BW. At this timepoint, 3500 ppm pups' BW is lower by 10% (compared to controls) and this difference persists along the entire treatment period (from day 0 on the Figure 1 to mating period). 3500 ppm F2 pups are also smaller by 5% compared to controls at PND14. The origin of this difference in BW is unclear as birth's BWs are similar among the different groups. This strongly suggests an impact of 3500 ppm-BPA exposure on lactation. Interestingly, this BW difference persists in males treated with 3500 ppm of BPA although they eat more than controls whatever the timepoint and the generation (food consumption of F1 parental sd 0 to 7 = control + 12.3%) but diseappears in females during direct treatment although treated females do not eat more than controls.

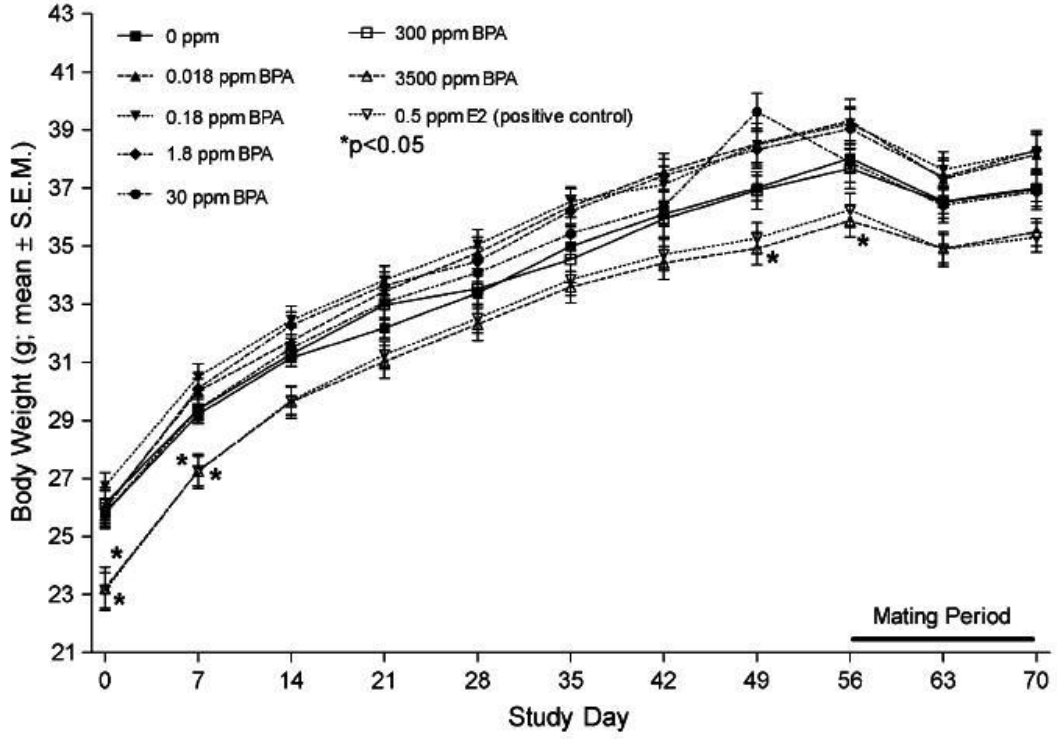


Figure XX from (Tyl et al., 2008): F1 parental body weights during the prebreed and mating periods.

Signs of toxicity were observed as increased kidney and liver weight from 300 ppm and onward for F0 males, from 0.018 ppm in F1 parental males, in F0 and F1 females and in F1 & F2 pups (male and females) at 3500 ppm. However, these results suggest rather a strong and direct effect of BPA on these organs than systemic toxicity.

Together with the effect of BPA on BW evolution depending on the sexe of the animals, another finding points out the potential endocrine effect of BPA: Pituitary relative weight is increased in F1 parental and retained male at all doses (significant at 300 ppm). Only F0 E2-treated males have this finding. Detailed brain dissection was not performed and brain global was weighted in pups, so we cannot confirm this finding in the next generation. Therefore, BPA exposure impacts the pituitary gland after an in utero exposure that might affect fertility through sexual hormone modifications.

In females, most of the reproductive parameters (i.e. reproductive organ weights, ovarian primordial follicles count, histopathology of ovaries and uterus, mating and fertility indices, litter size at birth, sex ratio, percent of post-implantation loss) were unaffected by the treatment. Effect of BPA on reproduction and the offspring were only observed at 3500 ppm. At this dose, BPA exposure increased the length of the gestation by 0.3 days, reduced the body weight of the pups during lactation, and F0 treated females were twice more in estrus compared to controls as shown in the supplementary table 22 p. 6/7, line 5. No effects on the female reproductive system were observed in the study in mice (Tyl et al., 2008).

When comparing all publications on BPA in female mice, the strain used might explain some of the differences through their genetic background together with the exposure period .

Hence the available data need to be evaluated by taking into account these parameters. Indeed, Tyl et al. study was performed on CD1 mice. CD1 stock is not as homogeneous as C57BL6 or BALB / c, which are stable lines. From non inbreed breeding , the stock CD1 is genetically labile and very heterogeneous, rendering this strain less sensitive (more animals needed due to strain inherent variability) (R Chia et al . Nature Genetics 2005) . In addition, CD1 have mutations that make them vulnerable to certain effects of carcinogens (Manenti G et al. , Carcinogenesis 2003).

Most of the studies performed in mice were performed on stocks such as CD1 , ICR and CF1 (Howdeshell et al 1999; . Honma et al 2002 . Nikaido et al 2004 and 2005 . Tyl et al 2008 . ; Nah et al 2011; . Xi et al , 2011; . Cabaton et al 2011) and not so much on lines.

➔ Rats

The effect of BPA on fertility was evaluated in an extensive oral two generation reproduction toxicity study (Copy of the RAR-UK, Final report 2003, study considered as key study) in Crj;CD (SD) IGS rats (Ema et al., 2001). The F0 generation consisted of groups of 25 rats per sex per group administered 0, 0.2, 2, 20 and 200 µg/kg/day BPA by gavage during a premating period of 10 weeks for males and 2 weeks for females and a 2-week mating period. Males and females from each group were randomly paired and co-habited for 2 weeks. Females were also administered the test material during gestation and lactation. F0 males and females were sacrificed after the mating period and weaning of F1 pups, respectively. Twenty-five male and female F1 generation offspring from each group were retained after weaning for

assessment of their reproductive capacity. F1 animals were administered bisphenol-A for a 10-week premating period and a 3-week mating period (see below). Again, females received the test material during gestation and lactation, and male and female parental animals were sacrificed at the same times used for the F0 generation. Twenty-five male and female F2 generation offspring from each group were retained after weaning for assessment of sexual maturation. Males and females were administered the test material until they were sacrificed at the age of 7 and 14 weeks, respectively.

For all F0 and all reared F1 and F2 animals, observations and weighings were performed regularly. In addition to determining reproductive capacity, various other parameters were assessed. Learning tests were conducted using a water filled multiple T-maze with 6 male and 6 female F1 animals per dose group at 5-6 weeks of age. Several reflex assessments were determined in 1 rat per sex per litter until successfully completed. Sexual maturation (vaginal opening and preputial separation) was determined in F1 and F2 parent animals, along AGD. After sacrifice, all F0 and F1 parent animals were subjected to a thorough macroscopic and microscopic examination. In males, this included examination and weighing of the epididymis, prostate and seminal vesicles (including the coagulating gland). Serum testosterone, oestradiol, prolactin, LH, FSH, T3, T4 and TSH concentrations were also determined in 6 animals per sex per group from the F0 and F1 generations. Seminal vesicles and coagulating gland were weighed and subjected to histological examination. The motility and morphology of sperm in the epididymis was also determined in F0 and F1 males. All pups that were not selected for further assessment were sacrificed and also underwent histopathological examination.

In parental animals, no clinical signs of toxicity, nor any effects on body weight gain, food intake or treatment-related deaths were observed in any generation. No effect on behaviour (i.e. performance in learning tests) was observed in F1 animals. Oestrus cycle, fertility index and the number of implantations in F0 and F1 females were not affected by treatment with bisphenol-A. (The mating period for F1 animals was extended for a week, as at the end of the first week mating was confirmed in only 19/25 females administered 0.2 µg/kg/day, compared to 24/25,22/25, 23/25 and 21/24 at 0, 2, 20 and 200 µg/kg/day respectively. At the end of the 3-week mating period no significant effect on the fertility index was observed between treated and control animals). No significant differences were observed between BPA and control animals for the time to vaginal opening. Compared to controls, a statistically significant decrease (<5%) in AGD was seen F1 and F2 females at 20 and 200 µg/kg/day. These decreases were not statistically significant when the ratio of the AGD to body weight was determined (the AGD is correlated with body weight). No treatment related changes were observed in any of the serum hormone levels measured. BPA had no effect on sexual maturation or the oestrus cycle in F2 animals and F2 females, respectively. At necropsy, no treatment-related macroscopic findings or organ weight changes were observed in F0 and F1 parental animals.

In the offspring (all live pups up to day 21), no clinical signs of toxicity or effects on body weight gain during lactation were observed in F1 and F2 pups. No treatment-related changes were seen in the litter size, survival, sex ratio, AGD and reflex ontogeny. At necropsy, no treatment related macroscopic findings were observed in F1 and F2 pups.

In the oldest study (Tyl et al., 2002), exposure of males and females CD Sprague Dawley rats to BPA (purity at 99.5%) administrated in the diet at 0, 0.015, 0.3, 4.5, 75, 750 or 7500

ppm (this doses were equivalent in actual intake to 0.0007-0.003, 0.015-0.062, 0.22-0.73, 4.1-15.4, 37.6-167.2 and 434-1823 mg/kg bw/day in males and females respectively) for three generations was evaluated under Good Laboratory Practice using the U.S. EPA OPPTS test guidelines (U.S EPA OPPTS 837.3800, 1998). 30 rats per sex and per dose were exposed 10 weeks prior to mating, and then for males continued through a 2-week mating period and for an additional 3 weeks after mating. Females were exposed from conception trough gestation and lactation. Males and females from a same group were mate together, 3-generations of males and females were then studied. For each generation 30 weanlings animals per sex and per dose were selected in order to become the parents of the next generation, and 3 animals per sex and per litter were necropised and undergo further analysis. Adult systemic toxicity were limited to reduced body weight due to lactational effects together with smaller body weight gains (-22% of the F1 7500ppm treated males). However, feed consumption did not show clear treatment-related effects. Although the data available for this study are less detailed than for the study above, we can affirm from the previous study that the slight to mild renal tubular degeneration and chronic hepatic inflammation observed in females for the three generations at 750 and 7500 ppm is a strong and direct effect of BPA on these organs rather than systemic toxicity.

Results show that there was no effect of BPA on estrous cycle length, paired ovarian primordial follicle counts or in reproductive organs histology. Similarly, many reproductive parameters including mating, fertility, pregnancy, dead pups per litter or percent post-implantation loss remain unaffected in F0, F1, F2 females. However, at 7500 ppm, the number of implants, total pups and live pups/litter at birth and on PND4 were reduced ($p < 0.001$) and the absolute and relative organ paired ovary weights were decreased in F1, F2 and F3 offspring and adult ($p < 0.05$ and $p < 0.001$ respectively). In female offspring, AGD was significantly increased in the F2 generation at all dietary doses, with the exception of the 75 and 7500ppm groups. The absolute age at vaginal patency (days) was significantly delayed in the F1, F2 and F3 generations at 7500ppm (and at 75 ppm only for the F2 generation).

This study was performed according to the US EPA (1998) guideline, presenting the following strengths: it is a third offspring generation, six treatment groups, examination for retained nipples and areolae in male preweanlings and retention of F3 offspring until adulthood with continuing exposure, with histopathologic and andrological assessments at their termination.

However, some weaknesses can be pointed out. The histopathological examination was performed only for the 30% of the BPA-exposed animals (10 animals per sex and per dose). No explanation were provided on the selection of the animals (randomly or if other selection criteria were applied). In addition to the 10 animals, BPA-exposed rats were examined histopathologically if showing "any gross lesions and reproductive tissues from unsuccessful breeders or animals suspected of reduced fertility". It is considered rather insufficient according to current OECD guidelines, reflecting that impaired fertility is an insensitive parameter in rats (a reduction of fertility in males is detected by a reduction of spermatogenesis well over 60%). In females, enumeration of ovarian primordial follicles was performed from step sections of both ovaries of ten females each at high dose and control, i.e. in 30% of animals only without any explanation.

Besides, delayed vaginal patency was observed only at very high doses in the study in rats but there were no other effects on female reproduction parameters in this study (Tyl et al., 2002), which contradict studies presented below also it is difficult to explain why. Some of the parameters were only not evaluated in Tyl study.

B.5.9.1.2.3 Data taken into account for reproductive tract hazard assessment

The studies in animals are presented below by window of exposure and then by effect. Among the studies presented, critical effects, key studies, and critical doses are selected and NOAEL or LOAEL are chosen.

B.5.9.1.2.4 Prenatal, perinatal and pre-pubertal exposure

Effects on the reproductive tract and ovaries

Oral exposure to 1.2 mg/kg bw/day of BPA in rats during pregnancy and lactation is suspected of inducing an increase in the thickness of the epithelium and stroma of the uterus in the offspring, a decrease in apoptosis of the uterine epithelium, disorders of the oestrus cycle, and a decrease in ERα receptor expression in the epithelial cells of the uterus during the oestrus phase (Mendoza, 2010). These results are in line with those obtained by Markey in 2005 (Markey, 2005). In that study, female CD-1 mice aged 3 months from mothers treated with very low doses (25 and 250 ng/kg bw by subcutaneous pump from GD9 to PND4) had decreased vaginal weight, impaired DNA synthesis in the uterine epithelium (250 ng) and a significant increase in the expression of ERα and PR (progesterone receptor) at the lowest dose (Markey, 2005).

In Balb-C mice, *in utero* exposure to BPA and before weaning (mothers treated at 100 and 1000 µg/kg bw/day by subcutaneous injection) was associated with the development of structures suggestive of endometriosis in the peri-uterine fat, an increased incidence of cystic ovaries, and endometrial hyperplasia (Signorile, 2010).

According to the studies reviewed, BPA is suspected to be linked to the development of ovarian pathologies, in particular polycystic ovaries, in CD-1 mice from mothers treated with BPA from PND1 to PND5 (10, 100, 1000 µg/kg bw/day by subcutaneous injection) (Newbold, 2007). In all groups treated with BPA and regardless of the dose, the animals developed ovarian and/or uterine pathologies (benign, pre-malignant, and neoplastic proliferative lesions of the uterus), with little or no representation in the control group. However, only the increase in the frequency of appearance of polycystic ovaries and cystic endometrial hyperplasia in the group treated with 100 µg/kg bw/day was statistically significant. The same team found similar results for exposure later in gestation (GD9 to GD16), from 10 µg/kg bw/day (Newbold, 2009). The increase in the frequency of occurrence of ovarian cysts was significant at 1 µg/kg bw/day. Similar lesions were found by Signorile *et al.* in offspring of Balb-c mice exposed to higher doses of BPA (100 and 1000 µg/kg bw/day) during gestation and lactation (Signorile, 2010).

Similarly, in rats, treatment with BPA subcutaneously in the neonatal period (0.25 to 25 mg/kg bw/day) was associated with the development of phenotypes similar to polycystic ovary syndrome. Although the effects were significant starting from the lowest dose, the doses used were high (Fernandez, 2010). Adewale *et al.* reported a reduction in age of puberty, an increase in the proportion of acyclic animals, and ovarian dysfunction among the descendants of females treated from PND0 to NDP3 with 50 µg/kg bw/day or 50 mg/kg bw/day of BPA (Adewale, 2009). The positive control used was oestradiol benzoate (25 µg – the unit was not

specified by the author). However, in the study of Nikaido *et al,* prepubertal neonatal exposure (15 to 19 days) to BPA (10 µg/kg bw/day subcutaneously) led to no change of the uterus or vagina or of mammary development, although over 80% of treated animals exhibited an anovulatory state (absence of corpora lutea) at 4 weeks (Nikaido, 2005). Moreover, in this study, exposure to BPA did not affect the age at puberty or ovarian cyclicity.

Similarly, Long Evans rats in gestation were treated with BPA (2, 20, and 200 µg/kg bw/day) or ethinyl oestradiol (EE2, 50 µg/kg bw/d) from GD7 to PND18 orally (Ryan, 2010). Unlike the positive control (EE2), the female offspring of mothers treated with BPA demonstrated no change in body weight, age at puberty, anogenital distance, fertility, or sexual behaviour. Finally, F1 offspring from Sprague Dawley rats treated with BPA in drinking water during gestation and lactation (estimated ingested dose from 0.1 to 1.2 mg/kg bw/day) showed no difference in age at puberty or anogenital distance at birth (Rubin, 2001). In contrast, the females after puberty had irregular ovarian cycles and decreased LH secretion after castration. This study provides excellent confirmation, and suggests that developmental exposure to BPA could induce, in rodents, impaired ontogenesis of gonadotropin function.

Moreover, the descendants of CD1 mice treated with subcutaneous osmotic pumps with very low doses of BPA from the eighth day of gestation until day 16 of lactation, studied over several successive pregnancies, presented reduced fertility and fecundity (number of pregnancies over 32 weeks and number of offspring per birth and total number of pups born over the 32 weeks of the study) at 25 ng/kg bw/day and 25 µg/kg bw/day, but not at 250 ng/kg bw/day. These effects are only apparent after 5-6 pregnancies (Cabaton, 2010). These results could be explained by a non-monotonic U-shaped dose-response curve. However, further studies are needed, including a greater number of doses to better characterise this type of dose-response relationship. According to the authors, BPA accentuated the "physiological" decline in the number of pups per litter as a function of age, similar to the DES control. This study is interesting, first because it has excellent safeguards in terms of control of experimental conditions, but also because it could explain the lack of effect in other studies where similar observations were limited to the first pregnancy in F1 offspring from exposed mothers, such as the Ryan study (Ryan, 2010) and the Zoeller study (Zoeller *et al.*, 2005) in rats. In the latter, the BPA administered to pregnant rats from gestation day 7 until the end of gestation at oral doses of 1-50 mg/kg bw/day did not seem to affect the *in utero* development of pups (Zoeller *et al.*, 2005).

Effects on the hypothalamic-pituitary-gonadal axis

In rodents, the neonatal period (PND1 to 10) is a critical period for development of the hypothalamic-pituitary-gonadal (HPG) system. Exposure to BPA during this period causes changes in the secretion of hypothalamic-pituitary hormones. These include the level and frequency of hormonal secretions and were responsible for disruption of reproduction in the long term.

Treatment of sheep during gestation over a period covering ontogenesis and sexual differentiation of the GnRH system (5 mg/kg bw/day intramuscularly for GD30-GD90) is associated with malfunctions of the HPG axis in female offspring: hypersecretion of LH in the prepubertal period, changes in the preovulatory LH surge (positive feedback of oestradiol) (Savabieasfahani, 2006). This same treatment induces a decrease in GnRH gene expression, an increase in expression of the ESR1 (ERα) oestrogen receptor, and decreased ESR2 (ERβ) receptor expression in the preoptic area (Mahoney, 2010 . The authors measured

unconjugated BPA plasma concentrations during treatment, and argue that these concentrations are close to the maximum plasma levels described in pregnant women (~ 10 ng/mL). The sampling period compared to the administrations was not specified, but it is likely that these concentrations correspond to residual levels and are not representative of the exposure of the animal over 24 hours. Similarly, treatment of prepubertal sheep intramuscularly 2 times/week for 5 weeks with diethylstilbestrol (DES; 0.175 mg/kg) or BPA (3.5 mg/kg) led to a decrease in the frequency and the amplitude of LH pulses after ovariectomy in these animals compared to controls (Evans, 2004). The treatment of prepubertal sheep with BPA for short periods (4 days) at different doses (intravenous infusion at 0.5 - 1 - 2.5 - 5 - 10 - 20 - 40 and 80 mg/kg bw/day), allowed detection of effects of BPA on the LH pulse generator system that are qualitatively similar to the effects of 17-β-oestradiol (positive control) and seem to obey two types of mechanisms: immediate inhibitory effects at high doses and delayed effects expressed at lower doses, resulting in plasma concentrations of about 38 ng/mL (double the highest values described in humans) (Collet, 2010). BPA is significantly less potent than oestradiol as an inhibitor of pulsatile secretion of LH. The lowest plasma concentration of oestradiol associated with an inhibition of pulsatile secretion of LH is 2 pg/mL, compared to 38 ng/ml for BPA.

In light of these studies in sheep, BPA is suspected to alter the ontogenesis of the GnRH/LH system controlling the pulsatile secretion of LH. In addition, short term effects are found on the neuroendocrine system controlling the pulsatile secretion of LH, with an EC_{50} close to the highest plasma levels described in humans. However, the relevance of the model remains questionable, as the prepubescent sheep is indeed particularly more sensitive than humans to oestradiol negative feedback.

Fernandez *et al.* reported increased secretion of GnRH, of progesterone (significant effect at the lowest dose) and increased secretion of oestradiol and testosterone in rats treated with BPA by subcutaneous injection (0.25 to 25 mg/kg bw/day) in the neonatal period (PND1 to 10) (Fernandez, 2010). However the effects are obtained for the two highest doses. Rubin *et al.* report an irregularity of the ovarian cycles and a decrease in LH secretion after castration of F1 offspring from SD rats treated with BPA in drinking water (estimated intakes of 0.1 to 1.2 mg/kg bw/day) (Rubin, 2001). However, Adewale *et al.* suggest that BPA disrupts ovarian development but not the sensitivity of GnRH neurons in the positive feedback of oestradiol at the origin of the genesis of the preovulatory LH surge (Adewale, 2009). The rats received 50 µg/kg bw/day or 50 mg/kg bw/day of BPA. The induction of the expression of the proto-oncogene C-Fos in GnRH neurons following a preovulatory dose of oestradiol (positive feedback) was not altered in animals treated with BPA, while it was reduced in positive control treated animals (oestradiol benzoate).

The KiSS neuropeptide is involved in the central control of reproductive function, especially in puberty. It is expressed in two structures, among others; the anteroventral periventricular (AVPV) and arcuate nuclei (ARC). Exposure to BPA during the postnatal period in young Wistar rats (100 to 500 µg/rat from day 1 to day 5) decreases the amount of mRNA of the KiSS peptide measured by RT-PCR in the whole of the hypothalamus in prepubescent males and females at 30 days. This effect persists in males at 75 days (Navarro VM, 2009**)**. Fifty µg/kg bw/day and 50 mg/kg bw/day of BPA were administered subcutaneously from the first to the fifth day of life for young Long Evans rats (Patisaul, 2009). Two positive controls were included: oestradiol benzoate (EB 25 µg/rat) and an ERα agonist (PPT 1 mg/kg). KiSS immunoreactivity was measured in intact pubescent males and ovariectomised females after

puberty and subjected to replacement steroid treatment (10 µg of oestradiol benzoate followed at 48 hours by 500 µg of progesterone). In the AVPV, the EB and PPT induced a decrease in KiSS immunoreactivity over untreated controls; BPA had no significant effects. In the ARC, only EB decreased KiSS immunoreactivity. In males, KiSS immunoreactivity was not affected by any treatment, regardless of the structure.

Effects on age at puberty

In animals, exposures limited to pregnancy (the second half in mice) demonstrate a fairly consistent advance of sexual maturation, assessed by age at vaginal opening and/or age at first oestrus (Honma, 2002) (Howdeshell, 1999) (Honma *et al.*, 2002; Howdeshell *et al.*, 1999; Nikaido *et al.*, 2004) (Nikaido, 2004). It should be noted that the age at vaginal opening is an indicator of sexual maturation and provides only indirect assessment of the degree of advancement of puberty. In the study by Howdeshell, for example, no significant effect of BPA on age at vaginal opening was observed, while a sizeable decrease in the time between the opening and the onset of first oestrus was recorded. In addition, this study clearly demonstrates that the effect of BPA on pubertal maturation can be largely modulated by the intrauterine environment. Thus, the effect of BPA is only slightly or not at all expressed in female foetuses having been surrounded by two male foetuses during pregnancy. This study clearly highlights a major limitation of the rodent model, and consequently other studies having estimated the age at puberty without incorporating the concept of the intrauterine environment. One single study has been conducted in another animal model without this limitation (sheep) with exposure to high doses (5 mg/kg bw/day) subcutaneously (2/5th of gestation) and showed no impact on age at first oestrous cycle (Savabieasfahani, 2006). However, it should be noted that the occurrence of the first cycle in sheep may be influenced by the photoperiodic environment, which in this study could have attenuated the effects of BPA.

Studies in rodents on early postnatal exposure also indicate a fairly consistent advance of the age at vaginal opening for a range of doses large enough for subcutaneous exposures (50 µg/kg bw/day to 6 mg/kg bw/day) (Adewale, 2009) (Fernandez, 2009).

Surprisingly enough, studies concerned with broader exposure, which include the second half of pregnancy and postnatal exposure until puberty in rats, reveal no effect of BPA on the age at vaginal opening and/or the first oestrus (Kwon S, 2000; Ryan, 2010; Ryan, 2010; Yoshida M, 2004; Takagi H, 2004). Similarly, a study of peripubertal exposure showed no BPA effect.

In summary, an acceleration of puberty in mice following exposure *in utero* and/or in the early postnatal period can be considered as an established fact. This effect is not expressed during extended developmental exposure comprising part of gestation and a postnatal and peripubertal period. However, most studies evaluated for exposures *in utero* have a major drawback: not taking into account the intrauterine environment, which could notably explain such a lack of effect. Such a bias is generally not expected in humans, where pregnancy with twins of the opposite sex is rather rare.

B.5.9.1.2.5 Adult exposure

Overall, data resulting from exclusive exposure of animals in adulthood are piecemeal, and rely on high doses for short periods, with the exception of studies on implantation and gestation.

Exposure during implantation in CD1 mice subcutaneously at high doses (minimum 100 mg/kg bw/day, 20 times the NOAEL) leads to a decrease in the number of implantation sites (200 mg/kg bw/day), histological alterations of the uterine wall (cell height) and a decreased expression of ERα and PR receptors only at the highest dose (300 mg/kg bw/day) (Berger, 2010). Oral doses of about 2 g/kg bw/day are necessary in order to observe an effect on gestation (Berger, 2007). Similarly, exposure to BPA at 10 mg/kg bw/day from GD0 to GD7 subcutaneously in ICR mice induced a significant decrease in the number of embryos at D10 and D12, associated with decreased weight of the uterus and marked alterations in placental structure (Tachibana, 2007). However, in C57BL6 mice, BPA at low doses in the diet (approximately 0.1 to 10 mg/kg bw/day) throughout gestation did not induce any modification of gestational parameters (duration, litter size, survival of young, etc.) (Kobayashi K, 2010). It is therefore likely that these low doses, pertinent in terms of human exposure, do not induce significant enough changes in the uterine wall to have a functional impact on gestation. In addition, BPA administered to pregnant rats from the seventh day to the end of gestation, at oral doses of 1 to 50 mg/kg bw/day, did not induce changes in litter size or pup weight at birth (Zoeller *et al.*, 2005).

Ovariectomy in rats induced changes in uterine morphological parameters (uterotrophic OECD TG 440) and an increased expression of oestradiol receptors in the uterus. The administration of BPA at doses of 0.5 to 50 mg/kg bw/day for 5 days by subcutaneous injection in ovariectomised Wistar rats did not restore these uterine parameters to a level similar to that of non-castrated rats. Similarly, BPA does not, unlike oestradiol, suppress the increased expression of ERα and β receptors induced by ovariectomy. On the other hand, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), a potential liver metabolite of BPA, is used to cancel the effects of castration on the uterotrophic test and the expression of oestrogen receptors like oestradiol (positive control) (Okuda *et al.*, 2010). The oestrogen-mimicking potential of BPA in this model appears quite moderate, compared to its potential metabolite MBP and oestradiol.

Table 18. Animal studies examining the effects of bisphenol A on the female reproductive tract: summary table

Reference	**Species**	**Routes**	**Dose** **Exposure period**	**Effects NOAEL/LOAEL**
Mendoza, 2010	Wistar rats	Oral	10 mg/L in drinking water, estimated intake of 1.2 mg/kg bw/day GD6 - PND21	**F1** ↗ thickness of the epithelium and uterine stroma ↘ apoptosis in the uterine epithelium disorders of the oestrus cycle ↘ of ER-α receptor expression in the epithelial cells of the uterus during the oestrus phase
Ryan, 2010	Long-Evans	Oral	2 - 20 or 200	No effect (F0 and F1 weight, primary sexual characteristics, fertility,

	rats		µg/kg bw/day GD7 - PND18	fecundity, sexually dimorphic behaviour) following a pre-and neonatal exposure to low doses of BPA Confirms the results of multigenerational studies (Tyl *et al.*, 2002) (etc.)
Adewale, 2009	Long-Evans rats	Sub-cutaneous	50 and 50,000 µg/kg bw/day PND0-PND3	**F1 as adults** ↘ age at puberty (advancing age of vaginal opening, stronger effect at lower doses) modification of ovarian morphology (cysts, ↘ number of corpora lutea, degenerate follicles) ↗ proportion of acyclic animals No change in sexual behaviour No change in the expression of FOS in the GnRH neurons for the two BPA groups
Fernandez, 2010	Sprague-Dawley rats	Sub-cutaneous	5 (0.25 to 0.62 mg/kg), 50 (2.5 to 6.2 mg/kg), 500 µg/50µL (25 - 62.5 mg/kg) PND1 - 10	↗ serum testosterone and oestradiol level and ↘ progesterone in adulthood and altered secretion of GnRH *in vitro* <u>50 µg/50 µL</u>: reduced fertility <u>500 µg/50 µL</u>: abnormal morphology of the ovaries with many cysts (morphology similar to that observed in the case of polycystic ovaries in women); all sterile females **LOAEL=2.5 mg/kg**
Markey, 2005	CD-1 mice	Sub-cutaneous	0.025 and 0.25 µg/kg bw/day GD9 - GD23	**F1** <u>0.025 and 0.25µg/kg bw/day</u>: Changes in ovarian morphology at 3 and 6 months and alteration of the uterus and vagina: ↘ dry weight of the vagina, ↘ endometrial volume, ↗ expression of ERα and PR in uterine epithelium, impairment of DNA synthesis in the uterine epithelium

				LOAEL=25 ng/kg bw/day
Rubin, 2001 (see description below)	Sprague-Dawley rats	Oral	estimate of 0.1 mg/kg bw/day to 1.2 mg/kg bw/day (drinking water) GD6 - pups were weaned	**F0:** Oestrus cycle disorders (longer than normal) ↘ secretion of LH in response to ovariectomy -> suggesting a neuroendocrine effect **F1** ↗ bodyweight (after birth and up to adult age) Alteration oestrogenic cyclicity and ↘ in LH at adult age after castration No difference in age at puberty nor difference in anogenital distance at birth
Newbold, 2007	CD-1 mice	Sub-cutaneous	10-100-1000 µg/kg bw/d PND1-PND5	- No difference between **body weight** of the treated and control animals, irrespective of the dose. **Ovaries** - Appearance of ovarian cysts, significant only at the dose of 100 µg/kg bw/d of BPA - Decrease in the observation of corpora lutea when the dose increases (NS) - Appearance of para-ovarian cysts of mesonephric origin (absence in the control group) NS - Appearance in the BPA groups of progressive proliferative lesions (PPL), absent in the control group (NS) **Uterus** - Increase in the incidence of endometrial hyperplastic cysts, but only the BPA100 dose causes a

				significant effect - Tendency, at the highest doses, towards an increase in atypical hyperplasia of the endometrium, a precursor for adenocarcinoma - Appearance (NS) of leiomyomas (absence in control group) - Upper stromal polyps in the BPA100 group - Increased incidence of enlarged Wolffian ducts in the treated mice **NOAEL: 10 µg/kg** **LOAEL: 100 µg/kg**
Newbold, 2009	CD-1 mice	Sub-cutaneous	0.1-1-10-100 and 1000 µg BPA/kg bw/d GD9-GD16	**Ovaries:** - No difference for the number of mice not having corpora lutea - Significantly increased incidence of ovarian cysts for BPA-1 only - Presence of prominent para-ovarian cysts (no associated statistical test) at BPA-10 - Neoplastic lesion in the ovary including cystadenoma present at BPA-10, 100 and 1000 (NS) - Progressive proliferative lesion observed in all the treated groups but not the controls (NS) **Uterus** - Cystic endometrial hyperplasia (CEH) incidence increased for all the groups except BPA-0.1 (even the control) at NS - Adenomyosis: In controls, BPA-0.1 and BPA10 - Adenomatous hyperplasia with CEH in BPA-1, BPA-100 but not in controls

				(NS) - Atypical hyperplasia of the uterus, considered to be a precursor for uterine adenocarcinomas, found in BPA0.1, BPA1 and BPA1000, not in controls (NS) - Wolffian remnants in the uterus comparable to those seen in the ovary and in the fallopian tubes in all groups except BPA-100 - Uterine polyps seen in BPA0.1, BPA1 and 10 (NS). Lesions of this type have been reported as being associated with the development of stromal cell sarcomas in rodents **Vagina** - One BPA-1000 mouse had a vaginal adenoma characterised by glandular structures at atypical locations **Premature death or euthanasia** - One BPA-1 mouse had a sarcoma which invaded the reproductive organs, but it was definitely a cancer of hematopoietic origin in view of the overall incidence of the lesions - There were significantly more lesions in the genital tract for BPA-0.1 than in the controls. - There were significantly more lesions (independently of location) for BPA-0.1 and BPA-1 than for the other doses. **$LOAEL_{est}$ = 0.1 µg/kg bw/d**
Nikaido, 2005	CD-1 mice	Sub-cutaneous	10 mg/kg bw/d PND15-PND19	No acceleration of the beginning of age at puberty No modification of the uterus nor of the vagina nor of mammary development Anovulatory state for 80% of the

				animals treated with BPA *versus* control group No modification of ovarian cyclicity
Signorile, 2010 **(see description below)**	Balb-C mice	Sub-cutaneous	100 and 1 000 µg/kg bw/d GD1 - PND7	Lesions of cystic hyperplasia type and atypical lesions of the endometrium <u>10 or 1000 µg/kg bw/d in F1 ♀ after 3 months:</u> ↗ frequency of appearance of structures (glands and stroma expressing ER and Hoxa10) of endometriosis type in the adipose tissue surrounding the genital tract. ↗ frequency endometrial hyperplasia, cystic ovaries and endometrial hyperplasia significantly more frequent in F1 ♀ BPA (free) in the liver of all treated F0 ♀ and F1 (no dose-dependant relationship) with no correlation with the occurrence of a pathological condition similar to endometriosis **LOAEL = 100 µg/kg bw/d**
Cabaton, 2010	CD-1 mice	Oral	25 ng, 250 ng or 25 µg/kg bw/d GD8 - PND16	↘ in fertility and fecundity (↘ in the number of gestations over a period of 32 weeks, in the number of young per birth and in the total number of young born over the 32 weeks of study) **LOAEL = 25 ng/kg bw/d**
Evans, 2004	Ewes	Intra-muscular	3.5 mg/kg twice a week 4-week-old ewes treated for 5 weeks	↘ in the frequency and amplitude of LH pulsatility after ovariectomy No modification of ovary weight
Navarro VM, 2009	Wistar rats	Sub-cutaneous	100- 500 µg/animal PND1-5	Suppression of KiSS-1 messenger RNA levels in the hypothalamus that may lead to a modification of the hypothalamic-pituitary axis and of gonadotropic hormone secretion

Savabieasfahani, 2006	Ewes	Intra-muscular	5 mg/kg bw/d GD30-GD90	Hypersecretion of LH in the prepubescent period Modification of the preovulatory peak of LH
Mahoney, 2010	Sheep	Sub-cutaneous	5 mg/kg bw/d G30-G90	↗ in the expression of ESR1 and ↘ in the expression of ESR2 ↗ in the expression of gonadoliberin
Collet, 2010	Ewes	Intra-venous	5-10-20-40 and 80 mg/kg bw/d Adult ewes treated for 4 days	Effects on the LH pulse-generating system qualitatively similar to the effects of 17β-oestradiol (positive control).
Berger, 2010	CF-1 mice	Sub-cutaneous	100 - 200 - 300 mg/kg bw/d GD1 - GD4	↘ implantation sites Histological modifications of the wall of the uterine cavity Decrease in ERα and PR receptor expression
Berger, 2007	CF-1 mice	Oral	Administration of BPA by addition to peanut butter in an amount of 0.11-9% or by addition to the feed in an amount of 3 and 6%. GD1-GD5	No modification of litter size or of parturition rate The dose of 68.84 mg of BPA/d/animal (corresponding to a BPA supplementation at 6%) causes the abortion of all gestations
		Sub-cutaneous	0.0005-0.0015-0.0046-0.0143-0.0416-0.125-0.375-1.125-3.375, and 10.125 mg/animal/ day GD1- GD4	↘ in litter size at 3.375 mg/d ↘ in the proportion of females to be parturient at 10.125 mg/d ↘ in the number of implantation sites at the dose of 10.125 mg/d
Tachibana,	ICR	Sub-	10mg/kg bw/d	↘ in the embryo number

2007	mice	cutaneous	GD0 - GD7	↘ in the weight of the uterus and marked modifications of placental structure
Kobayashi K, 2010	C57BL/ 6J mice	Oral	0.05-0.5 or 5 mg/kg bw/d GD6-PND22	No modification of body weight, of gain in body weight, feed consumption, duration of gestation, litter size, or survival of the young in the F0 animals No difference between the sex ratio and the viability in the F1 animals No modification of body weight, feed consumption, developmental parameters, anogenital distance, or organ weight (liver, kidney, heart, spleen, thymus, testis, ovaries and uterus) in F1 and F2 adults. No modification of sperm number or motility in F1 and F2 animals
Munoz del Toro, 2005	CD-1 mice	Sub-cutaneous	25 - 250 ng/kg bw/d GD9 - PND4	- No modification of TEB number, size and area - Increase in mammary gland sensitivity to oestrogen - Decrease in number of cells in apoptosis in the TEBs starting from 25 ng/kg bw/d - No proliferative effect - No increase in ERα receptors, but increase in progesterone receptors - Significant increase in side-branching of mammary glands at 25 ng/kg
Nikaido, 2004	CD-1 mice	Sub-cutaneous	0.5 and 10 mg/kg bw/d GD15-GD18	Acceleration of weight gain in F1 females Precocity of vaginal opening. Increase in oestrogen cycle duration Genital tract abnormalities (acyclicity, hyperplasia) Acceleration of mammary gland

				differentiation
Patisaul, 2009	Long Evans rats	Sub-cutaneous	50 µg/kg bw/d and 50 mg/kg bw/d PND1-PND5	No modification in immunoreactivity to KISS in the anteroventral periventricular nucleus, decrease in the ARC nucleus in females No modification in males

Table 19. Animal studies investigating the effects of bisphenol A on vaginal opening and on age at first oestrus: summary table

Exposure period	**References**	**Species**	**Routes**	**Exposure period**	**Exposure dose**	**Effect evaluated on vaginal opening and age at first oestrus**
Gestation	**Howdeshell, 1999**	CF-1 mice	Oral gavage	GD11-GD17	BPA: 2.4 µg/kg bw/d	Vaginal opening: no effect Interval between vaginal opening and age at first oestrus: decrease by 2-4d
	Nikaido, 2004	CD-1 mice	Sub-cutaneous	GD15-GD19	BPA: 0.5 or 10 mg/kg bw/d DES: 0.5 or 10 µg/kg bw/d	Vaginal opening: BPA 0.5 mg/kg bw/d: no effect BPA 10 mg/kg bw/d: advance of 1.2d DES: advance of 1.5 and 1.9d at doses of 0.5 and 10 µg/kg bw/d respectively
	Honma,	ICR Jcl	Sub-cutane	GD11-	BPA: 2 or	Vaginal opening and

	2002	mice	ous	GD17	20 µg/kg DES: 0.02-0.2 or 2 µg/kg	<u>age at first oestrus:</u> BPA 20 µg/kg: advance (~1d) DES: advance 1.5d minimum
	Savabieas fahani, 2006	Sheep	Sub-cutaneous	GD30-GD90 (2/5th of gestation)	BPA: 5 mg/kg	No effect: on age at first oestrus cycle determined by the progesterone level
Second half of gestation and postnatal	**Yoshida M, 2004**	Donryu rats	Oral gavage	GD2-PND21	BPA: 6 µg/kg bw/d 6 mg/kg bw/d	<u>Vaginal opening</u> No effect of BPA
	Takagi H, 2004	Sprague-Dawley rats	Oral feed	GD15-PND10	BPA feed: 60-600-3000 ppm, i.e. ~7-300 mg/kg bw/d Ethynyl E2 0.5 ppm	<u>Vaginal opening</u> No effect of BPA
	Kwon S, 2000	Sprague-Dawley rats	Oral gavage	GD11-PND20	BPA: 3.2-32-320 mg/kg bw/d DES 15 µg/kg bw/d	<u>Vaginal opening and age at first oestrus</u> No effect of BPA nor of DES
	Ryan, 2010	Rats	Oral gavage	GD7-PND18	EE2: 0.05-0.5-1.5-5-15-50 µg/kg bw/d BPA: 2-20-200 µg/kg bw/d	<u>Vaginal opening</u> EE2 at the dose of 5 µg/kg caused a vaginal opening advance of 4d.

						BPA did not cause any effect.
Early postnatal	**Adewale, 2009**	Rats	Sub-cutaneous	PND0-PND3	EB*: 25 µg BPA: 50 µg/kg BPA: 50 mg/kg PPT: 1 mg/kg	Vaginal opening: EB: Advance of 4d BPA: 50 µg/kg: advance of 2d BPA: 50 mg/kg: NS PPT 1 mg/kg: advance of 1d
	Fernandez, 2009	Sprague-Dawley rats	Sub-cutaneous Castor oil	PND1-PND10	1st BPA dose tested: 2.5-6.2 mg/kg bw 2nd BPA dose tested: 25 to 62.5 mg/kg bw	Vaginal opening: 2.5d advance 4.8d advance
Postnatal	**Nikaido, 2005**	CD-1 mice	Sub-cutaneous	PND15-19 prepubertal	BPA: 10 mg/kg bw/d DES: 10 µg/kg bw/d	Vaginal opening No effect with BPA 10 mg/kg bw/d DES 10 µg/kg bw/d: advance

Conclusion on the data on reproductive tract in animals

In animals, on the basis of the convergence of results from various studies carried out under various conditions and on various models, **the following effects on the female reproductive system can be considered to be "recognised in animals"** in protocols of exposure during development (pre- and postnatal exposure):

- **Increase in the occurrence of ovarian cysts,**
- **Hyperplastic modifications of the endometrium,**

- **Advancement of the age at puberty when there has been early pre- and postnatal exposure.**

The effects on the hypothalamic-pituitary-gonadal axis due to exposure *in utero* or to early postnatal exposure lead to variations in sex hormone levels, and modification of sex hormone receptor expression has been found in several studies. These effects are recognised in animals.

In animals, the potential effects of exposure in adults are observed for doses well above the NOAEL selected by EFSA.

Selecting the critical effect:

The results of key studies concerning these effects, as well as the dose levels for which the effects were observed, are represented graphically below.

Figure 10. Effects of BPA on the female reproductive system

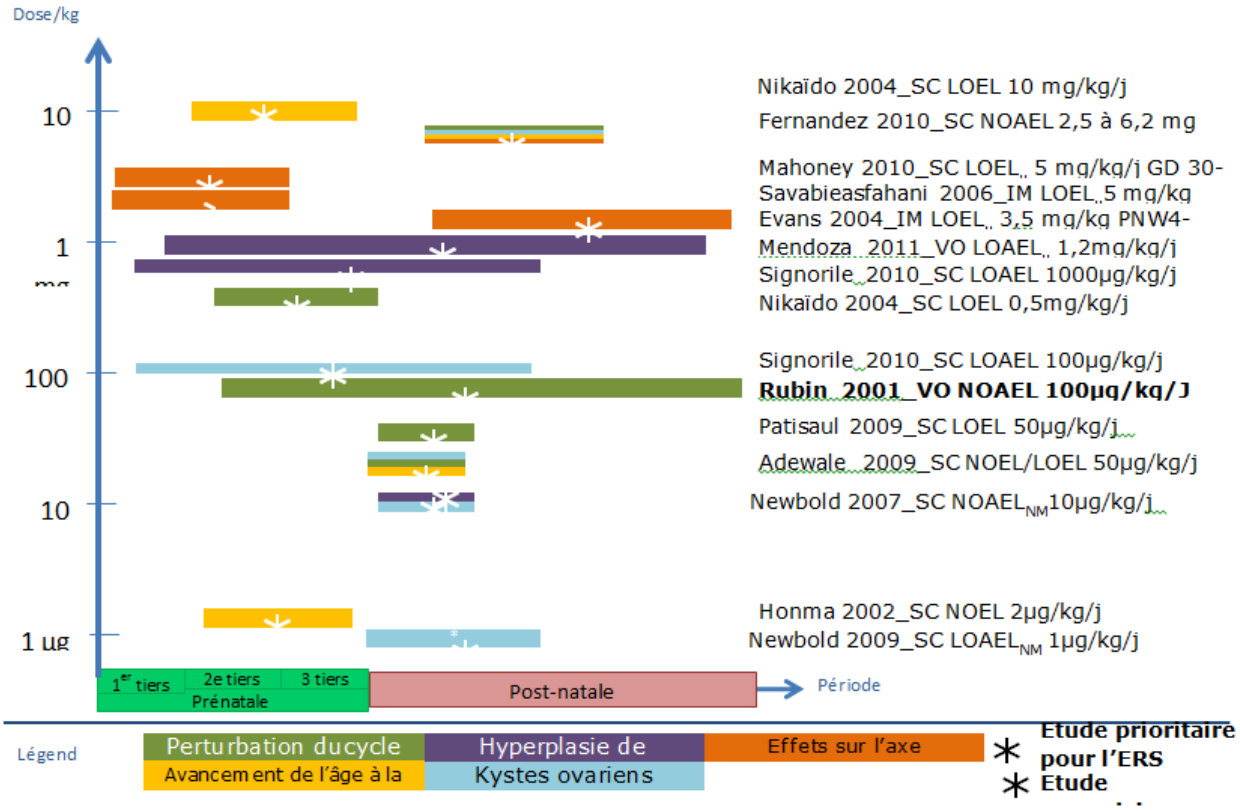


Table 20 Selection of studies of good quality showing effects on the female reproductive system

Disruption of the ovarian cycle	**Endometrial hyperplasia**	**Effects on the HT/HP/gonadotropic axis**	**Advancement of the age of puberty**	**Ovarian cysts**
Rubin 2001_VO NOAEL 100 µg/kg/D GD6 – Weaning **	Signorile 2010_SC LOAEL 1000 µg/kg/d GD1 - PND7 **	Femandez 2010_SC NOAEL 2.5 to 6.2 mg PND1 - PND10**	Nikaïdo 2004_SC LOEL 10 mg/kg/d GD15-GD18**	Femandez 2010_SC NOAEL 2.5 to 6.2 mg PND1 - PND10**
Nikaïdo 2004_SC LOEL 0.5 mg/kg/d GD15 - GD18**	Mendoza 2011_VO LOAELu 1.2mg/kg/d GD6 - PND21*	Mahoney 2010_SC LOELu 5 mg/kg/d GD30 - GD900 sheep*	Femandez 2010_SC NOAEL 2.5 to 6.2 mg PND1 - PND10**	Signorile 2010_SC LOAEL 100µg/kg/d GD1 - PND7**
Femandez 2010_SC NOAEL 2.5 to 6.2 mg PND1 - PND10**	Newbold 2007_SC $NOAEL_{NM}$ 10 µ/kg/d PND1 - PND5*	Savabieasfahani2006_IM LOELu 5 mg/kg GD30 - GD90 ewe*	Honma 2002_SC NOEL 2 µg/kg/d GD11 - GD17 **	Adewale 2009_SC NOEL/LOEL 50 µg/kg/d PND0 - PND3*
Patisaul 2009_SC LOEL 50 µg/kg/d PND1 - PND5*		Evans 2004_IM LOELu 3.5 mg/kg PNW4 - PNW9 ewe*	Adewale 2009_SC NOEL/LOEL 50 µg/kg/d PND0 - PND3*	Newbold 2007_SC $NOAEL_{NM}$ 10 µ/kg/d PND1 - PND5*
Adewale 2009_SC NOEL/LOEL 50 µg/kg/d PND0 - PND3*				Newbold 2009_SC $LOAEL_{NM}$ 1 µg/kg/d GD9 - GD16*

****Priority study for Health Risk Assessment considered of very good quality**

***Secondary study for Health Risk Assessment considered of less good quality**

The effects on the hypothalamic-pituitary axis are mechanistic adaptation aspects and regulations. These are to be regarded as mechanistic, but are particularly difficult to translate in terms of adverse effect. As such, it was decided not to consider this effect among the critical effects for HRA.

The parameters used such as the index of the puberty process in animals, the age of vaginal opening and/or the first estrus, show high sensitivity and are undoubtedly good markers of having reached reproductive function. However, certain methodological constraints relative to, in particular, the large numbers of animals required to reliably analyse this discontinuous type of variable, the low magnitude of the effects observed (often one or two days ahead for a parameter observed once daily) led ANSES to assert a few reservations as to the relevance of this parameter for the HRA. Lastly, given the difficulty of translating these observations in terms of harmful effects on health, it was decided not to retain these effects among the critical effects for HRA.

The following effects observed in animals resulting from pre or postnatal exposures were deemed to be of sufficient concern and relevance to be considered as part of the HRA:

- **Increase in the occurrence of ovarian cysts;**
- **Increase in the frequency of the appearance of endometrial hyperplasia;**
- **Disruption of ovarian cycles.**

Selecting the key study:

The studies deemed the most appropriate for the HRA were taken into consideration first. On the basis of this mode of graphical representation (see diagram above), the following studies appeared to be the most interesting:

- Rubin *et al.*, 2001 (Rubin, 2001-see detailed description below) – Disruption of the ovarian cycle with elongation of the estrous cycle - oral study leading to a NOAEL of 100 µg/kg bw/d and a LOAEL of 1200 µg/kg/day after treatment from GD6 until weaning in Sprague Dawley rats.
- Patisaul *et al.,* 2009 (Patisaul, 2009) – Disruption of the ovarian cycle - study by subcutaneous route leading to a LOEL of 50 µg/kg/day after treatment from PND1 to PND5 in Long Evans rats.
- Signorile *et al.*, 2010 (Signorile, 2010--see detailed description below) – Polycystic ovaries and endometrial hyperplasia - sub-cutaneous study leading to a LOAEL of 100 µg/kg bw/d based on the increase in the frequency of the appearance of polycystic ovaries and a LOAEL of 1000 µg/kg bw/d based on the increase in the incidence of endometrial hyperplasia after treatment from GD1 to PND7 in Balb-C mice.

The Rubin *et al.*, (Rubin, 2001) study was conducted orally, with administration of BPA in drinking water (1 and 10 mg/L) in Sprague-Dawley rats (n= 6 females/dose group). This study includes two exposure doses in addition to the control group, with an estimated intake of BPA of 0.1 mg/kg bw/day and 1.2 mg/kg bw/day. Six pregnant rats per dose group were exposed from GD6 until weaning of the young. This study did not follow the OECD guidelines or GLP. Precautions were taken in this experiment in order to reduce contamination due to the material used. In particular, drinking water was delivered in glass bottles and measurements with the E-Screen test conducted in order to monitor potential leaching of estrogenic compounds from used plastic cages. Many critical points were investigated in this study: LH secretion, frequency of estrous cyclicity estimated at PND28 and at the age of 4 and 6 months (n = 69) and uterotrophic test. The disruption of ovarian cyclicity observed in this study is supported by the studies of Patisaul et al., 2009 (Patisaul, 2009) and Nikaido et al., 2004 (Nikaido, 2004).

Study	**Rubin BS et al., Environmental Health Perspectives, 2001 vol.109 (7), p675-680**
Type of study like 1 or 2 generation with/without prenatal exposure	1G perinatal exposure
Objectives of the study	Assess the effects of BPA during development on female reproductive function and body weight Compare the sensitivity of adult vs developing animas
reports, scientific publications with original data or review	Peer-reviewed publication.
Fundings	Tufts Institute of the Environment & NIH-ES
Chemical, CAS number, purity, composition, vehicule	BPA and estrone sources non specified- BPA or estrone in solution in drinking water + 1% ethanol
Specie / age / weight	Dams : Rat Sprague Dawley-2 -3 months old pregnant for 5 days at arrival Ovariectomized (ovx) adults Sprague Dawley for uterotrophic assay (n=30)
Sex and number of animals per group	18 dams _ 30 OVX Male offsrping : newborn,n= 12 – 3 months old, n= 12 – 5 month old, n=18 Female offspring: newborn, n=12 – 8 months, n= 24 – 12 to 16 months, n= 34
Control group and number	Negative controls : n=6 dams water + ethanol (1%)
Positive control	For OVX uterotrophic test only : estrone in drinking water (1 and 0.1 mg/L)
Life conditions (humidity, light/dark cycle, Conditions de vie, diet, number of animals	L/D cycle : 14/10 : Light on from 5 :00 am to 7 :pm Plastic cages Water bottle: glass

per cage)	Alimentation: Purina Rodent Chow
Exposure route	Oral : drinking water
Frequency and period of exposure	Dams : from day 6 of pregnancy to the end of lactation OVX Uterotrophic test: 3 days
Doses / concentrations of BPA used	Nominal concentrations in drinking water : Dams: BPA 1 and 10 mg/L OVX: BPA 1-10 and 100 mg/L – estrone: 0.1 and 1 mg/L) Estimated ingestion: BPA: 0.1 and 1.2 mg/kg/day for the 1 and 10 mg/L concentrations, respectively
Observations / endpoints studied	Dams bodyweight gain –sex ratio and size of the liters- offspring bodyweight gain from birth to weaning male and female pooled-macrscopic abnormalities of the genital tract and mammary gland - anogenital distances in neonates- day of vaginal opening- estrous cyclicity at 4 and 6 months of age (daily vaginal cytology for 18 consecutive days)- LH plasma concentrations 3 months after ovariectomy – OVX uterotrophic: vaginal cytology before treatment and daily thereafter – uterine wet weight
Uncontrolled exposure (presence of phytoestrogens in the diet, polycarbonate cages in accommodation, composition of drinking water, litter composition, etc.)	Cages checked negative for estrogenic activity (ethanol extracts) by E-SCREEN assay Food and water checked neither for estrogenic activity, nor for BPA contamination Drinking water in glass bottle Control of Liter effect : no fostering , at least one male and one female from 4 different litters of each treatment group were included in the different evaluations at different time-point
Statistical analysis and quality criteria associated	Continuous variable: ANOVA followed by post hoc (t-test, Tukey or LSD) comparisons. Incidence of mammary tumors: Chi2 Non parametric test for the % of cycling females: Kruskall-Wallis followed by Mann-Whitney U test
Observed effects - general toxicity / Mother	None reported

Observed effects – reprotoxicity	Offspring body weight before weaning : • Body weight at PND 4,7 & 11: BPA treated group > negative controls • At PND11 & 22: low dose BPA> high dose BPA and vehicle • At PND28 : low BPA dose females > vehicle and high dose BPA females (not seen in males) Offspring body weight after weaning : • Female Body weight at PND 54, 87 & 110: low BPA> high dose BPA and vehicle Reproductive function and mammary gland • high dose BPA: % of females with regular estrous cycles at 4 and 6 months with prolonged diestrus or proestrus/estrus and decreased number of regular4-5 days cycles and LH levels in long-term OVX • A somewhat higher incidence of mammary tumors is mentioned by the authors but this did not reach significance given the low number of observations No effect of BPA on: • Liter size and sex ratio • Age at vaginal opening • Anogenital distance in neonates • Macroscopic abnormalities of the genital tract • Uterotrophic test in postpubertal ovx females (# estrone at 1 mg/L)
NOAEL / LOAEL values considered for determination of the critical effect	Estrous cyclicity: NOAEL: 100µg/kg/day/ LOAEL 1 mg/kg/day(estimated on the base of water consumption)
Conclusions of the authors	• Effect of BPA on bodyweight • Increased sensitivity to BPA during prenatal period (effect on in utero exposed animals but not on OVX adults used for uterotrophic tests) • BPA induces alterations od estrous cyclicity • Results on the lack of effect on the uterotrophic test highlight the needs for reevaluation of the endpoints used for the toxicologic assessment of BPA , of the acceptable levels of exposure to this compound and of other xenoestrogens in the environment
Comments and conclusions	• No descriptive data on individual litter sizes, this impedes any conclusion regarding the effect of BPA on bodyweight • Data regarding estrous cyclicity are OK

	• No possible comparison between uterotrophic test and effect resulting from in utero exposure since the duration of exposure is not the same: difficult to conclude about the shift in sensitivity to BPA • Lack of control of the estrogenic activity of food was not considered as a major issue since all group received the same food

The Patisaul *et al.,* 2009 study (Patisaul, 2009) (ranked 1* by the working group) conducted subcutaneously in Long Evans rats and ranked 1* by the working group, mentions a disruption of the estrous cycle with elongation of the estrus. This effect was not the subject of a very extensive description in the publication and the gap between the two doses tested is too significant (factor 1000). These findings led ANSES not to accept this study for the HRA. However, it should be noted that this result is consistent with the data of Rubin *et al*., (Rubin, 2001).

The Signorile *et al.*, 2010 (Signorile, 2010) study conducted subcutaneously in adult Balb-C mice (n = 6 females/dose group) and ranked 2 * by the working group confirmed the appearance of ovarian cysts with a LOAEL of 100 µg/kg bw/day and of endometrial hyperplasia with a LOAEL of 1000 µg/kg bw/day. This study includes two exposure doses: 100 and 1000 µg/kg bw/day in addition to the control group. Six pregnant mice per dose group were exposed from GD1 until PND7. This study did not follow the OECD guidelines or GLP. Nevertheless, precautions have been taken in this study in order to reduce contamination due to the material used in this experiment. In particular, drinking water was delivered in glass bottles and measurements with the E-Screen assay conducted in order to monitor potential leaching of estrogenic compounds from used plastic cages or the litter boxes used. The Mouse Chow food delivered to the animals was also controlled with the E-Screen test showing weak estrogenic activity. The BPA used presented an analytical purity greater than 99%. In addition, in order to minimise any effect, after birth, the young were grouped, by gender and then redistributed to mothers treated at the same dose level as the surrogate mother, 5 females and 5 males per mother. Female offspring were examined at 3 months of age (n = 20 per dose group). The study protocol is well described and many critical points in relation to the female reproductive system have been investigated: macroscopic and microscopic examination of the ovaries and of the endometrium. The results of this study are confirmed by (Mendoza, 2010) showing endometrial hyperplasia.

Study	**Signorile et al., 2010, General and comparative endocrinology, 168 : p 318-325**
Type of study like 1 or 2 generation with/without prenatal exposure	1G gestational and neonatal exposure (dams treated from GD1 to PND7)
Objectives of the study	Long-term effect of prenatal BPA exposure on the female reproductive tract with particular emphasis on endometriosis

reports, scientific publications with original data or review	Peer-reviewed publication
Fundings	Fondazione Italiana Endometriosi , Italiana Ministry of health
Chemical, CAS number, purity, composition, vehicule	BPA >99% pure Sigma-Aldrich Inc.
Specie / age / weight	Mice BALB-C adult , offspring studied à 3 months of age
Sex and number of animals per group	Dams:6 females/ treatment group Female Offspring : 20/ treatment group
Control group and number	Negative control: vehicle injection: 2% ethanol in sterile phsysiological saline
Positive control	none
Life conditions (humidity, light/dark cycle, Conditions de vie, diet, number of animals per cage)	14L/10D controlled temperature Food: Mouse chow (Mucedola, Milano, Italy) Tap watter in glass bottle
Exposure route	subcutaneous
Frequency and period of exposure	Daily from GD1 to PND 7
Doses / concentrations of BPA used	100 and 1000µg/kg/day nominal values
Observations / endpoints studied	• Internal exposure to BPA: BPA in the liver of dams at weaning (PND21) and of offspring at 3 months (i.e 14days and 3 weeks after cessation of BPA administrations for the dams and the offspring, respectively) • Histology of whole pelvic organs (serial sections) Er□ and HOXA-10 immunoreactivity within the endometriosis-like structures when present
Uncontrolled exposure (presence of phytoestrogens in the diet, polycarbonate cages in accommodation, composition of drinking water, litter composition, etc.)	Food cage and bedding tested negative for estrogenic activity with an E-Screen test Tap water not tested Liter effect was mimimized by fostering

Statistical analysis and quality criteria associated	Histological lesion incidence: One tail Fisher test comparing individually each BPA group to the vehicle control group. BPA concentrations in the liver: two way ANOVA (BPA doses 100 vs 1000 and physiological stage, mother vs offspring) followed by post hoc comparison with LSD test
Observed effects - general toxicity / Mother	No data
Observed effects – reprotoxicity	• Increased incidence of Cystic ovaries in 3 month old offspring in both BPA groups (10, 45 and 50 % in vehicle, BPA 100 and BPA 1000µg, respectively, p=0.008) • No effect on copora lutea number • Increased incidence of uterine adenomatous hyperplasia with cystic endometrial hyperplasia in 3 months old offspring in both BPA groups significant in the BPA 1000 group only (vehicle vs. BPA1000: 10 vs 50% in , respectively) • Increased incidence of endometriosis-like structure positive for Er□ and HOXA-10 expression (prototypical markers of genitals tissue) in the adipose tissue surrounding the uterus (5, 30 and 35% of incidence, in vehicle BPA 100 and BPA1000µg groups, respectively, p=0.024) • BPA was detected in the liver of both dams and their offspring from the BPA treated groups but not from the vehicle group. BPA concentration in the dam liver was approximately 3 fold higher in the BPA 1000 group than in the BPA 100.
NOAEL / LOAEL values considered for determination of the critical effect	Cystic ovaries: LOAEL: 100µg/kg/day sc Adenomatous enfometrium hyperplasia: NOAEL: 100µg/kg/day-LOAEL: 1000µg/kg/day Endometriosis-like structure: LOAEL: 100 µg/kg/day
Conclusions of the authors	Endometriosis-like phenotype in mouse can be exacerbated by in utero and peri-natal exposure to BPA

Comments and conclusions	Data regarding BPA internal exposure should be considered with cautions due to the absence of information regarding the performance of the assay . The comparison between mother and offspring does not make any sense since the livers were sampled at different time after cessation of the BPA treatment. Endometriosis observation are very interesting but need to be confirmed by others The number of animals for each treatment is satisfactory (20) as well as the measures taken to check for possible environmental and food contamination by estrogenomimetic substances, so that observation regarding ovarian cysts and uterine hyperplasia seem to be conclusive enough

The Newbold *et al.,* 2007 and 2009 studies (although less well ranked 1*) (Newbold, 2007; Newbold, 2009), appear particularly relevant. These were conducted with 3 or 4 doses and confirm the identification of ovarian cysts at low doses with a NOAEL of 10 µg/kg bw/d (see Newbold, 2007) subcutaneously, after a treatment from PND1 to PND5 and a LOAEL of 1 µg/kg bw/d from GD9 to GD16. These studies assess the impact of treatment with BPA on the frequency of the appearance of genital tract abnormalities. The groups included 14 to 16 animals and the frequency of these abnormalities remained relatively low, conditions which make the statistical analysis and interpretation very difficult. This study does not, therefore, allow the nature of the dose-response relationship to be assessed under satisfactory conditions. The only result that can be interpreted without too much uncertainty concerns the overall appearance of anomalies in the reproductive system, all diseases combined, with a LOAELnm of 0.1 µg/kg/d subcutaneously.

On the basis of this analysis, the Rubin *et al.*, (Rubin, 2001) and Signorile *et al.*, 2010 (Signorile, 2010) studies are proposed for the HRA on BPA for the effects on the female reproductive system.

The NOAELs / LOAELs selected for the HRA are reported in the summary table below:

Table 21. NOAELs/ LOAELs selected for the HRA on BPA for its effects on the female reproductive system

Reference	**Critical effects**	**NOAEL/LOAEL**	**Method of**	**Period of**

	observed		administration/Species	exposure
(Signorile, 2010)	Increase in the occurrence of ovarian cysts	LOAEL 100 µg/kg/d	Sub-cutaneous Balb-C Mice	GD1-PND7
(Signorile, 2010)	Endometrial hyperplasia	NOAEL 100 µg/kg/d / LOAEL 1000 µg/kg/d	Sub-cutaneous Balb-C Mice	GD1-PND7
Rubin, 2001	Disruption of ovarian cycles	NOAEL 100 µg/kg/d LOAEL 1.2 mg/kg bw/c	Orally Sprague Dawley Rat	GD6 – weaning of young

Recent studies on the effects on the female reproductive system:

The studies published after adoption of the report on the health effects of BPA concerning the effects on the female reproductive system result in a collection of converging data indicating that developmental exposure (*in utero* in the mouse and the monkey and early post-natal in the ewe) at low doses could be the cause of disruptions of the meiotic processes in the female and early follicullogenesis which could potentially lead to a reduction in the follicular reserve. The functional consequences of such changes to the reproductive life of the adult remain to be assessed.

Furthermore, a certain number of studies have recently reinforced the effects previously identified as proven in animals: disruption of the hypothalamo-hypophyseal gonadotrophic axis, histological changes and acceleration of the puberty process during early neonatal exposure.

Selecting benchmark doses:

The Signorile *et al.*, 2010 study (Signorile, 2010) in Balb-C mice after exposure *in utero* and before weaning to BPA (mothers treated with 100 and 1000 µg/kg bw/d subcutaneously) is associated with an increase in the incidence of cystic ovaries and endometrial hyperplasia.

The Rubin *et al.*, (Rubin, 2001) study, conducted orally with administration of BPA in drinking water (1 and 10 mg/L) in Sprague-Dawley rats, showed notable disruption of ovarian cyclicity. The animals in the group treated with the higher dose (1.2 mg/kg bw/day) showed a high proportion of cycle irregularity, with only 21% of animals with regular estrous cycles versus 80% in the controls. These results are supported by the studies of Patisaul *et al*., 2009 (Patisaul, 2009) and Nikaido *et al*., 2004 (Nikaido, 2004).

In conclusion, on the basis of the study by Rubin *et al.*, (Rubin, 2001), an **oral NOAEL of 100 µg/kg bw/d** and a LOAEL of 1.2 mg/kg bw/d for the disruption of ovarian cyclicity for a treatment from GD6 until weaning is identified and on the basis of the Signorile *et al.*, 2010 (Signorile, 2010) study by **subcutaneous route, a LOAEL of 100 µg/kg bw/d** is determined for the appearance of ovarian cysts and a **NOAEL of 100 µg/kg bw/d**, for endometrial hyperplasia for a treatment from GD1-PND7.

Other comments (uncertainties, confidence level, etc.)

The Rubin *et al.*, (**Rubin, 2001)** study shows certain limits:

- Statistical analysis using the offspring and not the litter as a statistical unit,
- Indirect estimate of the intake of BPA by animals on the basis of the animals' water consumption,
- Only two exposure doses were used.

The Signorile *et al.*, 2010 (Signorile, 2010) study shows the following limits:

- Statistical analysis using the offspring and not the litter as a statistical unit. However, in order to minimise any potential effect, the authors grouped the young after birth and then redistributed them by dose groups.
- Only two exposure doses were used.

However, these results are supported by other studies such as Patisaul *et al.*, 2009 (Patisaul, 2009) and Nikaido *et al.*, 2004 (Nikaido, 2004) and Mendoza *et al*., 2010 (Mendoza, 2010).

Critical analysis of Delclos et al, 2014 studies regarding the effect of BPA on the reproductive system[10]

This publication described a large scale study in Sprague Dawley rats aiming at investigating the scientific uncertainties about BPA health effect. A special effort was made to increase the range of BPA doses evaluated to establish a clear dose-response relationship more particularly within the low doses range, the number of animals involved and the characterization of potential background exposure and internal dosimetry of BPA. Regarding internal dosimetry, more detailed information is provided in a companion paper (MI Churchwell et al., 2014, Toxicological sciences 139(1), 4-20). Although this study is not evaluated in details in the present report, some of its key results appear to be critical for the analysis of the significance of the findings reported in the Delclos' publication and as so, are taken into account in the present analysis. The experiment described in this publication is part of a multiagency program known as the consortium linking academic and regulatory insights on BPA toxicity (CLARITY-BPA). The CLARITY-BPA program is conducted under the aegis of the NTP in collaboration with NIEHS and with support of the FDA and involved several academic partners. The program is based on a 2-year guideline rodent toxicity study on BPA using Sprague Dawley rats from the NTP breeding colony.

As far as reproductive toxicology is concerned the study evaluates classical parameters in F0 dams and their F1 offspring from birth to adult life.

The experiment involved 400 females mated with untreated males at 10 to 14 weeks of age. The liters were culled to five males and 5 females at PND1 with a minimum of 3 males and 3 females/liter. No clear statement is provided regarding the final number of liters and animals included in the study. According to data of table 1, 300 F0 females were involved in the

[10] This study was not investigated during the elaboration of the proposal since it was published subsequently. Quoted during the public consultation and considered by RAC as a key study, it is now analysed and incorporated in the BD.

experiment. It is noteworthy however that the number of observations for each parameter is systematically reported and is in the range of 7 to 26.

The study suffers from some limitations the impact of which depends on the evaluated parameter and the nature of the limitation.

Major limitations

Examination of the internal dosimetry (maximal concentrations) detailed in the paper of Churchwell and coll. Reveals that the controls both naïve and vehicle show some degrees of internal exposure to BPA (since both BPA-G and BPA were found) and that in some instance this contamination can be as high or even higher than the low dose groups. For example at PND21 and PND80, BPA aglycone in the vehicle controls was in average similar to the level recorded in the 80 µg/kg/day dose group. At the same time, it appears that total BPA maximal concentrations (aglycone + gluc) seemed to be linearly correlated to the doses between 25 µg/kg/d and 300 mg/kg/day, while they were very similar between controls and the 2.5 and 8 µg/kg/d groups. It can be concluded that the negative controls are totally inadequate as compared to the low doses groups up to 80µg/kg/days. Thus, from those data, it seems impossible to conclude that those low doses had no-effect. This highlights the absolute necessity to document internal exposure for the evaluation of such ubiquitous contaminant. This is unfortunately very rarely performed and one should acknowledge the special effort made in the CLARITY approach to clarify this point.

It is clear that the F0 animals were exposed to several sources of BPA (cages, water bottle and food) until weaning and thus were likely to have been exposed to BPA during sensitive periods such as in utero development and postnatal life. How this might have impacted observation on pregnancy parameters and F1 animals in particular is not clear. In addition, all animals including naïve and vehicle controls were exposed to BPA through food up to about 2.6 ppb (2.6 ng/g of food given for an adult an approximate dose of 52 ng/rat~260 ng/kg/day)

BPA was administered as an aqueous suspension, and although the suspensions have been carefully checked in order to make sure that the nominal concentrations were exact, there is no data allowing establishing how much of the BPA is under a soluble form potentially more bioavailable. That might be one reason to explain the discrepancy with other studies via oral route in which BPA was given in solution in oil. This difference in vehicle is very likely to modify toxicokinetic parameters in particular through the modulation of the digestive absorption. Once again, this highlights the need for monitoring internal dosimetry.

Samples for hormonal monitoring were taken in different conditions according to the period: post mortem.- in alive restrained animals at the tail vein-retroorbital puncture… All of these procedures are stressful and as so leave a large uncertainty on the validity of hormonal data in particular for hormones as responsive to stress as prolactin. For LH measurement, a single blood sample was collected in each individual at a period during which pulsatility is infrequent and characterized by high amplitude pulses. In those conditions the biological significance of this parameter is uncertain.

Minor limitations

Food, water and bedding were not tested for estrogenic activity. It was the position of the ANSES GT to consider that this is not a major limitation in terms of danger identification as it

is more representative of a "real life" scenario than protocole with no exposure to "natural" estrogens.

It might be argued that the strain of rat was not sensitive enough to estrogenic mediation. The real question here should be: is this strain more relevant for human than others even if not the best one to identify estrogenic properties.

Vehicle and BPA was administered through gastric gavage that can be considered as stressfull enough to alter the animal endocrine responses. However, this is a common practice and several studies evidencing BPA effects in rodents after oral administration have been using this mode of administration as well.

In conclusion: Whatever the reproductive parameter considered, in any case, does this study allow concluding that low doses of BPA have no effect. Indeed, the internal exposure status of the control animals both naïve and vehicle is too uncertain and too close to the one measured in some low doses group of the study.

As a complement, about the divergences in the scope of conclusions between ANSES and EFSA 2014 draft Opinion regarding the effect of BPA on the female reproductive system, see Annex 5 - *ANSES comments on EFSA draft opinion).*

B.5.9.2. Effects on the mammary gland

B.5.9.2.1. Effects on the mammary gland (according to prior work undertaken by expert assessment authorities)

According to the NTP-CERHR report, rodent studies have shown BPA to have an effect following exposure by subcutaneous perfusion at doses ranging from 0.0025 mg/kg bw/d to 1 mg/kg bw/d during gestation and support an increased susceptibility to developing mammary tumours (Durando, 2007; Murray, 2007) (NTP, 2008). Although these lesions were described as pre-neoplastic no evidence was provided of their progression to invasive carcinoma. As a result, it cannot be concluded that BPA carries a risk of breast cancer. Similarly, no effects have been reported in rodents after exposure during adulthood.

The EU RAR report cites three studies referring to investigation for pre-neoplastic lesions. The first study by Durando *et al.* (Durando, 2007) used Wistar rats exposed *in utero* between GD8 and GD23 to subcutaneous administration of 25 µg/kg bw/d (European, 2010). The study showed that BPA disrupts the histological structure of the mammary gland and increases its susceptibility to a carcinogen (N-nitroso-N-methylurea) administered 50 days after the end of BPA treatment. The second study by Murray *et al.* (Murray, 2007) involved foetal exposure to BPA (0.025 and 1 mg kg bw/d) which induced development of pre-neoplastic and neoplastic mammary lesions. The last study cited by Colerangle and Roy (Colerangle JB, 1997) assessed mammary gland growth in Noble rats treated subcutaneously with 0.1 and 54 mg/kg bw of BPA. The authors found a significant increase in conversion of immature into mature structures, a reduced average number of terminal ductules and terminal buds and an increase in the average number of lobules. The conclusions of the EU RAR report (EC, 2003) however criticised these 3 studies because of their methodological limitations.

In 2010, the FAO/WHO expert panel deemed that the conventional carcinogenesis studies on BPA in rodents using doses in the region of 75 to 150 mg/kg bw/d did not demonstrate any effects or showed only very weak effects. The panel questioned, however, whether the carcinogenic potential of BPA had been correctly investigated in these studies because the animals were not exposed during the prenatal period. Some studies have shown that perinatal exposure to BPA (at oral doses of between 10 and 250 µg/kg bw/d) can cause mammary duct epithelial proliferation in the F1 generation. BPA exposure during specific susceptibility windows may have an effect on the development of the mammary gland and make it more susceptible to the development of neoplastic or pre-neoplastic lesions after exposure to potent tumour initiators or promoters. These studies, however, have protocol weaknesses which limit their interpretation. The expert panel also reported that a carcinogenesis study in rodents was ongoing at the NTP in which oral exposure would begin from the foetal life period. This study intends to monitor internal free and conjugated BPA levels (FAO/WHO, 2010) .

According to the INSERM report, many studies consistently show that foetal or perinatal exposure to BPA changes the structure of the mammary gland in adulthood in rodents (INSERM, 2010). The report cites the work by Vanderberg *et al.* (Vandenberg, 2008) which reports an increase in density, branching and number of ducts and alveoli and terminal duct hyperplasia in mice. It also cites the work by Markey *et al.* (Markey CM, 2001; *in utero*, mouse), Munoz-de-Toro *et al.* (Munoz del Toro, 2005, *in utero* and neonatal, mouse), Murray *et al.* (Murray, 2007 - see detailed description as well as strengths and weaknesses below) (foetal, rat), (Moral, 2008 - see detailed description as well as strengths and weaknesses below) (*in utero*, rat) which report accelerated maturation of the adipose cushion, delayed lumen formation and increased density of terminal duct structures.

INSERM describes studies in which exposure to BPA either was or was not shown to be related to a risk of developing breast tumours (INSERM, 2010). The study by Murray *et al.* (Murray, 2007) in animals suggested an increased risk of mammary tumours in rats. INSERM also describes studies showing increased susceptibility of mammary cells exposed *in utero* to low doses of BPA to malignant change, notably the studies by Munoz-de-Toro *et al.* (Munoz del Toro, 2005), Durando *et al.* (Durando, 2007), Wadia *et al.* (Wadia, 2007) and Jenkins, 2009 . INSERM, 2010 describes one published epidemiological study by Yang *et al.* (Yang, 2009), which found no clear difference in blood BPA concentrations between cases (women diagnosed with breast cancer) and controls. INSERM ultimately concluded that although the data in rodents appeared to be convincing there was at present no study demonstrating BPA to have any developmental effects in humans.

B.5.9.2.2. Effects on mammary glands in Humans

Breast cancer								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Yang *et al.*, 2009) Effects of bisphenol A on breast cancer and its risk factors	Age-matched cross-sectional study	Study population: general population (women) N=70 cases (women with breast cancer) and 82 controls → The population size is difficult to assess as the expected difference is small	Blood (free and conjugated BPA) Conjugated BPA used as a biomarker (blood stored in Eppendorf tubes for over 10 years)	HPLC/FD	Age: Yes (age matching and adjustment) Sex: NA Medication: no Tobacco: yes BM: yes Other contaminants: No Other: age at menopause	Results: No significant difference in blood concentrations of BPA between the cases and controls.	Studies not taken into consideration since they have major methodological limitations This study was excluded for the following reasons: - The population size is difficult to assess as the expected difference is small, - blood samples stored in Eppendorf tubes for over 10 years, - population	Information from epidemiological studies Effects on the breasts

							recruited in 1994-97, - BPA analysed only in the blood (without specifying whether it was total blood or plasma) in a single sample - No urinary sampling.	

Only one epidemiological study has examined the relationship between BPA exposure and the risk of breast cancer (Yang, 2009). In this cross-sectional study, 152 Korean women (70 cases with breast cancer diagnosed between 1994 and 1997 and 82 controls recruited in the same hospital, matched for age) completed a questionnaire and had a blood BPA measurement (the biomarker of exposure used was the conjugated form). BPA levels did not differ between the cases and controls (p=0.42).

The major methodological limitations of this study, such as lack of statistical power (low numbers), undetectable BPA in half of the subjects with no details about any possible differences between cases and controls, a non-standardised questionnaire inappropriate for the question being asked (measurement of BPA, a substance which does not persist, after the diagnosis of breast cancer), prevent any conclusions being drawn about the association between BPA and breast cancer.

Conclusion in humans:

In conclusion in humans, the only study available does not enable a conclusion to be made on the link between BPA exposure and breast cancer.

B.5.9.2.3. Effects on mammary glands in animals

In most of the reproductive toxicology studies performed with females exposed *in utero* to BPA, it can be seen that either the authors did not analyse the mammary glands or the histological examinations were not suitable for showing carcinogenic effects. Also, studies analysing reproductive toxicity did not follow the animals for long enough after prenatal exposure to detect carcinogenic effects in adulthood.

Prenatal and perinatal exposure

Several *in utero* studies showed neoplastic and non-neoplastic effects on the mammary glands.

Munoz de Toro *et al.* looked at the extent to which perinatal exposure to BPA between GD9 and PND4 in CD-1 mice was able to induce a change in mammary gland development in F1s (Munoz del Toro, 2005). Using an Alzet osmotic pump, the authors exposed the mothers to concentrations of 25 and 250 µg of BPA/kg bw/day (BPA diluted in 50% DMSO). The mammary glands were sampled then analysed at 30 days. The analyses show that perinatal exposure to BPA significantly increases the response to oestrogens by increasing the number and size of breast buds and increases the expression of progesterone receptors. The authors suggest that this increase could be a precursor to an increase in the secondary branching of mammary ducts observed at 4 months and a significant increase in the percentage of mammary alveoli at the age of 6 months. Consequently, these correlations suggest that exposure to BPA in particular increases susceptibility to the development of cancer in the mammary glands.

In 2007, Murray *et al.* examined the extent to which prenatal exposure to BPA is sufficient to induce the development of preneoplastic lesions in the mammary gland in the absence of any additional carcinogenic treatment. They exposed pregnant Wistar-Furth rats to doses of 2.5, 25, 250 and 1000 µg/kg bw/day between GD9 and PND1 using an Alzet osmotic pump (Murray, 2007). The anatomical and histological observations were made in females at puberty (PND50) and on PND95. The results suggest that **prenatal exposure to BPA significantly**

increases the number of hyperplastic ducts in the mammary gland for all doses at puberty (PND50), whereas on PND95 the incidence of hyperplastic ducts is not significantly greater than that of the controls at the lower dose of 2.5 µg/kg bw/day. On PND50, the authors observed 1 case in 4 of CIS at the two BPA doses of 250 and 1000 µg/kg bw/day (1/4 at 250 µg/kg bw/day and 1/4 for 1000 µg/kg bw/day) and report that this incidence "increased" on PND95 with an incidence of 2 cases in 6 (non significant difference). The structures observed were of the "cribriform" type regarded as intraductal carcinomas (CIS) according to the criteria described by two authors (Russo, 1996) (Singh, 2000). In both rodents and humans, intraductal hyperplasia is regarded as a precursor of CIS (Singh, 2000). Several methodological limitations must be noted: the small number of animals used and the lack of information about the incidence of CIS in the controls. No investigation was made after PND95. It should be noted that the strain of rat used is very sensitive to chemical carcinogens.

Murray et al. 2007 study

As reported in the EU RAR (2008), Murray *et al.* (2007) examined the effect of prenatal BPA exposure on in situ induction of mammary tumours in rats. From GD 9 (GD 1 = day of vaginal sperm) through PND 1 (PND 0 = day of birth) Wistar-Furth rat dams received subcutaneous osmotic pumps of 0, 0.0025, 0.025, 0.250, or 1 mg/kg bw per day BPA. Number of dams treated was not reported. Based on a limited amount of information provided on the number of offspring examined, it appears that ≤ 6 dams/group were treated. Pup viability was assessed on PND 1. On PND 2 pups were sexed and litters were culled to 8 pups. Anogenital distance was measured on PND 4. Litters were weighed during the lactation period. Female offspring were monitored for body weight and vaginal opening in the post-weaning period. Female offspring were killed on PND 50 or PND 95. Mammary glands were collected and whole-mounted or sectioned for histopathological examination. Morphometric analyses were conducted to examine possible presence of preneoplastic lesions. Mammary glands were examined for ER-α and Ki-67 protein by an immunohistochemistry technique. One female/litter was included in the histological examinations. Apparently, ≤6 offspring/group were examined histopathologically. The number of offspring examined for the other endpoints was not reported. It was not clear if dams or offspring were considered the statistical unit. BPA exposure did not affect offspring viability, sex ratio, age at vaginal opening, or female anogenital distance. Anogenital distance was reduced on PND 4 in males from the 0.250 mg/kg bw per day group. Cribriform structures classified as carcinomas-in-situ were observed in the 0.25 and 1 mg/kg bw per day groups. The incidence of these structures in the controls and lower dose groups were not reported.

- **Strengths of the study:**

  ***** Rat (Wistar-Furth) study following previous work from the same team on mice with comparable results
  ***** number of doses (4)
  ***** Fetuses were exposed to BPA or vehicle from E9 (Embryonic day) until postnatal day (PND)1.
  ***** Histological evaluation performed during windows of susceptibility to initiator carcinogenic compounds (PND50) and also at PND 95; in the mammary gland, the peripubertal period is indeed characterized by intense ductal morphogenesis encompassing tissue remodeling, epithelial invasion of the stroma, increased rates of cell proliferation and cell death; hence, the pubertal mammary gland is particularly

prone to neoplastic development .
***** Morphometric evaluation performed with appropriate sensitive techniques
***** Accurate definition of intraductal hyperplasia: increase in the number of epithelial cells lining the ducts (3–4 cells thick).
***** Cages and bedding tested negligible for estrogenicity by the E-SCREEN assay; estrogenicity of the feed (Harlan Teklad 2018 was measured at 20 femtomoles of estrogen equivalents per gram, a negligible amount
* immunohistochemistry tests
* statistics: litter effects taken into account: For each histological measurement, only one individual from a given litter was assigned to each group and end point
* in addition to the increased number of hyperplastic ducts, cribriform-like structures were also observed in the mammary glands of BPA250 and BPA1000 rats at PND 50 which may be in favor of neoplastic transformation
* The fourth abdominal mammary glands were rapidly dissected from live ketamine/ xylazine anesthetized animals.The 'mammary tree'was trimmed free of excess fat and frozen for later microarray analysis. From another set of rats, mammary glands were dissected for whole mount preparation and gland differentiation analysis, and the contralateral mammary gland was paraffin blocked for cel proliferation studies

- **Weaknesses of the study:**
***** subcutaneous exposure with Alzet osmotic pumps
***** number of dams exposed not reported; apparently, ≤6 offspring/group were examined histopathologically.
***** no dose-response relationship
***** exposure to BPA was not measured in the rats

Vandenberg *et al.* published two articles in 2007 and 2008 about the mammary gland and BPA (Vandenberg, 2007 170 ;Vandenberg, 2008). In the first study of 2007, a single concentration of 250 ng of BPA/kg bw/day administered by continuous infusion from a subcutaneous pump was used between days GD8 and GD18 in CD-1 mice aged 8 weeks. In the foetus on GD18, BPA altered the general organisation of the mammary gland for all the morphological criteria studied. To validate these observations, these same authors performed a second study in 2008, in which mice were exposed to BPA (0, 0.25, 2.5 and 25 µg BPA/kg bw/day) from GD8 to PND16. The authors studied the characteristics of the mammary glands of the neonates at 3, 9 and between 12 and 15 months after birth. The results confirm the previous observations according to which exposure to BPA alters the morphology of the mammary glands in adult mice. The effects observed are hyperplasia, with the appearance of "polished" ducts with all doses of BPA at 9 months, but not at 12-15 months. The question of the reversibility of these effects was raised by the authors in their conclusion.

Doherty *et al.* propose a new mechanism to explain the effects of endocrine disrupters on mammary development (Doherty L, 2010). These authors exposed pregnant CD-1 mice to 10 µg of BPA/kg bw/day between GD6 and GD21. *In utero* exposure to BPA produced an increase in the expression of the histone "enhancer of zeste homologue 2" (EZH2), which suggests that BPA could be involved in the development of mammary lesions in adults. Expression of EZH2, a risk biomarker which is said to be involved in the development of breast cancer, was

assessed 6 weeks after birth. It should be noted that this protein is involved in stem cell renewal and is said to be activated by the mutant BRCA1 gene (Kunju LP, 2011). Durando *et al.* performed a prenatal study in Wistar rats exposed to 25 µg of BPA/kg bw/day by subcutaneous infusion between GD8 and GD23 (Durando, 2007). The low doses of BPA produced ductal hyperplasia, desmoplasia and the presence of mastocytes in the stroma, which suggests an increased risk of developing cancer, even 50 days after the end of exposure to BPA. This is in perfect agreement with other published results quoted above.

Moral *et al.* exposed female Sprague-Dawley rats to 25 or 250 µg of BPA/kg bw/day by gavage from GD10 to GD21 (Moral, 2008). The female neonates were sacrificed and the mammary glands sampled on PND21, 35, 50 and 100 to observe the morphological changes, and to assess gene expression and the cell proliferation index. **An increase in the number of undifferentiated epithelial structures** and changes in gene expression were observed. The results suggest that the effects on the mammary gland depend on both the dose and the period of exposure and that BPA affects the susceptibility of the mammary gland to undergo changes towards undifferentiated structures.

Moral R et al. 2008 study

In this study pregnant Sprague-Dawley rats were gavaged with 25 µg BPA/kg bw or 250 µg BPA/kg bw on days 10-21 post conception. Controls were given sesame oil vehicle only and there were 10 animals per group. The 4th pair of mammary glands from the offspring (8-10/group) was assessed for morphological changes in whole mount preparations and for cell proliferation in sections at days 21, 35, 50 and 100. Frozen mammary tissue was pooled from controls and each treatment group for gene expression analysis using microarrays and real-time (RT)-PCR.

High-dose BPA exposure was reported to induce small architectural modifications in the mammary glands, mainly in the number of undifferentiated epithelial structures but the proliferative index (as determined by BrdU) was not affected. Low and high doses of BPA were reported to alter the gene expression profile of mammary tissue but in a somewhat inconsistent manner: low dose had the highest effect by 50 days, while high dose had the most influence on gene expression by 100 days. At the low dose, up-regulated genes were related to the immune system and at the high dose, genes related to differentiation were upregulated.

- **Strengths of the study:**

  ***** Rat (Sprague–Dawley CD) study following previous work from the same team on mice with comparable results
  * Exposure of the fetuses from days 10–21 post-conception.
  ***** 10 animals per group
  ***** oral exposure (gavage)
  ***** Histological evaluation performed during windows of susceptibility to initiator carcinogenic compounds (PND21, 35, 50 and 90)
  ***** Morphometric evaluation performed with an appropriate sensitive technique
  ***** Accurate definition of intraductal hyperplasia:
  ***** phytoestrogen-free AIN-93G diet (Harlan Texlad, Madison,WS, USA).
  * determination of gene expression profile by microarrays

- **Weaknesses of the study:**
  ***** number of doses (2)
  ***** types of cages and drinking bottles not reported
  ***** exposure to BPA was not measured in the rats
  ***** Differences in architecture/histology was very small

Betancourt *et al.* studied the susceptibility to developing a mammary gland tumour after *in utero* exposure to BPA followed by postnatal exposure to a carcinogenic agent (dimethylbenzanthracene = DMBA) (Betancourt, 2010). The authors mention that the changes in the mammary glands are not accompanied by clinical signs such as premature vaginal opening or a variation in oestrogens and progesterone, which would, according to the authors, indicate that the changes are epigenetic alterations acting directly on the target organ. The highest dose of BPA (250 µg/kg bw/day) increased the incidence of breast tumours and changed the window of the mammary gland's susceptibility to DMBA, which moved from PND50 to PND100. In addition, the authors made a proteomic analysis in female rats treated by gavage with doses of 25 or 250 µg/kg bw/day of BPA during gestation (GD10-GD21) (Betancourt, 2010). The change in the expression of certain proteins that regulate cell proliferation which was observed on PND21 (weaning) and PND50 (puberty) could increase the susceptibility of the mammary gland to tumour development.

A study by Wadia *et al.* sought to show whether perinatal exposure to BPA between GD8 and PND2 could induce mammary gland sensitivity to oestradiol in adulthood in CD-1 and C57B16 mice (Wadia, 2007). The authors wanted to compare the sensitivity of each of these 2 strains of mice. Pregnant mice were exposed to 250 ng of BPA/kg bw/day from GD8 to PND2. On PND25 the neonates were ovarectomised, implanted with an oestradiol pump, exposed to concentrations of 0.5 or 1.0 µg of E2/kg bw/day for 10 days and sacrificed on PND35. The 2 strains showed a similar response. However, perinatal exposure to BPA altered several parameters in the 2 strains, and these effects were slightly more pronounced in the CD-1 strain. The results suggest that perinatal exposure to BPA alters the response to oestradiol at puberty in both strains, even though the effects are more pronounced in the CD-1 mice.

2014 studies showing adverse effects of BPA on mammary gland

It has to be noted that these studies were not available at the time of the submission of this proposal. They have been quoted during the public consultation on this dossier.

In the study of Acevedo et al. (2013) titled "Perinatally administered bisphenol a as a potential mammary gland carcinogen in rats", the authors aimed to examine the effect of duration of BPA exposure over a wide range of concentrations on the induction of preneoplastic lesions and DCIS in rats. They have also measured internal levels of BPA in serum of these animals (dams, fetuses and pups). Sexually mature virgin female and males Sprague-Dawley rats (Taconic, Germanton, NY/8-10 weeks) were exposed subcutaneously to BPA via Alzet osmotic pumps. Four BPA concentrations were administered: 0.25 µg/BW/d (BPA0.25), 2.5 µg/BW/d (BPA2.5), 25 µg/BW/d (BPA25), 250 µg/BW/d (BPA250) during 14 days from GD18 to GD21 (gestational exposure) or 28 days from GD9 to PND10 (Gestational

and lactational exposure). Control group were exposed to vehicle only with the same pumps. Female offspring were sacrificed at PND50, 90, 140 and 200 (n=9-12/dose/age at sacrifice per exposure group). Serum sample were collected in pregnant dams and their fetuses in the vehicle control group and in the BPA250 group. The following Biological studies of mammary gland pups were performed:

- Histological analysis at PND50:
  - Edge and Terminal end Buds (TEBs): count of ducts within a 4 mm² area around the most proximal TEB
  - Thick of the ductular epithelial cell layers was evaluate to looking for an Usual intraductal Hyperplasia (UDH)
  - Presence of Atypical Ductal Hyperplasia (ADH) and neoplastic lesions (DCIS)
- Whole mounts analysis at PND50, 90, 140 and 200 were performed to assess proliferative lesions and malignant tumor

The measures of serum BPA after continuous administration show that:

- in the "Gestational exposure group": total and unconjugated BPA was detectable in 100% (4/4) of pregnant dams and fetuses for BPA250 group. Total BPA in the exposed fetuses was > control (27.9±8.96 ng/mL vs mean<LOD; $p<0.05$). Same result were observed for unconjugated BPA but p-value approached the significance ($p=0.051$). Total BPA in fetuses was 4x greater compared to dams concentrations (27.9±8.96 ng/mL vs 6.13±3.88 ng/mL),
- in the "Gestational/Lactational exposure": total and unconjugated BPA were detectable in 100% (4/4) of pregnant dams but total BPA was detectable in only 33% (2/6) of pups (undetectable for unconjugated BPA concentrations). Total BPA in the exposed pups was > control (0.38±0.26 ng/mL vs mean <LOD; $p<0.05$). Total BPA in pups was << compared to dams (0.38±0.26ng/mL vs 16.5±13.0ng/mL)

Preneoplastic and neoplastic lesions in exposed mammary gland pups were reported:

- Histological analysis **at PND50** have shown: incidences for ADH of 0, 60, 20, 0 and 40% respectively in the control and BPA 0.25, BPA2.5, BPA25 and BPA 250 rats for gestational exposure and 0, 0, 20, 20 and 17% respectively in the control and BPA 0.25, BPA2.5, BPA25 and BPA250 rats for gestational/lactational exposure. UDH and DCIS (0-20% vs 0%) were not significantly different between exposed and control for all BPA doses and both exposure. Adenocarcinoma were observed in the BPA2.5, BPA25 and BPA250 groups (in one rat per group).
- **Whole mounts analysis**: incidences of lesions and tumors were not significant between exposed and control, for all BPA doses and both exposure

- Tumors were detected at **PND90, PND140, and PND200** in animals exposed to BPA across all doses and exposure times. A total of six mammary gland tumors were observed in females exposed perinatally to BPA at doses ranging from BPA0.25 to BPA250 (n = 230; Table 4). Five tumors were diagnosed histopathologically as adenocarcinomas and one was diagnosed as a benign fibroadenoma (Figure 3). No malignant tumors were detected in any vehicle treated control animals (n = 65).

The authors concluded that perinatal exposure to human-relevant internal doses of BPA, in absence of additional exposure to chemical carcinogens, was associated to induction of

malignant mammary gland tumors and other lesions in adult female rats. Subcutaneous doses of 250 µg/kg bw/day triggers changes in the postnatal (PND50) and adult mammary gland epigenome and alters gene expression patterns. Thus, based on this result of this study, BPA may act as a complete mammary gland carcinogen.

In the study of **Dhimolea *et al.* (2014) titled "Prenatal Exposure to BPA Alters the Epigenome of the Rat Mammary Gland and Increases the Propensity to Neoplastic Development",** the authors aimed to examine the effects of prenatal exposure to BPA on the genome wide DNA status of the mammary gland during postnatal development and explored potential cues linking epigenetic alterations to breast carcinogenesis during adulthood. They also monitor BPA serum concentrations in dams in order to relate internal dose to the effects of BPA in the mammary gland. Sexually mature female and male Wistar-Furth rats (8-weel old; Harlan Indianapolis, IN) were subcutaneously exposed via Alzet osmotic pumps from GD 9 to PND 1 at the dose of 25 or 250 µg bw/d. One control group for each stage included 4-5 litters. The litter was the exposure unit: 1 female pup per litter was included in each experimental group (control and treatment). Female offspring rats of ages PND4, PND21 and PND50: 8-11 litters were included in each stage groups. Total serum BPA were measured in dams at GD12:

- Total BPA: in the 25 µg/ bw/d animals: 67% (4/6) (1.02 ± 0.46µg/L (only detectable levels); in the 250 µg/BW/d animals: 100% (5/5) (9.76 ± 3.85µg/L)

- Unconjugated BPA: in the 25 µg/ bw/d animals: undetectable and in the 250 µg bw/d animals: 100% (5/5) (1.68 ± 0.74µg/L).

Mammary gland tissue was sampled from female pups at each stage to investigate: genomic DNA (gDNA) methylation and Gene expression: Transcriptom (mRNA), p57 and α-lactalbumin gene (only displayed results). Animals used in the epigenome and transcriptome studies were exposed to 250 µg BPA bw/d.

- Post-natal mammary gland development :
    - Epigenome studies: animals born from BPA-treated mothers displayed changes in methylation status
        - No significant trend toward hypo- or hyper-methylation at any of the tested time-points
        - Dynamic changes between hypo- and hyper-methylation over time
    - Gene expression studies:
        - Transcriptome: widespread changes in mRNA expression in mammary gland between BPA- and vehicule-treated groups at PND4 and 21
        - p57 gene: increase in the expression at PND50
        - α-lactalbumin gene: 2-fold increase in the expression at PND4 and significant enrichment of H3K4me3 at the promoter in treated mammary gland compared to control (epigenic levels of regulation)

The authors concluded that prenatal exposure to BPA alters the epigenome of the mammary gland of Wistar-Furth rats and increases the propensity to neoplastic development. Subcutaneous doses of 250 µg/kg bw/day triggers changes in the postnatal (PND50) and adult mammary gland epigenome and alters gene expression patterns.

Postnatal and/or pubertal exposure

Only a few recent studies look at postnatal exposure. The study of Jenkins *et al.* shows that female rats whose mothers were treated with BPA at a dose of 25 and 250 µg/kg bw/day during lactation (PND2 to PND20) develop more breast tumours and show a reduction in the latency period until the onset of those cancers after treatment by gavage on PND50 with a carcinogen, dimethylbenzanthracene (DMBA) (Jenkins, 2009). The type of tumours is not specified in the article. The highest dose of BPA produced an increase in cell proliferation and a reduction in apoptosis in the mammary glands on PND50 (but not on PND21) combined with overexpression of the proteins regulating cell proliferation. The time to the appearance of the tumours was significantly shorter in the group exposed to the highest dose. The authors conclude that BPA plays an amplifying role in the onset of mammary tumours after exposure to DMBA in the female offspring. This suggests that the effect of BPA could act via epigenetic mechanisms. This mode of action was recently demonstrated by (Yang, 2009).

Jones *et al.* assessed the impact of the loss of the function of the BRCA1 gene on cell proliferation induced by BPA (Jones LP, 2010). This study is open to criticism and interpretation of the results is difficult. It is a mechanistic study which cannot be used directly for the assessment of risk. Another study, that of Colerangle and Roy, assessed the growth of the mammary gland in female Noble rats treated subcutaneously with BPA at doses of 0.1 and 54 mg/kg bw/day (Colerangle JB, 1997). They noted a significant increase in the conversion of immature structures into mature structures, a reduction in the number of ductal buds and an increase in the mean number of lobules. The authors also noted an alteration in the cell cycle which was said to be an important factor in the development of genetic instability such as nucleotide errors in the synthesis of DNA.

Exposure in adulthood

As reported in the NTP study report, a study of carcinogenesis via the oral route in female rats (BPA: 74 and 135 mg/kg bw/day) and mice (BPA: 650 to 1300 mg/kg bw/day) did not show any neoplastic or non-neoplastic effect on the mammary gland (NTP, 1982).

Table 22. Studies examining the effects of bisphenol A on breast cancer: summary table

Reference	**Species / strain**	**Route**	**Dose** **Exposure period**	**Effects** **NOAEL/LOAEL**
Betancourt, 2010	Sprague-Dawley Rats	Oral	0 – 25 - 250 µg BPA/kg F0: Exposure in mothers to BPA from GD10 to GD21 followed by single dose of DMBA on PND50 or PND100 .	**Effects observed:** - *In utero* exposure to 250 µg/kg of BPA **associated with a single exposure to DMBA** at 100 days postnatally (but not on PND50), produced an increase in the incidence of mammary tumours and a shorter latent time compared to the control group. - **Without DMBA**, an increase in cell proliferation and

			F1: exposure not checked	overexpression of some proteins involved in cell proliferation was observed. **<u>Critical effect:</u>** - Amplification of breast tumour development (number/rat and time to occurrence) in a DMBA model - Expression of proteins involved in cell proliferation - Changes in proteins which influence cell proliferation on PND100 (250 µg/kg) - ERα, PR-A, Bcl-2, steroid receptor coactivators, (SRCs), EGFR, IGF-1R, and phospho-c-Raf. **Doses** are not known in the offspring and are possibly less than: **NOAEL 25 µg/kg bw/d** **LOAEL 250 µg/kg bw/d**
Betancourt, 2010	Rats	Oral	0 – 25 - 250µg BPA/kg GD10 - GD21. Female descendants were humanely killed on PND21 and PND 50.	↗ phospho-AKT, ↗ c-Raf, phospho-ERKs-1 and 2, ↘ TGF-β in breast tissues at 50 days postnatally Important signalling pathways are disrupted by BPA. Prenatal exposure to BPA results in deterioration of expression of proteins in breast glands postnatally.
Doherty L, 2010	CD1 Mice	Intra-peritoneal	0 - 10 µg/kg-5 m/kg	↗ histone H3 trimethylation ↗ of EZH2 (2X) expression in mammary tissues compared to

			GD9 to GD26	the control
Durando, 2007	Female Wistar rats	Sub-cutaneous pump	25 µg/kg GD8 to GD23	↗ proliferation/apoptosis ratio ↗ ductal hyperplasia ↗ sign of desmoplasia ↗ neoplastic lesion **No NOAEL/LOAEL**
Jenkins, 2009	Female Sprague Dawley rat pups	Oral	0 - 25 and 250 µg/kg bw/d, 5 d/week Administered to lactating mothers from PND 2 to PND 202 (equivalent to 15 administrations/mother). The female baby rats were treated with a single dose of DMBA on PND50.	↗ tumour incidence at high dose **NOAEL 25 µg/kg bw/d** **LOAEL 250 µg/kg bw/d**
Jones LP, 2010	BRCA1 deleted mice	Sub-cutaneous pump	250 ng BPA/kg bw/d	Difficult to interpret (transgenic mice) BRCA1 deletion followed by BPA exposure stimulates mammary glands leading to hyperplasia compared to the control
Moral, 2008 (see detailed description above)	Sprague-Dawley rats	Gavage	25 et 250 µg/kg pc GD10 à GD21	Increase in the number of undifferentiated epithelial structures (TEB and TD). No effects on proliferation; BPA exposure changes the gene expression signature: - altered gene expression, maximal at 100 d with the high dose (genes up-modulated at the two doses, including a cluster related to immune response; underexpressed genes including differentiation-linked genes at high dose). - At low dose, the expression

				profile is changed most at 50 d.
Munoz del Toro, 2005	CD1 mice	Sub-cutaneous pump	25 - 250 ng/kg bw dissolved in DMSO GD9 to PND4	↗ response to oestrogens ↗ expression of progesterone receptors.
Murray, 2007 (see detailed description above)	Wistar-Furth rats	Sub-cutaneous pump	2.5 – 25 – 250 – 1000 µg/kg bw GD9 to PND1	↗ number of intraductal hyperplasia in mammary gland at all doses (more pronounced at PND50 compared to PND95). CIS present in mammary glands of animals exposed to the highest doses at puberty and at 3 months.
Vandenberg, 2007	Female CD1 mice	Sub-cutaneous pump	250 ng BPA/kg bw/d GD8 to GD18	↗ ductal area ↘ cell size Delay in lumen formation Adverse changes in mammary gland phenotype
Vandenberg, 2008	Female CD1 mice	Sub-cutaneous pump	0 - 0.25 - 2.5 - 25 µg/kg bw/d GD8 to PND16	Deterioration in development of mammary glands ↗ proliferation indexes compared to control group
Wadia, 2007	Outbred CD-1 mice Inbred C57B16 mice	Sub-cutaneous pump	0 - 250 ng/kg bw/d Mixed exposure BPA and E2 GD8 to PND2	Perinatal exposure to BPA does not adversely affect the uterine response to E2 administered from PND25 to PND35 but does adversely affect the uterine response of the mammary gland.

B.5.9.2.4. Recent studies (2011-2012) on the effects on the mammary gland

Several *in vivo* experimental studies have recently supported the choice of critical effects used: effects on the terminal buds and terminal canals in the mammary gland during development in the monkey, development of intracanal hyperplasia, and increased sensitivity of the mammary gland to carcinogens (NMU, DMBA) following prenatal exposure in the rat and the mouse. The

development of neoplasic-type lesions with BPA alone is not always solidly backed up (Durando, 2007).

Summary articles on BPA highlight the vulnerability of the developing mammary gland to environmental agents as well as the inadequacy of the methodologies in standard toxicology in demonstrating the morphological changes, focusing in particular on the undifferentiated structures (terminal buds and terminal canals). The studies available are difficult to compare due to the different analysis methodologies used. The study by Tharp (Tharp, 2012 – see description below) has enabled the similarity of the effects on the terminal buds and terminal canals of the mammary gland during development between rodents and non-human primates to be demonstrated. However, the links between the morphological changes to the mammary gland and the functional (lactation) and lesional (tumours) consequences are not presently established, and require more in-depth research. A study in 2012 showed the impact of BPA on both the morphological development of the mammary gland and on lactation (Kass, 2012), while another study did not demonstrate changes to the post-natal development of the mammary gland preceding the increase of tumours induced in the DMBA after prenatal exposure to BPA (Weber, 2011).

Conclusion in animals:

In animals, although the studies are somewhat heterogeneous, some of the effects observed converge for the following critical effects:

- accelerated structural maturation of the mammary gland at adulthood, as a result of prenatal or perinatal exposure to BPA, is a **recognised effect in animals**;
- development of intraductal hyperplastic lesions from perinatal or prenatal exposure to BPA is a **recognised effect in animals**;
- development of neoplastic lesions (CIS: intraductal carcinoma *in situ*) after perinatal exposure to BPA is **a suspected effect in animals**;
- increased susceptibility of mammary glands to develop subsequent mammary tumours (following co-exposure with a carcinogen) from prenatal or perinatal exposure to BPA is a **suspected effect in animals**

Selecting the critical effect:

The effect of BPA on the mammary gland and on the risk of increased susceptibility during subsequent exposure to a carcinogen has been selected for the risk assessment (see studies presented hereabove).

At the end of this analysis, we firstly retained the effects considered "proven" in animals, namely:

The architectural changes to the mammary gland in adulthood in connection to pre - and perinatal exposure;

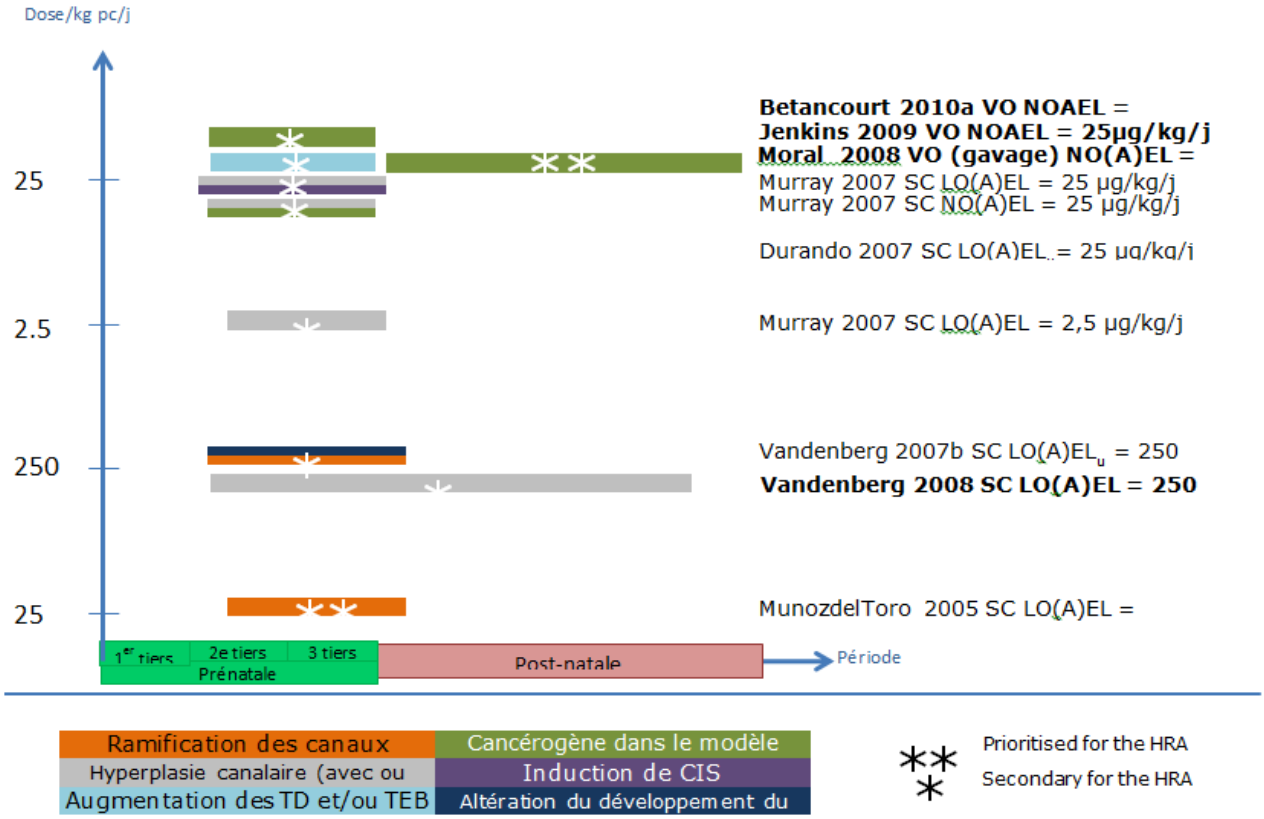
The **development of ductal hyperplastic lesions** in connection to **pre- or peri-natal exposure**.

In addition, the studies showing the **development of neoplastic-type lesions** (CIS; ductal carcinoma) or even an **increase in the likelihood of mammary glands subsequently developing mammary tumours** (during co-exposures to a carcinogenic agent) may be taken

into account insofar as these effects, although deemed "suspected" in animals, are part of a continuum of effect. The dose level at which these effects are observed will also be taken into consideration in order to determine the most appropriate NOAEL/LOAEL.

The NOAEL/LOAEL resulting from these studies are represented in the figure below.

Figure 11. BPA effects on the mammary gland



Branching of the ducts	Ductal hyperplasia (with or without inductor)	Increase in TDs and/or TEBs	Carcinogenic in the DMBA model	Induction of CIS	Alteration of the development of the stroma
Munoz del Toro 2005 SC LO(A)EL = 25 ng/kg/d GD9 - PND4**	Murray 2007 SC LO(A)EL = 2,5 µg/kg/d GD9 - PND1**	**Moral 2008 VO (tube feeding) NO(A)EL = 25 µg/kg/d GD10 - GD21****	**Betancourt 2010a VO NOAEL = 25 µg/kg/d GD10 - GD21****	Murray 2007 SC NO(A)EL = 25 µg/kg/d GD9 - PND1**	Vandenberg 2007b SC LO(A)ELu = 250 ng/kg/d GD8 - GD18*

Vandenberg 2007b SC LO(A)ELu = 250 ng/kg/d GD8 - GD18*	Durando 2007 SC LO(A)EL_u = 25 µg/kg/d GD7 - GD22		**Jenkins 2009 VO NOAEL = 25 µg/kg/d PND2 - PND20****		
	Vandenberg 2008 SC LO(A)EL = 250 ng/kg/d GD8 - PND16**				

Selecting the key study:

All of the studies evaluated in this dossier have been rated. The studies deemed to be of good quality were considered first. A way of representing the identified NOAELs/LOAELs graphically was developed (see figure hereabove) in order to help with the selection process.

Studies showing the **development of neoplastic-type lesions** (CIS; ductal carcinoma) – Murray *et al.*, 2007 (Murray, 2007-see detailed description above) study conducted subcutaneously or even an **increase in the likelihood of mammary glands subsequently developing mammary tumours** during co-exposures to a carcinogenic agent, (Jenkins, 2009 and Betancourt, 2010, conducted orally) are identified as key studies because, even though these effects are considered suspected and not proven in animals, they are considered of particular concern given their relevance to humans.

Selecting the benchmark doses:

The benchmark doses were based on a set of studies as no one study was robust enough to be identified as a critical study on its own.

Concerning the **effects deemed "suspected" in animals**, namely induction of CIS and promoting tumour effect in the presence of an initiator, a NOAEL/LOAEL couple of 25 / 250 µg/kg bw/d was identified:

- **Orally**: NOAEL/LOAEL of 25/250 µg/kg bw/d based on a promoting tumour effect in the presence of an initiator after postnatal exposure (Jenkins, 2009) and prenatal exposure (Betancourt, 2010).
- **Subcutaneously**: NOAEL /LOAEL of 25/250 µg/kg bw/d based on the induction of CIS after prenatal exposure (Murray, 2007),

With respect to the **effects deemed "proven in animals"**, it was deemed to consider the architectural changes of the mammary gland for the HRA (see Moral *et al.* (Moral, 2008)) study and ductal hyperplasia (see the Murray *et al.*, 2007 study; (Murray, 2007):

- Modification of the architecture of the mammary gland: identified NOAEL of 25 µg/kg/d in prenatal, orally in rats considering the most relevant structures for

carcinogenesis (TEB and TD). The **NOAEL/LOAEL couple of 25/250 µg/kg bw/d** is therefore selected on the basis of the Moral study, conducted orally in rats (Moral, 2008-see detailed description above). If one considers that biovailability of free BPA after oral exposure is 3%, then based on this study, the equivalent dose for free circulating BPA should be respectively 0.75 and 7.5 µg BPA/kg bw per day.

- Ductal hyperplasia induced subcutaneously in rats during prenatal exposure Murray *et al.*, 2007 (Murray, 2007-see detailed description above): LOAEL of 2.5 µg/kg bw/d. The NOAEL is not identifiable in this study. In mice, these hyperplasia were observed at even lower doses, namely 0.25 µg/kg/d (Vandenberg, 2008), during pre and postnatal exposure. If one considers 100% of the BPA administered subcutaneously is free-BPA reaching the systemic circulation, as one doesn't know the metabolism of BPA by this route, then by default this LOAEL could be considered as an internal LOAEL corresponding to unconjugated circulating BPA.

Among the "proven" effects, ductal hyperplasia are the effects observed at the lowest doses and are therefore the most sensitive effects. According to Russo, 1996 intra-ductal proliferations (or IDP) are ductal hyperplastic-type lesions which are part of a continuum of effect during a process of induced carcinogenesis. These lesions appear on the periphery of the mammary gland or dispersed depending on the carcinogen (DMBA or NMU). Furthermore, ductal hyperplasia are considered precursors of ductal carcinomas in rodents and in humans (Singh, 2000- see box on transposition to humans and more details about Singh et al 2000 study below).

According to Moral *et al.*, (Moral, 2008), the increase in the number of undifferentiated epithelial structures is associated with an increase in the likelihood of mammary gland tumour transformation. In addition, terminal buds (TEB) then the terminal ducts (TD) are considered the structures most sensitive to mammary carcinogens (Medina, 2007; Russo, 1996). The response to exposures to carcinogens is greater when exposure occurs during puberty or adolescence, the period when the TEBs are still numerous. Therefore, the increase in the number or persistence of the TEBs proliferating is considered of particular concern (see Birnbaum, 2003; Rudel, 2011; Fenton, 2006). Thus, the effect on the TEB seems most relevant for assessing sensitivity to carcinogens. This effect on terminal buds (TEB) was observed in non-human primates (Tharp, 2012-see description below), orally in a single dose of 400 µg/kg/d prenatally. The study on primates confirms the previous work carried out on rodents.

Tharp 2012 study

In this study pregnant rhesus monkeys were fed a dose of 400 µg BPA/kg bw/day. Deuterated BPA (in fruits) was administered daily during days 100–165. Day 165 corresponds to the full-term gestation. BPA was administered by food. The period of exposure corresponds therefore to the last trimester of gestation. All animals were trained to accept small pieces of fruit before beginning the BPA treatment period. Fruit was cut small enough that animals would take the fruit in one bite and would not try to pull it into smaller pieces before consuming. Preferences of each animal were noted. The BPA dose for each animal was calculated based

on body weight at weekly intervals. Maternal serum samples were taken near the time of spontaneous birth, ≈4 h after oral dosing. The serum samples were analyzed for conjugated and unconjugated BPA (LOD: 0.2 ng/ml) as described in Taylor et al. (2011). Exposure resulted in serum levels of 0.68±0.312 ng free BPA/ml and and 39.09 ± 15.71 ng/ml of conjugated BPA (range: 11.42–94.82 ng/ml). Levels of free BPA are comparable to those measured in the general population. The mammary glands of female offspring (n=4, controls = 5) were collected 1 – 3 days after birth. Effects similar to those seen in rodents were observed, e.g. increased number of terminal end buds, terminal ends, branching points, as well as total mammary gland area, ductal area and number of ductal units.

- **Strengths of the study**

  ***** primate study (female rhesus macaques), following previous work from the same team on rodents with comparable results
  ***** oral exposure (by food - small pieces of fruit)
  * morphometric evaluation performed with an appropriate sensitive technique and blind analysis of the mammary glands
  * cages made of stainless steel
  * exposure to BPA was measured in the treated and control monkeys; In the control mothers, the levels of conjugated and unconjugated BPA were below the level of quantification in three of four animals and four of four animals, respectively. Only one control mother had a quantifiable level of conjugated BPA (0.814 ng/mL); however, this level represents a mere 2% of the mean value found in exposed animals.
  ***** Morphometric evaluation performed with an appropriate sensitive technique

- **Weaknesses of the study:**
  ***** one dose only
  ***** low number of animals per dose (but quite usual for a primate study)
  * animal diet and phytoestrogen content not measured

LOAEL = 400 µg BPA/kg bw per day. If one considers that biovailability of free BPA after oral exposure is 3%, then based on this study, the equivalent dose for free circulating BPA should be respectively 12 µg BPA/kg bw per day.

At the end of this analysis, it was retained ductal hyperplasia and the effects on the architecture of the mammary gland, including effects on terminal ducts (TD) and the terminal buds (TEB) as critical effects for the HRA. For effects on these undifferentiated epithelial structures (TD and TEB), the Moral study (Moral, 2008) led to a NOAEL of 25 µg/kg/d, orally and a LOAEL of 250 µg/kg/day.

Thus, the NOAEL/LOAEL couple of 25/250 µg/kg/d orally, identified for the effect on the architecture of the mammary gland (TD and TEB) converges with that identified for the two other suspected effects, which means that these effects can be considered in the HRA. Moreover, the effects on ductal hyperplasia leads to a LOAEL of 0.25 µg/kg/d subcutaneously in mice (Vandenberg, 2008) during pre and postnatal exposure and to a LOAEL of 2.5 µg/kg/d

subcutaneously in rats during prenatal exposure (Murray, 2007-see detailed description above).

For further details on the mammary gland changes and the state of knowledge concerning preneoplastic lesions of the breast, see Annex 8.

As a complement, about the respective position of ANSES and EFSA regarding the effect of BPA on mammary gland, see Annex 5 - ANSES comments on EFSA draft opinion).

Transposition to humans: interpretation issues

Precancerous lesions of the breast are atypical epithelial proliferations which develop within the lactiferous duct tree and are of two types: ductal and lobular. These two types differ not in their location but in the type of their constituent cells. Histological diagnosis of precancerous lesions is difficult and inter-pathologist reproducibility is mediocre, as is shown by a number of studies. The classification of precancerous lesions in humans is based on the terms for ductal (DIN) or lobular (LIN) intraepithelial neoplasia. Ductal carcinoma *in situ* (DCIS) is a preinvasive cancerous lesion. In the United States, DCIS accounts for almost 20% of the cancers picked up in screening (1 case of DCIS per 1300 screening mammographies) (Ernster *et al.*, 2002).

When left in place, a preneoplastic or precancerous lesion can turn into a preinvasive carcinoma or an *in situ* carcinoma which can itself turn into an invasive carcinoma. The theory about the existence of a continuum between the normal mammary gland and invasive breast cancer, even if it may appear too simplistic, is based on direct and indirect arguments (Antoine *et al.*, 2010). Recent epidemiological studies have shown that women with a history of benign breast lesions had an increased risk of breast cancer.

Similarly, the natural development of low-grade ductal carcinomas *in situ* (DCIS) was determined by long-term follow-up studies in women who had undergone a diagnostic biopsy without any other treatment. After 10 years' follow-up, 14 to 60% of these women had a diagnosis of invasive cancer in the same breast (Page *et al.*, 1995). The natural development of high-grade DCIS or of clinically palpable DCIS, on the other hand, is not well characterised since, in most cases, the tumour is removed in its entirety by surgery which is also the case with atypical ductal hyperplastic (ADH) lesions.

The substantial increase in the number of biopsies performed on the basis of infra-clinical images and recent data provided by molecular study of the lesions have shed new light on the risk of hyperplastic lesions becoming cancerous.

When hyperplastic lesions turn into cancers *in situ* then into invasive cancers, imbalances are observed at chromosomal level with loss of heterozygosity in 40% of cases of hyperplasia and more than 70% of high-grade carcinomas *in situ* (Aubele *et al.*, 2000). Molecular markers of tumoral transformation in the breast such as the oestrogen receptor, expressed by normal epithelial breast cells, are expressed by more than 70% of ductal carcinomas *in situ* (DCIS) and the proto-oncogene HER2/neu is overexpressed in half the cases of DCIS but not in atypical hyperplasias (Allred *et al.*, 1992).

Rodents, i.e. rats and mice, have been widely used to study mammary carcinogenesis, in models of either spontaneous or induced tumours. The main advantage of the rat model is that the carcinoma most resembles human breast cancer; breast cancer in mice is often of viral and hormone-dependent origin (Cardiff *et al.*, 2000; Gould 1995). In CD-1 mice, spontaneous non-neoplastic and neoplastic lesions are not very common (less than 5%: (Gad 2007)).

The different strains of rats used have shown differing sensitivities to neoplasms induced chemically or by radiation, Sprague-Dawley or Wistar being more susceptible than the Fisher rat. In Sprague-Dawley rats, the incidence of spontaneous tumours is close to 50% in chronic studies (example, historical data (NTP, 2010)). Certain strains, such as Wistar-Furth, show increased susceptibility to mammary carcinogenesis via chemical carcinogens (Gould 1995).

The factors of mammary gland susceptibility include, in addition to genetic factors, the degree of differentiation of the breast tissue at the time of exposure, physiological and hormonal status, and diet. Susceptibility is increased in prepubertal females during the mammary development period: the ducts end in terminal buds (TEBs) which will progressively differentiate into alveolar buds and alveolar lobules. The greatest number of tumours was induced in female SD rats at between 40 and 46 days, the period of most active differentiation of the TEBs regarded as the target of chemical carcinogens (Russo and Russo, 1996). Breast carcinomas were induced in rats by chemical agents or ionising radiation. The most commonly used chemical carcinogens include the polycyclic aromatic hydrocarbon dimethylbenzanthracene (DMBA) or the alkylating agents N-ethyl-N-nitrosourea (ENU) and N-methyl-N-nitrosourea (NMU). After a single dose of DMBA or NMU, adenocarcinoma develops in 20 days in young rats. These cancers sometimes invade the surrounding tissue but rarely metastasise to distant sites (Gould 1995). A short-term carcinogenesis protocol involving the injection of NMU at 21 days made it possible to describe the chronology of the induction of preneoplastic and neoplastic breast lesions (Thompson *et al.*, 1998), and to compare these lesions with those observed in humans (Singh *et al.*, 2000). Thus, certain similarities were described regarding the lesions observed in humans and those induced in rodents.

- The similarities include:
  - Development in a multistage process
  - Most of the cancers induced by DMBA or NMU are hormone-dependent
  - A similar morphological pattern: hyperplasia, intraductal hyperplasia regarded as preneoplastic, adenomas/adenocarcinomas. Ductal carcinomas *in situ* (DCIS) are regarded as a morphological progression towards breast carcinoma from intraductal proliferative lesions.

Comparison of the histopathological preneoplastic and neoplastic lesions of the mammary gland induced in prepubertal rats with those described in humans

(Singh *et al.*, 2000)

	Humans	**Rats**
Benign lesions	Fibroadenomas that can exhibit carcinomas *in situ* (CIS) and	No ADH or CIS in the

	atypical ductal hyperplasias (ADH)	fibroadenomas
Hyperplasia	Possible atypical hyperplasias	No atypical hyperplasias
Carcinomas *in situ*	Lobular carcinomas *in situ* (LCIS) and ductal carcinomas *in situ* (DCIS) may be observed. Several histological subtypes. Possible microcalcifications.	Less histological diversity. DCIS are observed, particularly cribriform and papillary ones. No microcalcifications of the DCIS.
Invasive carcinomas	Elastosis and possible calcifications. Several types. Lymph nodes involved.	No elastosis or microcalcifications Absence of lobular carcinomas, etc. Much less histological diversity No lymph-node metastasis

- The differences include:
  - The morphology of most breast tumours in mice does not resemble that of human breast cancers (Cardiff *et al.*, 2000);
  - In rats, similarity of the histological lesions has been described, with less diversity than in humans: for example, no atypical hyperplasia, no microcalcifications, no lobular form of CIS or invasive lobular carcinoma have been described in the model of short-term carcinogenesis induced by the carcinogen NMU (Singh *et al.*, 2000).
  - The regional lymph nodes are not invaded in rats as compared to humans.

Other comments (uncertainties, confidence level, etc.)

As previously indicated, no study seemed robust enough to be identified as a critical study on its own. Previously identified uncertainties may be linked to the limited number of animals investigated in some studies (for example, the studies by Murray et al., (Murray, 2007) or by Tharp et al., (Tharp, 2012), or imprecision in the reporting of the data (for example, the Jenkins *et al.* study, (Jenkins, 2009).

The issue of the "adverse" nature of this type of effect was the subject of a discussion at the "Mammary Gland Evaluation and Risk Assessment Workshop, USA, November 16 and 17 2009" for which the proceedings were published by Rudel *et al.*, (Rudel, 2011). The principal findings of the working group are listed in the table below. This group believes that the effects on the mammary gland are "adverse" effects because they represent alterations in growth and development which are likely to pose a risk for lactation and/or cause carcinogenic effects.

The limits of toxicological studies covered by current OECD or US EPA guidelines were discussed in (Makris, 2011). The analysis by Makris (2011) highlights the absence of a histopathological examination adapted to the mammary gland in the current guidelines (US EPA, OECD, etc.). Moreover, the pre-natal period is not covered in the current exposure patterns in general toxicology with the exception of the recommendation made recently by the NTP, 2010 to carry out perinatal treatment during the 13 week and 2 year studies in rats. Furthermore, when an examination of the mammary glands is carried out, it is not carried out during the development phase of the mammary gland but in adulthood and preferentially in the female whereas the mammary gland in the male can be particularly susceptible to developmental disruption (see data reported by Rudel, 2011). Thayer and Foster, 2007 (Thayer, 2007) also recommend establishing experiment protocols including a treatment of animals during gestation and covering also the period of puberty.

Table 23. Conclusions of the "Mammary Gland Evaluation and Risk Assessment Workshop, USA, November 16 and 17 2009" from Rudel, 2011

Priority issues for the application of the risk assessment	**Current opinion**	**Pending issues**
Are rat and mouse models suitable for evaluating the development of the mammary gland in humans?	Current knowledge suggests that rats and mice are reasonable surrogates.	Lack of information on pubertal development in humans; the mechanisms involved may differ depending on the species.
What is the sensitivity of the developmental effects on the mammary gland?	In certain studies, exposures *in utero* lead to developmental effects at similar or even lower doses compared to the doses required to cause effects on reproduction or development for other systems or organs.	A few studies have assessed the effects on the development of the mammary gland and other critical effects "specific to endocrine disruptors"; there is a lack of human data to assess the dose-response relationship as well as a lack of standardised protocols to evaluate the effects on the mammary gland as well as the related assessment criteria.
Are the development changes to the mammary gland harmful effects?	The effects on the mammary gland are considered to be harmful because they have an impact on growth and development and can alter lactation and/or cause carcinogenic effects.	Definitions of the harmful character of a very different effect; depending on the context and the scientific discipline.

Uncertainties are raised as to the relevance of using the results of the studies on rodents (see Thayer, 2007).

Fenton *et al.*, (Fenton, 2006) recalls the basis of the analysis of the mammary gland and the interest of using the rodent model for the assessment of the effects on the mammary gland in humans. This author points out that induced mammary tissue tumours in rats are tumours that are similar to those observed in humans and that the sequence of development of the mammary gland in rodents and in humans are similar (see the table below) with a few exceptions. Although the incidence of mammary tumours in female F344/N rats, specifically fibroadenomas, is higher compared to the mouse, Thayer and Foster (Thayer, 2007) considers the rat model more suited to detecting the carcinogenic potential on the mammary gland. In addition, according to Rudel (Rudel, 2011), the rat is a better model than the mouse for the mammary gland.

Table 24. Summary of the stages of development of the mammary gland tissue in humans and rodents

Development stages	**Female**	**Rodent**
Mammary ridge	EW 4-6	GD10-11 (mice)
Formation of mammary epithelial bud	EW 10-23	GD 12-14 (mice) - GD 14-16 (rat)
Formation of the areola and the mammary papilla	EW 12-16	GD 18 (mice) - GD 20 (rat)
Ramification and formation of milk ducts	EW 20-32	GD16 – birth (mice) – GD 18 at birth (rat)
Possible secretion	EW 32-40	Birth (hormonal stimulation)
Isometric duct development	From birth until puberty	From birth until puberty
Presence of TEBs (peripuberty)	8 to 13 years of age in girls	PND 23-60 (rat)
Formation of lobular units	EW 32-40 or during the 1st or 2nd year of the 1st menstrual cycles.	Puberty and adulthood

EW: Embryonic Week

GD: Gestation Day

PND: Post Natal Day

Critical analysis of Delclos et al., 2014 **regarding the effect of BPA on the mammary gland[11]**

In this study of U.S. FDA/NCTR (2013), Sprague-Dawley rats were treated with BPA administered by oral gavage from gestation day 6 through the start of labor. BPA was then given directly to pups from PND 1 until termination at PND 90 ± 5 at doses of 2.5, 8, 25, 80, 260, 840, 2 700, 100 000, and 300 000 µg/kg bw per day. The number of litters per dose group was 18-23. The effects of BPA on a very wide range of pathological, physiological, endocrine, reproductive and developmental endpoints were investigated. Mammary gland samples were excised at PND21 and PND90. 1) At PND21: fifth mammary glands (inguinal glands; left gland for histopathology and right gland for whole mount). 2) At PND90: sacrifice in 'estrus', microscopically analysis of the fifth left mammary gland. At PND21, the authors report in the text statistically significant ductal hyperplasia as follows; 'ductal hyperplasia indicative a relative increase in the number (density) of branching ducts and alveolar buds per unit area'. These changes are claimed to be only statistically observed at high doses (2.7 and 100 mg BPA/kg/d) (Table 7). The absence of intraductal hyperplasia at PND21 agree with previous papers; intraductal hyperplasia only detected in cycling animals (PND50), a situation which was not reported in this paper. At PND90, intraductal hyperplasia was not mentioned, and one adenocarcinoma was reported but not discussed.This study provided some evidence for a BPA-related effect in the mammary gland of female rats at 100 000 and 300 000 µg/kg bw per day, and possibly also at the 2 700 µg/kg bw per day dose level (at PND 21).

General Comment.

Several weaknesses are present in the Materials and Methods sections and in the section that weakens the conclusions relative to the mammary gland.

Methods.

Several aspects relative to the Materials and Methods sections are difficult to assess.

-The number of female animals/group used in the study was not clearly indicated.

Only 400 female weaning (PND21) were mentioned to be F0 feeding (*Source and specification of animals* section), and 1 animal per sex litter evaluated at necropsy (*Animal breeding and maintenance* section). However, in the Result section, at PND21 (ductal hyperplasia, see Table 7), a rapid estimation indicates about 260 female rats (13 groups, 20 animals each). For estrous cycle evaluation (PND69- 90, see Table 4), a rapid estimation indicates about 1000 animals (13 groups of about 20 animals and 4 different stages estrus cycle. For female histopathology (at PND90, see Table 8), this number appears to be 260 female rats. These rats were supposed to be in estrus (see *In life data collection* section).

-Analysis of estrus cyclicity and estrus stage are performed using daily vaginal smears. No reference was given for this analysis which could explain the different stages (extended

[11] This study was not investigated during the elaboration of the proposal since it was published subsequently. Quoted during the public consultation and considered by RAC as a key study, it is now analysed and incorporated in the BD.

estrus, extended combined estrus, and extended E/D, which are only observed with EE2 and BPA) as compared to a normal rat cycle; as indicative, the estrous cycle is considered as a 4-5 days cycle.

-The analysis of hormones levels was mentioned to be performed 'on days when females were predicted to be in estrus based on the vaginal smear of the previous day of sacrifice'. Does it mean that the analysis of hormone levels (estradiol and progesterone) was performed on the day of sacrifice? Vaginal smears done the day before sacrifice is only indicative, but is not sufficient to conclude about the estrus stage at sacrifice; this should be confirmed by analysis of hormone levels (E2 and progesterone) on the day of sacrifice. *See also below (in the Result section) for the comment in the article from Churchwell.*

-Mammary gland samples were excised at PND21 and PND90. It can be acknowledged that:

1) samples taken at PND21 are correctly excised: fifth mammary glands (inguinal glands; left gland for histopathology and right gland for whole mount).

2) samples taken at PND90 also concern the fifth left mammary gland of rats in 'estrus' (see comment for estrus). The authors indicated that 'a flattened dorsoventral orientation provided a histological section with a similar profile to that of a routine mammary whole Mount'. However, it is very hard to clearly quantify the different structures in one paraffin section, as using the Whole Mount. **Details of the histopathology are lacking.** It is not indicated whether the analysis was performed by a certified pathologist? Was the analysis performed blindly? This point is very important. How many sections were analyzed for each paraffin block? How was detected and quantified mammary ductal hyperplasia)?. ***Same comment in the NTP report (see comment below in the Result section).***

3) However we can question the fact that samples at PND50 were not conducted although he peripubertal period is usually analysis because it is known as a critical period for mammary gland development (see references as Russo and Russo Russo IH, and Russo J. 1996. Mammary gland neoplasia in long-term rodent studies. Environmental Health Perspectives 104 (9): 938-967). Indeed, the response to exposures to carcinogens is greater when exposure occurs during puberty or adolescence, the period when the TEBs are still numerous. Knowing that terminal buds (TEB) and the terminal ducts (TD) are considered the structures most sensitive to mammary carcinogens (Medina D. 2007. Chemical carcinogenesis of rat and mouse mammary glands. Breast. Dis. 28: 63-68.; Russo, and Russo; 1996). The increase in the number or persistence of the TEBs proliferating is considered of particular concern (see Birnbaum LS, and Fenton SE. 2003. Cancer and developmental exposure to endocrine disruptors. Environmental Health Perspectives 111 (4): 389-394.; Rudel RA, Fenton SE, Ackerman JM, Euling SY, and Makris SL. 2011. Environmental exposures and mammary gland development: State of the science, public health implications, and research recommendations. Environmental Health Perspectives 119 (8): 1053-1061.; Fenton SE. 2006. Endocrine-disrupting compounds and mammary gland development: Early exposure and later life consequences. Endocrinology 147 (6): S18-S24.).

Results.

Table 4: estrus cycle evaluation. The terminology: extended estrus, extended combined

estrus, extended diestrus and extended E/D was very confusing; no data was given for estrus, combined estrus, diestrus and diestrus/estrus in control rats in a rat standard cycle (control animals).

Data on hormone levels should complete the Result section, especially data on ductal hyperplasia shown at PND90 (Table 8), since the animal experiment was claimed to concern rats in estrus.

Comment from the article (Delclos, Churchwell) and the NTP report: different methods were used for quantify the estradiol level, i.e. ELISA and LC/MS in the articles from Delclos and Churchwell, respectively. These methods do not have the same sensitivity, and Elisa may surestimate the E2 levels especially in low doses. Also, we don't know if the same animals were analyzed in the two papers. Data on hormone levels are given in the NTP report (NB. Je n'ai pas eu le temps de regarder la methode de dosage). More importantly, hormone levels were measured in blood samples from PND80, a period which does not correspond to the time of sacrifice (PND90) and cannot be used to confirm the stage of estrus cycle at sacrifice.

Results for mammary gland in Tables 7-8 only concern PND21 and PND90. No micrographs of the mammary lesions were produced.

- At PND21, the authors report in the text statistically significant ductal hyperplasia as follows; 'ductal hyperplasia indicative a relative increase in the number (density) of branching ducts and alveolar buds per unit area'; these endpoints do not correspond to lesions but are usually considered as morphological changes. In the article from Delclos, the 'severity profile' and the significance of the lesions, as seen in Table 7, are not clearly defined. Thus, statistical analysis are difficult to understand, but these changes were claimed to be only statistically observed at high doses (2.7 and 100 mg BPA/kg/d) (Table 7). Intraductal hyperplasia was not observed as a treatment-related lesion at this stage (at PND21), except in males with EE2 treatment? The absence of intraductal hyperplasia at PND21 with BPA treatment agrees with previous papers; in some previous papers on BPA, intraductal hyperplasia was detected in cycling animals (PND50), a situation which was not reported and/or examined in this study.
- At PND90, the same term 'ductal hyperplasia' (non neoplastic lesions) was used (Table 8) as in Table 7 (PND21). Again, how was defined the severity of these non-neoplastic lesions? Intraductal hyperplasia was not mentioned, and one adenocarcinoma was reported but not discussed.

Comment from the NTP report: quantitative data of glandular structures at PND90 are more 'semi-quantitative' if there is only one section/block and if the estimation on one paraffin section is made using 1-4 grades.

Discussion.

In the article from Delclos, there is no discussion focused on mammary gland result interpretation. In the NTP report, there is a short discussion concerning low doses of BPA, especially indicating the references from Maffini and Murray.

In conclusion, due to several weaknesses in the Material and Methods and Results analysis, it is thus difficult to accept the conclusions relative to mammary gland.

In summary, the strengths and weaknesses of the study can be summarized as follows:

- **Strengths**

***** GLP study
***** OECD guideline
***** oral exposure (gavage)
* high number of doses (7)
* high number of animals per dose
* Use of polysulfone cages for F1 generation
* Use of glass water bottles for F1 generation
* Fetuses exposed during gestation and after birth
* Litter was the unit for the statistical analysis
* both naïve and vehicle controls available

- **Weaknesses:**

* Use of polycarbonates cages for F1 generation
* Use of polycarbonate water bottles for F1 generation
* dose range-finding study, still not completed
* dose range study not designed to calculate or assess a NOAEL or a BMDL
* wide spacing between low and high dose levels
* results of morphometric analysis with whole mount examination were not reported
* no histopathological analysis were not performed during the window of sensitivity of the mammary gland to carcinogenic compounds namely around PND50
* animal diet with phytoestrogen content
* control animals also exposed to BPA at levels similar to the lowest exposed group

B 5.10 Other effects

B.5.10.1. Effects on the brain and behaviour in unborn child

B.5.10.1.1 Previous assessments of the effect of BPA on brain and behaviour

According to the FAO/WHO, a prospective cohort study in humans (Braun, 2009) showed changes in behaviour (aggressiveness, hyperactivity) in girls; this association was stronger when urinary concentrations of BPA at the start of pregnancy were higher in the mothers. This expert panel considers it a priority to undertake a prospective study in a large cohort using several urinary measurements, particularly at the start of pregnancy (FAO/WHO, 2010). A new study by Braun *et al.* confirming these results is under publication, according to this panel's conclusions. This study may also show a positive relationship between urinary concentrations of BPA measured in mothers during pregnancy and anxiety observed in children, which has also been reported in animals (Braun, 2011).

In animals, according to the expert panel that met in Chapel Hill in 2007 (Richter, 2007), exposure to low doses of BPA in the critical period of development can have persistent effects on cerebral structure and function, and on behaviour in rats and mice, including:

- Increased ERα and β receptors in various brain structures in response to development exposure (Ramos *et al.*, 2003; Khurana *et al.*, 2000; Ceccarelli *et al.*, 2007; Kawai *et al.*, 2007),
- Alteration of the hypothalamic-pituitary-thyroid axis (Zoeller RT, 2014),
- Effects on the cell signalling pathways,
- Effects on cerebral structure.

In adults, the onset of such effects appears to require exposure to higher doses of BPA and during a longer period.

According to the ECB (EC, 2010), more than 30 studies, including three on subcutaneous exposure, have assessed neurotoxicity in animals (locomotive and exploratory activity, sexual, cognitive, emotional, social, maternal behaviour, expression of genes and receptors and immunotoxicity, etc.) but their protocols had limitations (small number of animals, inappropriate statistical analysis, results and methods reported in insufficient detail, one single dose, etc.). Therefore, confidence in the reliability of the results is limited and the observed effects lack coherence.

The NTP-CERHR (NTP, 2008) has also expressed concern for humans ("some concern for adverse effects") as to the effects on cerebral development and behaviour related to BPA. According to the FDA (FDA, 2008), some studies suggest that exposure to BPA during development may, in rodents, alter brain development and have the following effects:

- possible effects on brain development and sexual differentiation,
- alteration of endocrine function in offspring: reduced testosterone in males, altered levels of thyroxine and genes responding to thyroxine, altered levels of retinoid receptors and thyroid hormone receptor coactivators,
- modulation of monoaminergic neural pathway development after exposure during development, suggested by significant changes in the behaviour of adult offspring.

According to the OEHHA, these effects, and particularly those involving changes in maternal behaviour, are consistent with BPA's oestrogenic potential; the statistical data analysis is appropriate and the doses seem to be consistent with those encountered in human exposure (around one µg/kg). The potential mechanisms of action behind developmental toxicity include regulation of gene expression in the embryo, action at membrane oestrogen receptor sites, and modulation of second messenger systems (OEHHA, 2009).

However, these effects at low doses remain controversial (NTP, 2008) (OEHHA, 2009) due to:

- the lack of study repeatability,
- doubts as to the relevance of the protocols used: a complete developmental neurotoxicity study of BPA has not been not undertaken despite routine protocols being available,
- the relevance of the animal model and its extrapolation to humans: the relationships between BPA exposure and neurological or neurodegenerative syndromes and behaviour in children have not been explored,
- a lack of consensus as to the harmful nature of the reported effects: for example, an observed effect in foetuses, newborns or prepubertal animals has generally not been

investigated in adult animals to determine whether or not it is reversible and establish its level of severity.

AFSSA, in its Opinion of 29 January 2010 and the corresponding Annex (Afssa, 2010), analysed several studies on the neurotoxic effects of BPA (Palanza, 2008; Nakagami, 2009; Stump, 2010; Braun, 2009; Monje, 2009; Ryan, 2010) and considered that some of these publications, and particularly those by (Nakagami, 2009) and Palanza *et al.* (Palanza, 2008), indicate alert signals after *in utero* and postnatal exposure to doses lower than the one used to establish the TDI (Afssa, 2010; Afssa, 2010). The reported effects included, firstly, feminisation in the behaviour of male offspring, and secondly, changes in exploratory behaviour and anxiety. However, other studies were not considered to be alarming (Braun, 2009, Monje, 2009). The studies by Stump and Ryan did not show effects at doses lower than 5 mg/kg bw/day.

EFSA considers that the data that are currently available do not provide sufficient proof that BPA affects behaviour at doses lower than 5 mg/kg bw/day EFSA, 2010.

Lastly, the expert panel that met in 2010 under the leadership of the FAO/WHO considered that exposure to BPA during development does not appear to affect the sensory organs, spontaneous behaviour or female sexual behaviour in laboratory animals (FAO/WHO, 2010). The available experimental data are not in favour of cerebral neuropathological effects after oral exposure during gestation, at doses lower than 164 mg/kg bw/day (Stump, 2010). Biochemical (monoaminergic, cholinergic, glutamatergic systems, etc.), morphometric and cellular changes, in the anatomical regions involved in sexual dimorphism and in certain neuroendocrine targets, have been reported after oral exposure during gestation to doses lower than 5 mg/kg bw/day. However, these studies had methodological limitations, and the observed effects had no functional equivalence, which means it is difficult to interpret them. On the basis of the available data, this panel considers that effects related to anxiety and sex differences in the brain, in both males and females, are potentially relevant critical effects in humans, but supplementary studies are required to reduce these uncertainties.

Data in rodents and sheep suggest effects on the organisation of the hypothalamic-pituitary-gonadal axis in females (>50 µg/kg bw/day for non-oral exposure) and its activity (>5 mg/kg bw/day for non-oral exposure).

This panel's experts recommend undertaking studies to examine specific effects related to stressful behaviour after exposure during pregnancy:

- by implementing various study protocols with several doses and in both sexes,
- by testing several ages,
- by examining the functional impact of changes in cerebral sexual differentiation,
- by undertaking dose-response analyses of anatomical changes linked to cerebral sexual differentiation.

B.5.10.1.2 Human data considered for the effect on brain and behaviour in unborn child in the ANSES assessment

All the studies in humans available until 2011 on effects of BPA on the brain and behaviour are presented below. More recent studies from 2011-2012 were also analysed and the result of this analyse is presented in the conclusion but the studies are not detailed because they do not contradict the obvious analyse of hazard assessment of BPA on the brain and behaviour.

Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Braun *et al.*, 2009) Prenatal Bisphenol A Exposure and Early Childhood Behavior	Prospective cohort study	Study population: Mothers and their 2-year-old children (included in the Health Outcomes and Measures of the Environment Study programme; use of an existing biobank, recruitment in 2003) N=249 mothers and their 2-year-old	Urinary (in mothers at 16 and 26 weeks of gestation and at birth), free and conjugated BPA	HPLC/MS / MS	Age: yes (age of the mother) Sex: NA Medication: NA Tobacco: yes BMI: NA Other contaminants: yes	Results: Positive association with externalising behaviour Comments: - no biological reliability - use of an existing biobank (recruitment in 2003) – the samples were stored for 4-5 years, *questionnaire - no direct urinary BPA measurements in children, The study was the subject of a highly critical analysis (Human Data on Bisphenol A and Neurodevelopment	Study of high quality or having no major methodological limitations	Information from epidemiological studies Effects on the brain and behaviour

		children -> Sufficient population size				doi:10.1289/ehp.0901610) whose comments are clearly justified.		
(Miodovnik *et al.*, 2011) Endocrine disruptors and childhood social impairment	Prospective cohort study	Study population: children between the ages of 7 and 9 years N=137 children	Urinary (in 404 mothers between the 25th and 40th weeks of pregnancy)	Not specified	Age: yes (maternal age and exact age of the child during the examination) Sex: yes (sex of children) Medication: no Tobacco: no BMI: no Other contaminants: no Other: urinary creatinine of children, marital status on the follow-	Results: No significant association was found between urinary levels of BPA and social impairment. BPA was positively correlated with the severity of social impairment (Social Responsiveness Scale), but this relationship was not statistically significant.	Study of high quality or having no major methodological limitations	Information from epidemiological studies Effects on the brain and behaviour

					up date, education of mothers, race, IQ of mothers and children			

The study by Braun *et al.* describes epidemiological monitoring of mothers exposed to BPA and their children at the age of 2 years (Braun, 2009). Exposure to BPA was determined by analysing residues in the urine of mothers at around 16 and 26 weeks of pregnancy and at their children's birth. Prenatal exposure to BPA was linked to externalised behaviours, especially in girls (hyperactivity, multiple aggressions). These behaviours are usually dominant in boys and may also be interpreted as increased anxiety in girls, and perhaps also in boys, but in the latter case they could be confused with behaviours in boys linked to behavioural sexual dimorphism. Regarding this study, Longnecker expresses reservations about absolute differences in the scores observed for externalised behaviours associated with BPA, which cannot be determined using the sex-standardised data presented in the study by Braun *et al.(*Braun, 2009); (Longnecker MP, 2009). Thus, the size of the association with BPA in girls cannot be compared with the size of the male-female difference. As such, it is impossible to know whether the girls developed masculine behaviour or whether they still behaved like girls. It should also be mentioned that due to the methodological limitations noted by AFSSA in its 2010 expert appraisal, the conclusions of the study by Braun *et al.* were not taken into consideration (Afssa, 2010). Furthermore, AFSSA pointed out that the authors concluded that BPA impacts behaviour on the basis of scores that fell within the normal range of individual variation. For example, the highest mean score was 53.9 (standard deviation of 1.3), whereas the score was normalised for the American population to a value of 50 with a standard deviation of 10. However, it should be noted that the FAO/WHO experts consider that replicating this study using a large cohort with several urinary measurements, particularly at the start of pregnancy, is a high-priority research need FAO/WHO, 2010.

Miodovnik *et al.* studied the correlation between urinary levels of BPA and phthalates analysed during pregnancy and the sociability of multiethnic urban children aged 7 to 9 years, in 137 children (Miodovnik A, 2011). Sociability was assessed using a Social Responsiveness Scale (SRS) that contained 65 items. Urinary concentrations of low molecular weight phthalate metabolites were associated with greater social deficits, with poorer social cognition, communication and awareness. However, no significant association was found between urinary levels of BPA and social impairment. BPA was positively correlated with the severity of social impairment (Social Responsiveness Scale), but this relationship was not statistically significant.

Thus, it was considered that the human data available to date are insufficient to reach a conclusion on the effects of BPA on behaviour.

B.5.10.1.3 Data considered by ANSES in animals for brain and behaviour effect:

The available studies until 2011 are presented below: firstly on the effects of BPA on behaviour, secondly on the effects on the cerebral development and then on the post natal exposure in animals. Then, the most critical effects, the key studies and the selected dose are identified and presented.

Effects on behaviour

Effects on exploratory behaviour

Changes in maternal, exploratory and emotional behaviour have been reported after *in utero* exposure. The results obtained by Poimenova *et al.* show that BPA modifies the behaviour of F1 females born to mothers who were orally exposed to BPA at 40 µg/kg bw/day in their diet during gestation and lactation (Poimenova, 2010). The F1 females had a sharp decrease in exploratory behaviour and a deterioration of spatial memory, but this study had methodological limitations (very small number of animals, one single dose, and number of animals not always specified in each trial, etc.) (Table 25). The developmental neurotoxicity study by Stump *et al.,* undertaken in accordance with OECD guideline 426 (tests, histopathologic evaluations, etc.) and with GLP (Good Laboratory Practice), established an NOAEL for developmental neurotoxicity effects at the highest tested dose of 2250 ppm (164 mg/kg bw/day for gestation and 410 mg/kg bw/day for lactation) (Stump, 2010). No effects on the exploratory behaviour of offspring were highlighted.

Effects on anxiety

Behavioural effects in mice were observed by Cox *et al.*, in the F1 offspring of mothers exposed during gestation (from E9 to the end of gestation) to doses of 50 mg/kg BPA administered in feed corresponding to 8 mg/kg bw/day (Cox, 2010). In this study, the offspring were weaned, either with their biological mother, or with a foster dam. The results show a clear increase in anxiety in the offspring of mothers exposed to BPA. In general, the type of mother weaning the offspring (biological mother versus foster dam) modified the effects of BPA. However, in order to be able to properly interpret the studies by Cox *et al.*, two additional procedures would have needed to be undertaken: (i) newborns born to control mothers and fed by foster dams exposed to BPA and (ii) newborns born to mothers exposed to BPA and fed by foster dams exposed to BPA. That said, these results can be compared with those of Poimenova *et al.* which also show that BPA alters behavioural coping to stress in a sex-dependent manner in F1 rats born to mothers which were exposed to 40 µg/kg bw BPA daily during gestation and lactation (Poimenova, 2010). For example, compared to males, F1 females exposed to BPA had increased anxiety and far lower exploratory behaviour.

In the study by Tian *et al.* using 100 and 500 µg/kg bw BPA daily in mice, prenatal and postnatal exposure (from GD7 to PND36) to BPA induces anxiolytic behaviour (at 100 µg/kg bw/day), unlike the anxiogenic effect reported by Cox *et al.* at the dose of 8 mg/kg bw/day (Cox, 2010) (Tian, 2010). It remains to be known whether an 80-factor dose difference can explain the differential anxiogenic/anxiolytic effects of BPA. Moreover, the studies by Tian *et al.* should be considered with caution since the experimental groups of individuals contained only two mothers (Tian, 2010).

Effects on behavioural sexual dimorphism

Exposure to BPA may result in a decrease or even loss of this dimorphism:

- in the locus ceruleus (Funabashi, 2004; Kubo K, 2001; Kubo, 2003);
- in the anteroventral periventricular nucleus, but with inconstant findings (Patisaul, 2006; Patisaul, 2007; Rubin, 2006).

The lowest *in utero* or perinatal exposure doses that have shown such effects are 0.03 mg/kg bw/day after oral exposure (Kubo, 2003), 0.000025 or 0.000250 mg/kg bw/day after subcutaneous perfusion (Rubin, 2006) and 100 mg/kg bw/day after subcutaneous injection (Patisaul, 2006).

In 2009, Nakagami *et al.* undertook a study examining the effects of prenatal exposure to BPA in monkeys by analysing infant-mother behaviour in F1 cynomolgus monkeys (*Macaca fascicularis*) (Nakagami, 2009). The behaviour of male and female offspring was studied during the early lactation period. The behavioural analysis in the offspring examined clinging to the mother, environmental exploration, outward looking, proximity and social exploration. In general, for the behaviours under study, the male F1 individuals behaved like females. After subcutaneous administration of BPA (using an osmotic pump) at doses of 10 µg/kg bw/day to gestational day GD20, the authors examined five types of behaviour: clinging behaviour, environmental exploration, outward interest, proximity and social exploration in the offspring, and approach, locomotion, orientation, outward interest and social exploration in the mothers. Each behaviour type was studied in detail in the male and female offspring and the mothers. The scores obtained for each behaviour type were summarised by a score encompassing the 5 discriminant behaviours. In general, BPA decreased maternal behaviour in a way that was distinguishable between the male and female offspring, and feminised behaviours in the male offspring exposed to BPA, often with the same behaviours as the female offspring. The following methodological limitations were reported:

- regarding exposure to BPA: plasma levels of BPA measured in mothers only on the 50th day of gestation, and lower than the Limit of Detection (12.5 ng/mL); no measurements were available for the offspring. Differences in metabolism between routes of administration were not taken into account. No dose/effect relationship could be established (only 1 dose).
- in terms of the interpretation of results: only 1 to 3 variables out of 14 were modified in the short-term (10-minute) recordings of the monkeys' behaviour. Their significance remains to be determined especially since, as affirmed by the authors, the results cannot be explained in psychological terms.
- it is difficult to interpret this study given the route of administration (non-oral), the lack of data on the offspring's actual exposure, phyto-oestrogen levels in food and BPA levels in water, the fact that only one dose was tested and doubts regarding the significance of the observed effects.

In conclusion, this study's results were considered to be an alert signal Afssa, 2010

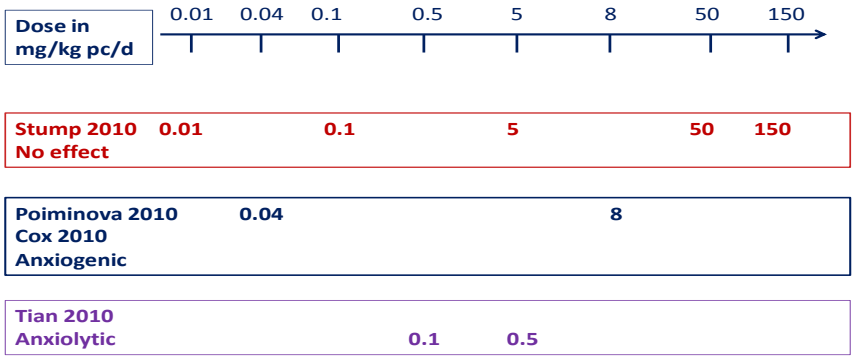
No studies have reported changes in the nucleus of the preoptic area, also a sexually dimorphic area in humans, up to doses of 320 mg/kg bw/day in rats. It therefore remains difficult to interpret these effects in rodents and their consequences.

In the recent study by Ryan *et al.* that was mentioned above, no effects on behavioural sexual dimorphism were observed with BPA at doses of 2, 20 and 200 µg/kg bw/day whereas, for comparable doses, ethinyloestradiol EE2 had the following notable effects: reduced lordosis behaviour, increased anogenital distance, reduced pup weight at PND2, early vaginal opening, reduced F1 fertility and reduced litter sizes (Ryan, 2010 117). This work does not necessarily indicate that BPA has no effects but rather that it may exert oestrogenic action at different exposure levels from those at which EE2 has effects.

Behavioural effects were highlighted in mice by Cox *et al.* when observing the F1 offspring of mothers exposed during gestation (from GD9 to the end of gestation) to doses of 50 mg/kg feed corresponding to 8 mg/kg bw/day (Cox, 2010). The study by Cox *et al.* showed a loss of behavioural sexual dimorphism in the offspring of mothers exposed to BPA during gestation.

Adewale *et al.* examined the effects of neonatal subcutaneous exposure to BPA in rats. Four injections of BPA were administered to female newborns at PND0, PND1, PND2 and PND3 at doses of 50 µg/kg bw/day and 50 mg/kg bw/day (Adewale, 2009). Two positive controls were used, one by injection of PPT (ERα agonist, 1 mg/kg bw) and the other by injection of oestradiol benzoate (EB 25 µg; the publication does not specify whether it was µg/per rat or per kg). BPA did not modify sexual behaviour at any dose, but increased body weight was observed at the age of 99 days, only at the dose of 50 mg/kg bw/day of BPA and also with EB. It should be noted that controls can be considered as positive only in relation to an expected effect, which here is oestrogenic action. In the absence of an expected action, the positive character of a control has no toxicological significance.

Figure 12. Effects of BPA on anxiety and exploratory behaviour according to exposure



Effects on cerebral development

Effects on neural development

The review by Hajszan and Leranth is particularly focused on how BPA affects synaptic remodelling (Hajszan T, 2010). It underlines that, in rats and non-human primates, BPA negates the 70-100% increase in the number of hippocampal and prefrontal spine synapses induced by both oestrogens and androgens.

Kim *et al.* undertook a prenatal exposure study in ICR mice and *in vitro*. In prenatal exposure, the mothers were exposed between embryonic stages GD 14.5 and GD 18.5 by subcutaneous administration of 0, 5, 10 and 20 mg/kg bw/day (Kim K, 2009). Studies of hippocampal neurogenesis were undertaken by exposing offspring for 3 days, from postnatal week PNW8, at a rate of two daily injections of one 20 mg dose of BPA/kg in the presence of BrDU to examine neurogenesis. The *in vivo* studies showed that at PNW3, an increase could be observed in body weight at the dose of 5 mg/kg and a decrease at the dose of 20 mg/kg. These changes were not observed at PNW8, which led the authors to suggest effects mediated by the mother. Formation of the dentate gyrus was accelerated at PND1 at the dose of 20 mg/kg. The authors suggest that BPA blocks the proliferation of neural stem cells and promotes cellular differentiation in a relatively early stage. However, at PNW3, BPA did not have any observed effects on the cortical structure of the hippocampal neuronal cells or cell density. In adult mice, BPA had no observed effects on hippocampal neurogenesis. In the *in vitro* studies, mouse neural progenitor cells were exposed to BPA at concentrations of 1 nM to 500 µM. BPA reduced the proliferation of neural progenitor cells, in a concentration-dependent manner starting at 200 µM, and induced cytotoxicity at the highest concentration (500 µM). At low concentrations, BPA stimulated the differentiation of neural progenitors into neuronal phenotypes.

Effects on aminergic systems[12]

Tian *et al.* reported that perinatal oral exposure (GD7 to PND36) in mice to BPA at doses of 100 and 500 µg/kg bw/day induced an increase in dopamine D2 receptors and a decrease in dopamine transporters (DAT) in the putamen (Tian, 2010).

BPA induces changes in cerebral development. Perinatal exposure in mice (embryonic day GD0-PND21) by subcutaneous injection at a dose of 20 µg/kg bw/day increases dopamine and its metabolites in the putamen and the dorsal raphe nucleus and increases serotonin and its metabolites in the putamen, dorsal raphe nucleus, thalamus and substantia nigra (Nakamura, 2010). No differences in the synaptogenic effects of BPA have been observed between oral and subcutaneous exposure (Hajszan T, 2010).

In rats injected intracranially with BPA at PND2 with doses of 0 – 0.1 and 1 µg/kg, significant changes in certain monoamines could be observed 7 days and 28 days after the injection (PND9 and PND30) (Matsuda S, 2010). Significant increases in 5-HT (serotonin) in the hippocampus, 5-HIAA (5-hydroxyindoleacetic acid) and 5-HT in the brain stem, and DA (dopamine) and DOPAC (3,4-Dihydroxyphenylacetic acid) in the striatum were observed 28 days after the injection. Seven days after the injection, increases in 5-HT and norepinephrine (NE) and decreases in DOPAC and 5-HIAA were observed in the hippocampus. In this study, the authors analysed the degradation speed of BPA in the brain. BPA disappeared from brain tissues within 5 hours of the injection, even at the highest dose of 1000 µg/kg. The authors concluded that BPA can have effects on cerebral monoamines over 28 days after its disappearance. The authors do not describe the analytical method used to assay BPA or the Limits of Detection and Quantification. Thus, residual levels of BPA, lower than the Limit of Detection, could induce effects within the 28-day period after exposure. However, this does not change interpretation of the results. This study should be considered with caution since the doses in relation to the individuals' body weights were administered in the brain, and therefore the size of exposure cannot be assessed. Furthermore, the *in situ* injection of BPA significantly modifies the toxicokinetics and consequently the potential effects of BPA.

In the study by Adewale *et al.* that was mentioned above, the effects of neonatal subcutaneous exposure to BPA in rats were studied (Adewale, 2009). BPA did not change serotonin fibre density in the ventrolateral subdivision of the ventromedial nucleus at any dose, whereas an increase was observed with EB and PPT which were used as positive controls.

Effects on the glutamatergic system

In the study by Tian *et al.,* which used 100 and 500 µg BPA/kg bw/day in mice, in perinatal exposure (GD7 to PND36), decreased NMDA receptors were observed in the frontal cortex, dentate gyrus (DG) and cornu ammonis 1 and 3 regions (CA1 and CA3) of the hippocampus (Tian, 2010). Xu *et al.* studied the effects of perinatal oral (intra-gastric) exposure to BPA (GD7-PND21) at doses ranging from 0 – 0.05 – 0.5 – 5 and 50 mg/kg bw/day in mice (Xu, 2010) and from 0 – 0.05 – 0.5 – 5 – 50 and 200 mg/kg bw/day in rats (Xu, 2010-see detailed description below). They showed that BPA negatively affects the expression of hippocampal

[12] The **dopaminergic system** plays a role in cognitive function as lesions of dopaminergic neurons reduce performance associated with various learning and cognitive tasks.

NMDA receptors in male rats and mice. BPA at doses of 0.05 to 50 mg/kg bw/day reduced the expression of hippocampal NMDA receptors (subunits NR1, NR2A and NR2B) in F1 males. However, in rats, compared to the lower doses, the effects of BPA on the NMDA receptor subunits NR2A and NR2 at the highest dose of 200 mg/kg bw/day were less marked, which suggests that BPA has differential action at low and high doses. These changes in NMDA receptor expression were associated with reduced learning capacities.

These results were supported by studies of hippocampal neurons cultured *in vitro* exposed to BPA at concentrations from 10 to 1000 nM (Xu, 2010). Changes in the dendritic morphology of the hippocampal neurons (enhanced filopodial motility and density) and enhanced NMDA receptor phosphorylation (subunit NR2B) via action exerted by BPA on the oestrogen receptors (effect suppressed by the oestrogen receptor agonist ICI 182780) were observed.

Developmental effects on NMDA receptors should be considered carefully knowing that these receptors are involved in memory and learning processes. They are also supported by the role of BPA in neural systems expressing nitric oxide synthase (NO synthase) with sex- and region-dependent effects in the hypothalamus and limbic system (Martini, 2010).

Effects on systems involving sex hormones

Adewale *et al.* showed that, in female newborn rats subject to postnatal subcutaneous exposure with 4 injections at PND0, 1, 2 and 3, BPA increased the number of oxytocin neurons in the paraventricular nucleus, a sexually dimorphic hypothalamic region responsive to oestradiol, at BPA doses of 50 µg/kg bw/day and 50 mg/kg bw/day (Adewale, 2009). This postnatal exposure did not affect sexual behaviour but was linked to increased body weight at the age of 99 days, only at 50 mg BPA/kg bw/day, which was also observed with oestradiol benzoate. No changes in ERα receptor density were observed in the ventrolateral subdivision of the ventromedial nucleus (VMNvl), the medial preoptic area (MPOA) or the arcuate nucleus (ARC).

In sheep, prenatal exposure to BPA (GD30-GD90) at 5 mg/kg bw/day has differential effects on the expression of hypothalamic oestrogen receptors ESR1 (ERα) and ESR2 (ERß), with increased expression for ESR1 and decreased expression for ESR2 (Mahoney, 2010). These changes were associated with increased gonadotropin-releasing hormone (GnRH) expression. In rats (Xu, 2010) and in mice (Xu, 2010), perinatal exposure to BPA (GD7-PND21) at doses of 0.05 to 50 mg/kg bw/day decreases oestrogen receptor ER□ expression and increases aromatase in the hippocampus. These studies confirm the work of Salian *et al.* which showed increased oestrogen receptor ERα expression and decreased ERβ receptors in the testes of rats whose mothers had been exposed during a period ranging from gestation (from GD12) to weaning (PND21) (Salian S, 2009). These results were observed in the F1 offspring of exposed mothers as well as in the untreated F2 and F3 generations.

A study undertaken in SD rats, in a protocol of perinatal exposure to a low dose (sc injection of 2 µg/kg bw/day) from GD10 to PND7, clearly indicates that this exposure could modify sexual differentiation of the GnRH system in male offspring, particularly through increased kisspeptin expression in the anteroventral periventricular nucleus (AVPV) of the hypothalamus (Bai, 2011). BPA increased the number of AVPV kisspeptin neurons at PND30, PND50 and PND90. BPA decreased the number of GnRH neurons by 40% at PND30, this was followed by a constant increase at PND50 and PND90. As a result, castrated adult males developed the ability to generate a pre-ovulatory surge-like LH release in response to a 'pre-ovulatory' dose

of oestradiol. In rodents, this ability was considered to be a characteristic sign of feminisation in the nervous component of the gonadotropic axis. This ability was fully expressed only in males after the age of 90 days. Furthermore, in non-castrated animals, exposure to BPA increased LH concentrations, decreased testosterone concentrations in adult offspring (PND30 and 50) and increased oestradiol concentrations at PND50 and 90. These endocrine effects are interpreted by the authors as indicative signs of long-term peripheral aromatase activity stimulation in animals exposed to BPA.

Postnatal exposure

Changes in maternal behaviour have been reported after oral exposure to 10 µg/kg bw/day of BPA from birth to adulthood (Palanza, 2008): F1 generation mice exposed in the postnatal period showed a decrease in nursing time and an increase in time spent away from the litter. However, no effects on body weight were highlighted in the offspring (which would suggest an adequate level of care). As the significance of the effects observed in mice (nursing and nesting time) for human health has been demonstrated by only one team, they can be considered as suspected.

Table 25. Studies examining the effects of bisphenol A on the brain and behaviour: summary table

Reference	**Species/ strain**	**Route**	**Dose** **Exposure period**	**Effects** **NOAEL/LOAEL**
Poimenova, 2010	Wistar rats	Oral	40 µg/kg bw/day GD1 - weaning (42 days)	↗ levels of corticosterone and ↘ levels of GR in males in basal state and in the 2 sexes after stress No effects on the MR receptor in normal conditions, but ↘ MR level in females in the 2 groups of females ↘ spatial memory in the 2 sexes ↘ exploratory behaviour in females and appearance of anxious behaviour
Stump, 2010	CD-SD rats	Oral	0.15 – 1.5 - 75 - 750 and 2250 ppm feed Gestation: 0.01 – 0.12 – 5.85 – 56.4 - 164 mg/kg bw/day	No effects on exploratory behaviour <u>For systemic effects</u>: NOAEL = 5.85 mg/kg bw/day for gestation and 13.1 mg/kg bw/day for lactation <u>For neurotoxic effects</u>: NOAEL = 5.85 mg/kg bw/day for gestation and 13.1 mg/kg bw/day for

			Lactation: 0.03 – 0.25 – 13.1 – 123 - 410 mg/kg bw/day GD0 - PND21	lactation
Nakagami, 2009	Cynomolgus monkeys	Subcutaneous	10 µg/kg bw/day (blood level equivalent to ingestion of 5 mg/kg bw/day in rats) PND31-60 and PND61 - 90	Univariate analysis: significant effects on 3 infant behaviours and 1 maternal behaviour: - <u>in ♂ F1 offspring</u>: 'embracing' and 'social exploration' behaviours ↘ at 2 months and 'outward looking' behaviour ↗ at 2 and 3 months. - <u>In mothers of ♂,</u> 'outward looking' behaviour ↗ at 2 and 3 months. Multivariate analysis: discriminant scores of F1 ♂ were closer to the F1 ♀ controls than the F1 ♂ controls. No effects in ♀. Regarding maternal behaviour, the mothers of F1 ♂: discriminant scores closer to those of the control mothers of F1 ♀ than those of the control mothers of F1 ♂.
Kubo K, 2001	Wistar rats	Oral	1.5 mg/kg bw/day GD0 - PND21	No sexual dimorphism compared to control No changes in reproductive organs or sex hormones
Kubo, 2003	Wistar rats	Oral	0.03 - 0.3 mg/kg bw/day GD0 - PND21	Effects on sexual dimorphism: elimination and reversal of differences in openfield behaviour (locomotive activity, hyperactivity, exploratory behaviour and anxiety) **LOAEL = 0.03 mg/kg bw/day**
Funabashi, 2004	Wistar rats	Oral	2.5 mg/kg bw/day GD0 - PND21	Difference in the number of CRH (corticotropin-releasing hormone-immunoreactive) neurons between females and males in the preoptic area but no difference in the BST (bed nucleus of the stria terminalis).No significant difference in the number of CRH neurons between exposed and

				non-exposed animals, all sexes combined
Patisaul, 2006	CD-SD rats	Subcut aneous	500 µg/animal/day PND1 - PND2	Demasculinisation of tyrosine hydroxylase immunoreactivity in the anteroventral periventricular nucleus of the hypothalamus
Patisaul, 2007	CD-SD rats	Subcut aneous	500 µg/animal/day PND1 - PND2	No change in SDN (sexually dimorphic nucleus) volume in the preoptic area Increased number of calbindin neurons in the SDN No demasculinisation of AVPV (anteroventral periventricular nucleus of the hypothalamus) volume but the neuron-dependent activation model was not affected
Rubin, 2006	CD-1 mice	Subcut aneous	0 – 25 - 250 ng/kg bw/day GD8 - PND16	↘ sex differences in the number of tyrosine hydroxylase neurons due to a ↘ in the number of TH neurons in females Altered sexual dimorphism in the exposed animals **LOAEL = 25ng/kg bw/day**
Ryan, 2010	Long-Evans rats	Oral	2 - 20 or 200 µg/kg bw/day GD7 - PND18	No effects on behavioural sexual dimorphism
(Cox, 2010)	Mice	Oral	8mg/kg bw/day (BPA administered in feed) GD9 - PND0	Suppression of behavioural sexual dimorphism in offspring exposed during embryogenesis No effects on dietary intake, caring behaviour or urinary marking in offspring irrespective of the mother's origin (treated or not). Increased anxiety (elevated plus maze) No effects of BPA exposure during gestation on the gonadal weight of

				male or female offspring No effects on corticosterone levels in male or female offspring **LOAEL 8 mg/kg bw/day (corresponding to 50 mg/kg feed)**
Adewale, 2009	Long-Evans rats	Subcutaneous	50 µg/kg bw/day and 50 mg/kg bw/day PND0 - PND3 (4 injections)	No change in sexual behaviour ↗ body weight at the age of 99 days, only at the dose of 50 mg/kg bw/day No change in serotonin fibre density or in the density of ERα receptors in the ventrolateral subdivision of the ventromedial nucleus ↗ in the number of oxytocin neurons in the paraventricular nucleus at BPA 50 µg/kg bw/day and 50 mg/kg bw
Kim K, 2009	ICR mice	Sub-cutaneous	5-10-20 mg/kg bw/day GD14.5 - GD18.5 then injection of 20mg/kg twice a day for 3 days from PNW8	**F1** At PNW3, ↗ body weight at 5 mg/kg and ↘ at 20 mg/kg but not at PNW8 Accelerated formation of the dendate at PND1 at the dose of 20 mg/kg. →BPA may block the proliferation of neural stem cells and promote cell differentiation in a relatively early stage. BPA has no observed effects on the cortical structure of the neural cells, hippocampus or cell density. In adult mice, BPA has no observed effects on hippocampal neurogenesis.
(Tian, 2010)	ICR mice	Oral	100 and 500 µg/kg bw/day GD7 - PND36	↗ dopamine D2 receptors and decreased dopamine transporters (DAT) in the putamen ↘ NMDA receptors in the frontal cortex, dentate gyrus (DG) and cornu ammonis 1 and 3 (CA1 and CA3) regions of the hippocampus
Matsuda S,	Rats	Intracr	0.1-1-10 µg/kg	significant ↗ in serotonin in the hippocampus, 5-HIAA and 5-HT in the

2010		anial	Single injection at PND2 (1st experiment) 1000 µg/kg single injection at PND2 (2nd experiment)	brain stem, dopamine and DOPAC in the striatum 28 days after the injection. Seven days after the injection, ↗ in 5-HT and norepinephrine (NE) and ↘ in DOPAC and 5-HIAA were observed in the hippocampus. BPA disappeared from brain tissues within 5 hours of the injection, even at the highest dose of 1000 µg/kg. → BPA may have effects on cerebral monoamine levels over 28 days after its disappearance
Xu, 2010 - see detailed description below	Mice and rats	Oral	0 – 0.05 – 0.5 – 5 – and 50 mg/kg bw/day in mice and up to 200 mg/kg bw/day in rats GD7 - PND21	BPA negatively affected the NMDA and ER□ receptor expression in the hippocampus in male rats and mice <u>Doses 0,05 to 50 mg/kg bw/day</u> ↘ expression of hippocampal NMDA receptors (subunits NR1, NR2A and NR2B) in F1 males. ↘ expression of ER□□oestrogen receptors and ↗ aromatase in the hippocampus
Mahoney, 2010	Sheep	Sub-cutaneous	5 mg/kg bw/day G30-G90	↗ expression of ESR1 and ↘ expression of ESR2 ↗ gonadotropin-releasing hormone expression
Palanza, 2008	CD-1 mice	Oral	10 µg/kg bw/day 3 scenarios 1) GD14 -GD18 2) during gestation and continued after birth until adulthood 3) only after birth until	Changes in maternal behaviour in F1 offspring only after *in utero* or adult exposure (scenarios 1 and 3), but not in scenario 2□ ↘ time spent by mothers caring for their offspring and ↗ time where they remained alone in the cage (isolated resting time). no effects on the weight of offspring at birth

			adulthood	

Recent studies from 2011-2012

Among the studies published since the adoption of the report on the health effects of BPA regarding the effects on the brain and behaviour, 7 review studies, 4 epidemiological studies and 25 experimental studies (rats and mice) have been published. These studies confirm the effects of BPA at low doses on exploratory behaviour, learning and memory functions, on anxiety and on alteration of sexual dimorphism. The periods of exposure in the studies on rodents most frequently cover the period *in utero*, or *in utero* and during breastfeeding. The effects are then observed on F1 offspring, even on the following generations (F2 and F4) in very recent studies. At the histological level, a number of these studies confirm the effects of BPA on brain development (effect on neurogenesis, on gene expression, on the morphology of certain brain regions, etc.). It should be noted, however, that several studies have reported effects on rodents exposed only in the early post-natal period (before weaning or before puberty) and on adult animals. If these effects were confirmed, they could justify considering the risks on the central nervous system for adults and children linked to exposure to BPA.

Conclusion

In animals, the effects on **cerebral development** linked to pre- or perinatal exposure to BPA have been confirmed by several studies that show, in particular, changes in neural differentiation, alterations of the NMDA aminergic and glutamatergic systems, changes in oestrogen receptor ERα and ERß expression, and changes in the number of neurons responsive to oxytocin and serotonin. These changes particularly occur in regions such as the hypothalamus (more precisely in regions involved in sexual dimorphism) and the hippocampus, a region involved in cognitive activities and anxiety, namely those associated with NMDA receptors. These neural effects could partly explain the behavioural effects of BPA and allow research to confirm or refute the effects of BPA on behavioural sexual dimorphism, anxiety and exploratory behaviour, and guide future research. **It is considered that these histological changes in neurogenesis are recognised effects in animals**. These histological changes in neurogenesis are critical effects considered for the health risk assessment.

In animals, studies examining the effects of pre- or perinatal BPA exposure **on anxiety** have been conducted with exposure levels that cannot be directly compared. BPA has been shown to have no effects (Stump, 2010), an anxiogenic effect (Cox, 2010); (Poimenova, 2010) and an anxiolytic effect (Tian, 2010). Thus, considering these results and those prior to 2010, the effects of pre- or perinatal exposure to BPA in animals on anxiety, exploratory behaviour and behavioural sexual dimorphism are considered to be controversial**.**

In animals, changes in maternal behaviour related to pre- or postnatal exposure to BPA are considered to be suspected effects.

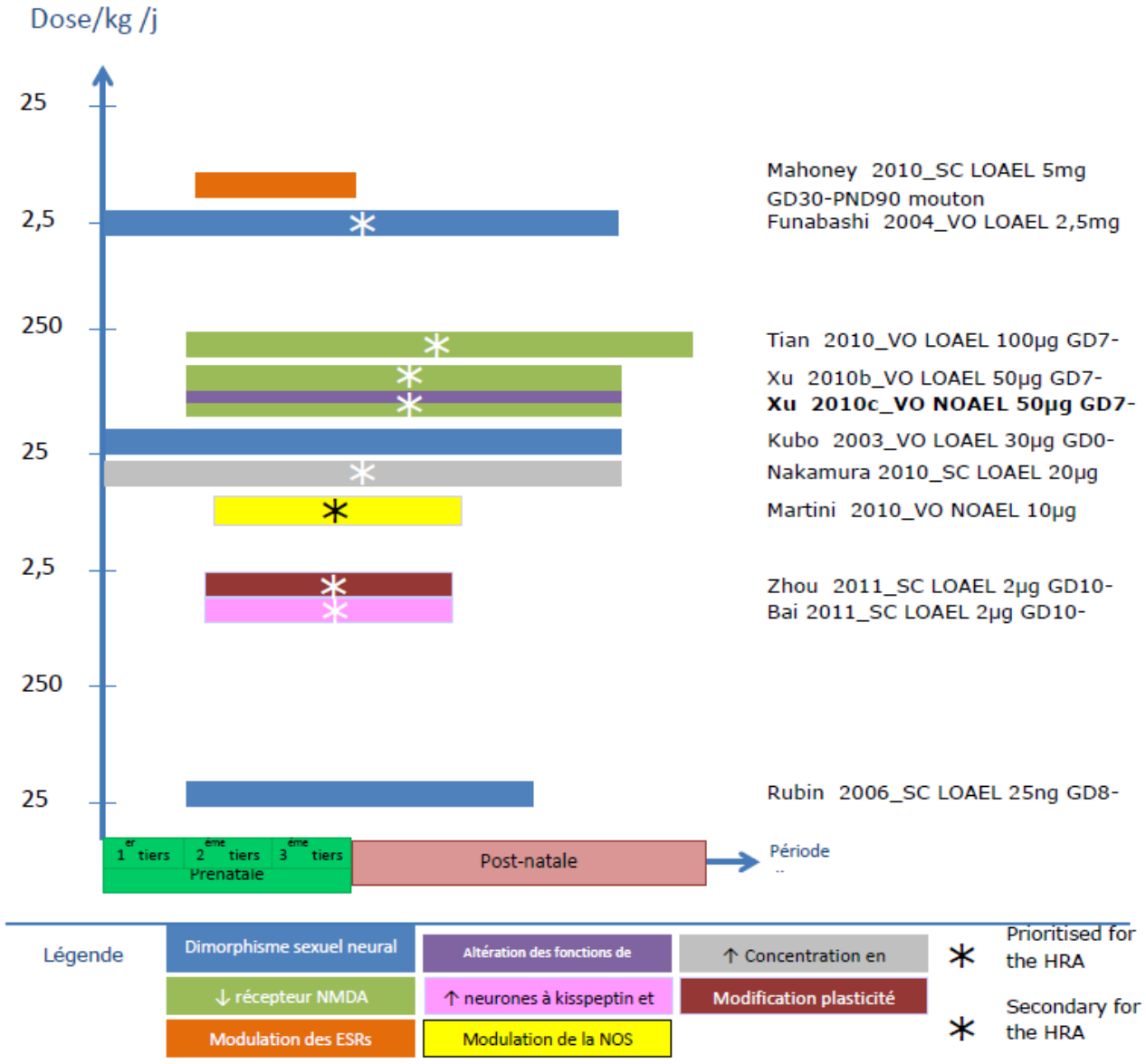
The effects of BPA in connection with damage to brain development resulting from pre and peri-natal exposure are retained for the HRA.

Selecting the critical effect:

Several types of effects were observed in animals in connection with changes in the neurodifferenciation profile (see study summary table on the effects of BPA on the brain and behaviour).

The NOAEL/LOAEL derived from these studies are represented in figure below.

Figure 13. Effects of BPA on the nervous system



Critical effect selected: decrease of NMDA receptor

Neural sexual dimorphism	↓ **NMDA receptor**	Modulation of ESRs	Alteration in memory and learning functions	↑ kisspeptin and GnRH neurons	NOS modulation	↑ in Monoamine concentration	Modification of neuronal plasticity
Funabashi 2004_VO LOAEL 2.5 mg GD0-PND21*	**Xu 2010c_VO NOAEL 50 ug GD7-PND21 mouse** ***	Mahoney 2010_SC LOELu 5 mg/kg/d GD30-PND90 sheep	**Xu 2010c_VO NOAEL 50 ug GD7-PND21 mouse** ***	Bai 2011_SC LOAEL 2 g GD10-PND7*	Martini 2010_VO NOAEL 10 µg GD11-PND8**	Nakamura 2010_SC LOAEL 20 µg GD0-PND21 **	Zhou 2011_SC LOAEL 2 µg GD10-PND7*
Kubo 2003_VO LOAEL 30 µg GD0-PND21	Xu 2010b_VO LOAEL 50 g GD7-PND21 rat**						
Rubin 2006_SC LOAEL 25 ng GD8-PND16	Tian 2010_VO LOAEL 100 g GD7-PND36*						

** Priority study for the HRA

* Secondary study for the HRA

Among all of the observed effects, the critical effect selected deals with the alteration of memory and learning functions concurrent to a decrease in the expression of NMDA (N(-methyl-D-aspartic acid) receptors specifically involved in neuronal plasticity and memory and learning processes. These effects are also supported by the action of BPA on neural systems expressing nitric oxide synthase (NO synthase) with sex and region dependent effects in the hypothalamus and limbic system (Martini, 2010).

Selecting the key study:

The Xu *et al.* study (Xu, 2010)[13] study was selected as the key study (see detailed description as well as strengths and weakenesses of the study and the corresponding reading assessment grid below). This study was conducted orally (gavage) in ICR mice (n = 10 animals/group) exposed to 0, 0.05; 0.5; 5 and 50 mg/kg bw/d. Ten pregnant mice per dose level were exposed from GD7 to PND21. This study did not follow OECD guidelines or GLP. Nevertheless, the study protocol is well described and many effects were investigated, both at the molecular level (NMDA, estrogen β receptor) and at the physiological level. The decrease in the expression of NMDA receptors at the level of the hippocampus in this study was reproduced by the same team in the Sprague Dawley rat (Xu, 2010)[14], under comparable conditions, as well as by other teams (Tian, 2010[15]).

It has to be noted that, in its 2014 draft Opinion, EFSA identified several weaknesses in the study by Xu et al. (2010) (page 303 of EFSA opinion) to which ANSES responded. For further details on ANSES' comments and the divergences between ANSES and EFSA regarding the effect of BPA on the central nervous system, see Annex 5 - ***ANSES comments on EFSA draft opinion).***

The choice of the Xu *et al*. (Xu, 2010) study is supported by studies whose results allow us to form a group of assumptions about neural damage induced by BPA in relation to cognitive effects. The Martini *et al.* (2010) study shows alterations in the expression of NO synthase (NOAEL 10 µg/kg/d) in mice exposed orally. The Tian *et al.* (Tian, 2010)study highlights the alterations of the aminergic and glutamatergic systems (NMDA), associated with cognitive impairment and an anxiolytic action in mice exposed orally (LOAEL 100 µg/kg/d). The Xu *et al.* (Xu, 2010) study shows an inhibition of the expression of glutamate NMDA and estrogen beta receptors ER in rats exposed orally (LOAEL 50 µg/kg/d), and the subcutaneous studies such as that of Zhou *et al.* (2011) highlight the link between alterations in neuronal plasticity and behaviour in rats with a LOAEL of 2 µg/kg/d.

[13] Xu XH, Zhang J, Wang YM, Ye YP, Luo QQ (2010c) Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus in male offspring mice. *Hormones and Behavior* **58,** 326-333.

[14] Xu XH, Wang YM, Zhang J, Luo QQ, Ye YP, Ruan Q (2010b) Perinatal exposure to bisphenol-A changes N-methyl-D-aspartate receptor expression in the hippocampus of male rat offspring. *Environmental Toxicology and Chemistry* **29,** 176-181.

[15] Tian YH, Baek JH, Lee SY, Jang CG (2010) Prenatal and postnatal exposure to bisphenol A induces anxiolytic behaviors and cognitive deficits in mice. *Synapse* **64,** 432-439.

Xu et al. 2010 study

Objective of the study

This study aimed to investigate the effects induced by a perinatal exposure of mice on cognitive functions (learning and memory) and expression of hippocampal glutamate NMDA receptors in F1 male offspring exposed during gestation and lactation.

Experimental design

ICR female mice were orally exposed to bisphenol A (BPA) at 0 (vehicle, sesame oil), 0.05, 0.5, 5 and 50 mg/kg bw/d from GD7 to PND21. Bisphenol A was incorporated in the diet (sesame oil) and mice were orally exposed by gavage. The purity of BPA was not indicated. The material used for cages and water bottles were not indicated. The mice were fed with a soy-free diet, which prevent exposure to soy phytoestrogens. The authors indicated that the presence of phytoestrogens in the diet was not checked. However, because food intake was equivalent between control and experimental mice (Varayoud et al., 2008), the authors assumed that all animals were exposed to the same levels of phytoestrogens.

For exposure, 10 females were randomly exposed for each group of treatment from GD7 to PND21. After parturition (PND 0), the pups were counted, weighed, and culled to 10 pups per litter, maintaining equivalent sex distributions if possible. The pups were identified individually on PND7 and weighed on PND21 and PND 56. At weaning (PND 21), the pups were separated into same sex littermates and housed. This study reports effects of BPA on males only.

Reference cited by the authors

Varayoud, J., Ramos, J.G., Bosquiazzo, V.L., Munoz-de-Toro, M., Luque, E.H., 2008. Developmental exposure to bisphenol A impairs the uterine response to ovarian steroids in the adult. Endocrinology 149, 5848–5860.

Effects observed

The investigations in the male offspring were made at PND0 (body weight) and at PND21 and PND56. Different endpoints were investigated: (i) **Effects on learning and memory.** Spatial memory was investigated by the behavior in the Morris water maze (n= 10), one group of 10 mice at PND21 and another group of 10 mice at PND56, for each modality of exposure. Avoidance memory was investigated by the step-down passive avoidance task (n= 10), one group of 10 mice at PND21 and another group of 10 mice at PND56, for each modality of exposure. Note, for this test PND21 corresponds to PND26, and PND56 corresponds to PND61 because the spatial test lasted 4 days and the avoidance task was tested after the spatial test. (ii) **Expression of receptors involved in learning and memory.** The expression of different subunits of NMDA glutamate

receptors was investigated in the hippocampus, a brain structure involved in learning and memory, and in long term memory.

Significant effects of the body weight

At PND21, BPA induces a decrease of the body weight at 0.05 mg/kg bw/d, and increase at 50 mg/kg bw/d. At PND 56, a decrease of the body weight is observed at 0.05 and 0.5 mg/kg bw/d. There is no dose dependence.

Significant effects on learning and memory

BPA impairs spatial memory (Morris water maze): the increase swam to find the hidden platform is increased (not due to a change in the swim velocity) and the escape length is extended at 5 and 50 mg/kg bw/d at PND 21, and at 0.5, 5 and 50 mg/kg bw/d at PND56. BPA elicits a decrease of time spent in the target quadrant (quadrant in which the platform was installed during training) at 0.5 and 5 mg/kg bw/d at PND21 and PND56.

BPA impairs learning and memory (step-down passive avoidance task) at 5 and 50 mg/kg bw/d at PND21, and at 50 mg/kg bw/d at PND56. BPA dose-dependently increases the error frequency to step down from a platform after receiving a footshock and decreases the step-down latency after the footshock.

Significant effects on the expression of hippocampal NMDA receptors

BPA elicits a dose-dependent decrease of the expression of the NR1 subunits of hippocampal NMDA glutamate receptors at PND21 and PND56. This effect is observed from 0.05 mg/kg bw/d with a c.a. 40% decrease.

BPA decreases the expression of the NR2A subunits of the hippocampal NMDA glutamate receptors at 5 and 50 mg/kg bw/d at PND21, and at all doses at PND56 (c.a. 41% decrease at 0.05 mg/kg bw/d, and 61% at 5 mg/kg bw/d). The decrease is globally dose-dependent.

BPA decreases the expression of the NR2B subunit of hippocampal NMDA glutamate receptors at 0.5, 5 and 50 mg/kg bw/d at PND21, and at all doses at PND56 (c.a. 42% decrease at 0.05 mg/kg bw/d)

Significant effects on the expression of estrogen receptors ERβ

A decrease of the expression of ERβ receptors is observed at 0.5, 5 and 50 mg/kg bw/d at PND21 (dose-dependent) and PND56.

Conclusions of the authors

BPA impairs learning and memory in mice, in association with a negative regulation of hippocampal NMDA receptors.

Comments from the reviewers

The effects observed on NMDA receptors are highly significant and of high amplitude.

Hippocampus is a brain structure involved in learning and memory that enables the consolidation of the mnesic trace and the transfer of information acquired during a learning process into the long-term memory. The long-term storage of information involves NMDA glutamate receptors. The literature shows that in human, alterations in the expression of NMDA receptors results in memory and cognitive impairments. In addition, alteration in the expression of NMDA receptors is greatly suspected to contribute to autism and schizophrenia. In schizophrenics, the alterations in expression of NR2 subunits mRNA in prefrontal cortex are potential indicators of deficits in NMDA receptor-mediated neurotransmission accompanying functional hypoactivity of the frontal lobes. Conversely, the hypoexpression of NR1 subunit seems to be involved in autism.

In this study, there is a strong association between impairment of learning and memory and the expression of hippocampal NMDA glutamate receptor subunits NR1 and NR2A. This association can be used as a biological basis to explain cognitive effects by modification of the brain development and plasticity. Because the decrease of NMDA receptor expression appears at 0.05 mg/kg bw/d and the cognitive effects appear at 0.5 and 5 mg/kg bw/d, effects on NMDA expression might be considered precursor effects on cognitive functions.

The decrease of the expression of ERβ should be considered with attention because these receptors facilitate the long-term potentiation (LTP, mechanism involved in memory) induced by the activation of NMDA receptors, and especially NMDA receptors containing the NR2B subunit.

Consequently, the reviewers agree with the conclusion of the authors, which are in accordance with the experimental results.

Moreover, the effects observed by Xu et al. (2010) are supported by the action of BPA on neural systems expressing nitric oxide synthase (NO synthase) with sex- and brain region-dependent effects in hypothalamus and limbic system (Martini *et al.*, 2010). The decrease in the expression of NMDA receptors observed in the hippocampus in the study of Xu et al. (2010) has been reproduced by the same research team in Sprague-Dawley rats (Xu *et al.*, 2010b), in comparable conditions, and by another team (Tian *et al.*, 2010).

Strengths of the study:

- The experimental protocol is described in details and numerous effects have been investigated, not only at molecular level (NMDA glutamate receptors and ERβ estrogen receptors) but also at physiological and neuro-behavioral (cognitive effects) levels.
- The mice were fed with a soy-free diet, which prevent exposure to soy phytoestrogens.

- The effects observed on NMDA receptors are highly significant and of high amplitude.
- Link between changes in synaptic and neuronal plasticity mechanisms in specific cerebral regions (hippocampus) and functional behavioural impairment (spatial learning and conditioning).
- The number of individuals is considered correct (n= 10 dams exposed)

Weaknesses of the study:

- This study does not follow OECD guidelines
- This study reports effects of BPA on males only.
- Lack of information about the selection of the pup in each litter.
- The material used for cages and water bottles were not indicated.

Tools for risk assessment

LOAEL = 0.05 mg/kg bw/d

Critical effect: Strong decrease of NMDA receptor subunits, associated with an impairment of learning and memory.

ANSES retained the impairment of memory as critical effect: NOAEL = 0.05 mg/kg/d

Reading assessment grid for the study from Xu et al., 2010	
Full reference	**Xu et al. (2010c) Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus in male offspring mice. Horm. Behav. 58, 326-333**
Type of study (1G, 2G, prenatal…)	Perinatal : GD7-PND21
Aim of the study	Investigation of the effects induced by a perinatal exposure of mice on cognitive functions and expression of glutamate NMDA receptors in the hippocampus of F1 male offspring
Compliance to GLP, OECD guidelines, standards…	- Academic study - Compliance with guideline, standards of GLP : not relevant - Compliance with Care and Use Standards of the Laboratory Animals
Chemical substances, purity, composition	BPA : No indication on the purity
Species / strain / Age - weight	ICR Mice
Gender and number of animals per category	- 10 mothers randomly exposed par modality of treatment (n = 10). Females were placed and exposed individually. - 10 pups selected per litter by trying to respect the male/female ratio. Pups individually identified at PND7 - At weaning (PND21), the pups were separated into same-sex littermates and housed
Control group and number of animals	Dose 0 mg/kg bw/d BPA (vehicle, sesame oil) – 10 mothers
Eventual positive control (if relevant)	No positive control
Life conditions	The materials used for the cages and the bottles are not indicated Soy-free diet
Route of exposure	Oral exposure (oral injection)

Frequency and duration of exposure	Daily : GD7-PND21 (weaning)
Exposure level	0 – 0.05 – 0.5 – 5 et 50 mg/kg bw/d
Observations / endpoints studied	Observation in the male offspring at PND21 and PND56 - **Effects on learning and memory** ▪ Spatial memory investigated by the behavior in the Morris water maze (n= 10), one group of 10 mice at PND21 and another group of 10 mice at PND56, for each modality of exposure. ▪ Avoidance memory investigated by the step-down passive avoidance task (n= 10), one group of 10 mice at PND21 and another group of 10 mice at PND56, for each modality of exposure. Note, for this test PND21 corresponds to PND26 and PND56 corresponds to PND61 because the spatial test lasted 4 days and the avoidance task was tested after the spatial test. - **Expression of receptors involved in learning and memory** ▪ Developmental expression of the subunits of NMDA glutamate receptors in the hippocampus
Confounding factors	- Soy-free diet (absence of phytoestrogens from soy) - It is indicated that the presence of phytoestrogens in the diet was not checked
Effects observed – General/maternal toxicity	**Significant effects of the body weight** - At PND21, decrease of the body weight at 0.05 mg/kg bw/d and increase at 50 mg/kg bw/d. - At PND56, decrease of the body weight at 0.05 and 0.5 mg/kg bw/d. No dose dependence. **Significant effects on learning and memory** - Impairment of spatial memory (Morris water maze): increase of distance swam to find the hidden platform (not due to a change in the swim velocity) and extension of escape length at 5 and 50 mg/kg bw/d at PND 21, and at 0.5, 5 and 50 mg/kg bw/d at PND56. Decrease of time spent in the target quadrant at 0.5 and 5 mg/kg bw/d at PND21 and PND56. - Impairment of learning and memory (step-down passive

	avoidance task) at 5 and 50 mg/kg bw/d at PND21, and at 50 mg/kg bw/d at PND56. **Significant effects on the expression of hippocampal NMDA receptors** - Dose-dependent decrease of the expression of the NR1 subunit of hippocampal NMDA glutamate receptors at PND21 and PND56. This effect is observed from 0.05 mg/kg bw/d with a c.a. 40% decrease. - Decrease of the expression of the NR2A subunit of the hippocampal NMDA glutamate receptors at 5 and 50 mg/kg bw/d at PND21, and at all doses at PND56 (c.a. 41% decrease at 0.05 mg/kg bw/d, and 61% at 5 mg/kg bw/d). Decrease globally dose-dependent. - Decrease of the expression of the NR2B subunit of hippocampal NMDA glutamate receptors at 0.5, 5 et 50 mg/kg bw/d at PND21, and at all doses at PND56 (c.a. 42% decrease at 0.05 mg/kg bw/d) **Significant effects on the expression of estrogen receptors ERβ** - Decrease of the expression of ERβ receptors at 0.5, 5 et 50 mg/kg bw/d at PND21 (dose-dependent) and PND56
Effects observed – Reprotoxicity	Not relevant here
Critical effect taken into account	Decrease of the expression of hippocampal NMDA glutamate receptor associated with cognitive effects
NOAEL / LOAEL for the critical effect	LOAEL : 0.05 mg/kg bw/d
Conclusions of the authors	BPA impairs learning and memory in mice, in association with a negative regulation of hippocampal NMDA receptors
Quality of the study (Klimisch criteria)	1d
Comments and conclusions of the reviewers	The effects observed on NMDA receptors are highly significant and of high amplitude. Hippocampus is a brain structure involved in learning and memory that enables the consolidation of the mnesic trace and the transfer of information acquired during a learning process into the long-term memory. The long-term storage of information involves NMDA glutamate receptors. The literature shows that alterations in the expression of NMDA receptors results in memory and cognitive impairments. In addition, alteration in the expression of NMDA

	receptors is greatly suspected to contribute to autism and schizophrenia. In schizophrenics, the alterations in expression of NR2 subunits mRNA in prefrontal cortex are potential indicators of deficits in NMDA receptor-mediated neurotransmission accompanying functional hypoactivity of the frontal lobes. Conversely, the hypoexpression of NR1 subunit seems to be involved in autism. In this study, there is a strong association between impairment of learning and memory and the expression of hippocampal NMDA glutamate receptor subunits NR1 and NR2A. This association can be used as a biological basis to explain cognitive effects by modification of the brain development and plasticity. Because the decrease of NMDA receptor expression appears at 0.05 mg/kg bw/d and the cognitive effects appear at 0.5 and 5 mg/kg bw/d, effects on NMDA expression might be considered precursor effects on cognitive functions. The decrease of the expression of ERβ should be considered with attention because these receptors facilitate the long-term potentiation (LTP) induced by the activation of NMDA receptors, and especially NMDA receptor containing the NR2B subunit. Consequently, the reviewers agree with the conclusion of the authors, which are in accordance with the experimental results.

Selecting the benchmark doses:

In the Xu *et al.* (Xu, 2010) study a slight decrease in body weight of the F1 mice is observed at PND21 at a dose of 50 µg/kg bw/d and a slight increase in the dose of 50 mg/kg bw/d(both statistically significant). An alteration in spatial memory and learning function is observed:

- at PND21 at doses of 5 and 50 mg/kg bw/d and at doses of 0.5; 5 and 50 mg/kg bw/d on PND56 for spatial memory
- at PND21 at doses of 5 and 50 mg/kg bw/d and at the dose of 50 mg/kg bw/d in PND56 for learning functions

At the tissue level, a statistically significant and dose-dependent decrease of expression of hippocampal NMDA receptors is observed from 50 µg/kg bw/d to PND21 and PND56 on the basis of the NR1 and NR2A and B subunits. Lastly, a significant decrease in the expression of ERβ receptors is observed at doses of 0.5; 5 and 50 mg/kg bw/d at PND21 and PND56.

In conclusion, a NOAEL of 50 µg/kg bw/d based on the alteration of memory and learning functions concurrent with a decrease in the expression of NMDA (N-methyl-D-aspartic acid) receptors is selected as the benchmark dose for the HRA.

Other comments (uncertainties, confidence level, etc.)

The weaknesses of Xu *et al.* (Xu, 2010) have been presented above.

Other recent studies show the effects of BPA on the brain and behaviour, at comparable dose levels, (Poimenova, 2010); (Cox, 2010); Funasbashi *et al.*, 2004), which supports this choice of key study. On the other hand the OECD study by Stump *et al.*, 2010 performed under GLP shows no effect on exploratory behaviour of the pregnant CD-SD rats exposed orally with doses ranging from 0.01 to 164 mg/kg bw/d. The Stump *et al.* (Stump, 2010) study and the other studies investigating the same effects of BPA were not used as key study for the HRA because the work on anxiety and exploratory behaviour shows conflicting results: no significant effect in the Stump (Stump, 2010) study, an anxiolytic effect in the Cox *et al.* ((Cox, 2010)) and Poiminova *et al.* (2010) studies, and an anxiolytic effect in the Tian *et al.* (Tian, 2010) study.

In its opinion dated January 29 2010 and its associated appendix (Afssa, 2010), Afssa reviewed several studies related to the neurotoxic effects of BPA (Palanza, 2008; (Nakagami, 2009); Stump, 2010; Braun, 2009; Monje, 2009; Ryan, 2010)[16] and considered that some of these publications, including (Nakagami, 2009) and Palanza, 2008), mention warning signs after *in utero* and post-natal exposure at doses below those upon which the TDI is based (Afssa, 2010 ;Afssa, 2010). The effects reported relate, on the one hand, to a feminisation in the behaviour of small males, and on the other hand, a change in the exploratory behaviour and anxiety. Other studies, on the other hand, were not deemed to be of concern (Braun, 2009, Monje, 2009).

[16] Ryan et al., 2010 In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. http://www.ncbi.nlm.nih.gov/pubmed/19864446 PMID: 19864446 Toxicol Sci. 2010 Mar;114(1):133-48.

B.5.10.2. Effects on metabolism and obesity

B.5.10.2.1 Previous assessments of the effect of BPA on metabolism and obesity

Metabolic syndrome, associated with a state of insulin resistance, is a combination of several criteria, including those that follow, in the same individual: central (abdominal) obesity, hypertriglyceridemia, low HDL-cholesterol, elevated blood pressure and fasting hyperglycaemia. It is a predisposing factor for cardiovascular risk and type 2 diabetes (see glossary).

The expert panel that met in Chapel Hill in 2007 considers that the *in vivo* results are contradictory. For example, certain studies show a decrease in body weight or no effect in response to developmental exposure to BPA. Other studies show an increase in postnatal growth after exposure during *in utero* development (Richter, 2007).

The NPT-CERHR also indicates that the available data are not sufficiently conclusive to link prenatal BPA exposure with obesity (NTP, 2007). It reports 2 animal studies that assessed disruption of the regulation of fat and carbohydrate metabolism. In male rats, sub-cutaneous doses of 0.01 and 0.10 mg/kg bw/day of BPA cause decreased glucose levels and increased insulin levels (Alonso-Magdalena *et al.*, 2006). Furthermore, increased insulin production by the pancreas and insulin resistance was described at 0.10 mg/kg bw/day (administered orally or by SC injection) after a 4-day period. The study by Miyawaki, 2007 reports effects on body weight, adipose tissue weight, serum leptin levels, triglyceridemia, non-esterified fatty acids and glucose (Miyawaki, 2007- see detailed description as well as the corresponding reading assessment grid further below). However, the NTP considered that these studies were non-admissible due to methodological problems.

Some studies have assessed mechanisms likely to interact with fat and carbohydrate metabolism: BPA has been found to stimulate the oestrogen receptors α found in the pancreatic beta cells (Richter, 2007; Ropero AB, 2008; Nadal A, 2009; Alonso-Magdalena P, 2006; Alonso-Magdalena P, 2008), while oxidative stress may contribute to insulin resistance (Hong, 2009). Likewise, the NPT-CERHR reports accelerated differentiation of fibroblast cells into adipocytes, and altered glucose transport in adipocytes (Masuno *et al.*, 2002 et 2005; Phrakonkham *et al.*, 2008; Sakurai *et al.*, 2004) (NTP, 2007).

According to Aschberger *et al.* (2010), epidemiological studies and *in vivo* and *in vitro* studies suggest that exposure to BPA is related to metabolic syndrome (Aschberger K, 2010). Liver enzyme abnormalities are also described (Takeuchi T, 2004; Lang IA, 2008); Newbold *et al.,* (Newbold, 2009); Elobeid *et al.,* (Elobeid MA, 2008) reported by Aschberger K, 2010).

The FAO/WHO experts considered that the two studies in humans that reported a positive relationship between urinary concentrations of BPA and cardiovascular diseases or diabetes (Lang IA, 2008; Melzer, 2010) have weaknesses that limit their interpretation (FAO/WHO, 2010). The experts consider that it is necessary to implement prospective studies linking BPA measurements during various windows of susceptibility and the onset of cardiovascular diseases or diabetes several years later. Two studies have examined birth defects and body weight index but the results are difficult to interpret (Padmanabhan V, 2008; Wolff, 2008); the experts recommend undertaking studies assessing the link between BPA exposure during pregnancy (urinary BPA levels sampled on several occasions) and body weight index and adipose mass at birth.

In animals, according to this panel, the available data do not clearly show that BPA has cardiovascular effects, and in particular, studies undertaken in accordance with GLP using large samples have not shown toxicity to the cardiovascular system. Changes in VEGF expression, NO

production and ion channels have been reported, but with no related adverse effects to date. These experts have been informed that studies examining the cardiotoxicity of BPA are in progress.

Regarding effects on metabolism, the available data are not sufficient to draw conclusions as to the effects of BPA. According to this panel, the 2008 conclusions of the NTP-CERHR (NTP, 2008) indicating that BPA does not affect obesity at doses < 5000 µg/kg bw/day remain valid. However, examining newborn weight is not sufficient to draw a conclusion regarding obesity, unlike a direct measurement of body fat and its distribution. Yet the available data on glucose intolerance, hyperinsulinaemia, adipose hypertrophy, etc. suggest that supplementary studies need to be undertaken to examine the effects of BPA on the regulation of fat, carbohydrate and insulin metabolism and other effects related to diabetes and metabolic disorders. These effects should be investigated in adult animals exposed during pregnancy, including older animals (FAO/WHO, 2010).

B.5.10.2.2 Data considered in the ANSES assessment for metabolism and obesity in Humans

All the studies available until 2011 on effects of BPA on metabolism and obesity in humans are presented below. Then, more recent studies from 2011-2012 were analysed and the result of this analyse is presented in the conclusion but the studies are not detailed because they do not contradict the previous analysis of hazard assessment of BPA on metabolism and obesity.

Effects on sex hormones								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Takeuchi *et al.*, 2004) Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	Cross-sectional study	Study population: general population: women N=7 patients with hyper-prolactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size	Serum	ELISA (validation of the assay method by HPLC)	Age: no Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	Results: correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels significantly increased in women with PCOS (obese or not) and obese women without ovulation dysfunction.	Study not taken into consideration since they have major methodological limitations This study was excluded in light of the following methodological weaknesses: - small population size, - statistical analysis lacking detail, - the final	Information from epidemiological studies Effects on metabolism and the cardiovascular system

						Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	comparison was made in relation to non-obese women, with normal cycles (considered as controls) - no adjustment for confounding factors, - plasma BPA measured using the ELISA technique (lower limit).	
Effects on metabolism / the cardiovascular system								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Hong *et al.*, 2009) Community level exposure	Cross-sectional study	Study population: general adult	Urinary	HPLC/MS	Age: yes Sex: yes	Results: Significant positive relationship	Study of high quality or having no major	Information from epidemiological studies

to chemicals and oxidative stress in adult population		population N=960 → Excellent population size			Medication: no Tobacco: yes BMI: no Other contaminants: yes Other: physical activity, medical and professional history, alcohol	between urinary concentrations of chemical contaminants, particularly phthalates and BPA, and markers of oxidative stress in a simple regression analysis (not significant if multiple regression analysis for BPA) Subjects with the highest levels of BPA were prone to fasting hyperglycaemia but no association with insulin-resistance indices	methodological limitations	Effects on metabolism and the cardiovascular system

(Lang *et al.*, 2008) Association of Urinary Bisphenol A Concentration With Medical Disorders and Laboratory Abnormalities in Adults	Cross-sectional study nested in the NHANES study (2003-2004)	Study population: general adult population (18-74 years) N=1455 adults -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic group/race, education, financial resources, abdominal circumference	Results: positive association between the highest urinary concentrations of BPA (5 and 13 ng/mL) and cardiovascular disease, diabetes and levels of liver enzymes in the blood Comments: This study warrants particular attention because: - powerful study with a solid design, - the associations are extremely robust,	Study of high quality or having no major methodological limitations	Information from epidemiological studies Effects on metabolism and the cardiovascular system

						- large sample size, - based on American national cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect <u>Note</u>: The studies by Melzer *et al.* and Lang *et al.*were undertaken 2 years apart with the same		

						type of protocol.		
(Melzer *et al.*, 2010) Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06	Cross-sectional study nested in the NHANES study (2003-2006)	Study population: general adult population (18-74 years) N=1455 (2003/04) and 1493 (2005/06) -> Sufficient population size	Urinary (free and conjugated BPA)	HPLC/MS	Age: yes Sex: yes Medication: no Tobacco: yes BMI: yes Other contaminants: no Other: urinary creatinine, ethnic group/race, education, financial resources, abdominal circumference	Results: - In 2005/2006: significant association between the highest urinary concentrations of BPA and coronary disease. No association between urinary concentrations of BPA and diabetes. - In 2003/06: significant association between the highest urinary concentrations of BPA and heart disease, diabetes, alkaline phosphatase	Study of high quality or having no major methodological limitations	Information from epidemiological studies Effects on metabolism and the cardiovascular system

						and lactate dehydrogenase. <u>Comments</u>: This study warrants particular attention because: - solid design and high power (80% for the 2003/2004 population and 74% for the 2005/2006 population) - the associations are robust, - large sample size, - based on American national		

						cohorts, However, the use of medication was not taken into account and contemporary exposure is not necessarily representative of past exposure, which was correlated with the observed effect. Note: The studies by Melzer *et al.* and Lang *et al.* were undertaken 2 years apart with the same type of protocol.		
(Takeuchi *et*	Cross-	Study	Serum	ELISA	Age: no	Results:	Studies not	Information

***al.*, 2004)** Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction	sectional study	population: general population: women N=7 patients with hyperpro-lactinemia, 21 with hypothalamic amenorrhea, 19 with PCOS (13 non-obese and 6 obese) vs 26 controls (7 obese and 19 non-obese) -> Small population size		(validation of the assay method by HPLC)	Sex: NA Medication: NA Tobacco: no BMI: no Other contaminants: no	correlation between plasma concentrations of testosterone (free and total) and BPA firstly and BPA concentrations and Body Mass Index secondly: levels were significantly higher in women with PCOS (obese or not) and obese women without ovulation dysfunction. Comments: The results remain difficult to interpret as is, due to the vagueness of the sampling plan, the lack of information	taken into consideration since they have major methodological limitations This study was excluded in light of the following methodological weaknesses: - small population size, - statistical analysis lacking detail, - the final comparison was made in relation to non-obese women, with normal cycles (considered controls)	from epidemiological studies Effects on the female reproductive system Effects on metabolism and the cardiovascular system

						on inclusion criteria and failure to take into account the pathologies of the control subjects in the results.	- no adjustment for confounding factors, - plasma BPA measured using the ELISA technique (lower limit).	
Effects on birth weight								
Reference **Article title**	**Study type**	**Study population**	**BPA measurement**	**Analytical method**	**Adjustments**	**Results / discussion**	**Study quality**	**Corresponding section(s)**
(Padmanabhan *et al.*, 2008) Maternal bisphenol-A levels at delivery: a looming problem?	Cross-sectional study	Study population: general population: women at delivery N=40 pregnant women → Small population size	Plasma (in mothers) (free)	HPLC/ESI-MS/MS	Age: no Sex: NA Medication: no Tobacco: no BMI: no Other contaminants: no	Results: No association between plasma concentrations of BPA and gestation period or birth weight Comments: - One single BPA	Studies not taken into consideration since they have major methodological limitations This study was excluded due to the following methodologic	Information from epidemiological studies Effects on metabolism and the cardiovascular system

					Other: no	measurement taken at birth and not at the start or middle of pregnancy	al weaknesses: - small population size, - no adjustment for confounding factors, - no measurement of conjugated BPA	
(Wolff *et al.*, 2008b) Prenatal Phenol and Phthalate Exposures and Birth Outcomes	Prospective study	Study population: general population (women) N=367 → OK population size	Urinary	HPLC	Age: yes (gestational age) Sex: yes (sex of children) Medication: NA Tobacco: yes (during pregnancy) BMI: yes	Results: no significant association between BPA and birth weight, infant size, head circumference or gestational age	Studies of high quality or having no major methodological limitations	Information from epidemiological studies Effects on metabolism and the cardiovascular system

					(pre-gestational) Other contaminants: yes Other: creatinine, race, maternal education	Comments: - Only one measurement taken, - Low plasma levels of BPA, - No association between plasma concentrations of BPA and effects on newborns		

Hong *et al.* studied levels of oxidative stress in an urban adult population in Korea exposed to various contaminants between April and December 2005 (Hong, 2009). A total of 960 (85%) people out of 1131 identified subjects (46% of men and 54% of women) were questionned. A questionnaire on lifestyle habits was developed and environmental exposure studies were undertaken. Furthermore, urine and blood samples were taken. The aim was to assess the relationship between chemical exposure and oxidative stress, and the potential role of certain environmental chemicals in insulin resistance. The authors found a significant positive relationship between urinary concentrations of chemical contaminants, particularly phthalates and BPA, and oxidative stress markers in a simple regression analysis. Nevertheless, this relationship disappeared for BPA in a multiple regression model after controlling for age, sex, smoking and exercise. Oxidative stress marker levels were correlated with levels of insulin resistance in peripheral tissues. A positive association was found between urinary levels of BPA and fasted glycaemia. The authors concluded that exposure to chemical contaminants is associated with oxidative stress in urban adult populations and suggested that exposure to certain environmental chemicals might contribute to insulin resistance.

In 2008, Lang *et al.* undertook a cross-sectional study in 5 adults aged 18 through 74 years in the United States. They used data from the 2003-2004 National Health and Nutrition Examination Survey (NHAHES) (Lang IA, 2008). Regression models were adjusted for age, sex, race/ethnicity, education, income, smoking, Body Mass Index (BMI) and waist circumference. Urinary concentrations of total (free and conjugated) BPA were measured using HPLC-MS and adjusted for creatinine. High BPA concentrations (5 and 13 ng/mL) were associated with a higher risk of cardiovascular disease, only after age and sex adjustment. An association with diabetes was found, but not with other types of diseases. A significant increase in alkaline phosphatase and γ-glutamyl transferase concentrations was associated with high BPA concentrations. The authors remain general in their conclusion and speak of a possible association between high BPA exposure and adult morbidity. The group of Melzer *et al.*, which was part of the Lang *et al.* team, used the data for the NHANES adult sub-population (Melzer, 2010). This new analysis partly confirmed the results of the 2003-2004 campaign. It showed that high BPA exposure, reflected by high urinary concentrations of BPA, were associated with cardiovascular diseases (coronary diseases) in the 2005-2006 campaign and in the two pooled campaigns, and with diabetes in the two pooled campaigns but not in the 2005-2006 campaign.

The mechanisms by which BPA results in cardiac disease are not yet absolutely known. However, Asano *et al.* reported a possible route of action that might involve the Maxi-K potassium channels (Kca1.1), which are sensitive to both oestrogens and BPA (Asano S, 2010). One of the limitations of the study by Asano *et al.* is that activation of the Maxi-K channels is observed at a pharmacological concentration (10 µM) of BPA, which is not compatible with environmental exposure levels for BPA (Asano S, 2010).

A cross-sectional study was undertaken in Japan in order to examine the influence of BPA, age and BMI on hormonal changes in the blood (Takeuchi T, 2004). In total, 73 women were recruited, then divided up after medical consultation into 6 groups including: women diagnosed as normal (normal weight; no related disease), obese (no related disease), with hyperprolactinemia, with hypothalamic amenorrhea and with polycystic ovary syndrome (PCOS) including a subgroup of obese and non-obese women. The authors identified a strong relationship between serum levels of BPA and the effects on androgen metabolism. More precisely, Takeuchi *et al.* (Takeuchi T, 2004) reported a positive correlation, in the group of

women diagnosed as normal, between serum BPA concentrations and free testosterone, androstenedione and dehydroepiandrosterone sulphate (DHEAS) concentrations. They also showed a positive correlation taking into account all of the women from the 6 groups and calculating a correlation between BPA and concentrations of testosterone (free and total), androstenedione and DHEAS. The authors concluded that there is a strong relationship between serum BPA and androgen concentrations, which they attribute to the effect of androgen on the metabolism of BPA. However, it remains difficult to interpret these results as is, due to the imprecision of the sampling plan and the lack of information about the inclusion criteria (particularly for the constitution of the control group). Moreover, the fact of taking into account all of the women in the 6 groups together introduces a selection bias on account of the various diagnosed diseases. The calculated correlations, even though they are significant, range from 0.391 and 0.684. These low correlation values could be due to the small population size, the variability of the measured parameters, biases linked to the summation of the diseases or the analytical technique that was used (ELISA).

Conclusion for hazard assessment in humans

In a cross-sectional study in humans (Melzer, 2010), a correlation was observed between the highest urinary levels of BPA and cardiovascular diseases (coronary diseases) and diabetes. These effects were considered as suspected effects and will not be taken into consideration in the HRA.

B.5.10.2.3 Data considered in the ANSES assessment for metabolism and obesity in animals

Prenatal and perinatal exposure

- **Effects on glucose metabolism**

Alonso-Magdalena *et al.* studied the effects of BPA on glucose metabolism in female mice, during gestation, and their male F1 offspring (Alonso-Magdalena, 2010). BPA was administered sub-cutaneously to the mothers, from GD9 to GD16, at doses of 0, 10 and 100 µg/kg bw/day. In the F1 offspring, 6-month old males had reduced glucose tolerance, increased insulin resistance, and higher plasma levels of insulin, leptin, triglycerides and glycerol. Moreover, the islets of Langerhans presented altered calcium signalling. The authors note that BrdU incorporation into insulin-producing β cells was reduced, yet their surface was unchanged. However, the latter results, although very likely, should be considered with caution, since they were obtained with cultured cells from exposed individuals. Therefore, taking into account isolation and culturing methods, cultured cells have different phenotypes than *in situ* cells. Such an approach is relevant when undertaking an instant analysis of the cellular state after rapid fixation and treatment of the tissues. However, it is not appropriate when examining differences in cell functioning between controls and individuals exposed to a stress agent.

Ryan *et al.* tested the hypothesis that perinatal exposure to BPA, at a dose consistent with environmental exposure (0.25 µg BPA/kg bw/day), results in increased susceptibility to high-fat diet-induced obesity and glucose intolerance in CD-1 mice (Ryan, 2010). F1 individuals were exposed to BPA in the perinatal period (1 µg/kg via the mothers' feed, equivalent to around 0.25 µg/kg bw/day) from the embryonic stage GD0 to weaning (PND21). In the weaned F1 individuals, increased body weight was observed in males and females at 3 weeks and increased body length was observed in males at 4 weeks, these biometric differences

disappearing in adulthood. No significant effects on glucose tolerance were observed. The authors concluded that the increased body length and weight were due to a faster rate of growth in the exposed mice rather than a state of obesity.

- **Effects on lipid metabolism**

Somm *et al.* studied the effects of BPA in F1 rats (Sprague-Dawley) subject to perinatal exposure (GD6 to PND21), by administering drinking water containing BPA at a concentration of 1 mg/L (corresponding to 70 µg/kg bw/day) to the mothers (Somm, 2009). In general, BPA did not alter sex ratio or litter size. The male and female F1 individuals exposed to BPA had higher weights than the controls at PND1. At PND21, body weight was increased only in females, whose white adipose tissue weight increased threefold, this was combined with adipocyte hypertrophy and overexpression of lipogenic genes such as C/EBP-α (CCAAT enhancer binding protein □), PPAR-γ (peroxisome proliferator-activated receptor □, SREBP-1C (sterol regulatory element binding protein-1C), LPL (lipoprotein lipase), FAS (fatty acid synthase) and SCD-1 (stearoyl-CoA desaturase). In addition, C/EBP-α, FAS and ACC (acetyl-CoA carboxylase) gene expression was also increased in the liver of exposed females at PND21, with no significant change in circulating glucose and lipid levels. After weaning, there was a sex- and diet-dependent predisposition to excess weight in F1 individuals exposed to BPA. Thus, no difference in body weight was observed between BPA-exposed individuals and control animals on a standard chow diet whereas exposed individuals fed a high fat diet were 7% overweight. This excess weight was not associated with increased food intake.

Miyawaki *et al.* studied the effects of BPA on hyperlipidemia, from gestation to PND10, and the development of obesity in mice (Miyawaki, 2007- see detailed description as well as the corresponding reading assessment grid further below). This group subjected mice to (1 µg/kg bw/day) (low dose = LD) or 10 µg (2.5 µg/kg bw/day) (high dose = HD) of BPA/mL in drinking water. They then measured anatomical and physiological changes at PND31. In females, they noted that the body weight of the mothers increased by 13% (LD) and 11% (HD) compared to the control group, adipose tissue weight increased by 132% in the LD group and cholesterol increased by 33% (LD) and 17% (HD). In males, body weight increased by 22% (LD) and 59% (HD) and the triacylglycerol level increased by 345% (LD) compared to the control group. In light of these results, they concluded that BPA, during pregnancy and in postnatal exposure during lactation, causes hyperlipidemia and the development of obesity.

Exposure in adults

Alonso-Magdalena *et al.* studied the effects of BPA on glucose metabolism in mice, considering mothers during gestation and their male F1 offspring (Alonso-Magdalena, 2010). BPA was administered sub-cutaneously on gestation days GD9 to GD16 at doses of 0, 10 and 100 µg/kg bw/day. In mothers, BPA exposure increased insulin resistance associated with gestation at the dose of 10 µg/kg bw/day versus the control group and had a tendency to increase insulin sensitivity at the dose of 100 µg/kg bw/day (not significant at p=0.05), and reduced glucose tolerance at 10 µg/kg bw/day. IT caused a dose-dependent increase in plasma levels of insulin at 10 µg/kg bw/day, leptin at 100 µg/kg bw/day, triglycerides at 100 µg/kg and glycerol at 100 µg/kg bw/day. At 10 µg/kg, BPA reduced insulin-stimulated Akt[17]

[17] Akt is a serine/threonine protein kinase that plays a role in glucose metabolism and is activated by the 3-phosphoinositide-dependent protein kinases PDK1 and PDK2. PI3K is involved in the signalling pathway associated with the synthesis and secretion of adiponectin.

phosphorylation in the liver and blunted it in the gastroc-nemius muscle. Long-term effects were also observed in the mothers, 4 months post-partum, with increased body weight and higher concentrations of insulin, leptin, triglycerides and glycerol in BPA-treated individuals.

In vitro studies

Effects on lipogenesis

In vitro, BPA at concentrations ranging from 100 pM to 1 µM promotes adipogenesis in mouse preadipocyte 3T3-L1 cells (Sargis, 2010). The activation of this lipogenesis is mediated by glucocorticoid receptors. BPA increases lipogenesis in differentiating adipocytes and activates the expression of specific adipocytic proteins (adiponectin, transcription factor CCAAT enhancer binding protein □□(C/EBP-α), a factor induced in the terminal phase of adipogenesis). However, the action of BPA on adiponectin induction shows a bell curve with a visible effect from 10 nM, peaking at 100 mN and disappearing at 1000 nM. An identical dose-response relationship was observed with dexamethasone. It should be noted that in this study, the other compounds under consideration, dicyclohexyl phthalate, tolyfluanid, troglitazone and triphenyltin had lesser effects at the highest concentration of 1 µM.

In the studies by Kidani *et al.*, 3T3-L1 cells were exposed to various forms of bisphenol (BP): BPA, BPB, BPE and BPF at concentrations of 0, 20, 40 and 80 □M. In a dose-dependent manner, BPA decreased the concentration of cellular adiponectin and was secreted in the extracellular medium (Kidani, 2010). Forms of BPA can be classified as follows according to their ability to reduce adiponectin secretion: BPB > BPA > BPE > BPF. BPA negatively regulates the Phosphatidylinositol 3-Kinase (PI3K)-Akt signalling pathway by reducing Akt and p-Akt expression.

However, the inhibition of adiponectin expression by BPA should be compared with the results obtained by Sargis *et al.* showing a bell-shaped dose-response relationship between BPA and adiponectin (Sargis, 2010). The negative effects on adiponectin expression observed by Kidani *et al.* are therefore not surprising in that they were produced at concentrations greater than 1 µM (Kidani, 2010). Thus, BPA may induce adiponectin expression at low doses and suppress it at high doses (which are already very low).

Asahi *et al.* undertook studies in cultured non-parenchymal hepatocytes, NCTC Clone 1469 cells (Asahi J, 2010). The cells were exposed to BPA at concentrations of 0, 1, 10, 50, 100 and 200 µM for 48 hrs. or at a concentration of 100 µM for a period of 120 hours, with an analysis of BPA's effects at various times. After having examined the cytotoxicity of BPA at various concentrations, the studies continued, exposing the cells to BPA at the concentration of 100 µM. At this concentration, BPA induced apoptosis which was expressed by DNA fragmentation, phosphatidylserine externalisation on the outer plasma membrane leaflet, an increase in caspase-12, the GRP78/BiP protein (involved in endoplasmic reticulum homeostasis) and transcription factor CHOP (C/EBP homologous protein, a transcription factor involved in stress-induced apoptosis in the endoplasmic reticulum), and a slight decrease in the anti-apoptic protein Bcl-2. These results strongly suggest that the endoplasmic reticulum plays a role in the apoptosis induced by BPA. The effects of BPA are accompanied by oxidative stress, with an increase in reactive oxygen species (ROS) counteracted by antioxidant N-acetylcysteine (N-AC). At the concentration of 100 µM, the effects of BPA do not appear to be mediated by oestrogen receptors; the oestrogen receptor inhibitors 4-OHT and ICI do not prevent the

cytotoxicity of BPA and 4-OHT enhances it (Note: 4-OHT has a partial agonist effect on oestrogen receptors).

Table 26. Summary table of the studies examining the effects of bisphenol A on metabolism and the cardiovascular system

Reference	**Species/strain**	**Routes**	**Dose** **Exposure period**	**Effects** **NOAEL/LOAEL**
(Alonso-Magdalena, 2010)	Mice	Sub-cutaneous	0 - 10 and 100 µg/kg bw/day GD9 to GD16	In F1 offspring, 6-month males had ↘ glucose tolerance, ↗ insulin resistance, and ↗ plasma levels of insulin, leptin, triglycerides and glycerol, altered calcium signalling in islets of Langherans ↘ BrdU incorporation into insulin-producing β cells , whereas their surface was unchanged. In mothers, ↗ insulin resistance induced by gestation and ↘ glucose tolerance. dose-dependent ↗ in plasma levels of insulin, leptin, triglycerides and glycerol. ↘ insulin-stimulated Akt phosphorylation in gastrocnemius skeletal muscle and liver. 4 months post-partum: higher body weight, higher concentrations of insulin, leptin, triglycerides and glycerol
Ryan, 2010	CD-1 mice	Oral	0.25 µg/kg bw/day	In F1 offspring, ↗ body weight in males and females at 3 weeks ↗ body length in males at 4 weeks, these biometric

			GD0 to PND21	differences disappearing in adulthood. No significant effects on glucose tolerance were observed.
(Somm, 2009)	Sprague Dawley rats	Oral	70 µg/kg bw/day GD6 - PND21	**At birth**: BPA treatment during gestation did not affect sex-ratio or litter size. Newborns (♀ and ♂): ↗ weight **PND21** ↗□body weight in females Increased parametrial fat associated with adipocyte hypertrophy and overexpression of lipogenic genes and lipogenic enzymes In the liver, increased RNA levels of C/EBP-α, SREBP-1C, ACC and FAS k. Circulating lipids and glucose were normal. **4 to 14 weeks**: no difference in body weight observed between BPA-treated males and control animals on standard chow diet. ↗ body weight in BPA-exposed males fed a high-fat diet. ↗ body weight in females for the 2 tested diets. In males fed a high-fat diet, normal glucose tolerance test results. <u>Conclusion</u>: Perinatal exposure to BPA.

				↗□Adipogenesis at weaning in ♀. In adult ♂, ↗ body weight observed if high-fat diet.

Recent studies from 2011-2012:

New experimental *in vivo* studies indicate that BPA exerts effects on the endocrine function of the pancreas (secretion of insulin). This effect is also reported in some epidemiological studies. The impact of BPA on lipogenesis, and therefore its influence on the risk of obesity, is reinforced by new studies which are experimental (*in vivo* and *in vitro*) as well as epidemiological. These new studies have therefore reinforced the observations initially made in the 2011 report as well as the selection of the key study for the health risk assessment by considering the effect on metabolism.

Conclusion for hazard assessment in animals:

In animals, studies examining effects on enzyme activity, growth and metabolism suggest that rodents exposed in adulthood or during gestation undergo metabolic changes in various organs such as the liver, adipose tissue and pancreas. Moreover, a few authors have noted changes in the expression of protein-coding genes intervening in the cell signalling pathways involved in lipogenesis and carbohydrate metabolism. There is a trend showing *in vivo* effects on lipogenesis. *In vitro* mechanistic studies support these observations.

However, the effects on carbohydrate metabolism cannot be confirmed on account of insufficient repeatability.

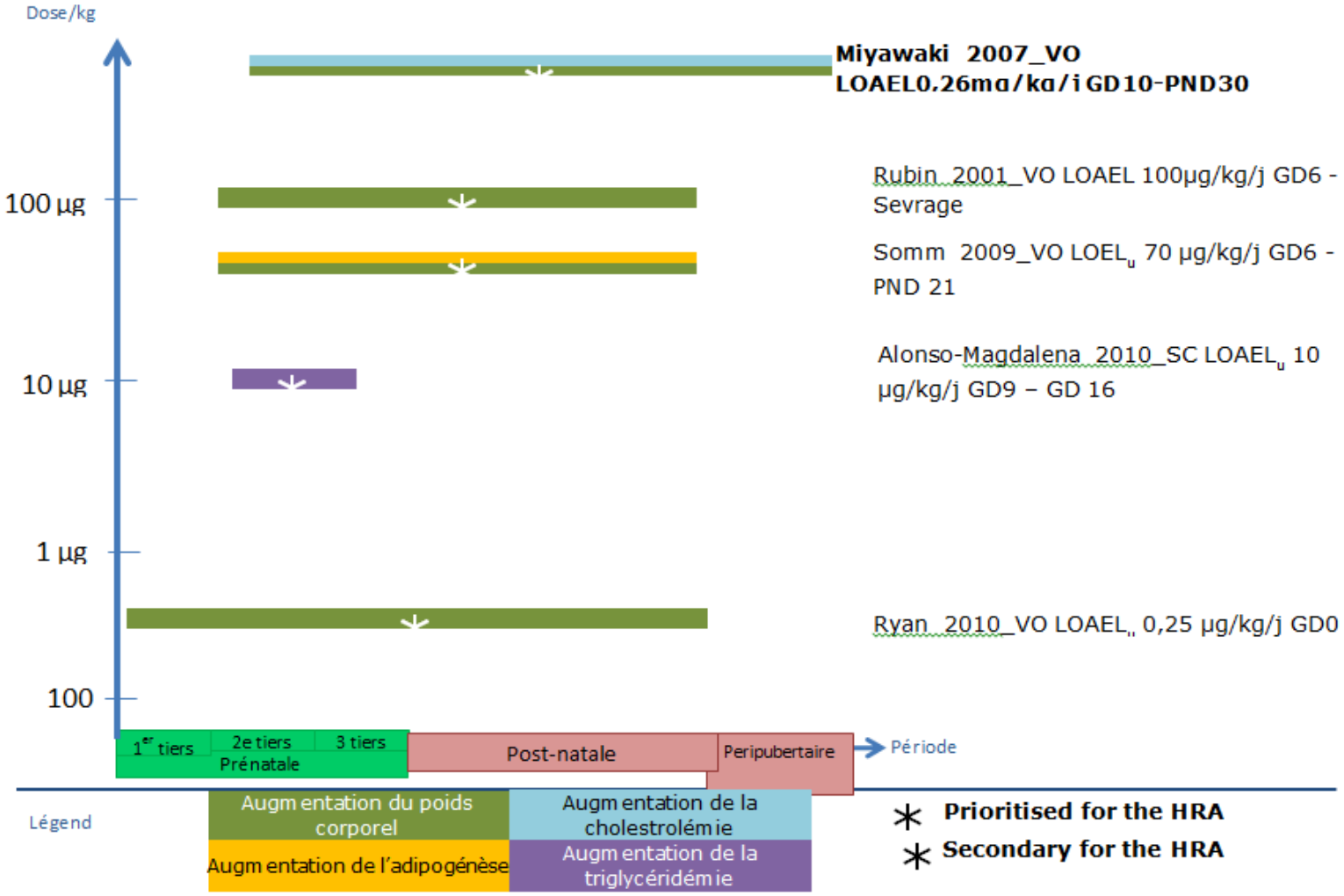
Thus, in animals, BPA increases blood lipid levels, leads to excess body weight and enhances lipogenesis. The effects on lipogenesis (*in vivo* and *in vitro* data), after pre- or perinatal exposure or exposure in adulthood, are considered to be recognised. The effects on glucose metabolism after pre- or perinatal exposure to BPA are considered to be controversial.

Changes in lipid metabolism are effects that are taken into account for the risk assessment.

Selecting the critical effect:

Several types of effects were observed in animals in relation to cardiovascular diseases (coronary artery disease) and diseases related to metabolism. The studies considered to be of good quality were considered first. A way of representing the identified NOAELs/LOAELs graphically was developed (see the figure below) in order to help with the selection process.

Figure 14. Effects of BPA on metabolism and obesity



Increase in body weight	**Increase in cholesterolemia**	**Increase in adipogenesis**	**Increase in triglyceridemia**
Miyawaki 2007_VO LOAEL 0.26 mg/kg/d GD10 - PND30**	**Miyawaki 2007_VO LOAEL 0.26 mg/kg/d GD10 - PND30****	Somm 2009_VO LOELu 70 µg/kg/d GD6 - PMD 21*	**Alonso-Magdalena 2010_SC LOAELu 10 na 2010 kg/d GD10 - P**
Rubin 2001_VO LOAEL 100 µg/kg/d GD6 – Weaning*			
Somm 2009_VO LOELu 70 µg/kg/d GD6 - PMD 21*			
Ryan 2010_VO LOAELu 0.25 µg/kg bw/d GD0-PND21*			

Among all of the observed effects, the increase in body weight, the increase in plasma lipids (such as cholesterol and triglycerides), and the increase in lipogenesis were retained as the critical effects.

Selecting the key study:

The Miyawaki, 2007 study is retained as the key study (see detailed description below). This study was conducted orally (by administration in drinking water) in pregnant ICR mice (n = 3 animals/group) and includes 2 exposure doses in addition to the control group: 1 µg/ml and 10 µg/ml (respectively equivalent to 0.26 mg/kg bw/d and 2.72 mg/kg bw/d). Pregnant mice were exposed from GD10 and then until weaning. This type of treatment allows exposure of the young through placental transfer and/or via breast milk. After weaning, the offspring were also treated with BPA through drinking water until PND30. This study did not follow OECD guidelines or GLP. Nevertheless, the study protocol is well described. The drinking water was delivered in glass bottles and the cages were made of polypropylene. A food containing 30% lipids (lipid-rich diet) was used in this study. Many biological parameters, in particular in relation to lipid metabolism, were investigated. Specifically, the total body weight of the male and female offspring, the weight of the perigonadal adipose tissue, the concentration of serum leptin, the total serum cholesterol, triacylglycerol, nonesterified fatty acids and glucose were measured.

Miyawaki, 2007 study

In order to investigate the effects of perinatal exposure to BPA on obesity/hyperlipidemia, Miyawaki et al. (2007) carried out a study on pregnant ICR mice fed a high-fat diet. The diet contained 30% kcal as lipids, 55% kcal as carbohydrates, and 15% kcal as proteins, with total energy of 4,353 kcal/kg. All mice (n=3 per group) *were* housed in *polypropylene* plastic *cages* with *ad libitum* access to water. Exposure to BPA was *via* drinking water, which was delivered in glass bottles. In addition to control group, two concentrations were tested: 1 µg/mL (LD

group) and 10 µg/mL (HD group), equivalent to 0.26 mg/kg bw/d and 2.72 mg/kg bw/d, respectively, as calculated by the authors on the basis of daily water intake on GD 13 and 16. Exposure period was between gestation day (GD) 10 and throughout the lactating period. After weaning, the pups were allowed free access to high-fat diet and to drinking water containing the appropriate concentrations of BPA. The number of females and males were 19 and 23 in the control group, 16 and 25 in the LD group, and 19 and 19 in the HD group, respectively. Thirty days after birth, the feed was removed from the cages and the following day all mice were sacrificed. In addition to total body weight and adipose tissue (parametrial and epididymal, for females and males respectively) the levels of serum total cholesterol, triglycerides, non-esterified fatty acids, leptin and glucose were measured. This study was not performed according to OECD guidelines or in compliance with GLP.

Thirty one day after birth, in female offspring, the mean body weight increased by 13% in the LD group and by 11% in the HD group, compared with the control group ($p<0.05$). The mean adipose tissue weight of the LD group increased by 132% compared with controls ($p<0.01$), whereas the increase was not significant for the HD group. In males, the mean body weight and the mean adipose tissue weight increased by 22 and 59%, respectively, compared controls, whereas the increase was not significant for the LD group.

Regarding serum analyses, a significant increase (123%, $p<0.05$) was observed in leptin levels, but only in females of the LD group. Compared to the corresponding control group, total cholesterol level increased by 33 and 17% in the females of the LD and HD groups, respectively ($p<0.01$). No changes were observed in males for this parameter. In males, the mean triglyceride and non-esterified fatty acid levels of the LD group increased by 34 ($p<0.05$) and 29% ($p<0.01$), whereas no significant differences were observed between the HD group and controls. No significant differences were observed in females with regards to these parameters as well as for blood glycaemia. The mean glucose level of the male LD group, but not the HD group decreased by 41% compared with the control group ($p<0.05$).

These data indicate that perinatal exposure to BPA of mice fed a high-fat diet increased the mean body weight and the mean adipose tissue weight, as well as serum lipid levels. The effect of BPA on adipose tissue mass was found to be more pronounced in females than in males.

- Weaknesses: limited number of animals, only two doses tested, nutritional conditions only based on high-fat diets, exposure estimated on the average amount of water consumed
- Strengths: Although this study shows some weaknesses it was considered as a key toxicological investigation to be used in the risk characterization of BPA. The protocol of the experiment and the technical quality of the analyses were considered as good. These data were supported by those provided by Alonso-Magdalena et al. (2010) carried out in mice injected subcutaneously with BPA at doses of 10 and 100 µg/kg bw/d. in which, 4 months after birth, an increase in body weight (significant at 100 µg/kg bw/d) as well as an increase in triglyceride (from 10/kg bw/d) and insulin, leptin, and glycerol concentrations at 100 µg/kg bw/d were observed. *In vitro* studies also support the observations that BPA increases lipogenesis in adipocytes. Put together, these data constitute a body of reliable, accurate and consistent evidence of metabolic disruption of BPA in mice.

In conclusion, a LOAEL of 0.26 mg/kg bw/d of BPA based on an increase in body weight and an increase in cholesterolemia in females was determined using this study.

Critical analysis of Delclos et al., 2014 **regarding the effect of BPA on metabolism and obesity[18]**

The paper recently published by Delclos et al. (2014) in rats provided additionnal information on different toxicological endpoints targeted by BPA. Although the primary goal of the study was to focus on reproductive and developmental toxicity of a large range of doses of BPA, some of the endpoints and clinical markers monitored were similar to those investigated previously Miyawaki et al. (2007). In particular, the body weight, the adipose tissue weight, the serum leptin concentrations and the serum lipid and glucose levels were reported.

Briefly, the experimental conditions described in the study from Delclos et al. were as follows : Sprague Dawley rat dams were dosed daily with BPA by gavage from gestation day 6 until the start of labor, and their pups were directly dosed from day 1 after birth to termination (postnatal day 90). In addition to control (vehicle) nine different doses were administered, namely 2.5, 8, 25, 80, 260, 840, 2700, 100,000 and 300,000 µg/kg bw/d. Two groups received ethinyl estradiol (0.5 and 5 µg/kg bw/d) as positive control. A minimum of 18 dams and 18 pups (males and females) was used per group.

Major differences between Delclos and Miyawaki studies were summarized in the box below.

[18] This study was not investigated during the elaboration of the proposal since it was published subsequently. Quoted during the public consultation and considered by RAC as a key study, it is now analysed and incorporated in the BD.

Study	**Miyawaki et al., 2007.** **Perinatal and postnatal exposure to Bisphenol A increases adipose tissue mass and serum cholesterol level in mice. Journal of Atherosclerosis and thrombosis Vol 14 N°5**	**Delclos et al., 2014.** **Toxicity evaluation of Bisphenol A administered by gavage to Sprague Dawley rats from gestation day 6 through postnatal day 90. Toxicological Sciences 139 (1) 174-197**
Type of study like 1 or 2 generation with/without prenatal exposure		Exposure from gestation day 6 (F0) through postnatal day 90 (F1)
Objectives of the study	The focus of the study was to investigate the effects of BPA on obesity and hyperlipidemia.	The focus of the study was on the reproductive toxicity of BPA. However, other endpoints that have been associated with BPA in animal and/or human studies were also evaluated including fat pad weights, serum levels of glucose, triglycerides, insulin, cardiac troponin, and histopathology of the heart and blood vessels.
reports, scientific publications with original data or review	Scientific publication with original data	Selected data are presented in tables and figures or as Supplementary data, whereas all data collected on the study are available in the NCTR Study Report (available upon request).
Respect of guidelines procedures	No	This study was conducted in compliance with the FDA Good Laboratory Practice Regulations (21 CFR, Part 58). All animal use and procedures for this study were approved by the NCTR Laboratory Animal Care and Use Committee and conducted in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. The health of the animals was monitored during the study in accordance with NCTR's Sentinel Animal Program and the sentinel animals were determined to be free of pathogenic organisms.

Fundings	Grants from the Japan Society for the promotion of science and from Ehime prefectural University of Health Sciences.	Study conducted under the auspices of the National Toxicology Program and funded by an Interagency agreement (IAG) between the Food and Drug Administration and the National Institute of Environmental Health Sciences/National Institutes of Health (FDA IAG: 224-12-0003; NIEHS IAG: AES12013).
Chemical, CAS number, purity, composition, vehicule		CAS no. 80-05-7 TCI America lot no. 111909/AOHOK (air-milled) with purity of 99.9% The vehicule for BPA and EE2 was 0.3% aqueous carboxymethylcellulose (CMC) from Sigma-Aldrich (St Louis, MO; catalog no. C5013, lot no. 048K0023 BPA and EE_2 doses were prepared in the vehicle, 0.3% CMC in water, and administered to the animals seven days a week at a rate of 5 ml/kg body weight
Species / strain investigated	ICR mice	Sprague-Dawley rats from the NCTR colony
Sex and number of animals per group	The number of dams is 3 per group, but statistical data are made with the number of pups ranging from 16 to 19 .	Four hundred males and 400 females weanling [*ca* postnatal day (PND 21)] NCTR Sprague Dawley cesarean-derived (CD) rats (strain code 23) were obtained from the NCTR breeding colony in four loads of 100 of each sex spaced three weeks apart. Approximately two weeks prior to mating, female breeders were randomized to treatment groups stratified by body weight to give approximately equivalent mean body weights in each group. Animals were mated in four loads spaced three weeks apart. For each load, there was a total of 13 groups of 6 to 12 dams per group making a total of 28 to 36 dams per group.
Control group and number	1 control group with 3 dams and a total of 16 pups	There were 2 negative controls, the naïve group (30 dams), the vehicle group (28 dams) and 2 positive groups with the EE2 (32 dams each)

Positive control	No	2 doses of Ethinyl estradiol (EE2) selected based on previous studies of the laboratory which are 5 and 0.5 microgr/kg bw/day
Life conditions (humidity, light/dark cycle, Conditions de vie, diet, number of animals per cage)	Mice were housed in a 12 h light/dark cycle and allowed free access to food and water	Throughout the study, animal rooms were maintained at 23 ± 3ºC with a relative humidity of 50 ± 20% and a 12 h light/dark cycle, with lights on at 6 a.m., and food and water were available *ad libitum*. There were at least 10 room air changes per hour.
Exposure route	Drinking water	Gavage
Frequency and period of exposure	Pups are exposed through their dam from GD10 to weaning and then directly through the drinking water after weaning to termination (PND 30)	Sprague-Dawley rats were dosed daily (gavage) from gestation day 6 until the start of the labor, and their pups were directly dosed from day 1 after birth to termination (day 90).
Doses / concentrations of BPA used	They were exposed to BPA in their drinking water at concentrations of either 1 µg/mL (LD group) or 10 µg/mL (HD group) beginning on day 10 of pregnancy. BPA was dissolved in absolute ethanol and the final concentration of ethanol was 0.2%. The control group received drinking water containing only 0.2% ethanol. Water intake on days 13 and 16 of gestation was determined by measuring the difference in the amount of water placed in the water bottle each day and the amount remaining the following day, and the levels of BPA consumed daily were estimated. Daily water intake on gestation day 13 and 16 was 11.9 ± 5.8 and 12.6 ± 1.1 mL, respectively, in the control group, 10.5 ± 3.9	The "low" [below the current no-observed-adverse-effect level (NOAEL)] BPA doses in this study (2.5–2700 µg/kg bw/day) were selected to cover a range where effects of BPA have been reported in various research studies and were spaced at an interval (approximately a half-log dose progression) such that a dose response could be established. This is the BPA dose range of regulatory concern. The two high doses of BPA selected were 100,000 and 300,000 µg/kg bw/day.

	and 13.5 ± 3.7 mL, respectively, in the LD group and 10.8 ± 5.6 and 16.3 ± 0.9 mL, respectively, in the HD group. Body weight on gestation day 13 and 16 was 42.5 ± 2.0 and 53.0 ± 2.3 g, respectively, in the control group, 41.1 ± 0.2 and 51.5 ± 1.1 g, respectively, in the LD group, and 42.9 ± 3.2 and 53.1 ± 4.4 g, respectively, in the HD group. Based on these values, the levels of BPA consumed daily were estimated to be 0.26 ± 0.09 (gestation day 13) and 0.26 ± 0.08 mg/kg body weight (gestation day 16) in the LD group and 2.42 ± 1.12 (gestation day 13) and 3.01 ± 0.89 mg/kg body weight (gestation day 16) in the HD group. The average BPA intake was 0.26 mg/kg body weight/day in the LD group and 2.72 mg/kg body weight/day in the HD group.	
Observations / endpoints studied	Female pup body weights, adipose tissue weight, serum leptin concentration, serum total cholesterol, triglycerides, non-esterified fatty acids and glucose	Gestational body weight gain from GD 6 and litter endpoints ; pup preweaning survival ; body weights with growth curves over the entire period of study for males and females; hormone dosages; markers of sexual development and reproductive endpoints; organ weights at termination
Uncontrolled exposure (presence of phytoestrogens in the diet, polycarbonate cages in	The diet contained 30% kcal as fat, 55% kcal as carbohydrate, and 15% kcal as protein with total energy of 4,353 kcal/kg. The composition of the diet (in g/kg) was as follows: lard 72.5, soybean oil 72.5, cholesterol 2, sucrose 109, dextrin 150, corn starch 340, cellulose 40, milk casein 163, vitamin mixture	The study diet was soy- and alfalfa-free to minimize phytoestrogen content [5K96 verified casein diet 10 IF, round pellets, γ-irradiated (catalog no. 1810069), Test Diets, Purina Mills, Richmond, IN]. Extracts of each lot of diet were monitored for daidzein, genistein, and BPA content by liquid chromatography/mass spectrometry. The mean levels of daidzein and genistein in the six diet lots utilized in the study were 0.249 ± 0.064 (SD) ppm and 0.374 ± 0.118 ppm,

accommodation, composition of drinking water, litter composition, etc.)	10, mineral mixture 40 and choline chloride 1. Glass water bottles were used to ensure that related compounds did not leach from plastic water bottles.	respectively. The mean level of BPA measured in the diet was 2.6 ± 0.8 ppb. Other study materials screened for BPA levels included animal bedding, polysulfone cage leachates, silicone water bottle stoppers, and drinking water. None of these materials had BPA levels detectable above the average analytical blanks.
Statistical analysis and quality criteria associated	Significant differences among three independent groups were evaluated statistically using one-way analysis of variance, and subsequent comparisons were performed using the Tukey-Kramer test, which allows for unequal sample sizes. Significant differences between two independent groups were analyzed by Student's t-test. Pearson r was used to calculate correlations between adipose tissue weight and body weight, and the levels of serum lipids and leptin. All statistical procedures were carried out using SPSS software (version 13.0). A p value of <0.05 was considered to indicate significant difference. All values were expressed as the mean± SE	The statistical methods used varied by endpoint. Treatment effects on dams during gestation: Gestational body weight was analyzed separately for each subgroup using ANOCOVA, with dose and number of pups in the litter as factors, dam body weight at time of mating as a baseline covariate, number of pups in the litter as a covariate, and an interaction between dose and number of pups. The interaction allows the dose to affect the impact that litter size has on body weight gain. Analysis of gestational length was performed within a one-way ANOVA. Metabolic efficiency is defined as the change in body weight divided by food consumption for a given time interval. Analysis of gestational metabolic efficiency was performed using ANOCOVA, with pup count as a covariate and the interaction of dose and number of pups. Litter endpoints : Litter sex proportions were analyzed using logistic regression. Pup counts (number alive, number of males, number of females, and number unsexed) were analyzed using Poisson regression. Due to sparse data for "born dead" counts, a Cochran-Mantel-Haenszel test was performed for overall differences between treatment groups. For litter body weight data, across and by sex, a one-way ANOVA was performed. For litter mean pup body weights, ANOCOVA was performed, adjusted for litter size. Numbers of implants and resorptions were analyzed separately using contrasts

		within a one-way ANOVA. F_1 pup pre-weaning survival and body weight : A modified Cox proportional hazards model was used to test for treatment differences in pup survival to the time of weaning. Pairwise comparisons were adjusted using Holm's method. Kaplan-Meier survival curves are presented, with uncensored defined as animals that were dead, moribund, or missing, and censored defined as terminal sacrifice, or any other planned removals (reallocated, discards, or surplus). Because there were three to five littermates per sex per dam, cross-sectional analyses of pre-weaning body weight were performed at PND 1, 4, 7, and 21 using a mixed model ANOVA accounting for litter correlation assuming a compound symmetric correlation structure. F_1 postweaning body weights, food consumption, and metabolic efficiency: Prior to analyses of body weight and food consumption, data from week 4 through 13 were regularized using LOESS smoothing for each animal's data to reduce outlier influence, normalize the data, and rescore to weekly intervals. For smoothing, a 12% window was used for body weight data and a 50% window was used for food consumption data. The window was larger for food consumption because food data were collected weekly compared to daily collection of body weight data. Using LOESS smoothed food consumption and body weight data, the average of metabolic efficiency was calculated for each animal by week. Average weekly body weight, food consumption, and percent metabolic efficiency were analyzed separately using the same statistical method. Pairwise comparison of means were performed using contrasts within a repeated measures mixed model ANOVA, with terms for dose group, week, and interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points.

		Age and Body Weight at Vaginal Opening, Testicular Descent, and Preputial Separation: For each subgroup, a two-factor mixed model was conducted with dose level and study arm as factors along with their interaction. Examination of the data indicated that a transform was needed for the event age. A log-transform of the left-shifted age was found to be appropriate: transformed age = log(age – left-shift). For vaginal opening, the left-shift was 25 days, for testicular descent, the left-shift was 20 days, and for preputial separation, the left-shift was 21 days. The variance was also not homogeneous among the dose levels for event age or body weight at occurrence. This was modeled within the mixed model by allowing each dose level a separate variance. Because littermates were present across the study arms, littermate correlation was modeled assuming a common correlation among littermates. The sample sizes given show the number of litters from which animals were derived. Organ weights Statistical analyses were conducted by ANOVA for absolute organ weight and by ANOCOVA with covariates brain and body weight. Separate analyses were performed with each covariate.
Observed effects - general toxicity / Mother		Treatment Effects on Dams During Gestation and Litter Endpoints: No treatment significantly affected gestation length or gestational metabolic efficiency. BPA doses ≤ 2700 µg/kg bw/day (n= 18-26) did not affect gestational body weight gain, but the 100,000 (n=24) and 300,000 (n=25) µg BPA/kg bw/day doses reduced body weight gain over the period from GD 6 to parturition by 16% and 11%, respectively. Similarly, both 0.5 (n=20) and 5.0 (n=21)µg EE_2/kg bw/day reduced gestational body weight gain by 7% and 14%, respectively. Controls, n=23; naïve, n=25. F_1 Pup Preweaning Survival : There was a significant difference in survival between the naïve and the vehicle males (100% vs. 88.3%, respectively). The only significant BPA effect on pup preweaning survival was a decrease by 35%, both in males and females, in the

		300,000 µg/kg bw/day dose group. EE_2 did not significantly affect this endpoint. The n value par group ranges around 20 more or less. Body weights : There were no differences in bw between controls and the low dose BPA groups in either sex. Body weights of females and males in the highest BPA dose group (300,000 µg/kg bw/day) of females and males were consistently lower than vehicle control throughout the study, varying from approximately 6–13% (average across weeks approximately 10%) lower. In females, low dose EE_2 significantly increased body weight (approximately 6–9%) on weeks 5–9 and the high dose EE_2 significantly increased (average across weeks approximately 10%) body weights from week 6. Male body weight was not affected by EE_2. The n value par group ranges around 20 more or less.
Observed effects – reprotoxicity	In female offspring (F1), • Increase in the average body weight of 13% (low dose) and 11% (high dose) • Increase in the average weight of adipose tissue of 132% (low dose) • Elevated cholesterol levels by 33% (low dose) and 17% (high dose) • Increase in serum leptin concentration of 123% (low dose) • No change in blood sugar. In male offspring (F1) • Increase in the average body weight of 22% (high dose) • Increase in the average weight of adipose tissue by 59% (high dose) • ↑ concentration of triacylglycerol (34%) and non-esterified fatty acids (29%) (low dose).	

	• Decreased glucose by 41% (low dose).	
Critical effects considered		
NOAEL / LOAEL values considered for determination of the critical effect	Loael 0.26 mg / kg / day of BPA / kg bw / day based on the body weight increase, increased cholesterol levels in females is determined from this study.	NOAEL = 2700 µg/kg bw/d
Conclusions of the authors	Non-monotonic dose response	The experimental model was sufficiently sensitive to detect clear effects of EE_2 at both dose levels tested, as well as of the high BPA doses. For example, F_1 females and males had body weights that differed from vehicle controls across the study period only at the highest BPA dose tested (300,000 µg /kg bw/day), with a treatment-induced depression of body weight in both sexes. In the present study, the low dose range of BPA did not significantly affect body or fat pad weights nor the serum levels of glucose, triglycerides, insulin, or leptin. Likewise, no effects on thyroid hormones, thyroid weight, or thyroid histology were observed in the low BPA dose region.
Comments and conclusions	**Effects on lipid metabolism and body weight gain, not connected with the dose.**	**The authors have either used BPA at doses at least 2-fold lower than the NOAEL of 5 mg/kg/day or doses 20 and 60-fold higher than the NOAEL. The authors should have at least tested the NOAEL and the 10 NOAEL doses.**

In contrast to the paper from Miyawaki et al., the data reported by Delclos et al. indicate that F1 females and males had body weights that differed from vehicle controls across the study period only at the highest BPA dose tested (300,000 µg/kg bw/d), with a treatment-induced depression of body weight in both genders. The effect on adipose tissue (reduction of mean weight) was confined to the highest dose tested in both males and females F1.

Regarding clinical chemistry measured at post-natal day 90, in females, the 100,000 µg/kg bw/d dose significantly depressed cholesterol and triglycerides by 16 and 30%, respectively, but there was no effect on these endpoints at 300,000 µg/kg bw/d. Leptin was significantly decreased (56%) in the highest BPA dose group. In males, 100,000 µg/kg bw/d and 300,000 µg/kg bw/d reduced cholesterol by 16 and 21% respectively. The 100,000 µg/kg bw/d dose group had a glucose level 8% lower than animals treated with the vehicle only, while the 300,000 µg/kg bw/d dose reduced leptin by 33%.There were no statistical significant differences between groups treated with a dose < 100,000 µg/kg bw/d and controls.

Several differences may explain the discrepancies between the data from Miyawaki et al. (2007) and Delclos et al. (2014).

- The study from Miyawaki et al. was performed on mice whereas Delclos et al. investigated the effect of BPA in Sprague Dawley rats. Differences in sensitivity of animal species/strains to BPA have been reported by several authors.

- The route of exposure was via drinking water for Miyawaki et al and by gavage for Delclos et al., which may result in significant differences in terms of toxicokinetics

- The period of exposure was from GD10 to PND 30 for Miyawaki et al and from GD6 to PND90 for Delclos et al.
- Sacrifice and sampling time were at PND 31 for Miyawaki et al and PND 90 for Delclos et al.

- Cages were in polycarbonate and polysulfone, in Delclos et al. and part of the experiment was performed with polycarbonate water bottles, whereas for Miyawaki et al only glass bottles and polypropylene cages were used. Polycarbonate and polysulfone are two types of plastics containing BPA.

As a complement, the study from Wei et al. 2009 (quoted in the Table of Annex 4 in "Miyawaki, 2007" line) and the recent study from Marmugi et al. 2014 are also described and analysed below.

Study	**Wei et al 2014** **Perinatal exposure to Bisphenol A exacerbates non-alcoholic steatohepatitis-like phenotype in male rat offspring fed a high-fat diet. Journal of Endocrinology 222,**

	313-325
Type of study like 1 or 2 generation with/without prenatal exposure	Exposure from gestation day 0 (F0) through postnatal day 21 (F1) and follow-up at the age of 27 weeks
Objectives of the study	The focus of the study was to describe the adverse effects of BPA on the metabolic functions of the liver.
reports, scientific publications with original data or review	Scientific publication with original data
Respect of guidelines procedures	No. However, all animal experiments were carried out humanely following the guidelines for the care and use of animals established by Tongji Medical College and was approved by the Ethics Committee of Tongji Medical College.
Fundings	This work was supported by the National Program on Key Basic Research Project of China (973 Program) (2012CB722401), the National Natural Science Foundation of China (81030051, 21177046, and 21207128), the R&D Special Fund for Public Welfare Industry (Environment) (201309048), the National Basic Research Development Program of China (2008CB418206), the Fundamental Research Funds for the Central Universities, Huazhong University of Science and Technology (2012QN240 and 2012TS072), and the Natural Science Foundation of Fujian Province, China (2013J05033).
Chemical, CAS number, purity, composition, vehicle	Not indicated. However, in their previous study (Wei et al., 2011), the authors indicated that BPA was from Sigma-Aldrich with a purity of >99% and a CAS number of no. 80-05-7. Vehicle was corn oil (Sigma Aldrich, CAS no. 8001-30-7
Espèce / souche étudiée / âge - poids	Wistar rats
Sex and number of animals per group	The number of dams is 6 per group. Offsprings were culled to five males and five females in each litter after delivery and were nursed with their own mother. Statistical data are made with n=3 to 6 rats depending on end-points with only one offspring selected per litter. In this study, only males are studied.
Control group and number	This study is a continuation of a former study (Wei et al., 2011) in which 3 doses of BPA were considered including the reference dose of 50 microgr/kg bw/day and two higher doses of 250 and 1250 with doses administered to dams through gavage throughout

	gestation and lactation. Dosages were adjusted daily for body weight changes of dams (2 ml/kg bw)
Positive control	No
Life conditions (humidity, light/dark cycle, Conditions de vie, diet, number of animals per cage)	Wistar rats were housed in polypropylene cages with free access to food and water under standard conditions (22±2°C, 12h light: 12h darkness cycles). Glass water bottles were used to avoid potential contamination from sources other than administration.
Exposure route	gavage
Frequency and period of exposure	Dams are exposed from gestation Day 0 throughout lactation through daily gavages. At weaning, the offspring is either fed a standard diet or a high fat diet until the termination of the experiment by week 27. This age was selected based on the findings of the former study.
Doses / concentrations of BPA used	Pregnant female rats were administered corn oil or 50µg/kg per day BPA by oral gavage from gestational day 0 to postnatal day 21. Offspring were culled to five males and five females in each litter after delivery and were nursed with their own mother. After weaning, offspring were supplied with a standard chow diet (SD) or a HFD and allowed to feed *ad libitum*.
Observations / endpoints studied	The study is a continuation of a former study with 3 doses of BPA and a comprehensive characterization of the metabolic phenotype of the offspring of dams orally dosed throughout gestation and lactation. The conclusions of the former study are the following: On a normal diet, perinatal exposure to 50 µg/kg · d BPA resulted in increased body weight, elevated serum insulin, and impaired glucose tolerance in adult offspring. On a high-fat diet, such detrimental effects were accelerated and exacerbated. Furthermore, severe metabolic syndrome, including obesity, dyslipidemia, hyperleptindemia, hyperglycemia, hyperinsulinemia, and glucose intolerance, was observed in high-fat-fed offspring perinatally exposed to 50 µg/kg/d BPA. No adverse effect of perinatal BPA exposure at 250 and 1250 µg/kg/d was observed no matter on a normal diet or a high-fat diet. These results suggest that perinatal exposure to BPA at reference dose, but not at high dose, impairs glucose tolerance in adult rat offspring on a normal diet and predisposes offspring to metabolic syndrome at adult on a high-fat diet. High-fat diet intake is a trigger that initiates adverse metabolic effects of BPA. Given the central role of the liver in lipid and glucose metabolism and the phenotype of insulin resistance and glucose intolerance in their former study, the authors have examined liver morphology and function, its sensitivity to insulin, lipid accumulation and steatosis, inflammation, fibrogenesis and oxidative stress.
Uncontrolled exposure	The SD contained 20.5% crude protein, 4.62% crude fat, and 52.5% nitrogen-free extract (total calories 3.45kcal/g, 12.05% calories in fat). The HFD contained 23.15% crude

(presence of phytoestrogens in the diet, polycarbonate cages in accommodation, composition of drinking water, litter composition, etc.)	protein, 13.14% crude fat, and 50.94% nitrogen-free extract (total calories 4.15kcal/g, 28.53% calories in fat). Both standard chow and HFDs were purchased from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). All experiments were carried out on male offspring from different litters in each treatment group.
Statistical analysis and quality criteria associated	Data are expressed as mean±s.e.m. Statistical analysis was performed using the SPSS 17.0 software (SPSS, Inc.). One-way ANOVA followed by Bonferroni's *post hoc* test was used for comparison of the relevant groups A *P* value of <0.05 was considered significant.
Observed effects - general toxicity / Mother	The authors reported no variation in litter size and sex-ratio at birth in their first study (Wei et al., 2011) . This suggests no gross general toxicity in the dams.
Observed effects – reprotoxicity	Development of fatty liver disease (abnormal accumulation of lipids in the liver) in agreement with the study of Marmugi et al (2012) as a result of disturbances between triglycerides (TG) delivery, synthesis, export and oxidation. The model recapitulates many of the features of the metabolic syndrome.
Critical effects considered	All events leading to NASH
NOAEL / LOAEL values considered for determination of the critical effect	Different doses were used in the former study. No effects were observed with the doses of 250 and 1250 µg/kg bw/day. The only active dose was 50 µg/kg bw/day.
Conclusions of the authors	Perinatal exposure to BPA at a reference dose of 50 µg/kg bw/day exacerbates non-alcoholic steatohepatitis-like phenotype in male rat offspring fed a high-fat diet. The authors propose a 2 hit model in which BPA impairs liver function in the foetus and during lactation. At weaning when rats are fed a high fat diet, then animals "primed" with BPA develop fatty livers and a non-alcoholic steatohepatitis-like phenotype.
Comments and conclusions	**The study of Wei & co-workers is a continuation of a former study (Wei et al 2011) in which a perinatal exposure to BPA at reference dose was described as predisposing offspring to metabolic syndrome in adult rats on a high fat diet. Thus the objective of the study was to characterize the mechanisms of the long-term effects initiated by a perinatal exposure to BPA. The rationale is based on**

	the developmental origin hypothesis known as the Barker's hypothesis which stipulates that many adult diseases find their origin during the maternal period. Indeed, this is a critical period for programming and false programming may thus result in the development of adult diseases. Because the liver is a key organ for glucose and lipid metabolism, the authors investigated the hepatic functions in the offspring of rats perinatally exposed to BPA at 50 µg/kg bw/day by gavage of the dams from Day 0 of gestation to weaning of the offspring. They conclusively described that the liver was insulin-resistant and steatotic with enhanced hepatic oxidative stress, inflammation and fibrosis. All these adverse effects were aggravated when rats were fed a high fat diet. Because BPA is a short-lived pollutant and exposure to BPA was terminated at weaning, the authors concluded that BPA could modify the programming of the metabolically active tissues including the liver through epigenetic mechanisms not described in the study. It will be of interest to examine the hepatic function in weaning rats and search for direct effects of BPA. This aspect is missing in the study. **Although the n value ranged from 3 to 6 depending on the end-point considered, each male originated from a different litter and all the end-points examined converged to the conclusion that the livers accumulated lipids and were insulin-resistant. Such feature is a metabolic risk factor for the progression to nonalcoholic steatohepatitis (NASH). In addition, these data corroborate findings published by other groups including the publications of Rubin & Soto 2009; Somm et al, 2009; Alonso-Magdalena et al 2010.**

Study	**Marmugi et al 2014** **Adverse effects of long-term exposure to bisphenolA durng adulthood leading to hyperglycemia and hypercholesterolemia in mice. TOXICOLOGY 325 (2014) 133-143**
Type of study like 1 or 2 generation with/without prenatal exposure	Exposure for 8 months starting with 6-week old male CD1 mice
Objectives of the study	The focus of the study was to describe the adverse effects of BPA on the metabolic functions of the liver as a continuation of the first study published in Hepatology 2012. Especially, this study aimed to investigate the effects of

	long-term BPA exposure (8 months) using a wide BPA dose range (0, 5, 50, 500, 5000 µg/kg/day), below or equivalent to the current NOAEL on CD1 adult mouse energy metabolism.
reports, scientific publications with original data or review	Scientific publication with original data
Respect of guidelines procedures	No. However, *In vivo* studies were conducted under E.U. guidelines for the use and care of laboratory animals and were approved by an independent ethics committee.
Fundings	This work was supported by grants from the ANR (CES; PerinaTox program). Alice Marmugi was funded by a grant from INRA Animal Health Department and the Midi-Pyrénées region (France)
Chemical, CAS number, purity, composition, vehicule	BPA : 4,4'-dihydroxy-2,2-diphenylpropane, CAS# 80-05-7, Sigma–Aldrich, France Control animals were given water containing 0.36% ethanol corresponding to the concentration used as a vehicle for BPA solutions. Of note, Miwayaki used 0.2% ethanol ; Wei used corn oil; Delclos et al 2014 used 0.3% aqueous carboxymethylcellulose.
Specie/age/weight	6 week-old male CD1 mice (Charles River, Les Oncins, France)
Sex and number of animals per group	The number of dams is 6 per group and there are 4 groups with different BPA doses in addition to the control group . Animals were bought after weaning. Consequently, no information is provided on the litters. In this study, only males are studied.
Control group and number	This study is a continuation of a former study (Marmugi et al 2012) in which a transcriptomic approach was performed in the livers of male CD1 mice exposed for 28 days to different doses of BPA (0, 5, 50, 500, and 5,000 µg/kg/day). Dosages were estimated to corresponding to BPA: 5, 50, 500, and 5,000 µg/kg/day based on water consumption and body weight per week. It is not indicated if water consumption was different between groups including the control group.
Positive control	no
Life conditions (humidity, light/dark cycle, Conditions de vie, diet, number of animals per cage)	Animals were kept in polypropylene cages and water bottles were used to avoid contamination of mice with free BPA. Animals were fed a standard diet (A04, from SAFE Diet, Augy, France) (housing at 22 ± 2 °C, 12 h light/dark).
Exposure route	Drinking water
Frequency and period of exposure	Animals are exposed daily through drinking water for 28 weeks starting at 6 weeks of age

Doses / concentrations of BPA used	Dosages were estimated to corresponding to BPA (0, 5, 50, 500, and 5,000 µg/kg/day based on water consumption and body weight per week.
Observations / endpoints studied	Plasma lipid profiles and liver analysis were performed in control and BPA-treated animals. Glycaemia, glucose tolerance and cholesterolemia are monitored so are genes involved in cholesterol metabolism using RT-qPCR. Genes regarded included *Mvd*, *Lss Hmgcr*, and *Sqle*, and the sterol regulatory element-binding proteins 2, a master regulator of hepatic cholesterol biosynthesis. All was performed at 34 weeks of age except for the Glucose tolerance tests realized at 2 and 4.5 months
Uncontrolled exposure (presence of phytoestrogens in the diet, polycarbonate cages in accommodation, composition of drinking water, litter composition, etc.)	Animals were fed the A04 from SAFE diet whom composition is available at http://www.aston-pharma.com/ productFiles/165/ds-a04.pdf. This is a standard diet with 3.1% lipids. Animals were kept in polypropylene cages and water bottles were used to avoid contamination of mice with free BPA.
Statistical analysis and quality criteria associated	All the data were analysed using R (www.r-project.org). Differential effects were analysed by analysis of variance (ANOVA) followed by Student's *t* tests with a pooled variance estimate. $P \leq 0.05$ was considered significant. Of note, all data are expressed with n=6 and there were 6 animals per group. It means that no value has been discarded indicating that protocols were carefully handled.
Observed effects - general toxicity / Mother	There is no apparent liver toxicity based on lack of changes in liver weight and lack of changes in the concentrations of the ASAT and ALAT.
Observed effects – reprotoxicity	Not concerned
Critical effects considered	hepatic and plasma metabolic markers
NOAEL / LOAEL values considered for determination of the critical effect	First metabolic changes were seen with the first dose corresponding to 1/10 of the TDI dose in agreement with the former study of the authors (Marmugi 2012)
Conclusions of the authors	In the present study the authors demonstrate that BPA exposure during 8 months in adult mice results in metabolic disorders consisting in increased adipose tissue mass, hyperglycaemia, glucose intolerance, hypercholesterolemia and increased cholesterol biosynthesis by the liver.
Comments and conclusions	**The study is a continuation of a former study (Marmugi 2012) in which the authors have suggested that exposure to low doses of BPA may influence *de novo* fatty acid synthesis through increased expression of lipogenic genes, thereby contributing to hepatic steatosis. The second important point of that study was that effects observed showed a non-monotonic dose response since a stronger**

	response was seen in the liver of mice receiving 50 µg/kg bw per day than those receiving mice receiving 5000 µg/kg bw per day. In the present study, the long-lasting effects are being described focusing on hepatic and plasma metabolic markers using the same range of doses from 1/10 TDI up to the NOAEL dose. **It is interesting to note that while the maximally effective dose of BPA depended of the studied metabolic pathway, its effects on cholesterol metabolism cannot be assigned to one specific dose. Worthy to note, the increase of cholesterol levels, observed from 5 to 5000 µg/kg/day BPA is a recognized risk factor for ischemic heart disease and coronary mortality.** **In addition, the highest dose used in this study corresponds to the no adverse effect level dose and chronic exposure to the NOAEL dose resulted in impaired glucose tolerance and hyperglycaemia. Hyperglycaemia is one of the 3 characteristics leading to diabetes with hyperinsulinemia and hypertriglyceridemia. The authors did not report hyperinsulinemia while this feature was reported in Marmugi 2012 with a 28-day exposure. This point is not discussed in the discussion and it may be of interest to give a look at the pancreas to clarify why insulin levels did not increase to face enhanced glycaemia.** **In the present study, the response profiles whether linear or non-linear with the doses of BPA used differ depended on the metabolic end-points considered. It is likely that this reflects different sensitivity of the end-points measured and whether the end-points measured are direct or indirect targets of BPA exposure.**

Selecting the benchmark doses:

In the Miyawaki, 2007 study, an increase in body weight of F1 females and male offspring is observed at PND31 at a dose of 0.26 mg/kg bw/d and a slight increase in body weight at a dose of 2.72 mg/kg bw/d (statistically significant in high doses and in females at the lowest dose):

In the female offspring (F1):

- Increase in mean body weight of 13% (low dose) and 11% (high dose),
- Increase in the mean weight of fatty tissue 132% (low dose),
- Increase in cholesterolemia of 33% (low dose) and 17% (high dose),
- Increase in serum leptin concentration of 123% (low dose),
- Absence of glycemic change.

In the male offspring (F1):

- Increase in mean body weight of 22% (high dose),
- Increase in the mean weight of fatty tissue 59 % (high dose),
- Increase in the concentration of triglycerides (34%),

- Increase in nonesterified fatty acids (29%) (low dose),
- Decrease in glycemia of 41% (low dose).

In conclusion, **a LOAEL of 0.26 mg/kg bw/d of BPA** based on an increase in body weight and an increase in cholesterolemia in females was determined using this study.

Other comments (uncertainties, confidence level, etc.)

As explained above, the Miyawaki, 2007 study presents certain limits. The number of animals investigated is restricted with 3 pregnant females treated solely by dose level. In addition, the amount of BPA administered relies on an estimate of the average amount of water consumed and weight gain per dose group. A monotonic dose-response relationship was observed in this study for body weight, but seems non-monotonic for change in triglycerides. No study to investigate the effects on lipid metabolism and covering the pre and postnatal period, conducted in accordance with good laboratory practices, was recorded. Other studies such as the Alonso-Magdalena, 2010 study, conducted subcutaneously at doses of 10 and 100 µg/kg bw/d show, 4 months after birth, an increase in body weight (significant at 100 µg/kg bw/d) as well as an increase in triglyceride (from 10/kg bw/d) and insulin, leptin, and glycerol concentrations at 100 µg/kg bw/d. *In vitro* studies support the observations that BPA increases lipogenesis in adipocytes.

As a complement, about the divergences between ANSES and EFSA regarding the effect of BPA on metabolism, see Annex 5 - *ANSES comments on EFSA draft opinion).*

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The table below provides a summary of the NOAELs/LOAELs selected for the HRA on BPA

Table 27. NOAELs / LOAELs selected for the HRA on BPA

Critical effects	**Study reference**	**Animal population**	**Exposure period**	**Route of exposure**	**Type of effect**	**LOAEL/ NOAEL**	**Population to be considered in the HRA**
Brain and behaviour							
	Xu, 2010	ICR mice	GD7-PND21	Oral (forced feeding)	**Decrease in the expression of NMDA receptors in connection with a**	NOAEL = 50 µg/kg bw/d	Pregnant women and their offspring

					disturbance in spatial memory and learning functions		
Female reproductive system							
	Signorile, 2010	Balb-C Mice	GD1-PND7	Sub-cutaneous	**Increase in the occurrence of ovarian cysts**	LOAEL 100 µg/kg bw/d	Pregnant women and their offspring
	Signorile, 2010	Balb-C Mice	GD1-PND7	Sub-cutaneous	**Endometrial hyperplasia**	NOAEL 100 µg/kg bw/d / LOAEL 1000 µg/kg bw/d	Pregnant women and their offspring
	Rubin, 2001	Sprague Dawley Rat	GD6 – weaning of young	Oral (drinking water)	**Disruption of ovarian cycles**	NOAEL 100 µg/kg bw/d / LOAEL 1.2 mg/kg bw/d	Pregnant women and their offspring
Metabolism and obesity							
	Miyawaki, 2007	Pregnant ICR mice	Treatment of mothers from GD10 until weaning of the young then treatment of the young from the day of weaning until PND30	Oral (drinking water)	**Increase in body weight, increase in cholesterol in females from 0.26 mg/kg bw/d**	LOAEL 0.26 mg/kg bw/d	Pregnant women and their offspring
Mammary							

gland							
	Moral, 2008	Sprague Dawley Rat	GD10-GD21	Oral (forced feeding)	**Effect on the buds and terminal breast ducts (Tan & TD)**	NOAEL 25 µg/kg bw/d	Pregnant women and their offspring
	Jenkins, 2009	Sprague Dawley Rat	PND2-PND21 (treatment of mothers with BPA and treatment of young with DMBA on PND50)	Orally	**Promoting effect in the presence of an initiator**	NOAEL 25 µg/kg bw/d / LOAEL 250 µg/kg bw/d	Juvenile
	Betancourt, 2010	Sprague Dawley Rat	GD10-GD21 and administration of DMBA at PND50 or PND100	Orally	**Promoting tumour effect in the presence of an initiator and shift of the period of susceptibility to DMBA**	NOAEL 25 µg/kg bw/d / LOAEL 250 µg/kg bw/d	Pregnant women and their offspring
	Vandenberg, 2008	CD1 female mice	GD8-PND16	Sub-cutaneous	**Ductal hyperplasias**	LOAEL 0.25 µg/kg bw/d	Pregnant women and their offspring
	Murray, 2007	Wistar Furth Rat	GD9-PND1	Sub-cutaneous	**Ductal hyperplasias**	LOAEL 2.5 µg/kg bw/d	Pregnant women and their offspring

B 5.11 Derivation of DNEL(s)/DMEL(s)

The toxicological benchmarks are derived for pregnant women consumer and her descendants and for pregnant women worker as cashier and her descendants, by cutaneous route and for the long term.

The table below summarises the effects and the selected LOAEL/NOAEL which are used to conduct the risk characterisation (RC).

Table 28. Summary of the effects and selected LOAEL/NOAEL for the RC

Critical effects on	**Reference study**	**Route of exposure**	**LOAEL**	**NOAEL**
			(µg/kg/d)	**(µg/kg/d)**
Brain and behaviour	**Xu, 2010**	Orally		**50**
Female reproductive system	**Rubin, 2001**	Orally	**/**	**100**
Metabolism and obesity	**Miyawaki, 2007**	Orally (drinking water)	**260**	**86.7***
Mammary gland	**Moral, 2008**	Orally (tube feeding)	**/**	**25**

*: NOAEL calculated using the LOAEL.

For a detailed description of these reference studies, see previous sections above.

The NOAEL/LOAEL resulting from the experimental data correspond to the external doses administered to animals. Concerning BPA, for which we know the significance of the effect of the first hepatic passage, only the unconjugated fraction of BPA is considered to be active and responsible for the effects observed. According to the data available, discussed here above (cf toxicokinetics by oral route), this fraction is estimated at 3% of the oral exposure dose. This factor is therefore used to firstly convert the NOAEL/LOAEL into equivalent internal doses.

The table below summarises the effects and the internal NOAEL calculated with a bioavailability factor of 3%.

Table 29. Summary of effects and the internal NOAEL calculated with a bioavailability factor of 3%

Critical effects on	Reference study	Route of exposure	LOAEL	NOAEL	Internal NOAEL by application of a bioavailability factor of 3%
			(µg/kg/d)	(µg/kg/d)	(µg/kg/d)
Brain and behaviour	**Xu, 2010**	Orally		**50**	**1.5**
Female reproductive system	**Rubin, 2001**	Orally	**/**	**100**	**3**
Metabolism and obesity	**Miyawaki, 2007**	Orally (drinking water)	**260**	**86.7***	**2.6**
Mammary gland	**Moral, 2008**	Orally (tube feeding)	**/**	**25**	**0.75**

*: NOAEL calculated using the LOAEL.

Secondly, an **assessment factor of 300** is applied if the starting critical dose is a NOAEL and an **assessment factor of 900** is applied if the starting critical dose is a LOAEL. This overall factor can be divided into several factors commonly applied in a QHRA (quantitative health risk assessment) and detailed in the Agency report on the "Method of construction of TRV based on the toxic effects on reproduction and development" (Afsset, 2007) and also justified by the guidance R8 of REACH (p.36). Within the context of this assessment, the following factors were considered:

- **Assessment factor relating to the use of a LOAEL: A factor of 3 is applied** when the critical dose corresponded to a LOAEL and not to a NOAEL (as a miminum and in a majority of cases according to the R8 guidance), considering that the effects identified as "critical" (an increase in body weight and an increase in the cholesterol levels in females at 0.26 mg/kg bw/d of BPA) occurred already at levels of low doses, and that when a NOAEL/LOAEL couple was available in certain studies, the LOAEL/NOAEL relationship was less than 10.

- **Assessment factor relating to the inter-species variability**: This factor takes into account the transposition from animals to people, and the value identified for this factor in the absence of specific data on the substance considered is generally **10**. This factor may be divided into a toxicokinetic component (factor of 2.5) and a toxicodynamic component (factor of 4). The justification of the use of this factor is in the R8 guidance of REACH, (p.30 R8.4.3.1.): "If no substance specific data are available, the standard procedure for threshold effects would be, as a default, to correct for differences in metabolic rate (allometric scaling) and to apply an

additional factor of 2.5 for other interspecies differences, i.e. toxicokinetic differences not related to metabolic rate (small part) and toxicodynamic differences (larger part)". The table R.8-3 of the guidance show the allometric scaling factors for different species as compared to humans: a factor of 4 is applied for the extrapolation from the rat to humans. So, **a factor of 10 (2.5*4) is applied** in the case of BPA.

- **Assessment factor relating to the intra-species or inter-individual variability**: this factor takes into account the variability within the human population.
    - **For consumers/the general population:** By default, the assessment factor is set at 10 for the general population to protect the larger part of the population, including the children and the elderly (R8 REACH guidance). This AF of 10 was chosen for consumers by default in the absence of toxicokinetic or toxicodynamic data which would enable the uncertainties regarding humans to be reduced.
    - **For workers:** By default, the assessment factor is set at 5 for workers (in comparison with 10 for the general population) (R8 REACH guidance) because this sub-population does not cover the very young, the very old, and the very ill. However, the scope of this restriction is the foetus of pregnant women; and developmental effects concern effects upon the foetus. Indeed, the DNEL derived from developmental effects for workers does not cover the worker himself but covers the foetus of the worker who finally belongs to the general population group. Moreover, the foetus of workers and of the general population have the same specific sensitivity, indeed, developmental effects used for selecting the NOAEL and deriving the DNEL are the same whether it is for the foetus of the workers or for the foetus of the general population.

Thus, the default factor of 5 for workers, implicitely considering a population with less variability amongst the worker population, does not include the unborn child. Unborn child is part of the general population and its default factor for the general population, which includes the unborn child, is taken forward for (prenatal) developmental effects, to cover for intraspecies differences.

To summarize, an assessment factor of 10 is taken for (prenatal) developmental effects, to cover for intraspecies differences. However, the risk assessment has also been calculated with an assessment factor of 5 for professional women in order to demonstrate that the risk still exists for the foetus of women exposed.

- **An additional assessment factor in connection with the corpus of data available and the severity of the effect:** this factor enables, either through lack of data on a substance or (for substances which have been well studied such as BPA) difficulties in interpreting all the data, the severity of the effects considered and any other residual uncertainty not covered by the preceding factors to be taken into account. When used, this factor is generally between 3 and 10. Within the framework of this assessment of BPA, a factor of 3 may be justified by all of the uncertainties relating to the effects of BPA in lower doses

than those used (Martini *et al.* 2010 ; Kubo, 2003) for effects on the brain and the central nervous system; Rubin *et al.*, (Rubin, 2001), Somm *et al.*, (Somm, 2009) for the effects on metabolism and obesity, etc.), the existence of a non-monotonic dose-response relationship as referenced in the summary work conducted as part of a study proposed by the French Agency (Lagarde, 2013) and which may concern some of the studies on metabolic syndrome (Marmugi, 2012), used in this assessment of BPA (ref : on metabolism), the existence of data *in vitro* and *ex vitro* showing a greatly increased sensitivity (above a factor of 3, already considered in the inter-species variability factor) of human tissue to BPA compared to animal tissue.

The table below summarises the effects and the associated internal DNELs which were used to conduct the risk characterisation (RC) for the general population (intraspecies assessment factor of 10).

Table 30. Summary of effects and associated internal DNELs used to conduct the RC for the general population

Critical effects on	**Reference study**	**Route of exposure**	**LOAEL**	**NOAEL**	**Internal NOAEL by application of a bioavailability factor of 3%**	**Internal DNELs by application of an assessment factor of 300 on the internal NOAEL for the general population (AF intraspecies = 10)**
			(µg/kg/d)	**(µg/kg/d)**	**(µg/kg/d)**	**(µg/kg/d)**
Brain and behaviour	**Xu, 2010**	Orally		**50**	**1.5**	**0.005**
Female reproductive system	**Rubin, 2001**	Orally	**/**	**100**	**3**	**0.01**
Metabolism and obesity	**Miyawaki, 2007**	Orally (drinking water)	**260**	**86.7***	**2.6**	**0.009**
Mammary gland	**Moral, 2008**	Orally (tube feeding)	**/**	**25**	**0.75**	**0.0025**

*: NOAEL calculated using the LOAEL.

The table below summarises the effects and the associated internal DNELs which were used to conduct the risk characterisation for the workers (intraspecies assessment factor of 5).

Table 31. Summary of effects and associated internal DNELs used to conduct the RC for the workers

Critical effects on	**Reference study**	**Route of exposure**	**LOAEL**	**NOAEL**	**Internal NOAEL by application of a bioavailability factor of 3%**	**Internal DNELs by application of an assessment factor of 150 on the internal NOAEL for the workers (with an AF intraspecies = 5)**
			(µg/kg/d)	**(µg/kg/d)**	**(µg/kg/d)**	**(µg/kg/d)**
Brain and behaviour	**Xu, 2010**	Orally		**50**	**1.5**	**0.01**
Female reproductive system	**Rubin, 2001**	Orally	**/**	**100**	**3**	**0.02**
Metabolism and obesity	**Miyawaki, 2007**	Orally (drinking water)	**260**	**86.7***	**2.6**	**0.0173**
Mammary gland	**Moral, 2008**	Orally (tube feeding)	**/**	**25**	**0.75**	**0.005**

*: NOAEL calculated using the LOAEL.

B 5.12 Pitfalls while using rodent data for human risk assessment

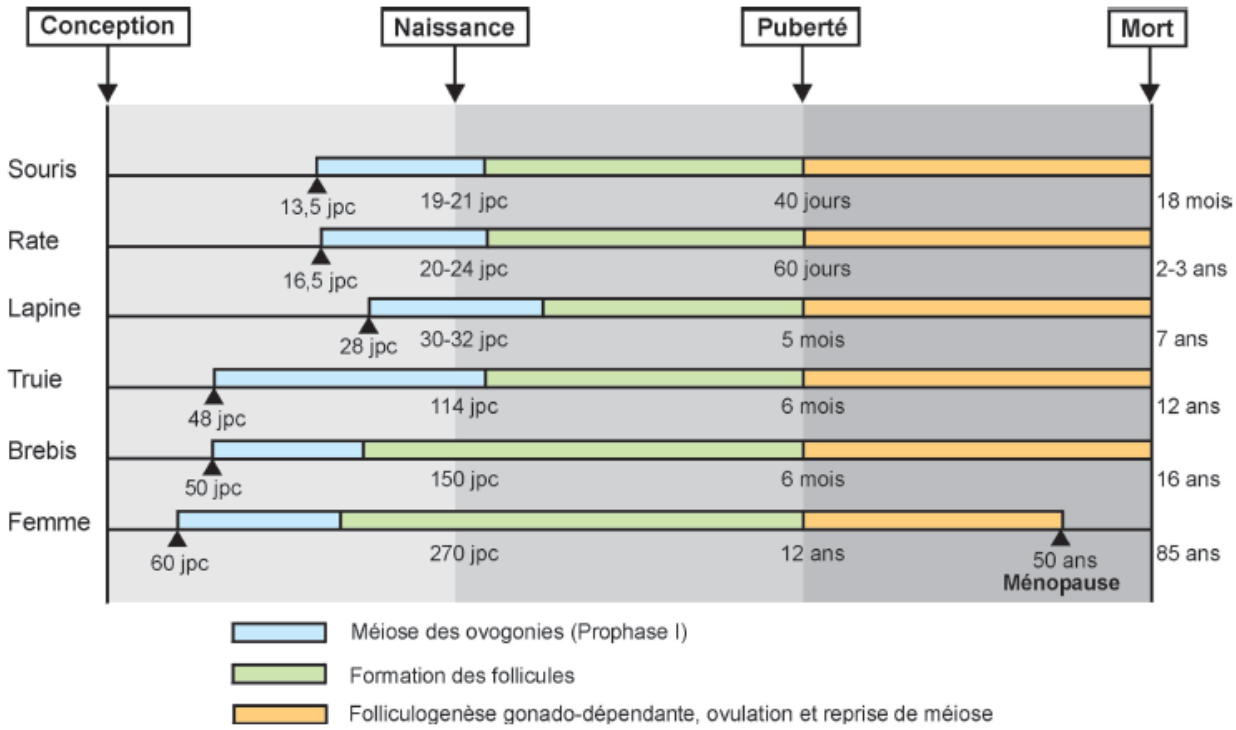
The major differences between humans and animals in terms of kinetics make it difficult to transpose to humans the effects observed in animals. The BPA biotransformation pathways are also different in nature and proportions according to species. The data collected in humans show that BPA glucoronide is the major metabolite, whereas BPA sulphate is more rarely identified and quantified. While glucuronic acid conjugation is the major pathway in rodents, the aglycone is not exclusively unchanged BPA, but part of it is hydroxylated BPA (Zalko *et al.*,

2003). Several other metabolites have also been identified, such as BPA diglucuronide, or methoxylated conjugates (Zalko *et al.*, 2003). Furthermore, the BPA metabolisation enzymes differ between animals and humans. Indeed, in rats, the 2B1 isoform of UDP-glucuronosyl transferase (UGT2B1) is mainly responsible for BPA glucuronidation (Yokota *et al.*, 1999). In humans, it is mainly UGT2B15 and 2B7 that are responsible for this glucuronide conjugation (Hanioka *et al.*, 2008). Finally, extrapolation of the pharmacokinetic data from animals to humans is unreliable owing to the various inter-species differences with regard to the existence or not of an enterohepatic cycle in the glucuronide-conjugated BPA elimination process (INSERM, 2011).

Rodents are born in a relatively immature state compared with humans, and their development continues after birth. In order to induce similar developmental effects, the exposure must be carried out in the neonatal period in rodents and the prenatal period in humans. The newborn rodent would be more vulnerable to this exposure than the human foetus, which is partially protected by the placental barrier. For example, prostate differentiation occurs around the time of birth in rodents (predominantly after birth), whereas it takes place during intrauterine life in humans. Other major differences are also noted in terms of maturation of the central nervous system (CNS) and thyroid function (Howdeshell 2002).

Moreover, the same effect can be initiated by different mechanisms of action which will not necessarily be disrupted by the same factors. For example, the masculinisation of the hypothalamic-pituitary-gonadal (HPG) axis occurs around the time of birth in the male rodent and is partially mediated by oestradiol produced locally in the brain from circulating testosterone. In humans, on the other hand, this developmental stage is initiated in the 3rd trimester of pregnancy and is brought about essentially by androgens, without oestrogens being involved.

Finally, the results must be extrapolated over time in order to adjust for the differences in longevity: the earliest stages of spermatogenesis in rodents are initiated shortly after birth and come to an end at six to eight weeks, whereas these events occur around the age of 12 to 15 in boys. In the same way, the maturation of the organs forming the HP axis, which regulate the oestral cycle, is complete at 15 days in rodents whereas this event occurs at the age of 10 to 12 in girls. Error! Reference source not found. 7 lists the periods of ovarian differentiation in arious mammals and Figure 16 represents the principal periods of development of the male genital tract in humans and rats, in relation to the level of testosterone production (INSERM, 2011).



*** jpc: days post-conception**

Figure 15: Comparison of ovarian differentiation periods in various mammals (INSERM, 2011)

Legend:

French	English
conception	conception
naissance	birth
puberté	puberty
mort	death
souris	[female] mouse
rate	[female] rat
lapine	[female] rabbit
truie	sow
brebis	ewe
femme	woman
mois	months
ans	years
ménopause	menopause
méiose des ovogonies (prophase I)	meiosis in oogonia (prophase I)
formation des follicules	follicle formation
folliculogenèse gonado-dépendante, ovulation et reprise de méiose	gonad-dependent folliculogenesis, ovulation and resumption of meiosis

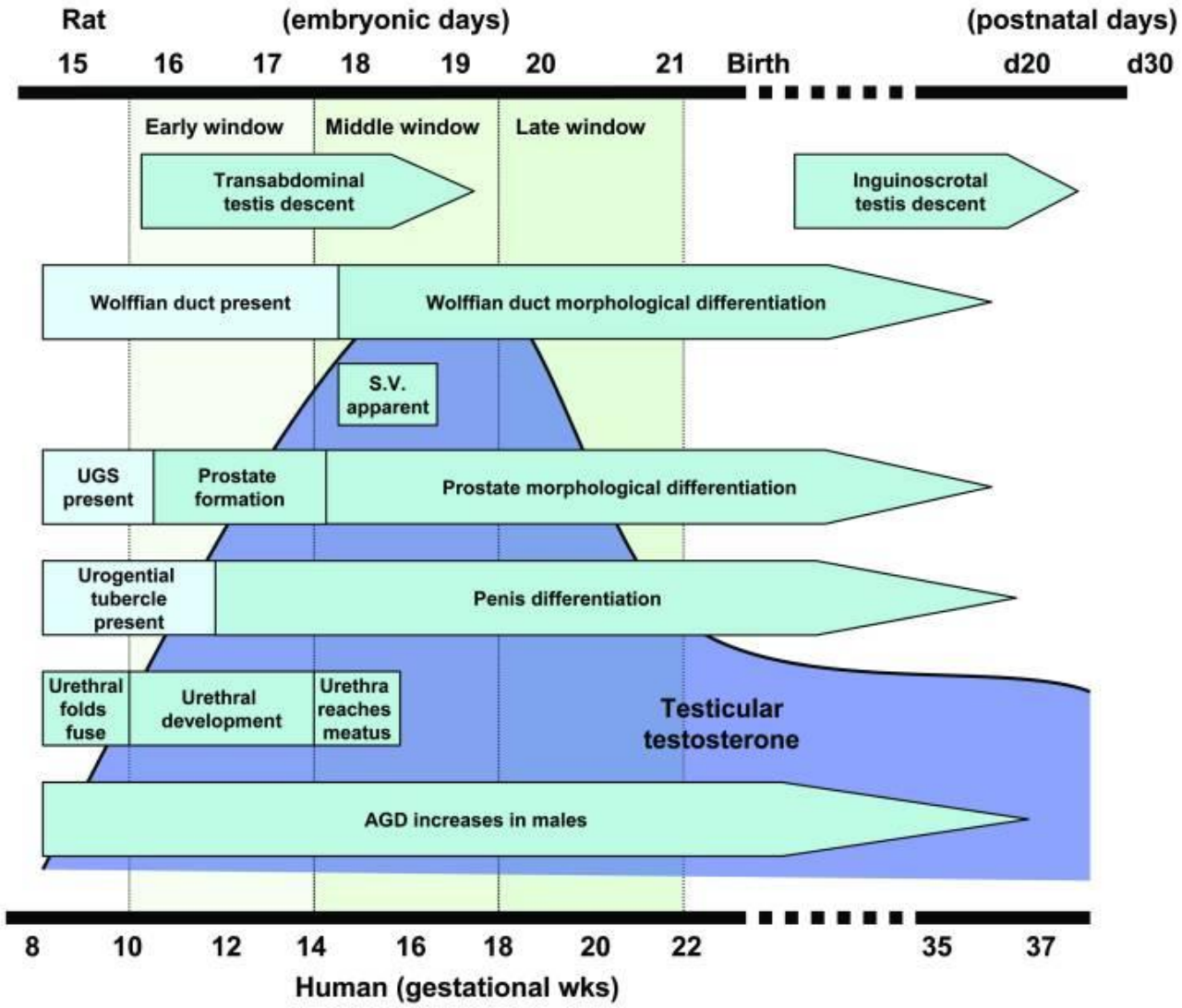


Figure 16: Diagram showing the main developmental periods of the male genital tract in humans and rats in relation to the level of testosterone production (INSERM, 2011) and according to (Welsh *et al.*, 2008)

Consequently, when transposing to humans the results obtained in animals, it is important to consider, as far as possible, the differences in periods of development influencing sexual differentiation and also to consider the role of the various hormones involved in this process.

B.6 Human health hazard assessment of physico-chemical properties

Not relevant for this proposal.

B.7 Environmental hazard assessment

Not relevant for this proposal.

B.8 PBT and vPvB assessment

Not relevant for this proposal.

B.9 Exposure assessment

B.9.1 General discussion on releases and exposure

A conceptual exposure diagram has been developed which takes into account all of the information collected during the study on the uses of BPA. The objective of this conceptual exposure diagram is to represent the possible compartments and routes of exposure of the population, based on the uses identified by Anses.

This diagram identifies the categories of products or goods that may lead to direct BPA exposure by virtue of their use or handling. They are the following categories:

- cosmetics;
- medical devices;
- dental cements;
- various supplies (household appliances, electrical elements, computer products, protective equipment, etc.);
- **glues, lacquers, varnishes, paint, etc.;**
- movable equipment, construction elements;
- thermal paper.

The "cosmetic product" and "fungicide product" uses, listed in the bibliography but not concerning the European Union, are mentioned on the conceptual diagram but do not, *a priori*, result in situations of exposure for the European population.

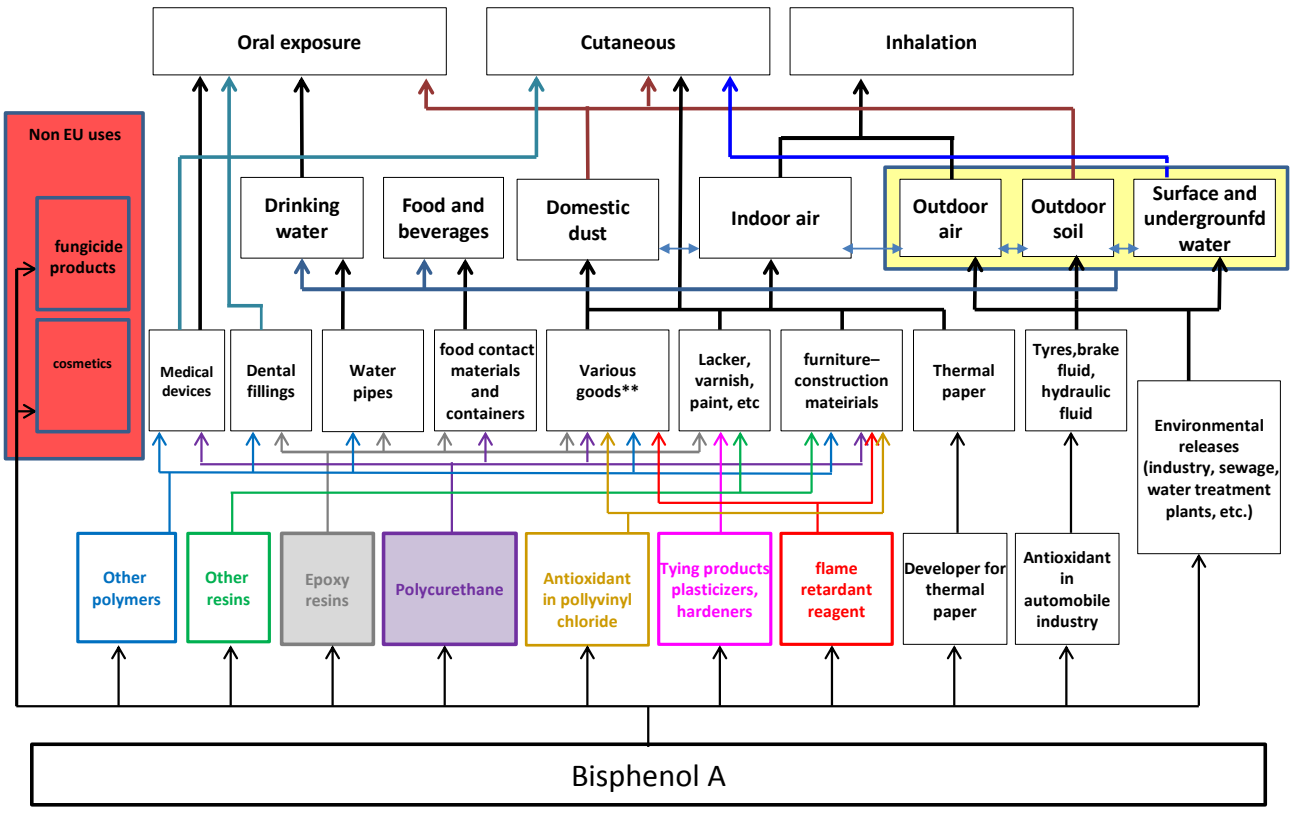
The survey on uses and the resulting conceptual diagram highlights the following points:

- For most of the identified uses, BPA acts as a synthetic intermediate in the manufacture of polymers and resins. The scope of use and/or application of such compounds, and, in particular, polycarbonates and epoxy resins, indicate that BPA is found in a variety of preparations, consumables and goods that the general population is in the presence of, or in permanent contact with.
- Given BPA's range of use and application in every day consumables, several means of exposure are potentially involved, and thus, may contain BPA:
    - The air compartment (outdoor and inside) - *a priori*, preferentially the particulate phase - due to leaching and/or extraction of nanoparticles related to the phenomena of friction, abrasion,
    - Sedimented dust, due to the deposit of particulates from the air or transfer from contact surface of various objects,

- Drinking water distributed through the network,
- Food and drink that has come into contact with containers (food cans, drink cans, etc. with epoxy resin-based coating) or polycarbonate food containers (tableware items, jugs, etc.)
- The Earth's exterior crust and groundwater and surface waters due to industrial or individual waste (industries producing or using BPA, WWTP effluents and sludge, leaching, "uncontrolled" direct release into the natural environment, etc.).

- The entire population is likely to be exposed to BPA regardless of age: infants, children and adults.
- In fact, the population can be exposed to BPA through 3 routes of exposure including inhalation, ingestion and skin contact.

Figure 17 Exposure diagram on the uses of BPA



** : CD, DVD, computers, screens, household electric appliances, small electric equipment, cell phones, optical equipment, sportswear, etc.

Existing national legal requirements for the uses of BPA in Europe

B.9.1.1. Summary of existing legal requirements

Danish EPA provided in 2013 an overview of national legislations on BPA in EU and EFTA countries (Danish E.P.A., 2013). The information given in the tables below comes from this report.

Food contact materials

Table 32. Overview of the EU MS national legislations on BPA for food contact materials

Country	**Legal Act/Provision**	**Scope**
Denmark	Statutory Order on food contact materials No. 822 June 26th 2013 (Bekendtgørelse om fødevarekontaktmaterialer nr 822 af 26/06/2013) Formerly Statutory Order No. 579 June 1th 2011 (BEK nr 579 af 01/06/2011)	Ban on BPA in food contact materials intended to come into contact with food for 0-3 year olds
Belgium	Law of 4 September 2012 modifying the Law of 24 January 1977 concerning protection of consumers in relation to BPA in food contact materials (4 Septembre 2012.—Loi modifiant la loi du 24 janvier 1977 relative à la protection de la santé des consommateurs en ce qui concerne les denrées alimentaires et les autres produits, visant à interdire le bisphénol A dans les contenants de denrées alimentaires)	Ban on BPA in food contact materials intended to come into contact with food for 0-3 year olds
Sweden	Swedish Food Decree (2006:813) (Livsmedelsförordning (2006:813))	Ban on BPA in varnish and coating in the packaging of food intended for 0-3 year olds

France	Law 2012-1442 of 24 December 2012 on the suspension of BPA in food contact materials (LOI n° 2012-1442 du 24 décembre 2012 visant à la suspension de la fabrication, de l'importation, de l'exportation et de la mise sur le marché de tout conditionnement à vocation alimentaire contenant du bisphénol A)	Banning BPA in any food packaging by 1 January 2015 Banning BPA in food packaging for infants and young children by 1. January 2013 Also provides for labelling/warning advising against the use by pregnant women, breastfeeding women and infants and young children of the above packing until such packaging is suspended from the market (NB! The decree with modalities for implementing this provision is discussed further down in this table) Finally, also BPA in pacifiers and teething is banned via this legislation. As this provisions is not related to food contact materials, it is addressed under "Other legislation".
Germany	Recommendation XXXVI (Paper and board for food contact) from the Federal Institute for Risk Assessment (BfR* Empfehlung XXXVI. Papiere, Kartons und Pappen für den Lebensmittelkontakt (Stand vom 01.06.2013)). *BfR: Bundesinstitut für Risikobewertung	Migration limit of 0.6 mg/kg foodstuff for recycled fibres used as raw materials for the production of paper and board for food contact materials
National legislation in the pipeline		
France	In preparation/consideration Proposed to be implemented as a decree, specifying the modalities for affixing the	Concerning health warnings against the use of packaging containing bisphenol A intended to enter into direct

	health warnings as specified in Article 2 of the LOI n° 2012-1442 (see above)	contact with foodstuffs
Belgium	In preparation/consideration	"Some measures for the protection of pregnant women"

Source: Danish E.P.A., 2013

Occupational Exposure Limits

Table 33. Overview of the national OELs in the EU MS concerning BPA

Country	**Legal Act/Provision**	**Scope**
Denmark	Statutory order 507 of 17/05/2011 (Bekendtgørelse om grænseværdier for stoffer og materialer, nr. 507 af den 17. maj 2011 med senere ændringer)	National OEL: 3 mg/m^3 (8h TWA, as inhalable dust fraction)
Germany	Standards for Hazardous Substances (TRGS* 900) (Arbeitsplatzgrenzwerte (TRGS* 900)) TRGS: Technischen Regeln für Gefahrstoffe	National OEL (MAK*): 5 mg/m3 (8h TWA, as inhalable dust fraction) *MAK: Maximale Arbeitsplatz-Konzentration
Austria	Austrian OEL regulation as adapted in 2011 (GKV 2011) (Verordnung des Bundesministers für Arbeit, Soziales und Konsumentenschutz über Grenzwerte für Arbeitsstoffe sowie über krebserzeugende und über fortpflanzungsgefährdende (reproduktionstoxische)	National OEL (MAK): 5 mg/m3 (8h TWA, as inhalable dust fraction) (= German MAK)

	Arbeitsstoffe (Grenzwerteverordnung 2011 – GKV 2011 - BGBl II Nr. 429/2011))	
Finland	Act 1213\2011 (Social- och hälsovårdsministeriets förordning om koncentrationer som befunnits skadliga, 1213/2011)	National OEL: 5 mg/m3 (8h TWA, as inhalable dust fraction)
Switzerland	Fact sheet - Swiss occupational exposure limits (latest edition: January 2013) (Grenzwerte am Arbeitsplatz" (German) or "Valeurs limites d'expositions aux postes de travail" (French), according to Article 50, §3 VUV (Ordinance regulating accident prevention and occupational diseases))	National OEL (MAK): 5 mg/m3 (8h TWA, as inhalable dust fraction) (= German MAK)

Source: Danish E.P.A., 2013

Other legislations

Table 34. Overview of other legislations on BPA in the EU MS

Country	**Legal Act/Provision**	**Scope**
Austria	Federal law gazette – Part II No 327/2011 (Bundesgesetzblatt für die republic Österreich - Teil II - Ausgegeben am 6. Oktober 2011) (BGBl. II Nr. 327/2011)	Ban of BPA in the manufacture of pacifiers and teething rings
France	Law 2012-1442 of 24 December 2012 on the suspension of BPA in food contact materials specifying	Banning BPA in pacifiers and teething rings

	that Article L.5231-2 from the Code of Public Health should be adapted (LOI n° 2012-1442 du 24 décembre 2012 visant à la suspension de la fabrication, de l'importation, de l'exportation et de la mise sur le marché de tout conditionnement à vocation alimentaire contenant du bisphénol A and Code de la santé publique - Article L5231-2)	
National legislation in the pipeline		
Sweden	In preparation/consideration. Suggested to be implemented in the Swedish Environmental Code 1998:808 (Miljöbalken – SFS 1998:808) Proposal for legal text on p. 48 of the background report mentioned in the column "Background information collected"	Ban of BPA in Thermal paper
France	In preparation/consideration	BPA in medical devices
Sweden	In preparation/consideration (early phase)	BPA in relining of tap water pipes

Source: Danish E.P.A., 2013

Existing/Proposed EU legal requirements for the uses of BPA

Table 35. Overview of the existing/proposed legal requirements for the uses of BPA (other than thermal paper) at EU level

EU Legislation	**Provision**	**Scope/year**

Directive 2011/8/EU	EU ban prohibiting the use of BPA for the manufacture of polycarbonate infant feeding bottles	Babies bottles (adopted in January 2011)
EU Regulation 10/2011/EU	Restriction for use in food contact materials relating to plastic materials and articles intended to come into contact with foodstuffs (from now on "the plastic food contact material regulation"). Annex 1 of the regulation specifies a maximum migration limit of 0.6 mg BPA/kg food	Plastic materials and articles intended to come into contact with foodstuffs (2011)
Directive 2009/48/EC	The European Commission has notified the World Trade Organization (WTO) for the proposed inclusion of BPA (and 3 flame retardants) under the Toy Safety Directive 2009/48/EC. The proposed enforcement date is 18 months after publication in the Official Journal of the European Union. The EC proposed a migration limit of BPA ≤ 0.1 mg/L (migration) [EN 71 Parts 10-11]	Toys (under consideration, adoption expected early 2014)
IOEL	An indicative OEL of 10 mg/m3 (8-hour TWA; as inhalable dust) is in place based on a SCOEL (Scientific Committee on Occupational Exposure Limits) recommendation from 2004 (SCOEL/SUM/113, May 2004). In 2013, SCOEL has updated its recommendation and recommends an OEL of 2 mg/m3 for BPA (8-hour TWA; as inhalable dust) in a draft document which was for consultation until September 2013 (SCOEL/SUM/113; March 2013).	Workers exposure / 2004 Workers exposure / 2013

<u>In the pipeline regulatory actions to address BPA under REACH and CLP regulations</u>

Table 36. Overview on current regulatory actions under REACH and CLP regulations in Europe concerning BPA

	Substance Evaluation	**Classification & Labeling**
	Concerns regarding hazard and exposure	**C+L reprotoxic**
Description	Evaluation of concerns with respect to hazards and environmental exposure took place 02/ 2012-02/2013.	A harmonised and stricter classification is proposed to address reprotoxicity of BPA (reprotoxic 1B) – now adopted
Deadline	Draft Decision to be decided upon in MSC 32 (11/2013).	Public consultation ended on 11 October 2013
Area	ENV and HH	HH
Member State	DE	FR

Existing legal requirements for the use of BPA in thermal paper outside the EU

Table 37. Overview on existing legal requirements for the use of BPA in thermal paper in the world

Country	**Legal Act/Provision**
Japan	Ban the use of BPA in thermal paper in 2001
US Connecticut State	Connecticut Senate Bill 210 prohibits the manufacture, sale or distribution of thermal receipt paper or cash register receipt paper containing BPA – adopted July 2011 / entry into force in

(**Maine** and **Illinois** are considering the adoption of the same position)	October 2013 (unless no alternative, in which case the restrictions take effect by July 2015)
Taiwan	BPA was banned in thermal papers in 2011

B.9.1.2 Summary of the effectiveness of the implemented operational conditions and risk management measures

As there is no current EU-wide restriction of BPA in thermal paper in force in the EU and no adopted national regulations in EU Member States neither, no RMM related to this particular use is assessed herein.

B.9.2 Manufacturing of BPA-containing thermal paper

Information of manufacturing (and the whole supply chain of thermal paper) of BPA-containing thermal paper is given in section B.2.

B.9.2.1 Occupational exposure

Not relevant for this proposal since it is focused on the use of thermal paper specifically by workers and consumers downstream. However, it may be expected that workers can be exposed to BPA while producing BPA-containing thermal paper.

B.9.2.2 Environmental release

Not relevant for this proposal since it is focused on the use on thermal paper specifically by workers and consumers downstream. However, it could be noted that the environment (water in particular) might be contaminated by BPA releases during the production of BPA-containing thermal paper. Some BPA releases may also occur during recycling of thermal paper, as explained in section B.2.

B.9.3 Use of BPA in thermal paper

B.9.3.1 General information

The following will adress the exposure of workers and consumers from BPA contained in thermal paper. Indeed, at the time of the elaboration of this proposal, there was no available

biomonitoring data investigating the exposure through thermal papers. The exposure of BPA through thermal paper has thus been modeled.

It has to be noted that, since the submission of this proposal, several studies have been published and are now analysed on RAC request further below in section B.9.3.2.2.2.

Here below is presented a summary of the key methodological elements of the exposure assessment performed. For a detailed and extensive description and justification of the methodology used, see Annexes 6 and 7.

- **Probabilistic characterisation of the exposure: general framework**

Within the scope of works relating to BPA, it has been chosen to model the doses of exposure in accordance with a probabilistic approach for optimum management of the variability. By contrast with a conventional deterministic approach, for which only occasional estimations of exposure are calculated, a probabilistic approach takes into account all of the possible modalities of an entry variable through the intermediary of its distribution of probabilities. So any possible modality of an entry variable of a model can be combined with the modalities of the other entry variables depending on their probability of occurrence.

Random samples using the Monte Carlo approach (10000 iterations) are then done on each of the entry distributions of the model to define distribution of the exposure doses, represented in the form of histograms or accumulated distributions.

The probabilistic approach presents numerous advantages:

- It enables the percentage of overrun of the reference toxicological doses to be determined.
- The sensitivity analyses enabling the identification and ranking of the most influential exposure parameters on exposure models are facilitated (Pouillot *et al.*, (2002) or Cullen & Frey (1999)).

- **Specification of the probability distributions**

As previously mentioned the probabilistic approach is based on allocation of a distribution of probabilities to each of the variables to then carry out a random draw in these distributions using the Monte Carlo method.

The major difficulty of this appraoch is defining the distributions of probabilites of the entry variables of the models. This information is generally not given in the literature and not all the variables collected in the population are available. The theoretical distribution of probabilities attributed to an exposure parameter and the underlying hypotheses to this choice therefore rely on the level of information available on the data (literature, subsidiary survey, etc.). It is therefore advisable to establish hypotheses to be able to define a theoretical distribution which is as close as possible to distribution of the data observed and to check these hypotheses using

statistical tools such as the tests of Kolmogorov-Smirnov or Anderson-Darling and graphs (quantile-quantile graphs or comparison of histograms or of distribution functions), which give information on the validity and plausibility of this adjustment. However, a limit of the theoretical adjustment of a distribution of probabilities is that, although a distribution "sticks" correctly to the data, the latter will never be perfectly adjusted, notably at the level of the distribution queues. Therefore, it is advisable to integrate the discrete distributions within the models of exposure, constructed from all of the possible modalities and their probability of respective occurrence.

The different levels of information available for an entry variable as well as the hypotheses made in order to be able to allocate it a distribution are presented below. Four different situations are encountered, in addition to the case where only a single value is available and is integrated as in the model. They are ranked below from the one with the least information to the one with the most information, the ideal situation for specification of a distribution of probabilities, thus resulting in a different strategy to be adopted.

- A variation interval

Certain studies only give the variation interval of the parameter studied. In this case, a uniform distribution is allocated with the interval given as all of the possibilities of the parameter studied, characterised by the fact that all the intervals of the same length included in this variation interval have the same probability of occurrence.

- A variation interval and a central value

Other studies may give more and more info on the variation interval of the parameter, a central value, central, average or median, of the sample. In this situation, the distribution of probabilities specified is a triangular distribution which is characterised by a central value which has the highest probability and minimum and maximum values which have a zero probability.

- A set of percentiles

In the majority of the studies selected, several percentiles are given, notably the percentiles 0 (minimum) and 100 (maximum), thus giving the variation interval. It is then a case of creating a distribution function from the couples $(x_i; p_i)$ of cumulative data available, with p_i the probability of obtaining a value lower than or equal to x_i. The following step consists of simulation of a sample of values taken randomly on this cumulative distribution and integrating it as "input" in the model for the exposure parameter.

- A set of raw data

It may be that the raw data from a survey are available. In this situation, it is necessary to have an occurrence percentage of each individual. This weighting is at times specified by the study. If this is not the case, it is allocated an appearance percentage of $100/n$, n being the individual number of the survey. Then the different values taken by the parameter are organised and the probabilities are added in the box where the same value is measured on several occasions. A set of couples $(X_i; p_i)$ with $\{X_1, X_2, \ldots, X_m\}$ is then obtained with all of the possible modalities of the parameter, and $\{p_1, p_2, \ldots, p_m\}$ their respective probability of

occurrence. However, it is at times preferable to group possible issues by class, so as to avoid having too large a number of different modalities with a percentage of low combined occurrences. Lastly, a sample of values taken randomly on the discreet distribution is simulated, defined from the occurrence probabilities of each of the realisations or classes of possible realisations for the parameter studied.

Comment: all the distributions of probabilities used in the exposure calculations are constructed from a Monte Carlo simulation of 10,000 iterations carried out using the software @Risk 5.0.

Results: In the final simulated sample of each of these doses, the percentile 95 was used as the value to which the DNELs shall be compared with a view to characterising the health risk. This choice is justified by the fact that the values beyond this percentile are the result of the combination of random draws in the distribution queues of each of the parameter of exposure and may in this way be judged as extreme cases, not representative of the study population.

B.9.3.2 Exposure estimation

With respect to thermal papers, the review of the uses (section B.2.4) showed that the use of thermal papers containing BPA is ubiquitous in different countries where studies were conducted (with the exception of Japan). In "eco-paper"-type thermal paper used mainly for receipts and credit card receipts, BPA is present in the free monomer form and offers no significant resistance to abrasion (Mendum, 2010). It can also be transferred through skin contact (Biedermann, 2010; Zalko, 2011) and, thus, constitutes a potential source of exposure to BPA; cashiers can represent an intensely exposed population.

Thermo-printed receipts are therefore a source of potential BPA exposure, and cashiers may represent a particularly vulnerable population. However, at the time of the elaboration of this proposal, there was no biomonitoring data evaluating specifically exposure through thermal paper available neither for workers nor for consumers. Thus, within the framework of this restriction, the development of a BPA exposure scenario has been proposed through the handling of thermo-printed receipts for 2 categories of the population: the worker (cashier) and the consumer. As already mentioned above, it has to be noted that, since the submission of this proposal, several biomonitoring studies have been published and are now analysed on RAC request further below in section B.9.3.2.2.2.

This work evaluates the health risks for one target population only exposed cutaneously to BPA contained in thermal paper: workers and consumers' pregnant women and their offspring. The foetus of cashiers and consumers pregnant women is the targeted population.

B.9.3.2.1 Workers exposure

For a detailed and extensive description and justification of the methodology used, see Annexes 6 and 7.

The scenario of occupational exposure is focused on exposure via the cutaneous route of cashiers handling receipts with a particular focus on pregnant women. So, other professions exposed to thermal papers (lottery tickets, self-adhesive labels) were not taken into account.

Other routes of exposure to BPA such as hand-mouth contact are possible but were not able to be modelled taking into account the insufficiency of the available data. Hand mouth contact

route could be considered as took into account by default with studies using oral route by diet and drinking water. But this exposure route has not been deeply analysed.

Only contact with the skin of the pads of the fingers was taken into account and not a surface in greater contact (inner side of the hands) which may not be excluded during changing of the roll or folding or receipts for example.

The exposure is assumed to be continuous and constant for the entire work duration on the basis of the observations of Biedermann, 2010 which show a constant quantity of BPA transferred to the surface of the skin of the finger, whatever the duration (between 5 and 60 seconds) and repetition (between 3 and 10 contacts) of contact with the receipts.

The equation used to model the dose of exposition via the handling of thermal receipts for a professional is based on the hypothesis of skin exposure to BPA over the work period. This hypothesis is based on the works of Biedermann, 2010 which show a constant quantity of BPA transferred to the surface of the skin of the finger, whatever the duration (between 5 and 60 seconds) or the repetition (between 3 and 10) of contact with the receipts. The exposure depends on the percutaneous absorption flow; the duration of exposure assimilated to the work duration, the surface in contact with the paper and the body weight. In the situation of pregnant women, the distribution of probabilities attributed to body weight was different from the one specified for consumers, a pregnant woman having to stop her job around the 7th month and a ½ of pregnancy. Thus, reduced periods were taken into account for calculation of the average body weight of pregnant women.

The study retained for the **percutaneous absorption flow** parameter was the one by Marquet *et al.* (Marquet, 2011) which aimed to determine a percutaneous absorption flow for BPA. It gave 15 data readings of BPA crossing the cutaneous barrier in $\mu g.cm^{-2}.h^{-1}$. On the basis of this information, it was then decided to allocate a **uniform distribution** with, as the distribution variation interval, the **minimum (0.026 $\mu g.cm^{-2}.h^{-1}$) and maximum (0.331 $\mu g.cm^{-2}.h^{-1}$)** values measured.

For **the exposure duration**, the specified distribution was based on the assessment of experts from the data from the collective agreement of the retail trade and the wholesale trade with dietary predominance. It was decided to allocate a **triangular distribution** of probabilities with as a **minimum value 3 $h.d^{-1}$** and as a **maximum value 10 $h.d^{-1}$**, corresponding respectively to the minimum and maximum values of the daily work duration on the days worked, and lastly as an average value, taken as a mode of distribution, **6.5 $h.d^{-1}$.**

The distribution allocated to the **surface in contact** with a thermal receipt was based on the assessment of experts who proposed taking a total surface area corresponding to the cumulated surface area of the pads of the ten fingers (last phalanxes). It was decided to allocate a value of **12 cm^2,** relying on US EPA (1986) which gives by default a surface area of **2 cm^2 for the thumb** and **1 cm^2** for each of the other fingers.

To determine the distribution of probabilities illustrating the **body weight for the pregnant woman**, the entire period of pregnancy must not be taken into consideration in calculation of the average weight of each of the individuals. The study of pre- and post-natal determinants of

development and health of the Child (EDEN[19]), gives the body weights of the pregnant women at different stages of the pregnancy and was used to document this parameter, with the similar exception of the weights measured taken into account in order to calculate the average weight of the women are those given from the start of the pregnancy until the **7th month and a ½,** i.e. up to the **35th** week of amenorrhea.

The internal daily dose by contact with thermal receipts for professionals was estimated using the following model:

$$DI_{ticket_{trav}} = \frac{F \times D \times S}{PC_{trav}}$$

With:

$DI_{ticket_{trav}}$	: Internal daily dose	[µg.kg_{bw}^{-1}.d^{-1}]
F	: Absorption flow	[µg.cm^{-2}.h^{-1}]
D	: Duration of exposure to the till receipt	[h.d^{-1}]
S	: Surface in contact with the till receipt	[cm²]
PC_{trav}	: Body weight	[kg_{BW}]

In this paragraph the results of calculations of the internal dose linked to the handling of thermal receipts are presented for a professional population of pregnant women.

Figure 18. Histogram and descriptive statistics of the internal DNEL *via* the handling of thermal receipts for a population of pregnant women workers

–Internal daily dose of BPA (µg.kg_{bw}^{-1}.d^{-1})	Descriptive statistics	
DI TICKET THERMIQUE TRAVAILLEURS FEMMES ENCEINTES 0.014 0.434	Minimum	0,014

[19] The EDEN study was initiated by several teams of epidemiologists from the Institut Fédératif de Recherche 69, as well as participating clinicians from the CHU *(University Hospitals)* of Poitiers and Nancy. Their aim was to better define the characteristics of foetal development and the first few months of life which influence the development and the subsequent health of the child. 2002 women agreed to participate. Amon the very large amount of data available from this study, a distribution of discrete probabilities was simulated from the pairs "average weight/probability of occurrence".

	P5	0.05
	P25	0.11
	P50	0.20
	P75	0.29
	P90	0.38
	P95	0.43
	Maximum	0.71
	Average	0.21

For the "professional pregnant women handling thermal receipts", the internal dose varies from 0.01 to 0.71 $\mu g.kg_{BW}^{-1}.d^{-1}$. The 95th percentile is 0.43 $\mu g.kg_{BW}^{-1}.d^{-1}$.

The estimation exposure is in the same range or below other estimations:

In a report from KemI (Kemi, 2013), it is reported that BPA can be released from the cash receipts and up to 660 µg BPA were evaluated to be released to the skin. Considering the absorption rate from the skin this may lead to an internal BPA worst case exposure of 1 µg BPA/ kg bw day (Kemi, 2013).

In a Danish report, the BPA worst case exposure from cash receipts was calculated based on Danish experimental measurements when exposed to 8 µg/kg bw /day (corresponding to an absorbed intern dose of 0.8 –4 µg/kg bw day) for consumers and to 43 µg/kg bw day (corresponding to an absorbed dose of 4.3-20µg/kg bw day) for cashiers (Lassen et al, 2011 in Danish E.P.A., 2011).

B.9.3.2.2 Consumer exposure

Other uses of BPA, such as in printing inks and thermal paper, are considered to result in negligible potential for consumer exposure in comparison with the other sources considered (EC, 2010). New estimates have been given on exposure to BPA migration from cash receipts.

B 9.3.2.2.1 Biomonitoring data

General considerations related to biomonitoring data in urine and blood are exposed here below for information.

1. **Urine**

a. Urinary concentrations reported by the different studies

The biomonitoring studies aim to assess human exposure to bisphenol A from urine (the majority of the studies mesure total BPA ie unconjugated BPA + conjugated BPA). Moreover, these data represent the total exposure to BPA through all the route and media the consumer are exposed to (food, consumer products, dust in the air...). The urine samples were analysed using different techniques (liquid or gas chromatography with detection via mass spectrometry, via fluorimetry, or electron capture) after an enzymatic deconjugation stage.

The results reported show a strong disparity within each of the populations studied with ranges of concentration generally covering several orders of magnitude and ranging from "non-detected" (i.e. concentrations lower than the lowest detection limits ranging from 0.1 to 0.4 ng/ml) up to values of several hundred ng/ml for the highest (Figure 20). However, the average values are quite similar between the different studies, including between the different geographical zones (Figure 20), and are generally comprised between 1 and 5 ng/ml.

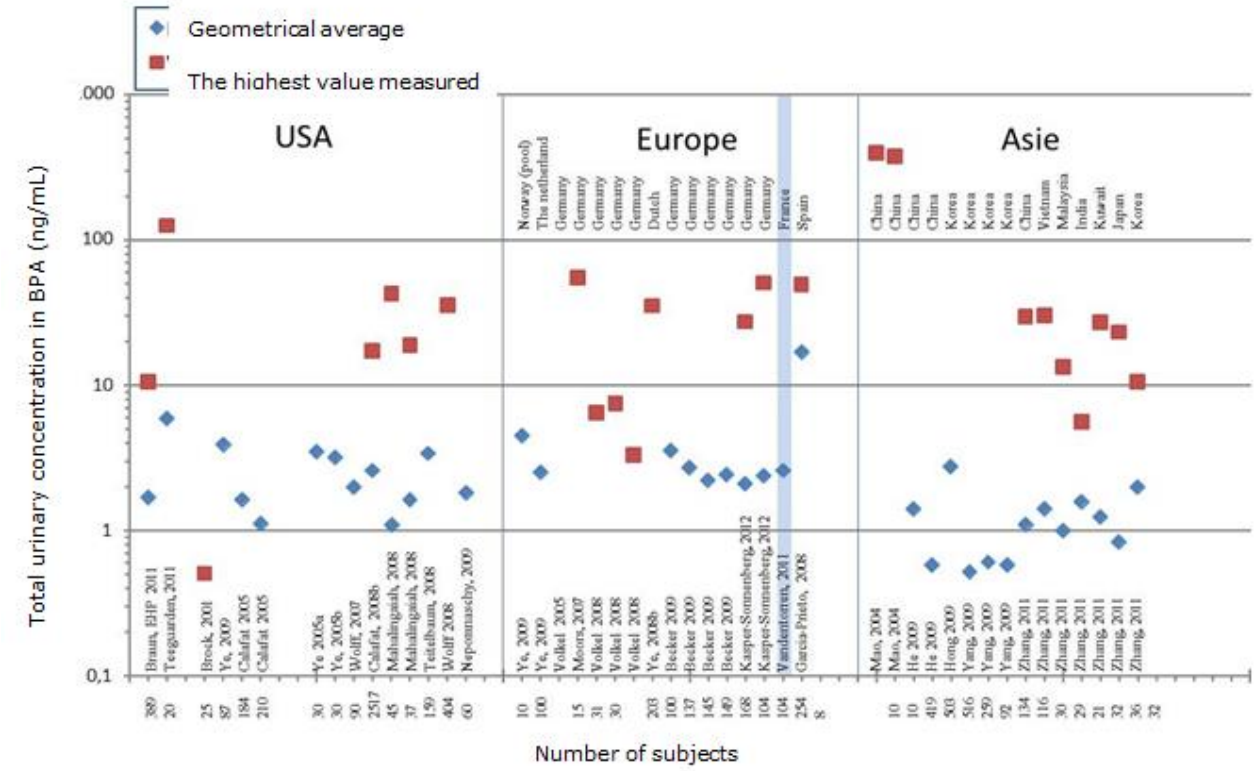


Figure 19 Urinary concentrations in total BPA reported in the literature for studies published between 2001 and 2012.

The multiple citations correspond to the values given for different categories within the same study: Calafat et *al.*, 2005 (184 men, 210 women)**, Mahalingaiah et *al*., 2008 (45 women, 37 men), Volkel et *al*., 2008 (31 women, 30 children aged 5-6 years, 21 adults), Becker et *al*. 2009 (137 children aged 3-5 years, 145 children aged 6-8 years, 149 children aged 9-11 years, 168 children aged 12-14 years), Kasper-Sonnenberg et *al*., 2012 (104 mothers, 104 children), Mao et *al.*, 2004 (10 men, 10 women), He et *al*., 2009 (419 men, 503 women), Yang et *al*., 2009 (259 men, 92 women pre-menopause, 134 women post-menopause), Zang et *al.*, 2011 (different countries).**

b. Sensitivity of the analytical methods and positive detection rates

The different analytical methods used for the dose of BPA in urine present variable sensitivity levels according to the studies, ranging from a detection limit (DL) of 3 ng/ml or the least sensitive to DLs lower than 0.1 ng/ml for the most sensitive methods. Generally, very few of these studies satisfy the criteria recommended today for validating an analytical method, which are required to guarantee the quality of the results obtained. In the majority of the studies analysed here, the quantitative results are given above the DL instead of only being given above the quantification limit (QL). A possible and probable consequence of this "misuse" is that the results shown are in reality accompanied by a significant degree of uncertainty which could increase the level of variability of the readings, particularly for the lowest concentrations.

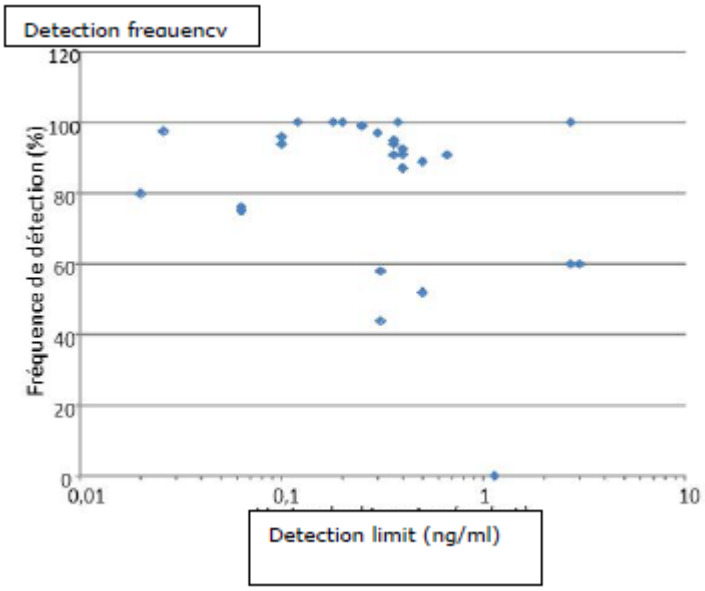


Figure 20: Positive detection limit of the total BPA in urine depending on the detection limit shown in different studies.

The positive detection rates of the total BPA shown in the different studies are most frequently higher than 80 %. The only study which reported a detection rate of 0% (Völkel *et al*., 2005) was subsequently called into question (Vandenberg et al., 2010), due specifically to its relatively high DL (1.14 ng/ml) and the small sample size (n=6). The fact that other studies presenting even higher DLs (2.7 and 3 ng/ml) had reported detection levels ranging from 60 to 100% (Mao *et al.*, 2004; Moors *et al.*, 2007) for adult populations in China and Germany appears to support the assumption that the different detection rate in the Völkel *et al*., 2005 studies was due to the small sample size. Similarly, positive detection levels of 100% were only reported when the methods used were relatively sensitive (from 0.12 to 0.38 ng/ml), or in the case for a higher DL (2.7 ng/ml) but on a reduced sample (n=10) (Mao et al., 2004).

Figure 17 does not show the marked relationship between the detection limit and the positive detection rate, in contrast to what may be observed for other substances. This lack of relationship, although surprising, is probably linked to the rapid elimination of BPA. The urinary concentration does not reflect the average level of exposure but only the recent exposure and

may therefore rapidly fall back down under the analytical sensitivity limits for the subjects which have not been exposed to BPA very recently (hours).

c. Elimination of BPA and variability of the results

The particularly rapid kinetics of elimination of BPA has a direct influence on the use of urinary doses for the assessing impregnation of individuals with BPA.

The Teeguarden et al., 2011 studies, focusing on a group of 20 subjects subjected to controlled feeding over a period of 24 hours have shown a urinary concentration peak in total BPA at t=2.75h (0.75 – 5.75) after ingestion of the meal, which was considered to be the source of exposure to BPA. For the people who consumed the same meal, the variability in the quantity of total BPA eliminated during the same time lapse ranged from 15% to 60%.

The rapid elimination of BPA is in principle responsible for the high variations in urinary concentration observed inter- and intra-individually. In the works carried out on 8 individuals followed over the course of one week, Ye *et al*., 2011 showed variations of several orders of magnitude for the same individual over the course of one day, with coefficients of variation (CV) intra-day ranging from 9% to 117%. Similarly, the intra-day CVs of total urinary BPA for the same individual ranged from 63% to 235%. The CVs observed for the first urination ranged from 53% to 120% between the different days for the same individual. The CVs on the average urinary concentration over 24 hours ranged from 25% to 85% between the different days. In another study conducted on 389 pregnant women, Braun et al., 2011 showed an absence of correlation through time between the concentrations in total BPA in the urine collected at 16 weeks of pregnancy, at 26 weeks and at birth. The creatinine adjustment did not increase the correlations.

These different elements tend to show that 1) a single sample of urine taken at random over the course of a day does not account for the level of exposure of an individual, 2) the collection of urine over 24 hours does not account for the average level of exposure for a longer period (weeks or months), and 3) the concentration in the first morning urination is not representative of the average concentration over the course of the day.

d. Unconjugated or total urinary BPA?

The majority of biomonitoring studies of exposure to BPA generally present results expressed in total BPA, corresponding to the sum of unconjugated BPA and conjugated BPA (glucuronide or sulphate) obtained by analysis after enzymatic deconjugation. Some studies also report results of unconjugated BPA, generally presented alongside the total BPA. These studies show values of unconjugated urinary BPA markedly lower than the total BPA (Table 39) and the positive detection levels (values above the sensitivity limit of the method) are always noticeably lower for the unconjugated BPA than for the total BPA. However, the variability of the total BPA/unconjugated BPA ratio, which may range from 2 to over 100 depending on the studies, limits any interpretation.

For the first time, a recent study conducted by Liao and Kannan, 2012 presented results on the different forms of urinary BPA (unconjugated and conjugated) in which the forms of conjugated BPA were analysed directly and not indirectly, as is generally determined by the difference in unconjugated BPA before and after enzymatic deconjugation. The values presented show that glucuronide BPA represents 57±34% of the total, followed by unconjugated BPA (32±31%), disulphate BPA (7±14%) and lastly the forms substituted by 1,

2 or 3 chlorine atoms which represent a proportion of several percent of the total BPA. The absence of deconjugation linked to the analytical procedure was verified.

Table 38: Summary of concentrations of total BPA and unconjugated BPA in urine expressed in ng/ml

1 References	2	3 Age	Unconjugated BPA (ng/ml)				Total BPA (ng/ml)			
			Tx detected	Range of concentrations observed	Average geo.	Med	Tx detected	Range of concentrations observed	Average geo.	Med.
Ouchi & Watanabe, 2002	48	Adults	2				100			
Brock, 2001	5 pools		0	<0.12			100	0.11 ls2001		
Kim, 2003	15 h		100		0.58	200	100		2.82 2003	
	15 f		100		0.56	200	100		2.76 2003	
Ye, 2005	30	Adults	0		<0.3		97		3.2	
Sch3ts05301ana	12	Adults	75			0.3	100			1.1

b2007										
Volkel, 2008	31	Adults		ND ltsar				ND ltsar		
	30	5-6 years		ND ltsar				ND ltsar		
	203	Adults		ND ltsar				ND ltsar		
Calafat, 2009	41	Premature babies	92	ND mature	1.8	1.7		1.6 ature	30.3	28.6
Kasper-Sonnenberg, 2012	104	Adults (mothers)	15	<0.15 en oth	<0.15	<0.15	100	0.3 5 en o	2.1	2.1
		Children	16	<0.15 en ot	<0.15	<0.15	100	0.3 5 en o	2.4	2.3
Koch, 2012	30	Adults	6.7	<0.1 s2012t	<0.1	<0.1	96.7	<0.1 s2012t	2.11	1.32
	30	Adults	13.3	<0.1 s2012t	<0.1	<0.1	96.7	<0.1 s2012t	1.77	1.04
Liao & Kannan, 2012	31	Adults	97	<0.01 Kanna	0.701		87	<0.05 Kan	2.16	

While the conjugated forms of BPA are considered as having been inactivated by the organism during the metabolisation processes, free BPA (unconjugated) is the form considered as potentially responsible for toxic effects.

The ratio of the different forms of BPA presented is moreover likely to vary between different individuals (differences in metabolism) but also depending on the route of exposure (ingestion, inhalation, skin contact).

This ratio could therefore represent a marker of the detoxification capacity of the organism following an exposure to BPA, but may also be considered as an indication of the potential effects of the latter. Exact knowledge of the proportion of the different forms of BPA present in the organism is therefore much more relevant information than the concentration in total BPA alone.

Although studies conducted on different animal models appear to indicate that unconjugated BPA represents a minor proportion of the total BPA (generally lower than 3%) (Doerge *et al*.,

2010; Collet *et al.*, 2012), not all the studies conducted on human urine confirm this hypothesis, specifically the studies by Kim *et al.,* 2003 and by Liao and Kannan, 2012, which indicate a proportion of unconjugated PBA which may represent up to 20 to 30% of the total BPA (Table 39).

e. BPA and age

Vandenberg *et al*., 2010 suggest a higher exposure in children compared to adults based, in particular, on the values of total urinary BPA reported in several studies which analysed different age groups (Calafat *et al*., 2008, Volkel *et al*, 2008 and Becker *et al.*, 2009) and presented higher values in children compared to adults. Kasper-Sonnenberg *et al.,* 2011 also report slightly higher values for children than for adults, but with no statistically significant differences. However, the values presented in these studies, as well as those reported in the studies which analysed urine from children (Martin *et al.,* 2005; Wolff *et al.*, 2007, and Teitelbaum *et al.,* 2008) show values comparable to those observed in adults. More recently, Li *et al*., 2013 showed concentrations of total urinary BPA statistically lower in the youngest children compared to the oldest. Lastly, LaKind *et al*., 2012 showed variable results depending on the year and the region (USA *vs.* Canada) (Figure 18).

The most significant results are those reported by Calafat *et al.,* 2009, who analysed urine from 41 premature children and presented average concentrations of total BPA around 10 times higher than the generally reported values.

No difference was observed between men and women but Liao and Kannan (2012) reported that the concentrations in total BPA were significantly higher in Caucasians compared to Asians, with, nevertheless, a similar profile of the different forms of BPA.

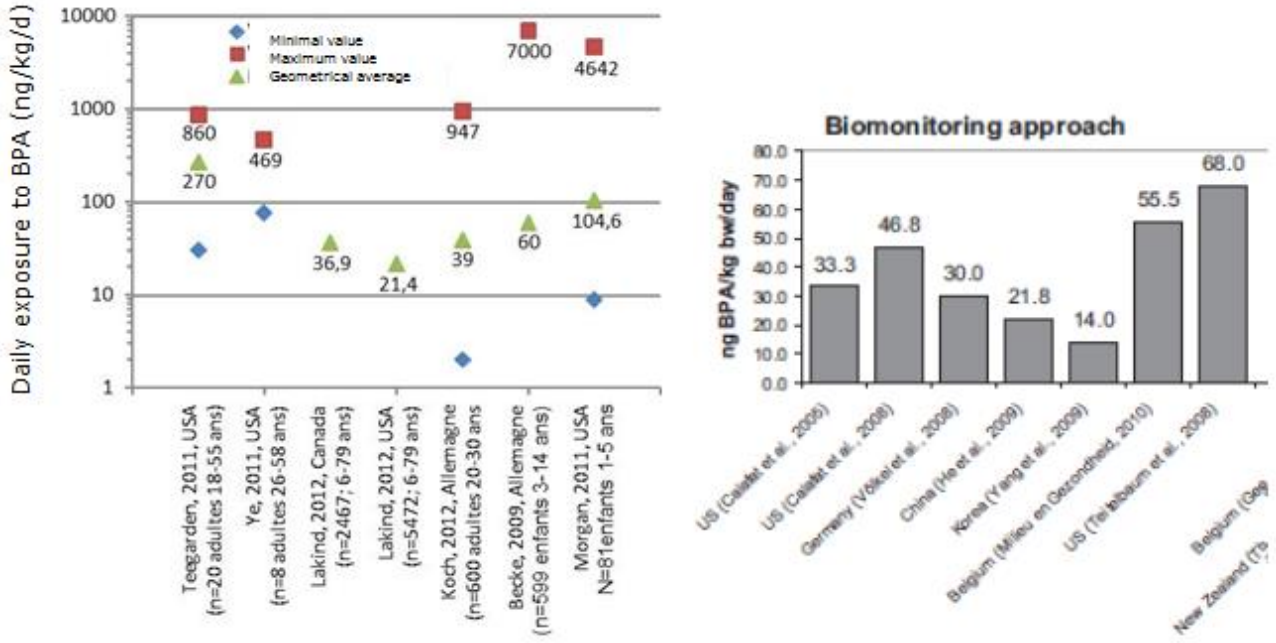


Figure 21 Left: Level of daily exposure to BPA calculated from urinary excretion over 24h.

Legend: **The values of Ye *et al*., 2011 are extrapolated by considering an average body mass of 70 kg. The other values are those presented in the studies mentioned.**

Figure 22 Right: Level of daily exposure to BPA reported by Geens *et al.,* 2011.

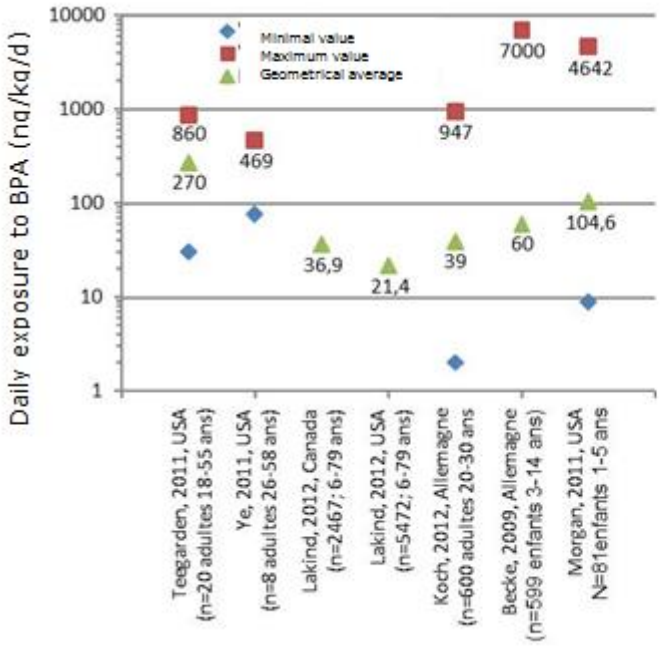
Legend: Level of daily exposure to BPA reported by Geens *et al.,* 2011. In 5 of the 7 studies shown, the daily exposure is extrapolated from the urinary concentration from a single sample compared to a theoretical daily urinary volume of 1500 ml and a body mass of 60 kg for adults (Calafat *et al*., 2005; He *et al*., 2009; Yang *et al*., 2009 and 30 kg for children (Teitelbaum *et al.,* 2008).

Although the majority of the studied presented in figure 22 do not enable definitive conclusions to be drawn on the effect of age on the concentrations of BPA found in the urine, it should be noted that none of the studies which carried out a comparison between children and adults or between children of different categories of age looked at children aged under 3 years. Therefore, these studies do not enable a comparison to be made between individuals with an immature metabolic system and individuals with a mature system, nor do they demonstrate a higher exposure in newborns linked, for example, to their particular diet or to their more frequent contact with the floor. The only studies concerning children under 3 years (Calafat, 2009 and Morgan 2011) reported slightly higher concentrations in urinary BPA (Morgan *et al*., 2011, children of 2 to 5 years), markedly higher even (Calafat *et al*., 2009, premature babies), than other studies. However, as these two studies do not present values obtained in adults subjected to the same levels of exposure, it is not possible to draw definitive conclusions as to the influence of age on the concentrations of urinary BPA. Additional studies are required in order to determine the urinary concentrations of BPA in children of a very young age (infants) in comparison with adults, and to potentially compare the proportion of unconjugated and conjugated BPA in the two populations.

f. Estimation of the dose absorbed daily

Based on the doses of urinary BPA, an estimate of the daily dose absorbed may be made by comparing the concentration measured to the volume of urine produced, considering that the totality of BPA absorbed is eliminated in the urine.

Such an estimation was done by Geens *et al*., 2011 using bibliographical data and on the basis of a single urinary dose (average values in populations) considered as representative of the average urinary concentration and compared with a theoretical volume of urine produced and the body mass of the individuals (Figure 25, right). Other studies which considered the high variability of the concentration of urinary BPA over time favoured the total collection of urine over 24 hours or 48 hours with assays of BPA carried out independently on each of the samples of urine or on the pooling of all the samples of urine collected (Figure 24, left).



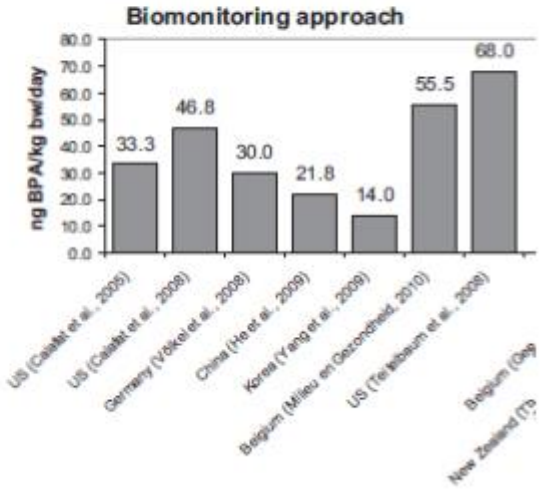


Figure 23 Left: Level of daily exposure to BPA calculated from urinary excretion over 24h.

Legend: **The values of Ye *et al*., 2011 are extrapolated by considering an average body mass of 70 kg. The other values are those presented in the studies mentioned.**

Figure 24 Right: Level of daily exposure to BPA reported by Geens *et al.,* 2011.

Legend: Level of daily exposure to BPA reported by Geens *et al.,* 2011. In 5 of the 7 studies shown, the daily exposure is extrapolated from the urinary concentration from a single sample compared to a theoretical daily urinary volume of 1500 ml and a body mass of 60 kg for adults (Calafat *et al*., 2005; He *et al*., 2009; Yang *et al*., 2009 and 30 kg for children (Teitelbaum *et al.,* 2008).

However, these two approaches provide average values of daily exposure to BPA relatively similar between the different studies, generally comprised between 10 and 100 ng/kg day (Figure 26) and the maximum values could reach 7000 ng/kg day (Becker *et al*., 2009).

Having analysed all the fractions of urine collected in the course of one full week from 8 individuals, Ye *et al*., 2011 clearly demonstrate the high variability in exposure for the same individual between the different days, with inter-day CVs ranging from 23 to 97%. On the other hand, taking the exposure of each individual averaged over one week, the inter-individual values were relatively similar and ranged from 3.5±1.3 µg/d to 6.7±2.3 µg/d.

Comparing the exposure of children calculated from the assay in the urine of 24 hours with an indirect estimate based on the concentrations measured in their environment (internal air and external air, dust, floor) and their diet, Morgan *et al*., 2011 were able to show quite a good concordance of the average values obtained by the two approaches (Figure 22). The results presented in these studies also suggest the major role of diet (mainly solid) which may account for more than 95% of the total exposure. However, the low correlation between the urinary BPA excreted and the estimated ingested dose (r=0.23, p=0.07) shows that if the

indirect approach proves relevant for assessing the average level of exposure of a population, it proves to be lower for assessment at the individual level.

The predominant role of diet in exposure to BPA is confirmed by the studies of Teeguarden *et al.*, 2011, which have shown that, following the ingestion of a controlled diet rich in BPA, a group of 20 volunteers presented a level of daily exposure ranging from 30 to 860 ng/kg (average of 270±230 ng/kg) which placed them above the 95th percentile of the American population over 6 years of age (according to Lakind and Naiman, 2010).

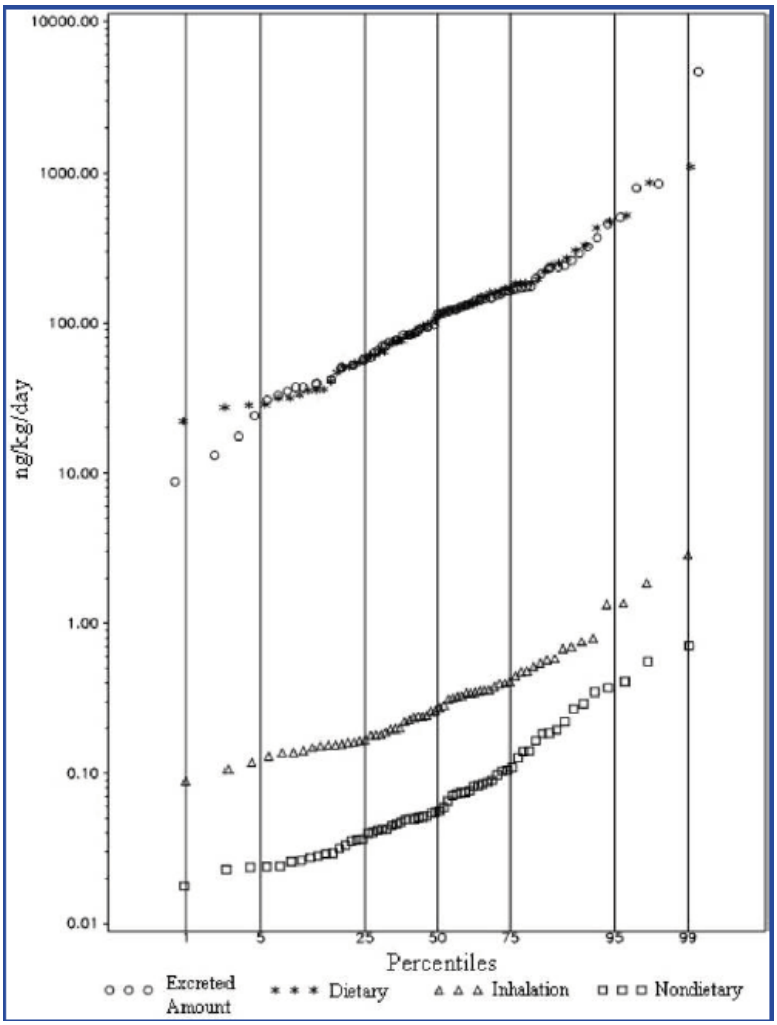


Figure 25: Estimate of exposure of 81 children aged from 1 to 5 years (Ohio, USA) to BPA via the different routes of exposure compared to the quantity of total BPA excreted in the urine (according to Morgan *et al.,* 2011 and Wilson *et al*., 2007).

Legend: routes of exposure concerned - dietary, respiratory and non-dietary.

g. Conclusions on the use of urinary assays for the biomonitoring of exposure to BPA

The different research studies conducted clearly show that due to the rapid elimination of BPA from the body, the urinary concentration of BPA is only representative of recent exposure (hours preceding the sample only) in individuals. An increase in the representativeness of the results may be obtained by multiplying the samples (total urine samples taken over several days and even weeks), but the use of a matrix providing access to more extensive windows of detection (tissues, skin appendages, etc.) would be preferable.

Due to the rapid elimination of BPA, single samples of urine may not *a priori* enable the suspected sources of exposure in the general population to be correctly identified. Such research on sources of exposure to BPA by means of urinary analyses would require the use of two populations (assumed exposed vs. control) in identical conditions (diet, environment) except for the suspected source of exposure, and systematic sampling of all the urine samples produced during the period studied.

A study on the actual determination of the different forms of BPA (conjugated and unconjugated) present in the organism appears to be required.

Additional research would also be required to determine the exposure of infants as well as their BPA metabolisation capacity.

2. Blood

Although not the matrix of choice for assessing exposure to BPA, several studies focused on the assay of BPA on blood samples (serum, plasma). Teeguarden *et al*., (2011) have shown that the serum concentration peak of total BPA was observed 1.63 ± 0.47 h after ingestion of a meal considered as the source of exposure, and therefore preceded by the urinary peak of around one hour.

Generally, the values found in blood are lower than the urinary concentrations (Figure 27).

Teeguarden *et al.* 2011 showed that the serum concentration of total BPA was between 3 and 250 times (average 42) lower than in urine and they highlighted the high variability of the urinary BPA / serum BPA ratio, including for the same individual throughout the day (which could vary from 3 to 8:00 up to 56 to 18:00, or from 5 to 14:30 up to 215 to 22:30). Similarly, Koch *et al*., 2012, did not observe any relationship between urinary BPA and serum BPA. This study also reported that the serum BPA detected was essentially in the unconjugated form and suggested the possibility of external contamination (Koch *et al*., 2012). Conversely, Liao and Kannan 2012, who conducted a metabolic profile of the different forms of serum BPA, reported that the major form is glucuronide BPA (43±41%), followed by disulphate BPA (38±38%), and lastly unconjugated BPA which represents 19±30% of the total BPA.

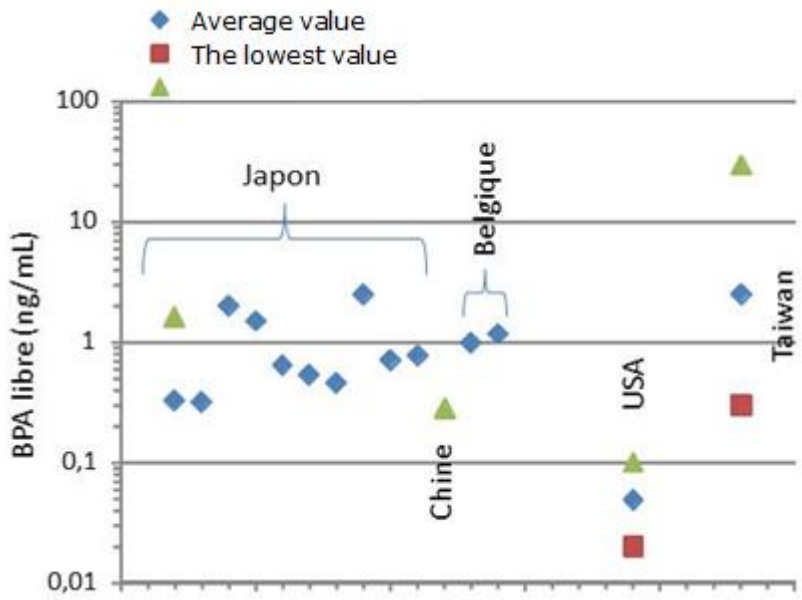


Figure 26: Serum concentration values of unconjugated BPA reported by different studies published between 1999 and 2012.

3. Breast milk/Colostrum

Several studies have demonstrated the presence of BPA (unconjugated and conjugated) in breast milk or in human colostrum. The studies which analysed breast milk involved a limited number of subjects (n=3 to 23). In these studies unconjugated BPA was detectable in the majority of cases (60% or more) (Otaka *et al*., 2003; Sun *et al*., 2004; Ye *et al,* 2006; Ye *et al.,* 2008), with average concentrations ranging from 0.61 to 1.3 ng/ml, but which could reach up to 6.3 ng/ml (Ye *et al*., 2006). The total BPA was detectable in nearly all of the studies, with the average concentrations comprised between 1.0 and 1.9 ng/ml, but which could reach up to 7.3 ng/ml (Ye *et al*., 2006). It should be noted that the methods of assay used in the studies mentioned previously (specifically those by Ye *et al*., 2006 and 2008) did not present satisfactory validation criteria (*e.g.* no quantification limits), which could have resulted in an overestimation of the declared sensitivity and therefore an under-estimation of the actual number of samples, the concentration of which was higher than the value used as a detection limit.

Kuruto-Niwa *et al.,* 2007 analysed the BPA in colostrum collected within the 3 days after birth (n=101). They report concentrations of total BPA of 3.4 ng/ml on average, which could reach up to 7 ng/ml. The method used (immuno-assays), although presented as detecting both unconjugated and conjugated BPA, could however have under-estimated one (or more) of the different forms present.

More recently, using the LC-MS/MS method, Cariot *et al*., 2012, analysed three samples of milk taken several days after birth (without further clarification). The data obtained showed concentrations of free BPA of 0.80; 3.29 and 3.07 ng/ml.

These studies indicate that the concentrations of BPA in colostrum (collected within 3 days after birth) and breast milk are of the same size.

A daily exposure dose, calculated on the basis of a volume of 600 ml breast milk consumed for an infant of 3.5 kg, would result in the ingestion of 171 ng/kg for milk containing 1 ng/ml of BPA, and 1200 ng/kg for milk containing 7 ng/ml of BPA. These values place the exposure of infants to higher levels than those shown on average for adults. In addition it should be noted that the majority of the BPA detected in breast milk is in the unconjugated form (up to 80%) and that it is likely that a significant proportion of the conjugated BPA absorbed is deconjugated by acid hydrolysis during passage into the stomach and/or by the intestinal flora.

4. Amniotic fluid/placenta/blood of the umbilical cord/follicular fluid

Assays of unconjugated BPA were carried out on the blood of the umbilical cord taken from 152 male newborns after 34 weeks of pregnancy via radio-immunological assay (RIA), (n=106 control group not presenting with cryptorchidism or abnormality of testicular descent; n= 46 cryptorchid newborns). The serum concentrations of unconjugated BPA were 0.14 – 4.76 ng/ml (average of 1.12 ng/ml ± 0.86 ng/ml and median of 0.9 ng/ml) versus 1.26 ± 1.13 ng/ml (Fenichel *et al.*, 2012) respectively. After sampling, the samples were stored at -80°C and contamination by BPA was limited as far as possible by the use of glass tubes and laboratory materials and by checking the absence of leaching of BPA from the laboratory equipment used. Calibration of the RIA method compared to a GC-MS assay was done. In addition, according to the authors, the extraction method used enabled the unconjugated BPA to be separated from the conjugated BPA ensuring specificity of the method for unconjugated BPA. However, although the values obtained from analysing the samples with both methods (RIA versus GC-MS) were correlated together, the values of BPA obtained by RIA were on average around 30% higher than those obtained by GC-MS for the same samples. This tends to suggest that the RIA method plus extraction is not quite specific for unconjugated BPA. This study showed levels of impregnation of around ng/ml in the blood in the umbilical cord. These results are supported by other studies published previously (see table below).

Table 39: Summary table of data of BPA concentration in the blood of the umbilical cord (according to Fenichel *et al.,* 2012)

References	**Method**	**n**	**Sex of the foetus**	**Interval**	**Average +/- SD**
Ikezuki *et al.*, 2002	ELISA	32	m et f		2,2 +/- 1,8 ng/ml
Kuroda *et al.*, 2003	HPLC-FD	9	m et f	0,45 – 0,76	0,62 +/- 0,13 ng/ml
Lee *et al.*, 2008	HPLC-FD	300	m et f		0,65 +/- 1,06 ng/ml
Tan et Mohd, 2003	GC-MS	180	m et f	ND – 4,05 ng/ml	
Schonfelder *et al.*, 2002	GC-MS	37	m	0,2 - 9,2	2,9 +/- 2,5 ng/ml

Fénichel *et al.*, 2012	RIA	106	m	0,14 – 4,76	1,12 +/- 0,86 ng/ml

ND: non detectable

The assay of free and conjugated BPA was carried out on amniotic fluid taken during the second trimester of pregnancy (n=20) and during the third trimester (n=20) by liquid chromatography coupled with mass spectrometry (Edlow *et al.*, 2012). During the second trimester of pregnancy a total BPA was detected in 16 samples out of 20 with values ranging from the detection limit (DL at 0.1 ng/ml) to 0.75 ng/ml with a median of 0.47 ng/ml. Unconjugated BPA was found in 9 samples out of 20 with concentrations of around 0.31 to 0.43 ng/ml with a median of 0.38 ng/ml. During the third trimester a total BPA was found in 2 samples out of 20 and free BPA was detected in a single sample out of 20 (0.42 ng/ml). The authors suggest that this study shows a predominance of the unconjugated form of BPA in the amniotic fluid compared to the conjugated form. However, the very low number of observations showing detectable concentrations and the very low level of these concentrations often close to the quantification limit of the assay moderate this interpretation. The authors explain their interpretation through the capacities of BPA deconjugation by placentary □glucuronidases and the low capacity of hepatic glucuronoconjugation.

Vandenberg *et al.,* 2012 cite two studies in which assays of BPA were carried out in follicular fluid. In the first study (Ikezuki *et al.*, 2002), average concentrations of 2.4 ng/ml were reported. In the second study (Kaddar *et al.*, 2009), 11 out of 28 (39 %) samples taken from infertile women following an IVF protocol show free BPA at a concentration comprised between 0.15 ng/ml to 1 ng/ml). However, Vandenberg and colleagues emphasise that these studies are limited, in particular with regard to the analytical methods used and the type of population investigated.

5. Adipose, liver and brain tissue

Unconjugated BPA was assayed in different types of tissues: fat, liver and brain (Geens *et al.*, 2012). The average concentrations were around 3.78 ng/g for fat, 1.48 ng/g for the liver and 0.91 ng/g for the brain for unconjugated BPA. Glucuronide BPA was not detected. Analysis of the samples was conduced on human tissues taken from patients who died in hospital. The samples were taken in 2002 and the tissues were kept at -20°C. Analysis of the samples was carried out in 2011. No detection of conjugated BPA could be attributed to the instability of glucuronide BPA over time. However, Geens *et al.* highlight that contamination of samples on its own cannot explain the presence of aglycone BPA. In their review, Vandenberg *et al.*, 2012 refer to the Fernandez *et al.*, 2007 data which indicates concentrations comprised between 1.8 and 12 ng/g (3.16 ng/g on average) in free PBA in samples of adipose tissue of human origin.

6. Discussion

These data came from biomonitoring studies. They show large fluctuations in urinary concentrations of BPA depending on the type of diet. They clearly show that due to the rapid elimination of BPA from the body, the urinary concentration of BPA in the individuals is only representative of recent exposure (hours preceding the sample only). The urinary sampling also shows high variability, and while collection over 24 hours significantly represents the quantity of BPA excreted daily, it does not reflect the hourly excretion (Ye et al., 2011).

Sweat was also identified as a fluid of elimination of BPA (Genuis et al. 2011).

Care should be taken when considering the data used as often data lower than the QL are used and values lower than the DL are replaced by DL/1.414.l-type values.

The efficacy of the enzymes used during hydrolysis of conjugated BPA into free BPA also needs to be considered. According to Lakind et al., 2012 (Lakind et al., 2012), the enzymes used by the CDC (Centre for Disease Control and Prevention) to deconjugate the sulphoconjugate are more effective compared to those used by the INSPQ. Some authors such as Koch et al., 2012 (Koch et al., 2012) estimate that the assays of free BPA should be taken into consideration when the studies were carried out in conditions limiting the contamination of BPA or carried out with radiolabelled BPA. If these conditions are not met, these authors recommend using the value in total BPA accompanied by the ratio of total/unconjugated BPA.

Biomonitoring data have been interpreted and used differently in various assessments. Below is a summary of the comments that have been made on EFSA biomonitoring data also analysed by ANSES in response to the consultation of the European Food Safety Authority on its draft Opinion regarding the assessment of risks to human health related to dietary exposure to Bisphenol A.

Although exposure is generally determined by assaying BPA in urine, where it is mainly found in conjugated form, a number of studies also report blood concentrations of BPA in adults and in the umbilical cord blood of newborns. In its expert appraisal report on BPA (ANSES, 2013), ANSES thus devoted a paragraph to blood assays and particularly the share of the various forms of BPA (conjugated and unconjugated) in this matrix. Since the toxicity of BPA has been attributed to its unconjugated form, the share of this form in the blood, related among other things to the individual's metabolising capacity, is an essential parameter to be taken into account when assessing the potential effects of exposure.

In its expert appraisal report, ANSES presented mean values of blood concentrations of unconjugated BPA reported by various studies undertaken between 2002 and 2012 in Asia, Europe and the USA ranging from 0.32 to 2.5 ng/mL in adults. A study carried out in Taiwan in a sample of 97 pregnant women (Chou et al., 2011) reported a maximum value of 29.4 ng/mL.

In umbilical cord blood, the study by Fénichel et al. (2012) cited in the section B.9.3.2.2.1 on "Biomonitoring data" (under the title "Amniotic fluid/placenta/blood of the umbilical cord/follicular fluid") and in the ANSES report (ANSES, 2013) presented, for a population of 152 newborns, blood concentrations of unconjugated BPA ranging from 0.14 to 4.76 ng/mL, with a mean greater than 1.1 ng/mL.

In its report (Section 3.1.2.4, pages 42 to 44), EFSA concludes that the data published since 2010 confirm the fact that, after oral exposure to BPA, the unconjugated form of BPA in the plasma is so low that it cannot be detected/quantified with analytical methods having a limit of detection below 0.3 ng/mL. These conclusions, at odds with the ANSES report (ANSES, 2013), are based on a single study (Teegarden et al., 2011) undertaken in the USA in 20 subjects in whom successive blood assays over a 24-hr. period had shown concentrations of unconjugated BPA below the 0.3 ng/mL limit of detection for all of the 320 serum samples analysed.

The study by Teegarden et al. (2011), also taken into account in ANSES's expert appraisal, was the only one of the studies that reported such low values. The other studies cited in the ANSES report are not taken into account in the EFSA report.

In the paragraph devoted to BPA in the blood of pregnant women and umbilical cord blood, the EFSA report cites the study by Kosarac et al. (2012), reporting serum concentrations of total BPA in 12 pregnant women ranging from <0.026 ng/mL to 10.4 ng/mL (median = 0.548 ng/mL, detection frequency: 67%) at mid-pregnancy and from <0.026 ng/mL to 3.05 ng/mL (median = 1.46 ng/mL, detection frequency: 58%) at delivery. Umbilical cord blood concentrations ranged from <0.026 ng/mL to 2.57 ng/mL (median = 1.82 ng/mL, detection frequency: 42%). Most of the detected total BPA was considered unconjugated BPA since conjugated BPA was only detected in two out of 12 serum samples at concentrations of 0.12 ng/mL and 0.22 ng/mL respectively (this last point is not specified in the EFSA report).

However, the EFSA experts consider that, despite the good quality of the analytical methodology, the data in the study by Kosarac et al. have low credibility due to a lack of information with respect to sample collection and handling, and discrepancies with the study by Teegarden et al. (2011), in which free BPA was never detected and total BPA was only detected in six out of 20 subjects who had peak concentrations of 0.6 to 1.3 ng/mL. In Appendix II of the EFSA report, the low number of subjects in the Kosarac study is also considered a weakness.

In general, the conclusions of the EFSA report on blood concentrations of total BPA and free BPA and the ratio of these two forms are based only on the results of the study by Teegarden et al. (2011). The few studies cited in the report that present high concentrations of unconjugated BPA in biological fluids are all considered as having many methodological shortcomings. This position is particularly questionable insofar as the study by Teegarden et al. ultimately appears to be an exception in the literature compared to the vast majority of other studies, most of which are not covered in the EFSA report.

Biomonitoring studies analysed by EFSA in its scientific opinion (on the risks to public health related to the presence of bisphenol A in foodstuffs, EFSA, 2013) and also analysed by ANSES are reported here below for information. These studies focused on European biomonitoring studies from 2006 and onwards.

European human biomonitoring (HBM) data on urinary total BPA are available from the German Environmental Survey for Children (GerES IV) (Becker et al., 2009; Kolossa-Gehring et al., 2012), the German Environmental Specimen Bank (ESB) study (Koch et al., 2012; Kolossa-Gehring et al., 2012), the Duisburg birth cohort study (BCS) (Kasper-Sonnenberg et al., 2012), two Munich studies (Völkel et al., 2008, Völkel et al., 2011), the Austrian HBM study (Hohenblum et al., 2012), the Flemish and Liege HBM studies (Milieu en Gezondheid, 2010; Pirard et al., 2012; Schoeters et al., 2012), the Generation R (Rotterdam) study (Ye et al., 2008a), the Norwegian mother and child birth cohort (MoBa) study (Ye et al., 2009a), the Spanish environment and childhood (INMA) project (Casas et al., 2011), the French Elfe pilot study (Vandentorren et al., PUBLIC CONSULTATION Draft opinion on BPA exposure 2011), the Italian InCHIANTI study (Galloway et al., 2010) and the European-wide pilot study DEMOCOPHES (Joas et al., 2012).

The fourth German Environmental Survey (GerES IV) is a representative study focussing on the chemical exposure of children (Becker et al., 2009; Kolossa-Gehring et al., 2012). Morning urine samples were collected from 3–14 year old children in 2003–2006. The concentration of total BPA was measured by GC-MS/MS with a LOQ of 0.15 µg/l. BPA was detected in 98.7 % of the n = 599 samples with a geometric mean of 2.7 µg/l and a 95th percentile of 14.0 µg/l (Becker et al. 2009) (Figure 2). The uncertainty in the geometric mean as expressed by the

95th percentile confidence interval corresponded to a relative margin of error of 8–9 %. An analysis by age groups revealed a significantly higher BPA concentration (GM: 3.55 µg/l) in the age category 3–5 years compared to the 6–8 yrs, 9–11 yrs, and 12–14 yrs age categories (GM: 2.22–2.72 µg/l).

By using historical samples from the German Environmental Specimen Bank (ESB), Koch et al. (2012) analysed retrospectively the extent of BPA body burden in the German population from 1995–2009 based on a total of 600 24-h urine samples. According to the ESB concept, samples were taken annually from approximately 60 male and 60 female students (20–30 years old) at each of four university cities (two from East Germany and two from West Germany). Total and unconjugated BPA was determined by HPLC-MS/MS with an LOQ of 0.1 µg/l. In the stored urine samples, total BPA was quantifiable in 99.8 % with a geometric mean of 1.6 µg/l (relative margin of error: 7 %) and a 95th percentile of 7.4 µg/l (Koch et al., 2012). Unconjugated BPA was quantifiable in <15 % of the samples. Total BPA concentrations (geometric mean) decreased over time from 1.9 µg/l in 1995 to 1.3 µg/l in 2009, but 24-h urine volumes (mean) increased from 1.6 litres in 1995 to 2.1 litres in 2009. The derived daily exposures therefore remained rather constant at a geometric mean of 39 ng/kg bw/day (95 % confidence interval (CI): 37–42 ng/kg bw/day) and a 95th percentile of 171 ng/kg bw/day.

Within the framework of the Duisburg birth cohort study (Duisburg BCS), 208 morning urine samples of 104 mother-child pairs (29–49 and 6–8 years old) were collected in 2006–2009 (Kasper-Sonnenberg et al., 2012). Total BPA was measured by LC-MS/MS with an LOQ of 0.1 µg/l. Total BPA was quantifiable in all samples. The geometric mean concentration was 2.1 µg/l (95 % CI: 1.8–2.5 µg/l) in the mothers and 2.4 µg/l (95 % CI: 2.0–2.8 µg/l) in the children (Figure 2); the relative margin of error was 14–19 %. The 95th percentile of total urinary BPA was 8.4 µg/l for the mothers and 9.7 µg/l for the children. The BPA concentrations between children and mothers showed a low but significant correlation ($r_{Spearman} = 0.22$, p-value ≤ 0.05).

The second Munich study (Völkel et al., 2008) analysed spot urine samples from different sources, comprising 62 (multiple) samples from 21 co-workers (19–52 years old) as well as single samples from 31 women (18–41 years old) and 30 children (5–6 years old). The samples were collected in 2005–2008. Total BPA was measured by HPLC-MS/MS with a LOQ of 0.3 µg/l. The median concentration and 95th percentile of this heterogeneous data set was 1.2 and 4.0 µg/l, respectively.

The French longitudinal study of children (Elfe: Etude Longitudinale Française depuis l'Enfance) is a national cohort study examining the effects of environmental exposure on children's health (Vandentorren et al., 2011). Prior to this study, a pilot survey was conducted in two regions for validation purposes, which included the collection of spot urine samples from parturient women having a natural delivery (n = 164) or a Caesarean/forceps delivery (n = 79) in hospital maternity units. Total and unconjugated BPA was quantified by GC-MS with an LOQ of 0.3 µg/l. Total BPA was quantifiable in 96.9 % of all samples. The geometric mean concentration was 2.0 µg/l (95 % CI: 1.6–2.5 µg/l) in the natural-delivery group and 4.5 µg/l (95 % CI: 2.8–7.1 µg/l) in the Caesarean/forceps-delivery group. The higher values in women who had Caesarean sections (or forceps delivery) suggest a contamination from medical devices either from catheterisation or urine probes when biomonitoring at delivery (Vandentorren et al., 2011).

Both North American surveys used spot urine samples and measured the concentration of total BPA. The surveys differed slightly in their analytical procedures (Lakind et al., 2012). For example, the NHANES analysed the samples by HPLC-MS/MS with a LOD of 0.4 µg/l and a LOQ of 1.2 µg/l; measurements below the LOD were assigned a value of LOD/√2. The CHMS used GC-MS/MS with a LOD of 0.2 µg/l and a LOQ of 0.82 µg/l; missing values (<LOD) were assigned a value of LOD/2. Both surveys performed reagent-blank checks, but only the CHMS found results slightly above the LOD that were subtracted from the reported data.

Given the survey differences in geometric means and 95th percentiles of the urinary BPA levels, it can be speculated whether analytic differences such as CHMS-specific background subtraction could have led to a bias in the results. Lakind et al. (2012) examined this issue as well as the differences in the survey methodologies (e.g. participant selection, urine sampling, fasting time) and concluded that the survey differences are unlikely to have substantial impacts on inter-survey comparisons of BPA exposures.

B.9.3.2.2.2 Modelised exposure for the consumers

For a detailed and extensive description and justification of the methodology used, see Annexes 6 and 7.

In the event of exposure of a consumer to thermal papers, it was decided to model the exposure according to two different approaches, using on the one hand an **absorption flow** expressed **in µg.cm^{-2}.h^{-1}**, and on the other an **absorption rate** expressed **in percentage absorbed** of the quantity of BPA transferred onto the skin. Unlike the professionals, the consumer will touch relatively few receipts over the course of a day and it is likely that the quantity of bisphenol A on the fingers is not constant through time. It appeared therefore justified to use an approach based on the level of absorption combined with contact with a thermal receipt with BPA.

Given the uncertainties associated with each of these two approaches, and with a view to being more conservative for the health of consumers, the internal daily dose were calculated *via* the two models, but only distribution of the highest doses was retained to undertake the HRA (corresponding to the approach by level of absorption).

The model using an absorption flow (model a) depends on this flow, on the absorption duration, on the surface in contact with the thermal receipt and on body weight. The one using an absorption level (model b) depends on this level, on the quantity of BPA deposited onto the fingers by contact, on the number of fingers in contact with the receipt, on the absorption duration and lastly on body weight.

Handling of thermal receipts: example of consumers – model with absorption flow

The data used for calculation of the **absorption flow** are the same as those used for the workers scenario. It concerns the study by Marquet *et al.* (Marquet, 2011) from which a **uniform distribution** is allocated with as interval of variation of the distribution, the **minimum (0.026 µg.cm^{-2}.h^{-1})** and **maximum (0.331 µg.cm^{-2}.h^{-1})** values measured.

For the **absorption duration**, it was decided to allocate a **uniform distribution** with an interval varying at least from the **daily duration** of contact with the receipt (produced from the duration of the contact with the daily frequency of contacts) **at 2 hours** at the most. It was therefore considered that the duration of absorption of BPA corresponded *at least* to the

duration of the contact with the receipts and at the most at 2 hours. This maximum value was retained; other evaluations of BPA/receipts exposure purport to be protective using moreover the Biedermann rate obtained after 2 hours of absorption.

The distributions of probabilities specified for the contact time with a receipt as well as the frequency of daily contact with a receipt for a consumer were issued by assessment of experts based on the study by Danish E.P.A., 2011 where a contact time was considered varying from 5 to 66 seconds per contact and a daily frequency of 1 to 5 contacts. This frequency of contact was estimated by the Danish EPA from data on the number of transactions by bank card in Denmark, on distribution of the payment methods, and on the percentage of thermal paper receipts containing BPA (EU data).

The **surface in contact** with a thermal paper is also based on the report by US EPA (1986) which gives by default a surface area of **2 cm²** for the thumb and **1 cm²** for each of the other fingers, it was decided by assessment by the experts to allocate a uniform distribution over a variation interval of **1 to 12 cm².**

The distributions of probabilities used to represent the **body weight** parameter for the different populations studied were based on the following study:

The study of pre- and post-natal determinants of development and health of the Child (EDEN[20]) gives the body weights of the pregnant women at different stages of the pregnancy and was used to document this parameter.

The model with "the absorption flow" is presented below:

$$DI_{ticket\_CF} = \frac{F \times D_{abs} \times S}{PC} \qquad \text{(model)}$$

With:

DI_{ticket_CF} : Internal daily dose by contact with thermal receipts for consumers with a flow [µg.kg$_{pc}^{-1}$.d^{-1}]

F : Absorption flow [µg.cm^{-2}.h^{-1}]

D_{abs} : Absorption duration [h.d^{-1}]

S : Surface in contact with the till receipt [cm²]

PC : Body weight [kg_{BW}]

Handling of thermal receipts: example of consumers – model with absorption rate

The model of exposure linked to the handling of thermal receipts for a consumer using a rate of absorption, depends on the rate and duration of absorption of BPA, of the quantity of substance deposited, onto a finger after contact, onto the number of fingers in contact with a till receipt and on body weight.

Absorption rate

The distribution of probabilities allocated to this parameter relies on the assessment of experts from two bibliographical sources, the risk assessment report by the European Commission (2008) and the study by Biedermann, 2010. It was then proposed to specify a triangular distribution with a minimum of 10%, the value used by default in the RAR, a mode of 27% which corresponds to the rate estimated in the study by Biedermann, 2010 from the quantity of BPA transferred onto the skin of the finger after a single contact of 5 seconds with a receipt, and the quantity of BPA which was no longer removable from the skin by soap and water 2 hours after this contact., and lastly a maximum of 60% which corresponds to the rate estimated by Biedermann, 2010 2 hours after immersion of the finger in a BPA/ethanol solution.

Quantity of BPA deposited by contact

For this parameter, it was decided by assessment by the experts to specify a uniform distribution in which the limits were defined from the two source studies. It concerns the studies by Biedermann, 2010 and by the Danish E.P.A., 2011 the measurements of which were carried out by a similar protocol. The first of these studies carried out 14 readings over five types of thermal papers and obtained quantities of BPA deposited, on the finger varying from 0.035 µg to 3 µg. The second measured over four thermal receipts quantities of BPA varying from 0.58 µg to 3.75 µg. Thereby; it was decided to allocate a uniform distribution with a variation interval going from 0.035 to 3.75 µg per finger.

Number of fingers in contact with a thermal receipt

The uniform distribution specified for this parameter also relies on the assessment of experts. Its minimum limit is one finger, corresponding to the fact that the receipt can be held with just the thumb in contact with the single side containing BPA, and with a maximum limit of ten fingers.

Absorption duration

The same distribution of probabilities was used as the one used in the scenario of exposure *via* a thermal receipt for a consumer by using a flow.

Body weight

The same distribution of probabilities was used as the one used in the scenario of exposure *via* a thermal receipt for a consumer by using a flow.

The study of pre- and post-natal determinants of development and health of the Child (EDEN = Study of pre and postnatal determiners of the development and the health of the child) gives

the body weights of the pregnant women at different stages of the pregnancy and was used to document this parameter.

The model with "the absorption rate" is presented below:

$$DI_{ticket\_CT} = \frac{T_{abs} \times Q_{subs} \times N}{D_{abs} \times PC} \quad \text{(model)}$$

With:

DI_{ticket_CT} : Internal daily dose by contact with thermal receipts for consumers with a level [µg.kg_{pc}^{-1}.d^{-1}]

T_{abs} : Level of absorption (established for an absorption duration of 2 hours) [%]

Q_{subs} : Quantity of substance deposited by contact [µg.$finger^{-1}$]

N : Number of fingers in contact with the till receipt [finger]

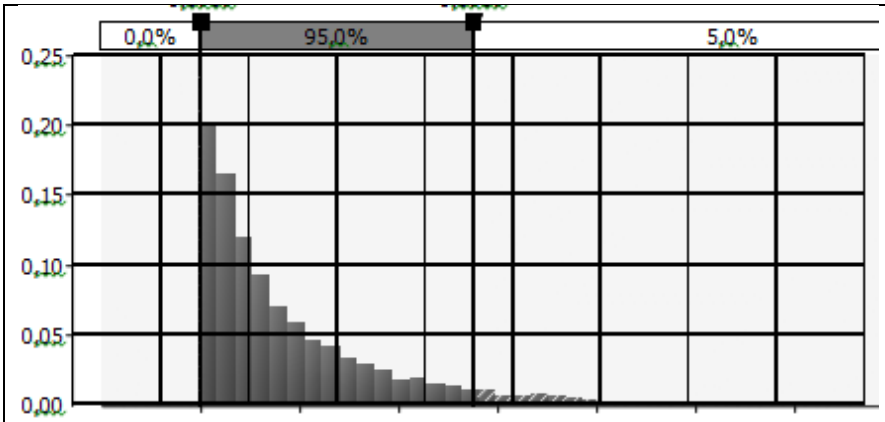
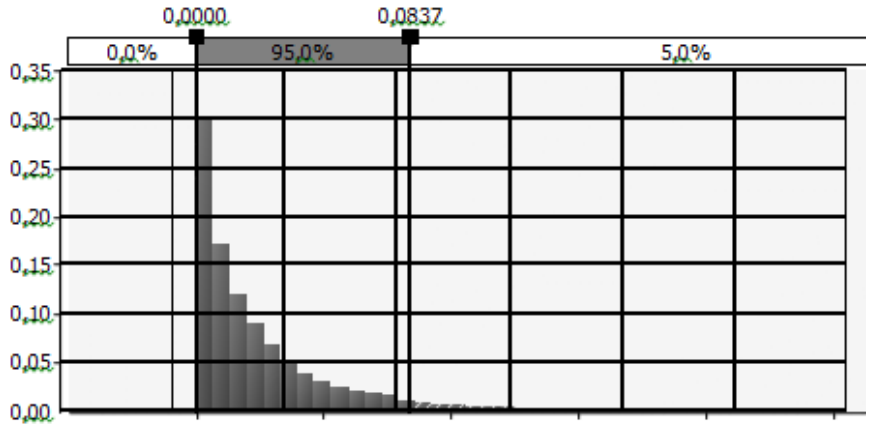
D_{abs} : Absorption duration [$h.d^{-1}$]

PC : Body weight [kg_{BW}]

In this paragraph the results of calculations of the internal dose linked to the handling of thermal receipts are presented for a population of pregnant women consumers.

Figure 27. Histograms and descriptive statistics of the internal doses *via* the handling of thermal receipts for a general population of pregnant women

Histograms - µg.kg_{bw}^{-1}.d^{-1}	Descriptive statistics	
Distribution of internal doses for consumer pregnant women - FLOW	Minimum	$2.90.10^{-5}$
	P5	$9.67.10^{-4}$
	P25	$4.44.10^{-3}$
	P50	0.01
	P75	0.02
	P90	0,04
	P95	0,05

	Maximum	0,14
	Average	0,02
Distribution of internal doses for consumer pregnant women - FLOW 	Minimum	$9{,}13.10^{-6}$
	P5	$8{,}82.10^{-4}$
	P25	$5{,}12.10^{-3}$
	P50	0,01
	P75	0,03
	P90	0,06
	P95	0,08
	Maximum	0,26
	Average	0,02

For "pregnant women consumers handling thermal receipts", the internal doses vary from:

$2.90.10^{-5}$ to 0.14 $\mu g.kg_{bw}^{-1}.d^{-1}$ for the exposure model using a **flow,**

$9.13.10^{-6}$ to 0.26 $\mu g.kg_{bw}^{-1}.d^{-1}$ for the exposure model using a **rate.**

The percentiles 95 are 0.05 $\mu g.kg_{bw}^{-1}.d^{-1}$ and 0.08 $\mu g.kg_{bw}^{-1}.d^{-1}$ respectively.

In a conservative approach, the assessment of the health risks of the population of pregnant women consumers handling thermal receipts is done from the exposure model using a **rate of absorption**.

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Analysis of Some Recent Biomonitoring Publications on Exposure Assessment Concerning Cutaneous Exposures to Bispenol A (BPA) or Substitute in Thermal Paper[21]

Ehrlich et al. (2014)

Ehrlich, S., Calafat, A. M., Humblet, O., Smith, T., and Hauser, R. (2014). Handling of thermal receipts as a source of exposure to bisphenol A. *JAMA* **311**(8), 859–860.

Description of the Paper (Ehrlich et al., 2014)

This group conducted a simulation experiment to test the hypothesis that handling thermal receipts significantly increases bisphenol A (BPA) exposure, but using gloves during handling minimizes exposure. Participants of this study handled BPA receipts continuously for 2 hours. The experimental design included three experimental groups. The first group consisted of 24 volunteers with a mean age of 35 ±12 years (age ± sd) who provided at least two urine samples for the simulation without gloves. The second group consisted of 12 volunteers with a mean age of 33 ±12 years who provided additional sequential samples, and another 12 participants with a mean age of 34 ±13 years who completed the simulation with nitrile gloves. The geometric mean of the BPA concentration in the urine collected from the volunteers before exposure was 1.8 µg/L (95% confidence interval [CI] 1.3–2.4 µg/L; n=23). Four hours after handling thermal papers without gloves, the BPA concentration in their urine was 5.8 µg/L (95% CI 4.0–8.4 µg/L; n=23). In the group wearing the nitrile gloves, the authors observed no significant increase in urinary BPA concentration after handling receipts. The third group of 12 volunteers, which were from the first group of 24 without gloves, provided additional sequential urine spot samples at 8, 12, and 24 hours after the experiment. The mean BPA concentration in their urine was 11.1 (95% CI 5.5–22.8) at 8 hours, 10.5, at 12 hours and 4.7 µg/L at 24 hours. The authors concluded that in this pilot study, they observed an increase in urinary BPA concentrations after continuously handling receipts for 2 hours without gloves, but no significant increase when using gloves. In addition the authors mentioned the clinical implications of the height of the peak level and the chronicity of exposure are unknown, but these factors may be particularly relevant to occupationally exposed populations such as cashiers, who handle receipts 40 or more hours per week.

Analysis of the Paper (Ehrlich et al., 2014)

This paper presents some interesting information because through the test, the authors implicated thermal paper as a significant source of exposure. However, an analysis of this paper reveals several advantages and disadvantages.

The first advantage is that the consistency between the baseline concentration and the general population is strong, suggesting that the population used during the experiment reflects the general population. A second advantage is that a Centers for Disease Control and Prevention laboratory measured the BPA concentration in the urine. Located in the United

[21] These studies were not available at the time of the elaboration of the proposal. Quoted during the public consultation and considered by RAC as important studies, they are now analysed and incorporated in the BD.

States, this federal laboratory is internationally recognized. A third advantage is that the BPA concentrations reported were adjusted for specific gravity. This adjustment is important to control the dilution of the urine with the period of the day. Another interesting point is that the authors compared the exposed group to the *National Health and Nutrition Examination Survey* (NHANES) level of exposure in the general population.

Despite these advantages, the study does present some disadvantages. The first disadvantage is that the time of exposure limitation of 2 hours restrained the concentration, as can be observed in the urine. However, during those 2 continuing hours, we did not expect that meals could interfere with the cutaneous exposure. The urine samples were taken 4 hours post-exposure, but the authors did not indicate whether the volunteers ate during that time (the question should be asked to the authors). This missing information can be a bias. In addition, the rationale is not reported why the samples were collected 4 hours post-exposure. We are not sure if this information is enough to establish an overall picture of the elimination and to determine what is relevant for this route of exposure and the potential health impacts. A second disadvantage of this study is the loss of participants during the second simulation. Additional disadvantages of this study are the spot urine samples and the authors did not report information regarding when the meals were consumed pre- and post-exposure. However, because the authors did not collect total urinary volume, it is difficult to estimate total daily excretion based on these figures reported. This information may be important, but my understanding of this paper is that the authors wanted to determine whether holding thermal paper for 2 hours significantly increases the urinary BPA total concentration after 4 hours. Based on that objective, the study was well done with enough volunteers.

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Porras et al. (2014)

Porras, S. P., Heinala, M., and Santonen, T. (2014). Bisphenol A exposure via thermal paper receipts. *Toxicol Lett.* **230**(3), 413–420.

Description of the Paper (Porras et al., 2014)

This paper reported a human cutaneous exposure from thermal paper receipts. The objective of this work was to assess whether handling the thermal paper (e.g., as a cashier) significantly affects daily urinary excretion of BPA. More specifically, this research was used to evaluate the background levels of urinary BPA concentrations from members of the Finnish working age population who were non-occupationally exposed and to set a reference value for non-occupationally exposed Finns. An international thermal paper manufacturer donated the BPA–containing and BPA–free thermal papers for this study. The authors analyzed the papers, which were found to contain 0.9% (w/w) of BPA; however, they were not able to measure any relevant amount of BPA in the BPA–free paper.

For the first experiment, three volunteers collected spot urine samples the day before the test (e.g., first morning void, end of shift, and evening samples). The participants provided full volumes of urine on the day of the experiment and on the following day. Participants washed their hands after collecting the urine sample. The authors used the collected urine to determine the volume of each individual sample. For this scenario, it was assumed that the cashier meets a customer every 3 minutes and holds a paper receipt for 5 seconds while handing it to the customer. The working day was set to 8 hours, including lunch and

refreshment breaks. During this scenario, the paper receipt was handled approximately 140 times, and the total time of the paper's contact with the fingers was approximately 11 minutes. BPA was firmly held by three fingers including the thumb; the BPA–containing side of the paper was in contact with the pads of the forefinger and the middle finger. The results of this experiment showed that the total BPA excreted from 0 to 24 hours ranged from 4.2 to 9.1 µg, corresponding to urinary concentrations ranging from 0.065 to 0.152 µg/kg of body weight.

Regarding the second experiment, before this test began, three volunteers thoroughly rubbed their hands with a hand cream. The cream allowed the BPA to be more easily absorbed into the skin, which is different approach than the first experiment, because this was expected to increase skin penetration. The participants used three fingers including the thumb to hold the thermal papers (15-cm long and 8-cm wide) for 5 minutes. During that time, the fingers moved the thermal papers around to improve the exposures. This process was replicated three times with a 5-minute break, during which the volunteers repeatedly rubbed their hands with hand cream. The authors conducted the total exposures for a total of 15 minutes. This experiment was replicated on the second day. The participants collected urine samples until approximately 24 hours after the experiment began. Volunteers 1 and 2 collected full urine volumes, and Volunteer 3 collected spot samples. The BPA concentrations were normalized to a specific gravity of 1.021 (based on measurements at the Finnish Institute of Occupational Health [FIOH]). The authors used a specific gravity method to normalize the urine sample. The result showed a total BPA excretion of less than 7.8 µg for 0 to 24 hours (less than 0.12 µg/kg of body weight) for Volunteers 1 and 2.

The authors concluded that, according to the results of this study, normal handling of BPA–containing thermal paper receipts (either professionally or as a consumer) only has a minor effect on our systemic BPA exposure. Although this study was limited to only a small number of participants (corresponding to n=3), the results may be of value when the risks of BPA exposure from thermal paper are assessed under regulatory frameworks. The results are in line with the few available studies on BPA exposure via thermal papers; however, further studies may be still needed to confirm these findings.

Analysis of the Paper (Porras et al., 2014)

An analysis of this paper reveals many advantages. First, this experiment was relatively well conducted. Second, the authors tried to control the experiment and the potential bias. Third, the authors also normalized their sampling by gravimetric method or creatinine, which is good. Fourth, the urinary concentrations are reported individually in Figures 2 and 3 for each volunteer (Experiment 2). Lastly, it seems that each micturition is represented.

Despite these advantages, this paper presents many disadvantages. First, an important disadvantage is that the number of volunteers was limited to three, which limited the sampling and increase the variability observed. Second, Food was discussed in the paper, but this was not measured as a contribution of BPA. However, the authors believe that the food exposures are the major contributors, which are well recognized and documented in the literature. Third, the variability might be important information, but this is not discussed in this paper. Fourth, the mean of the BPA concentration (referred to Table 3 in the paper) is

interesting for a long life exposure; however, for a short half-life, the maximum concentration represents the major information that needs to be considered. Fifth, the maximum BPA concentration in the urine provides information about the concentration in blood and possibly about when the maximum concentrations were previously present; however, there is no indication about a level of this maximal reach in blood.

It is believed that this experiment is interesting by itself, but certainly not enough to confirm whether the European Food Safety Authority (Efsa) level is adequate. The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) made its analysis by considering the windows of sensitivity. ANSES demonstrated that during pregnancy, the sensitivity is higher, particularly for the fetus, compared to the general population.

The major contribution of this paper is to show that thermal papers significantly contribute to increasing BPA exposure and that this contribution is added to other sources of exposure. In general, this paper provides interesting pieces of information, but there are still some limitations regarding the number of volunteers and simplifications of the discussion and the conclusion based on this limited experimentation. This paper expresses a false impression of safety, which is not necessarily a good idea. Thus, the strong conclusion, based on a very small group, is probably the major concern of this paper. I highly agree with one remark made by the authors in the Conclusion section of the paper that says more experiments are required to confirm these observations.

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Thayer et al. (2014)

Thayer, K. A. et al. (2014). Bisphenol A, Bisphenol S and 4-hydroxyphenyl 4-isoprooxyphenylsulfone (D-8) in urine and blood of cashiers pre- and post-shift (Submitted).

Description of the Paper (Thayer et al., 2014)

The objective of this research was to study a sample of receipt paper for the presence of BPA and BPA alternatives listed in the U.S. Environmental Protection Agency's Design for the Environment report titled *Bisphenol A Alternatives in Thermal Paper* (US EPA 2014). Results of the screening-level analysis showed that the receipts contained BPA, BPS, or BPSIP (4-hydroxyphenyl 4-isoprooxyphenylsulfone, also called D-8, CASRN 95235-30-6), a bisphenol S (BPS) analogue with an isopropyl ether on one of the phenolic hydroxyls. This paper presents four groups of participants: three cashiers groups exposed to BPA (n=34), to BPS (n=32), or to BPSIP (n=12) and a non-cashiers group (n=25). All participants were sampled pre-shift and 2 hours post-shift. The authors determined the concentrations of BPA_{total}, BPS_{total}, and the $BPSIP_{total}$ but also measured other bisphenol in a specific categorie of thermal paper. The authors mentioned that they did not measure the serum aglycone of BPA or BPS because the participants' levels in the serum were small. The median concentrations in the thermal receipts were as follows: 19.3 mg of BPA/g of paper, 14.6 mg BPS/g and 13.9 mg/g of paper for BPSIP. The total urinary BPA concentrations post-shift were significantly higher compared to pre-shift levels in cashiers with the medians as follows: pre-shift = 2.09 ng/mL and post-shift = 4.37 ng/mL (p=0.01). For BSPIP, the authors observed a similarity of the concentrations for pre- and post-exposures. For all volunteers, the authors used a

questionnaire to gain an understanding of the importance of inter-individual variations. Blood samples were also measured for BPA, BPS, and BSIP. For BPA, the authors measured this more often post-shift than pre-shift. Similar results were found for BPS. In conclusion, there were higher post-shift levels or detection frequencies of BPA, BPS, and BPSIP in urine from cashiers who handled receipts containing these compounds compared to pre-shift. Urinary levels of BPA and BPS concentrations and detection frequencies of BPSIP concentrations were higher compared to samples from the non-cashiers group.

Analysis of the paper (Thayer et al., 2014)

In this paper, the authors measured 3 groups of cashiers exposed to BPA, BPS, or BSIP and a non-cashiers exposed group. The size of these groups are relatively interesting with n=34 for BPA, n=32 for BPS, n=12 for BSIP, and n=25 for non-cashiers. According to the results, the contribution of BPA and BPS through the thermal papers on a cutaneous exposure route was not a negligible route of exposure. However, a very careful analysis is required to explain the important individual and inter-individual variabilities. The authors mentioned that they are undertaking a human pharmacokinetic study of deuterated BPA following a cutaneous exposure of 100 µg/kg. In the human pharmacokinetic study, the authors are using many of the same volunteers who participated in our recently completed oral pharmacokinetic study using the same dose level. This study should help to explain the variability and the uncertainty observed here.

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General conclusions on these studies

After the review of these three papers, it is important to mention that new documents available that attempt to characterize this source of exposure (thermal papers) to the cutaneous route contribute substantially to improve the knowledge route of exposure by transfer of BPA. These papers go in the same direction, concluding that the cutaneous route of exposure from thermal paper sources is not negligible based on the significant increase of urinary concentrations measured after testing cashiers during exposure scenarios. Nevertheless, the fraction attributable to the cutaneous route exposed to thermal papers is lesser compared to the oral food source. In addition, to characterize the risk, it is needed to specify the windows of sensitivity. As one knows, the most susceptible groups of the population regarding BPA exposure are foetuses and new-borns. A pharmacokinetic study is required to explore the variability and the uncertainty observed in this last study. It is considered that these new references might not change the conclusion of the Anses 2013 risk assessment of health for Bisphenol A, but confirm the importance of this route of exposure.

2014 biomonitoring studies

It has to be noted that these studies were not included in the dossier at the time of the submission of this proposal. They have been quoted during the public consultation.

- **Hormann et al 2014 study**

The Hormann et al paper discusses the contribution of dermal exposure to bisphenol A (BPA) in to the global internal exposure. The objective of the study was to determine whether using hand sanitizers increase the dermal penetration-of BPA from the surface of BPA–coated thermal paper receipts than dry hands. For this work, the authors reviewed typical and realistic exposure scenarios in which participants were aged between 20–40 years (average age was 27.0 years) and had been in contact with the thermal paper receipts. The results from four experiments were presented in this paper. Experiment 1 measured the concentration of BPA and bisphenol S (BPS) in 50 thermal receipt papers. Experiment 2 determined whether BPA–coated thermal paper receipts held for different lengths of time transferred more BPA to dry hands versus volunteers who used a hand sanitizer. Experiment 3 measured serum and urine BPA concentrations in men and women before and after transdermal and oral exposures to BPA after using a hand sanitizer. Experiment 4 examined the serum and urine BPA concentrations in men and women after transdermal exposure to BPA from holding thermal receipt papers with dry hands.

The results revealed that after applying hand sanitizers and then holding the thermal receipt papers the amount of BPA deposited onto the skin and potentially available for thermal absorption increased the order of magnitude by 2. The authors also provide data showing that thermal receipt paper may significantly increase of BPAfree levels in human serum. The findings conclude that the use of hand's sanitizer significantly increase the transfer of BPA on to the skin and increase the rate of absorption.

- **Ndaw et al 2014 INRS study** (confidential data – under publication process)

INRS provided during the public consultation the preliminary results of their urinary biomonitoring study on occupational exposure to Bisphenol A via thermal paper where cashiers and printers were monitored. These preliminary data concern internal exposure to total and free BPA in cashiers and also in workers from a printing company compared to non exposed group. These results support the scenarios and figures used by the DS but are kept confidential since not published yet.

Considerations by the RAC Rapporteurs, in addition to the views expressed in the opinion of RAC

Description of biomonitoring studies

Hormann et al. (2014) performed research simulating behavior of consumers in a fast-food establishment who are using hand sanitizers before handling the thermal receipt and then eating French fries subject in this case both to dermal exposure and oral exposure from BPA contaminated French fries. It is supposed that the use of hand sanitizers and other skin-care products, including soaps, lotions and sunscreens can contain mixtures of chemicals that are dermal penetration enhancers, for example, propylene glycol. Furthermore, the transfer of a chemical directly from hand-to-mouth (mouthing behaviour) has been considered to be an important variable for estimating total chemical exposure in humans.

A number of experiments were carried out:

- Estimation of the transfer of BPA from thermal paper to hands wetted by applying hand sanitizer and to dry hands. 2 men and one woman each held the thermal paper for different lengths of time: 2, 15, 30, 45, 60 or 240 sec (in 6 separate trials for each subject). In addition, 2 men and 2 women held the receipt with dry hands for 60 or 240 sec (2 separate trials for each subject).
- The transfer of BPA from thermal paper receipts to hands, and the amount of BPA remaining on the surface of a hand 90-min later, after using hand sanitizer in 5 male and 5 female subjects, the amount of BPA transferred from a BPA-contaminated hand to 10 French fries, and measured blood and urine concentrations of unconjugated BPA (uBPA) and its conjugated metabolites BPA-glucuronide (BPA-G) and BPA-monosulfate (BPA-S) before and after ingestion of the French fries and BPA absorption through skin.
- Estimation of the amount of BPA transferred to a clean dry hand and then present in serum and urine without using hand sanitizer was examined. In this study 12 adult men and 12 adult women participated.

In all experiments an 8 x 12 cm portion of thermal paper cut from an unused receipt roll that was obtained from a local merchant (previously identified as containing 27.2 mg BPA/g paper) was placed BPA-coated (print surface) side down into the hand for 4 min. during the main exposure experiments. The sanitizer was not allowed to dry prior to holding the receipt paper. In urinary samples only the total BPA was measured.

It must be remarked that principles of GLP were carefully followed - the possibility of BPA leaching from each piece of equipment used in the collection or processing of samples was determined by passing BPA-free water through all collection equipment, all equipment and sample handling was determined to not leach detectable BPA before any sample collections occurred, baseline measure of BPA on the hands prior to holding a thermal receipt was determined, etc.

All data are presented as mean ± SEM (Standard error of the mean). Total daily intake µg/kg/bw of BPA was not determined.

The data reveal that transfer of BPA from thermal paper to hands wetted with hand sanitizer is much more than to dry hands. The decrease in BPA swiped from the wet hand between 45 sec and 4 min may have been due to absorption into skin occurring at a greater rate than transfer to the skin from the thermal receipt. For dry hands the BPA level increased over time likely due to a reduced rate of absorption with dry relative to wet hands.

Increase in BPA blood serum levels compared to background level prior to the beginning of the experiment was detected after holding BPA-containing receipt paper for 4 min with a wetted hand and followed by picking up and eating 10 French fries over about 4 min with a BPA contaminated hand.

Remarkable increase in total urinary concentration of total BPA was determined in persons who held thermal receipt paper and ate French fries after using hand sanitizer (Table 40). The BPA remained on the contaminated hand throughout the following 90-min period of the experiment.

On the contrary, minor or no increase in BPA urinary and blood serum levels was observed for persons who held thermal receipt paper with dry hands for 4 min.

Table 40. Urine total BPA concentrations for 4 male and 2 female subjects who held thermal receipt paper and ate French fries after using hand sanitizer (from Hormann et al. 2014).

Analyte	Parameter	Male	Female	All
Urine Total BPA	Baseline (µg/l)	0.15±0.04	1.10±0.58	0.46±0.24
	Baseline (µg/g creatinine)	0.20±0.09	1.22±0.24	0.54±0.19
	90 min (µg/l)	23.36±6.66	10.62±3.16	19.11±4.32
	90 min (µg/g creatinine)	18.20±5.33	40.93±22.56	25.77±8.56

Conclusion

The study suggests that transfer of BPA from thermal paper to hands wetted with hand sanitizer is much more than to dry hands which seems to influence the dermal absorption after hand sanitizer is applied. In addition, hand-to-mouth contact plays crucial role in this experiment due to contaminated food taken by hand.

Porras et al (2014) studied BPA exposure via thermal paper receipts in simulation experiments performed by three volunteers, and examined urinary excretion of BPA. Background BPA excretion among the Finnish working-age population was also evaluated. The geometric mean BPA excretion among non-occupationally exposed working-age Finns (n = 121) was 2.6 µg/l, the range being 0.8–18.9 µg/l. The 95th percentile of the non-occupationally exposed people was **8 µg/l**, and this was set as the reference limit for the non-occupationally exposed population.

The first simulation experiment was conducted under conditions representing the most likely exposure associated with the work of a cashier in a supermarket. It was assumed that the cashier meets a customer every 3 min and holds a paper receipt for 5 s while handing it to the customer. A working day was set to 8 h, including lunch and refreshment breaks. A thermal paper receipt containing 0.9% (w/w) BPA was firmly held by three fingers, the BPA-containing side of the paper being in contact with the pads of the forefinger and the middle finger. The paper was held for 5 s. After a 3-min break, the same procedure was repeated with a new paper receipt. This was continued for 8 h, excluding two 15-min refreshment breaks and one 30-min lunch break. During the experiment, the paper receipt was handled about 140 times, and the total time of the paper's contact with the fingers was approximately 11 min. The urinary excretion of BPA was followed from 30 h before and 50 h after the experiment started.

BPA excretion remained below the reference limit in all participants. Calculated total excreted amount of BPA per day (from the beginning of the experiment to 24 h after the experiment) were 0.065, 0.051 and 0.152 µg/kg bw for volunteers 1, 2 and 3, respectively. It should be noted that these values represent total BPA intake from diet and receipts.

The experiment was also repeated with BPA-free paper. Respective amounts in BPA-free paper-experiment were 0.043, 0.017 and 0.103 µg/kg bw.

In the second experiment hands were thoroughly rubbed with a hand cream and the cream was allowed to absorb into the skin. BPA-containing thermal paper receipt was firmly held with

three fingers and the fingers were moved so that they touched all parts of the paper. Occasionally, the ring finger and the little finger also touched the paper. After five minutes, the paper was put into a waste bin and the volunteers' hands were thoroughly rubbed with cream again. The same procedure was repeated two more times. Urine samples were collected until about 24 hours after the experiment began. Urinary excretion also remained at or below background levels (highest value being **10.3 µg/l**). The calculated excreted amounts were 0.12 and 0.093 µg/kg bw for volunteers 1 and 2 (volunteer 3 provided only spot samples - no calculation could be done).

The calculated maximum BPA excretion per day after handling thermal paper was less than **0.2 µg/kg of body weight** which, according to the study authors, suggests a total daily intake over 25 times lower than the European Food Safety Authority's (EFSA's) proposal for a temporary tolerable daily intake (temporary TDI) of 5 µg/kg/day[22].

Ehrlich et al (2014) made a simulation experiment in which participants handled BPA receipts continuously for 2 hours (conditions of the experiment not specified). The geometric mean urinary BPA concentration of the volunteers before exposure was 1.8 µg/l (95% confidence interval 1.3–2.4 µg/l; n=23) and 4 h after handling thermal papers without gloves 5.8 µg/l (95% confidence interval 4.0–8.4 µg/l; n=23). When nitrile gloves were used, no increase was seen (**Error! Reference source not found.**). Half of the participants (n=12) provided sequential spot samples 8, 12 and 24 h after the experiment with mean urinary levels of 11.1 (95% CI 5.5-22.8), 10.5 and 4.7 µg/l, respectively. However, because total urinary volume was not collected it is difficult to estimate total daily excretion based on these figures. The authors indicate that the peak level (5.8 µg/L) was lower than that observed after canned soup consumption (20.8 µg/L) (Carwile et al. 2011).

Thayer et al (2014) studied cashiers prior to shift and after shift (preliminary, unpublished results). The authors assigned each cashier to a receipt-type group based on the results of the receipt analysis (34 cashiers who handled BPA receipts, 32 cashiers who handled BPS receipts and 12 cashiers who handled BPSIP (4-hydroxyphenyl 4-isoprooxyphenylsulfone) receipts). Post-shift urine BPA_{TOT}, $BPS_{TOT,}$ and $BPSIP_{TOT}$ in cashiers was compared to pre-shift levels and to a sample of 25 non-cashiers. Post-shift samples were collected within 2 hours following the end of the shift.

The authors found higher post-shift levels or detection frequency of BPA, BPS, and BPSIP in urine from cashiers who handled receipts containing these compounds compared to pre-shift. Urinary levels of BPA and BPS and detection frequency of BPSIP were higher compared to a sample of non-cashiers. The results of the study are summarized in the Table 41. It shall be noted that those are preliminary results not published and not peer-reviewed yet.

Table 41. Urine total BPA, total BPS and BPSIP concentrations in cashiers and non-cashiers (µg/l)

	cashiers, BPA receipts (n=34)	**cashiers, BPS receipts (n=32)**	**cashiers, BPSIP receipts (n=12)**	**non-cashiers (n=25)**
BPA urine mean ± SD [median, range]				

[22] It is to be noted that EFSA in 2015 proposed a revised tTDI value of 4 µg/kg bw/day

pre-shift	7.48 ± 17.97 (n=34) [2.09, <LOD - 96.70]	4.55 ± 10.59 (n=32) [1.49, 0.40 – 58.12]	1.27 ± 1.29 (n=12) [0.82, <LOD – 4.58]	n/a
post-shift	17.69 ± 63.63 (n=34) [4.37, 0.36 – 372.17]	4.26 ± 6.47 (n=31) [2.20, 0.39 – 36.48]	1.61 ± 1.64 (n=12) [0.84, 0.11 – 4.81]	n/a
non-cashiers	n/a	n/a	n/a	2.01 ± 2.35 (n=21) [0.84, 0.13 - 8.04]
BPS urine mean ± SD [median, range]				
pre-shift	0.87 ± 1.28 (n=34) [0.39, <LOD – 6.68]	0.95 ± 1.50 (n=32) [0.26, <LOD – 5.63]	1.14 ± 1.97 (n=12) [0.49, <LOD – 7.12]	n/a
post-shift	1.03 ± 1.81 (n=34) [0.40, 0.03 – 9.61]	2.24 ± 3.17 (n=32) [0.87, 0.19 – 15.15]	0.64 ± 0.85 (n=12) [0.19, <LOD – 2.53]	n/a
non-cashiers	n/a	n/a	n/a	2.12 ± 5.90 (n=25) [0.24, <LOD – 29.26]
BPSIP urine (% detect)				
pre-shift	4/34 (11.8%)	6/32 (18.8%)	10/12 (83.3%)	n/a
post-shift	6/34 (17.6%)	8/32 (25.0%)	9/12 (75.0%)	n/a
non-cashiers	n/a	n/a	n/a	9/25 (36.0%)

Ndaw et al (2014) (not peer-reviewed) studied cashiers (90), workers from a printing company handling BPA containing thermal paper on rotary press (4) and non-occupationally exposed workers (44) in France. Spot urine samples, including pre-shift and post-sift samples and first morning void were collected from each participant, during 1 or 2 days. Samples collections were performed between July 2013 and June 2014. Free (unconjugated form) and total (unconjugated and conjugated BPA forms) were measured in each urine sample. Exogenous contamination due to releasing of BPA from the materials and solvent contamination was minimized during the overall procedure.

The median urinary total BPA concentration was 3.5 µg/l (2.9 µg/g creatinine adjusted) for non occupationally exposed workers and 8.9 µg/l (6.8 µg/g creatinine adjusted) for cashiers. For the free BPA, the median urinary concentration was 0.22 µg/l (0.21 µg/g creatinine adjusted) for non occupationally exposed workers and 0.28 µg/l (0.22 µg/g creatinine adjusted) for cashiers.

The median urinary total BPA concentration of 4 workers of printing company was 80.7 µg/l.

As the results of this study are not given as geometric average values, it is difficult to compare them with some other biomonitoring investigations showing exposure levels of general

population.

B 9.3.2.3 Indirect exposure of humans via the environment

The exposure of the general public to BPA through their environment has been analysed, considering:

The air compartment (internal air and external air) – exposure through inhalation;

Sedimented dust – exposure through ingestion;

Food and drinks (including drinking water) – exposure through ingestion.

Internal exposure doses related to the environment were first calculated separately for each of the means of exposure considered, then combined to reflect a total exposure.

The exposure doses to BPA were characterised using a probabilistic approach in order to take account of the maximum variability of exposures.

Therefore, a distribution of internal exposure doses (IED) was modelled for each of the target populations considered.

Figure 28. Characterisation of the risks associate with BPA contained in the air

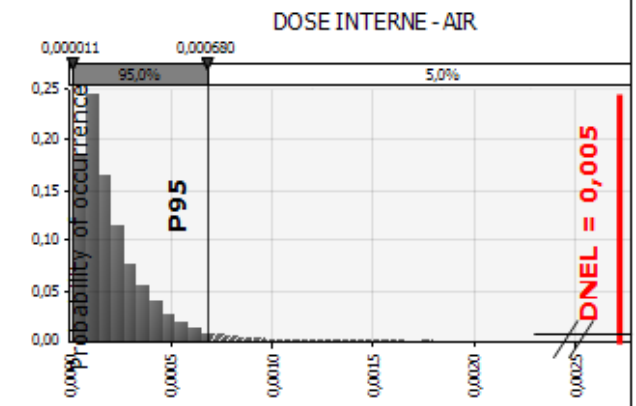
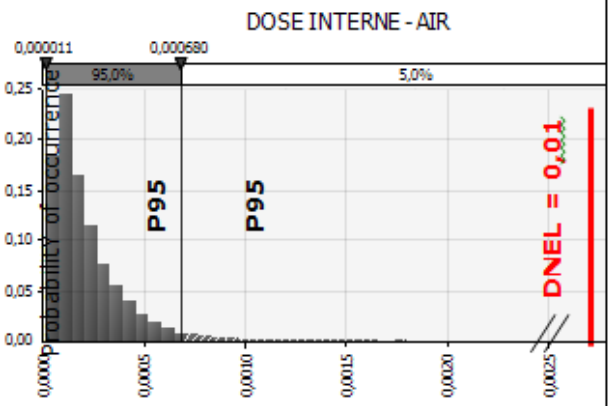
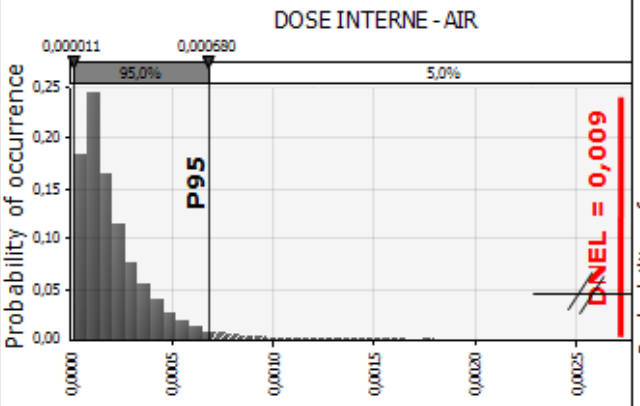
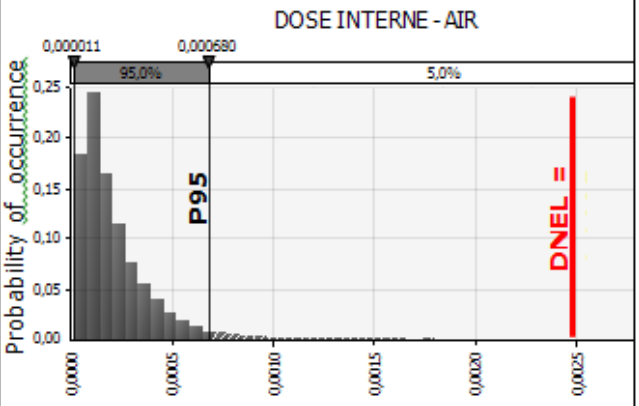
Critical effect on the brain and behaviour	**Critical effect on the female reproductive system**
INTERNAL DOSE - AIR 	INTERNAL DOSE - AIR 
Critical effect on metabolism and obesity	**Critical effect on the mammary gland**
INTERNAL DOSE - AIR 	INTERNAL DOSE - AIR 

Figure 29. Characterisation of the risks related to BPA contained in sedimented dust

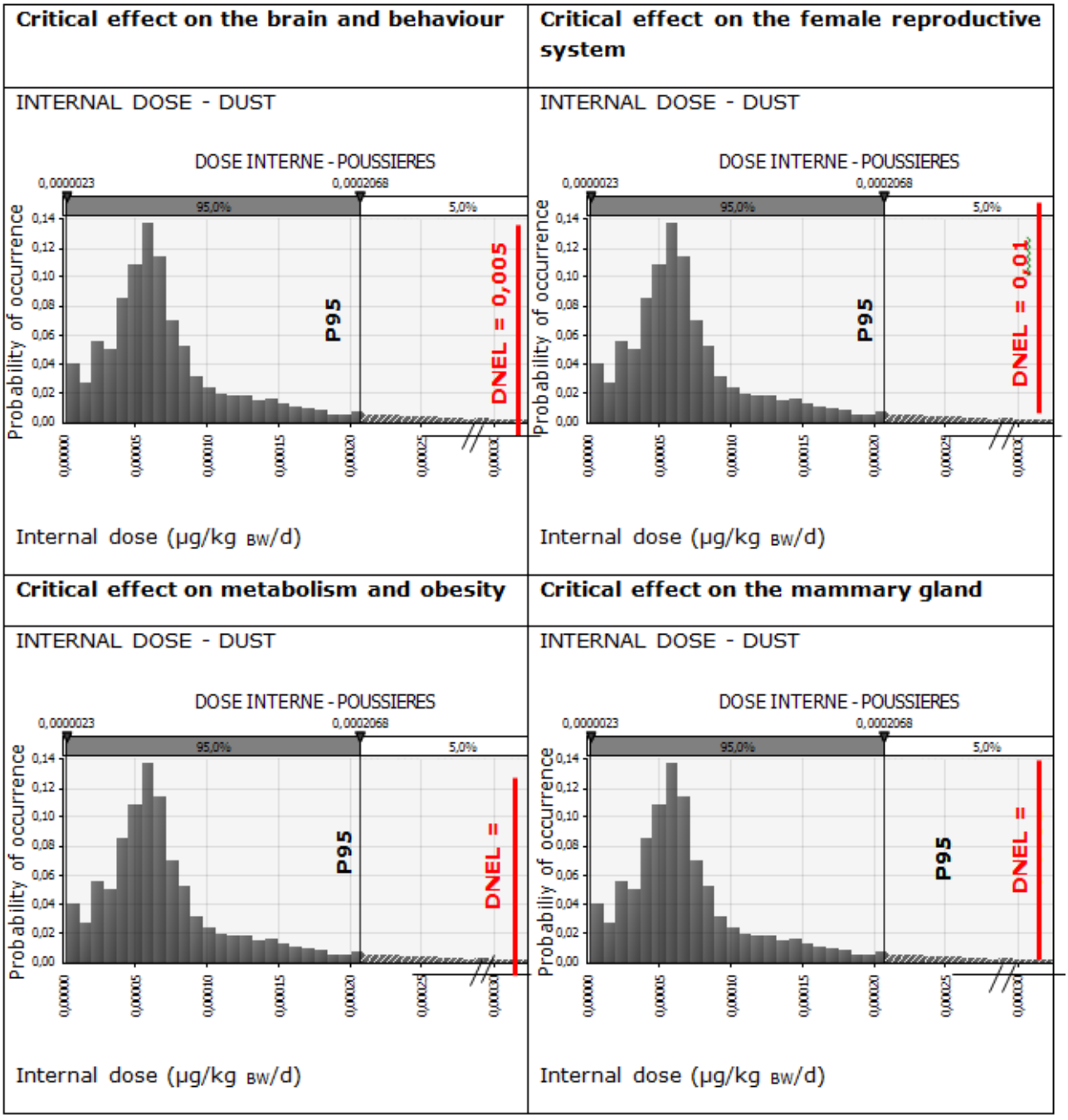
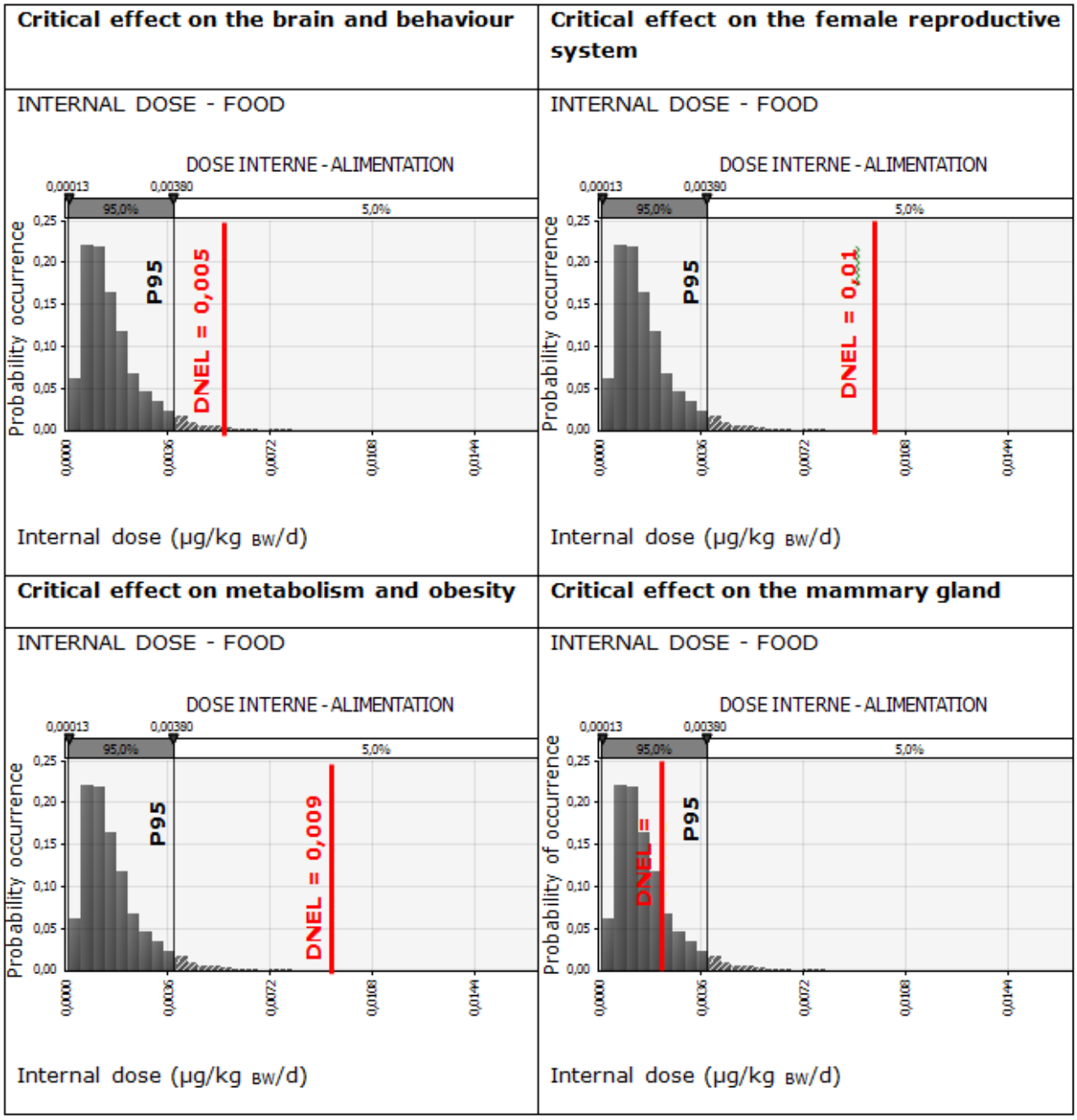


Figure 30. Characterisation of the risks related to BPA in food



B.9.3.2.4 Environmental exposure

As explained in the section B.2, BPA can be present in recycled paper products such as mentioned above (napkins, toilet paper, paper towels, newspapers, magazines, etc.). This hydrolysis treatment allows removing up to 95 % of BPA composing the thermal paper. Traces of BPA may in principle remain in the products produced from recycling. According to a German study, thermal paper is the main contributor to the BPA contaminating these products, given the concentrations in this type of paper, and despite their very small share in the total production of papers (Gehring, 2004). Moreover, the recycling of thermal paper containing BPA is suspected to be one of the main sources of contamination of the environment via aqueous

effluent recycling containing BPA-chlorinated derivatives or sludge from sewage purification plants (UBA, 2010). From EU RAR 2008 and (OECD, 2009), the estimation of BPA quantity likely to enter the recycling supply chain is about 500 tons/year. This figure is consistent with the quantity of BPA released provided for the paper recycling sector by the European Commission, be it 350 tons/year. This stand for 70% of total annual aquatic releases (INERIS, 2010).

B.9.4 Other sources

BPA contamination data in food and beverages

The French Total Diet Studies (TDS) based on a standardised method recommended by the World Health Organization (WHO) examined the different substances that may be found in food "as consumed" and sought to determine the "background levels" of exposure to which populations are subjected. ANSES commissioned a study on reserve samples from the second French study (TDS 2) that has enabled the characterisation of BPA levels in all foods in the diet of the targeted populations. The CES on the Assessment of physico-chemical risks in food (ERCA) points out that these data were produced from food collected throughout France between 2007 and 2009. Nearly 85% of the composite samples (i.e., each consisting of fifteen samples) had a low level of contamination that is due to the ubiquitous nature of BPA. A significant share of samples contaminated with higher levels (>5 µg/kg) was highlighted. This primarily concerns canned goods (vegetables, prepared meals, fishery and meat-based products) and fishery and meat products not packed in cans.

The study conducted by the ANSES Nancy Laboratory for Hydrology on water intended for human consumption (WIHC) supplemented the data available for food. This study is the first in France to investigate the levels of BPA in the public water supply system across the country and in various packaged waters (still, carbonated, spring and natural mineral waters – packaged in bottles, cans and refillable water containers).

A review of the international literature has revealed low levels of BPA contamination in the public water supply and has also pointed to the possibility of BPA migration from polycarbonate containers into drinking water. The results obtained at the conclusion of the ANSES study indicate low levels of contamination, with the exception of water in polycarbonate refillable containers. Thus, for water bottled in refillable water containers, the French data obtained in 2011 on 28 samples of water container available on the French market have confirmed that the levels of BPA concentrations reach 4 µg/L.

Exposure by ingestion of food and beverages

Exposure *via* ingestion of food and beverages is characterised from data on individual consumption and BPA contamination in each food. Due to the comprehensive nature and limits of analytical sensitivity of the TDS 2 study, covering the entire diet, and the study on WIHC (336 results for mainland France), it was decided to use these recent French data on BPA concentrations to characterise exposure of the general population.

The results of the probabilistic assessment of the ID of the general population to BPA *via* its environment shown below have helped to define the median value of the exposure and the 95th percentile used for the characterisation of risk to pregnant women:

Table 42. Internal doses (ID) associated with other media than thermal paers: air, settled dust and food exposure media, for pregnant women and their unborn child.

Exposure scenario	Internal exposure dose by $\mu g.kg^{-1}.d^{-1}$	
	median	95th percentile
Exposure by inhalation	$1.63.10^{-4}$	$6.8.10^{-4}$
Exposure by ingestion of settled dust	$6.23.10^{-5}$	$2.07.10^{-4}$
Exposure by ingestion of food and beverages	$1.36.10^{-3}$	$3.8.10^{-3}$
Total exposure resulting from the aggregation of these three routes of exposure	**$1.68.10^{-3}$**	**$4.18.10^{-3}$**

The relative contribution of each other source of exposure to the total internal dose was also calculated from each medium and is shown in the graph below. It was not possible to combine thermal paper exposure with these sources for the following reasons: certain data have been measured whereas others are modelised; the specific model for thermal paper exposure takes into account numerous parameters that were not taken into account for the other sources (number of finger, flux or rate of absorption...), a few data available taken into consideration for demal absorption and finally, the lack of available data for validating the systemique bioavailability.

B.9.5 Overall environmental exposure assessment

Not evaluated in this dossier.

B.9.6 Combined human exposure assessment

As explained in section B.9.4, it has to be emphasized that the combined human exposure assessment herein has been performed on food, dust and air and does not include exposure to thermal paper for the following reasons: certain data have been measured (for food , dust and air) whereas others are modelised (for thermal paper); the specific model for thermal paper exposure takes into account numerous parameters that were not taken into account for the other sources (number of finger, flux or rate of absorption...), a few data available taken into consideration for demal absorption and finally, the lack of available data for validating the systemique bioavailability.

Internal exposure doses related to the environment were first calculated separately for each of the means of exposure considered, then combined to reflect a total exposure.

The exposure doses to BPA were characterised using a probabilistic approach in order to take into account the maximum variability of exposures.

The choice of populations used for the characterisation of exposure doses is based on the data available for quantifying exposure associated with the presence of BPA in food intended for human consumption. In the ANSES report (ANSES, 2013), exposure doses were calculated for pregnant women, adults (both men and women) and children over three years of age. The probability distributions and descriptive statistics of the internal doses (IDs) were provided for each medium considered and analysed (air, settled dust and food) and the total internal dose, as a result of the aggregation of these three routes of exposure.

Calculation of internal doses is based on a consideration of knowledge on the absorption or bioavailability of BPA in the body. On the basis of a critical analysis of the available toxicokinetic data, the bioavailability factor used by oral route of unconjugated BPA is 3% and by inhalation is 100%.

In view of the hazard characterisation of BPA and the available dose-response relationships, the HRA was conducted only for the pregnant woman, in order to protect her unborn child. This choice reflects the identification of a window of susceptibility during pregnancy. Only the results for pregnant women who were the subject of the HRA are detailed in this note. The exposure models used based on BPA contamination data are summarised below.

The results of the risk assessment carried out by ANSES for the combined human exposure to BPA **via the air, sedimented dust and food** are summarised in the table below (ANSES, 2013).

Table 43. Environmental and food exposure: health risks for the offspring of the human species assessed using critical effects observed in animals

Population exposed: pregnant women **Target population: offspring**	**Critical effect on:**			
	Brain and behaviour	**Female reproductive system**	**Metabolism and obesity**	**Mammary gland**
Air	Negligible risk	Negligible risk	Negligible risk	Negligible risk
Sedimented dust	Negligible risk	Negligible risk	Negligible risk	Negligible risk
Food	Negligible risk	Negligible risk	Negligible risk	Situations of exposure to risk exist
All means	Negligible risk	Negligible risk	Negligible risk	Situations of exposure to risk exist

On average, the major contribution of the internal exposure dose is derived from food (84% for pregnant women). The ingestion of dust or inhalation of air contaminated by BPA contributes a small amount to the internal dose (4% and 12% respectively).

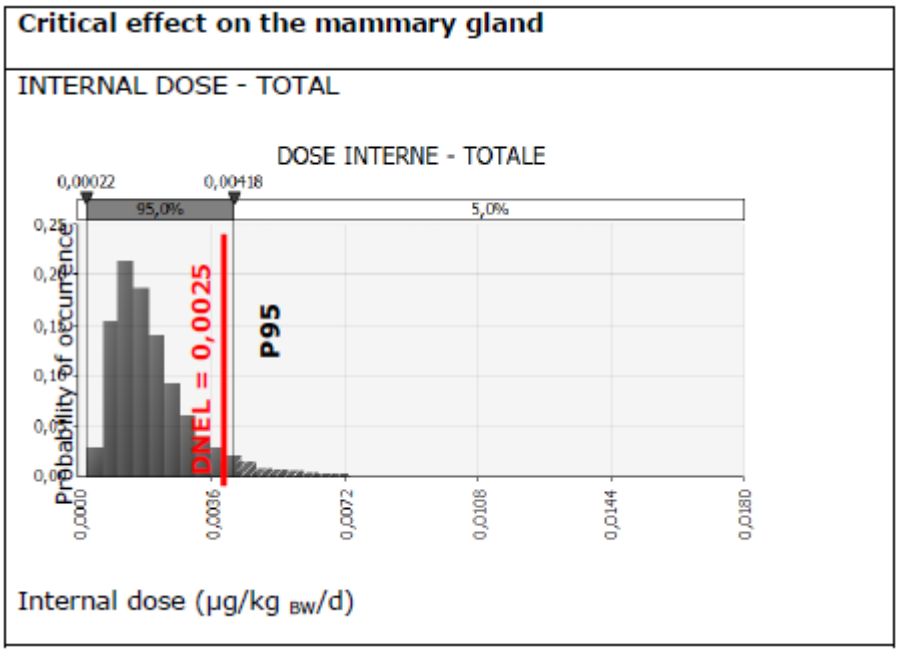
Lastly, following the methodology used, the results of the HRA for a total exposure through air, sedimented dust and food show that some exposure to BPA situations presents a risk to the mammary gland of the embryo and the foetus through maternal exposure.

For the critical effect on the mammary gland, and on the basis of the simulations of internal exposure doses carried out (which taken into account the variability of the parameters used in the calculation of the exposures as much as possible), we cannot exclude the appearance of this critical effect in 23% of the exposure situations (exceeding the toxicological benchmark).

It should be noted that exposure through food only led, for this same effect, to the observation of potential situations of risk (a probability of around 16%). Taking into account this element and furthermore considering the major contribution of food to the total levels of internal dose of BPA, a supplementary study was conducted specifically on food exposure which aimed to use the data on food contamination in greater detail. Under these specific developments, an identification of the principal contributors to food exposure to $BPA_{unconjugated}$ in pregnant women, and also in adults and children over 3 years old, could be conducted. Furthermore, different types of exposure scenarios have been developed in connection with the identification of the principal contributors.

For the other 3 types of effects, the 95thpercentile of the distribution of internal exposure doses is less than the respective toxicological benchmarks, which, according to the methodology used, leads to the classification of the negligible risk. However, it should be noted that for the critical effect relating to the brain and behaviour, the probability of observing risk situations is not zero and is in the order of 2.

Figure XX. Characterisation of the risks associated with BPA via all media, air, settled dust and food, with respect to effects on the mammary gland.



B.9.7 Analysis of uncertainties related to exposure estimation

The uncertainties linked to assessing exposure to BPA of consumers and professionals via handling of thermal-printed receipts are described here above.

Uncertainties linked to the scenarios:

The scenario of occupational exposure is centred on exposure via the cutaneous route of cashiers handling receipts with a particular focus on pregnant women. So, other professions exposed to thermal papers (lottery tickets, self-adhesive labels) were not taken into account.

Other routes of exposure to BPA such as hand-mouth contact are possible but were not able to be modelled taking into account the insufficiency of the available data.

Only contact with the skin of the pads of the fingers was taken into account and not a surface in greater contact (inner side of the hands) which may not be excluded during changing of the roll or folding or receipts for example.

The exposure is assumed to be continuous and constant for the entire work duration on the basis of the observations of (Biedermann, 2010 which show a constant quantity of BPA transferred to the surface of the skin of the finger, whatever the duration (between 5 and 60 seconds) and repetition (between 3 and 10 contacts) of contact with the receipts.

Uncertainties linked to the structure of the models:

For the scenario "consumers" linked to the handling of thermal receipts, two models were developed:

The first which consisted of evaluating the internal dose from the flow of percutaneous penetration (as is the case for professionals) and with a non-continuous exposure ration over one day,

The second which consisted of evaluating the internal dose from a percutaneous absorption rate (in % of the quantity of the surface of the skin) and the quantity of BPA on the surface of the skin linked to contact with receipts over one day.

It has been decided to carry out calculations according to the two approaches by considering the limits and the advantages of each one. Due to the uncertainties linked to use of either model, the most conservative results of exposure for the HRA orientated towards the choice of the second model (by use of a percutaneous absorption rate).

For the scenario "workers", the model retained cannot take into account the absorption of residual BPA in the skin tissue after the working day, which constitutes a factor of under-estimation of exposure.

The models considered do not take into account the processes of metabolisation, distribution and elimination by the body. This constitutes a major uncertainty. One of the principal limits is that they consider by default that 100 % of the dose absorbed by the skin is then bioavailable in the absence of robust toxicokinetic data for the cutaneous route, and contrarily to the oral route which included the effect of initial hepatic passage. This hypothesis contributes to overestimate exposures calculated in connection with the handling of thermal receipts. The metabolisation of BPA linked strictly to passage through the skin barrier may be considered as an insignificant overestimation factor. The rate metabolised of the absorbed dose is estimated at 6 % after 10h of exposure according to the data from a study on explants of human skin (Zalko, 2011). These choices tend to increase the estimation of the internal dose.

More generally, knowledge of exposures via the cutaneous route is limited: contrarily to concentrations in the air, generally measurable by sampling and chemical analysis, there is not, to date, a standardised method, enabling sampling and therefore quantifying of the surface deposits of chemical products onto the skin. The only data of exposure that we have therefore comes from physical and toxicological models or from studies implementing experimental protocols.

Uncertainties linked to the input data of the models:

Concerning the value of the entry variables, the data of percutaneous penetration flow comes from 15 data *in vitro* on explants of human skin (Marquet, 2011). Extrapolation to a situation *in vivo* is reinforced by effective coherence of the estimations *in vitro* and *in vivo* in rats obtained according to the same experimental protocol (Marquet, 2011).

For the scenario of exposure of consumers using an absorption rate (in % absorbed of the dose transferred onto the skin), the percutaneous absorption of BPA corresponds to the least probable rate values of 10 % at the least and 60 % at the most, encompassing the most probable value of 27 % (Biedermann, 2010). This rate of 27 % was retained from an experimental study (Biedermann, 2010), the data of which cannot also be considered as representative to a population scale. However, the experimental protocol is considered as similar to conditions of exposure of a consumer who handles till receipts on an occasional basis during the day, different to cashiers. With the absorption rates therefore being estimated by Biedermann, 2010 for a duration of exposure of the skin to BPA of 2 hours, they were then weighted in the model of calculation by an exposure duration in the consumer varying at least from the daily duration of contact with the receipt (produced from the duration of contact with the daily frequency of contacts) to 2 hours at the most.

Taking into account the data and hypotheses used, the scenario of exposure of consumers handling till receipts appears subject to more uncertainties that the scenario of exposure of cashiers.

B.10 Risk characterisation

B.10.2 Analysis of uncertainties related to risk characterisation

No analysis of uncertainties related to risk characterisation was made.

B.10.1 Use of BPA in thermal papers

B.10.1.1 Human health

B.10.1.1.1 Workers

Here below, the tables show that the risk characterisation ratio (RCRs) for workers, with an intraspecies assessment factor of 5 and of 10 is always superior to 1 for all the critical effects. Thus there is still a risk for all the critical effects, whether the AF is 5 or 10. For the following of the analysis, the risk characterisation and the human health impact assessment, the values of Table 43, based on the AF of 5, are used, in accordance with REACH Guidelines.

Table 44. Calculation of risk characterisation ratios for workers pregnant women with an intraspecies assessment factor of 5.

Critical effects	DNELs for workers pregnant women with an intraspecies assessment factor of **5**	RCRs calculations with P95 = 0.43 (toxicological benchmarks)
Brain and behaviour	**0.01**	43
Female reproductive system	**0.02**	21.5
Metabolism and obesity	**0.0173**	24.85
Mammary gland	**0.005**	86

Table 45. Calculation of risk characterisation ratios for workers pregnant women with an intraspecies assessment factor of 10.

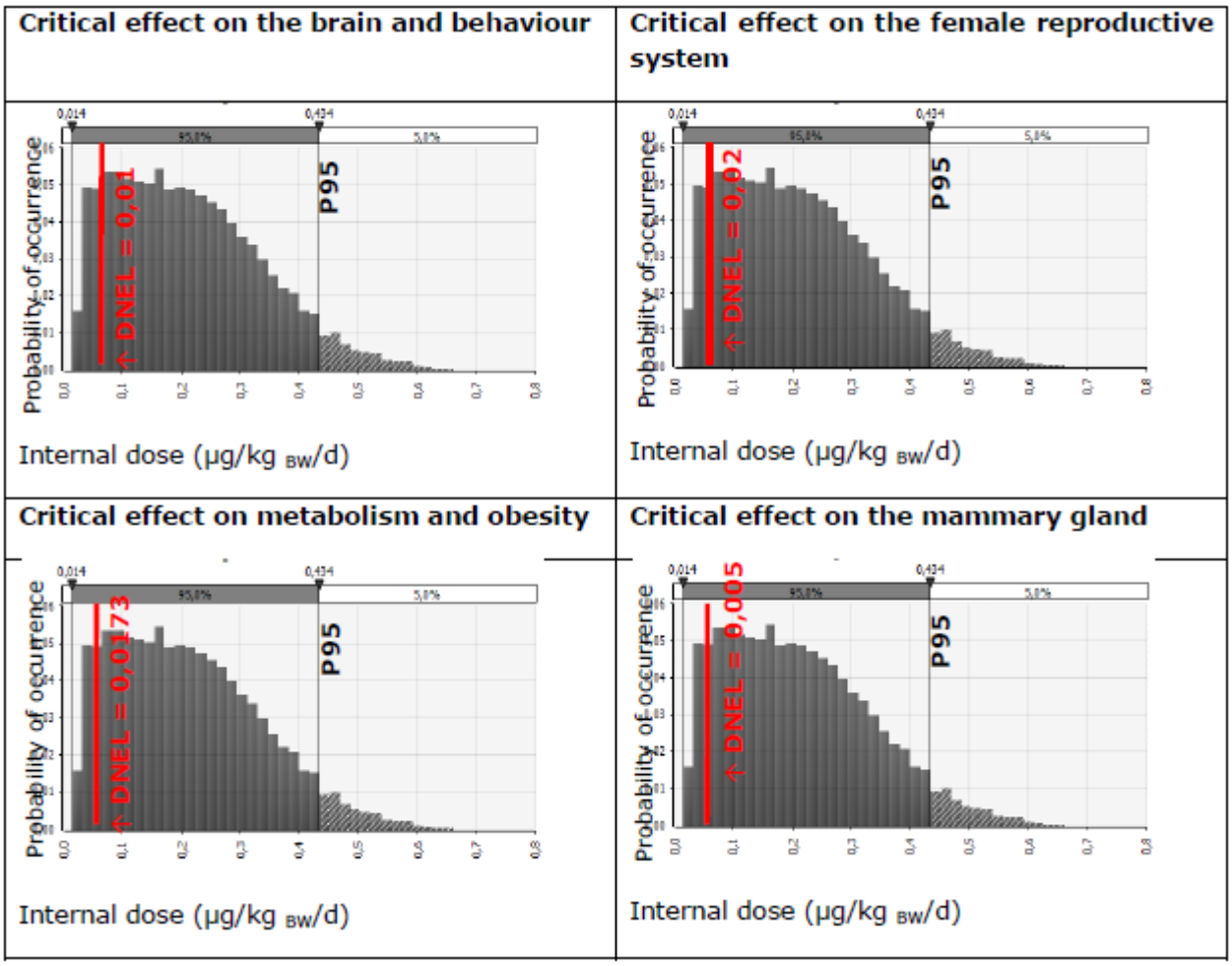
Critical effects	DNELs for workers pregnant women with an intraspecies assessment factor of **10**	RCRs calculations with P95 = 0.43

		(toxicological benchmarks)
Brain and behaviour	0.005	86
Female reproductive system	0.01	43
Metabolism and obesity	0.009	47.78
Mammary gland	0.0025	172

The handling of thermal tickets led to situations of presumed risk for the 4 types of effects considered, for pregnant women working as cashiers. This was the case for the distribution of all the exposure doses modelled.

Figures herebelow enable the position of P95 of the distribution of internal doses to be visualised in relation to the internal DNEL associated with each effect considered.

Figure 31. Characterisation of the risks associated with handling thermal receipts containing BPA – "Cashier/Teller" scenario



B.10.1.1.2 Consumers

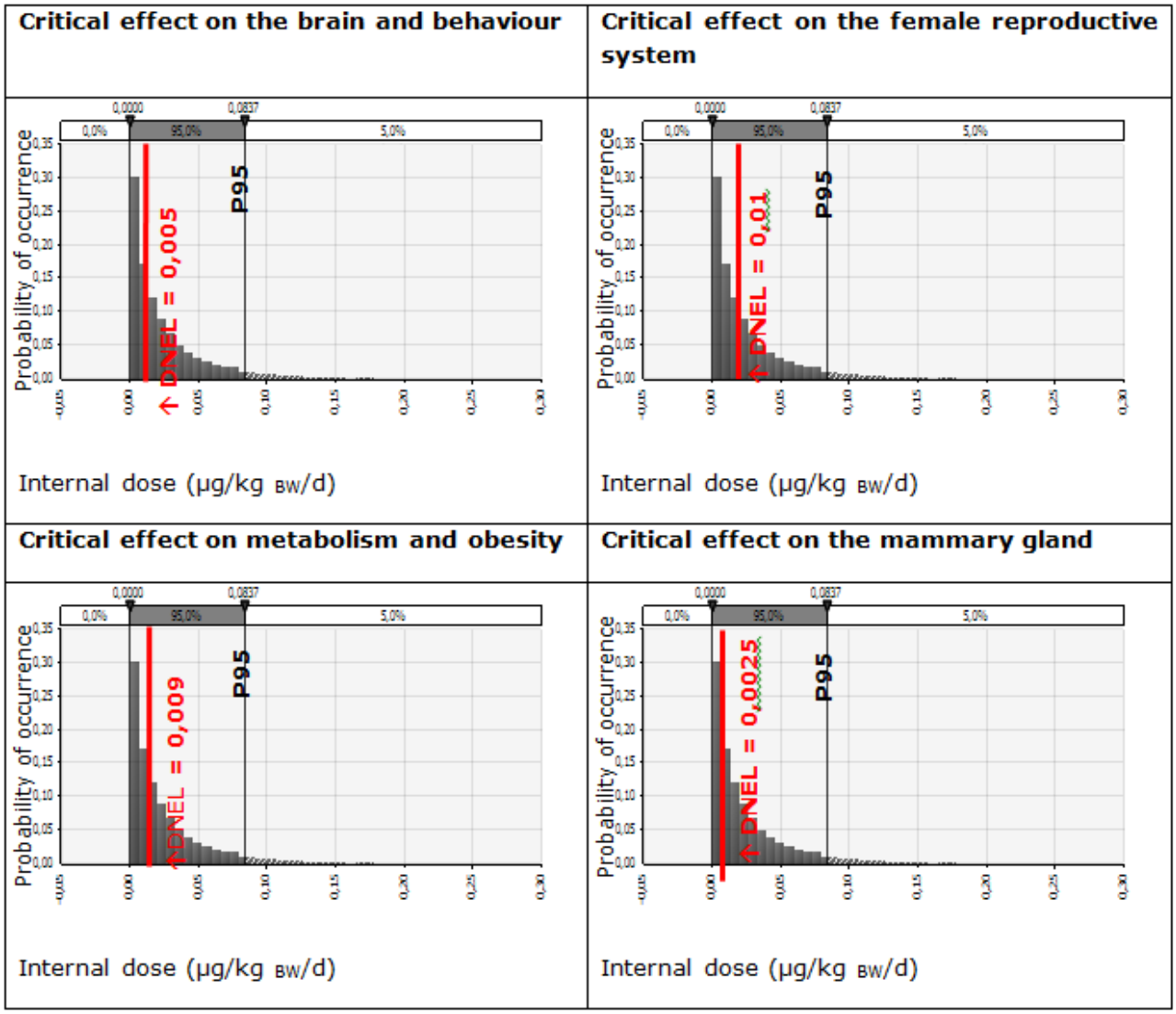
Here below, the tables show that the risk characterisation ratios (RCRs) for pregnant women in the general population, with an intraspecies assessment factor of 10 is always superior to 1 for all the critical effects. Thus there is a risk for all the critical effects.

Table 46. Calculation of the risk characterisation ratios for pregnant women in the general population with an intraspecies assessment factor of 10.

Critical effects	DNELs for pregnant women in the general population with an intraspecies assessment factor of 10	RCRs calculations with P95 = 0.08 (toxicological benchmarks)
Brain and behaviour	0.005	16
Female reproductive system	0.01	8
Metabolism and obesity	0.009	8.89
Mammary gland	0.0025	32

The handling of thermic tickets led to situations of presumed risk for the 4 types of effects considered, for pregnant women in the general population. This was the case for the distribution of all the exposure doses modelled.

Figure 32. Characterisation of the risks associated with handling thermal receipts containing BPA – "Consumer" scenario



Sensitivity analysis (Anses, 2013; annex 21, tome 2):

In view of these results, a sensitivity analysis was carried out:

to identify the influence of the parameter variability on the variability of the internal dose calculated at the output;

to test the influence of the systemic bioavailability after the cutaneous absorption.

The analysis was carried out for the 2 situations:

female pregnant cashiers at their workstation during the course of one day;

a pregnant female handling thermic tickets containing BPA during one day, as a consumer.

The analysis is presented below.

Scenario for female pregnant cashiers at their workstation during the course of one day

1)Parametric uncertainty

In view of the results of the risk characterization for pregnant women handling thermal receipt containing BPA at their workplace, an analysis of sensitivity by tornado graph was made to prioritize the "influence of different parameters of the exposure model". The tornado graphs are in fact a representation of the "influence of the variability of different input probability distributions in the model on the variability of the output". The software "@Risk 5.0" offers two types of statistical analysis to calculate the indices measuring the impact of each parameter on the model output: regression analysis and calculation of rank correlation. In the case of this work, the sensitivity analysis is based on the calculation of rank correlation coefficients of Spearman. The main factors influencing the result are presented first. Indeed, the link between each value of distributions and the result of the model is analyzed by the correlation coefficient. The rank correlation coefficients of Spearman were then preferably used as oblivious to the fact that the distributions of parameters follow or not a normal distribution, whereas the "hypothesis" of a normal distribution is underlying the use of correlation coefficients conventionally used.

The model used to calculate the exposure dose through handling thermal tickets for a professional is:

$$DI_{ticket_{trav}} = \frac{F \times D \times S}{PC_{trav}}$$

With :

- *DI ticket_trav* : Daily internal dose by contact with thermal tickets for the professionals [µg.kg bw-1.j-1]

- *F* : Flow of absorption [µg.cm-2.h-1]

- *D* : Exposure duration to the receipt [h.d-1]

- *S* : Contact area with the receipt [cm²]

- *PC trav* : Body weight [kg bw]

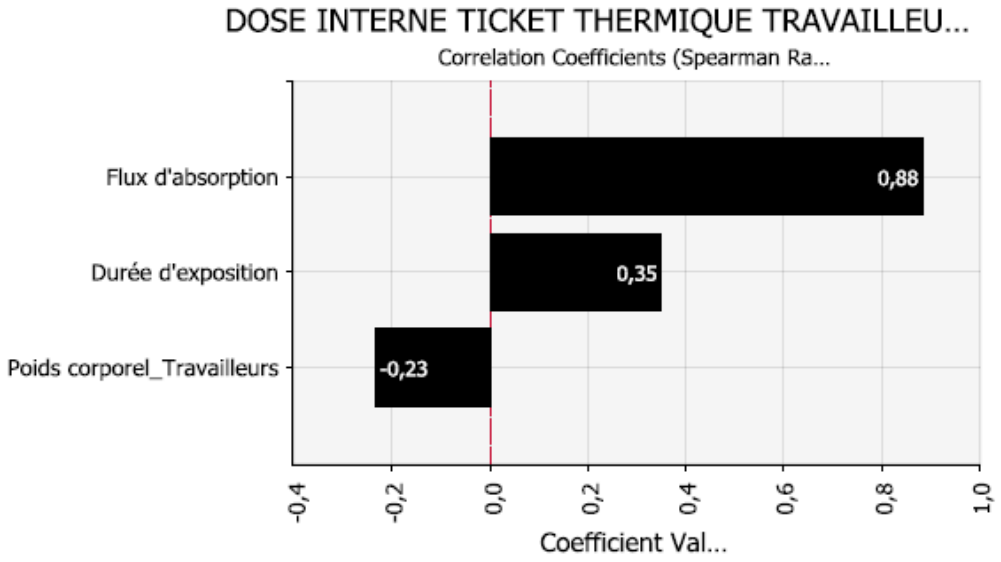
The probability distributions of the model parameters are as follows, the contact surface being fixed in a deterministic way to 12 cm²:

Absorption flow	Uniform distribution [0.026 µg/cm²/h – 0.331 µg/cm²/h]
Exposure duration to the receipts	Triangular distribution

	3h/j – 6.5 h/j – 10h/j
Body weight	Discrete distribution

The graph below shows the sensitivity analysis by tornado graph of modelled internal dose for pregnant women handling thermal receipt containing BPA in the workplace (cashiers) by application of the model mentioned above:

Figure 33. Sensitivity analysis by tornado graph of the modelled internal dose for pregnant women cashiers handling thermal paper containing BPA.



This graph reflects that the flow of percutaneous absorption is the most influential parameter on the internal dose calculated, given the variability of different probability distributions included in the model.

ii) <u>Uncertainty on the value of systemic bioavailability factor after dermal absorption:</u>

As described in the report, this model includes the skin absorption but does not involve the systemic bioavailability factor after the absorption. Indeed, there is no data to determine this bioavailability factor in the scientific literature, thus it was considered by default that BPA absorbed through the skin was then bioavailable in the body, with a systemic bioavailability of 100% absorption.

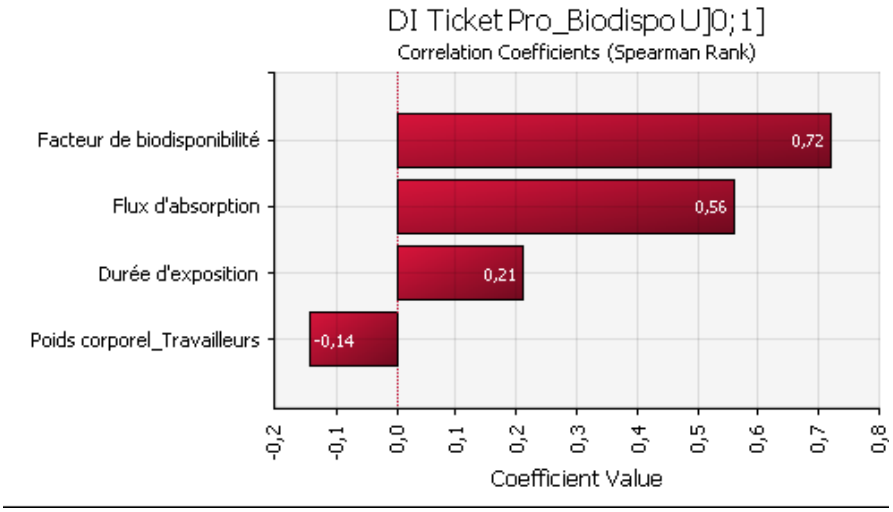
In the following, the influence of the bioavailability factor F on the daily intake ("1st exercise") and on the risk assessment ('2nd exercise") was tested.

The first exercise conducted no longer considers a factor of systemic bioavailability after dermal absorption of 100% by default but introduces into the model for this factor, a uniform distribution of probabilities ranging from 0.01% to 100%.

$$DI_{ticket\_trav} = \frac{F \times D \times S}{PC_{trav}} \times F_{biodisponibilité-cutanée}$$

This exercise is only a theoretical exercise conducted to assess, via a sensitivity analysis by tornado graph on the obtained results, the extent to which factor of systemic bioavailability after dermal absorption is an influential parameter. The sensitivity analysis shows that the factor of systemic bioavailability after dermal absorption is the most influential parameter on the result of the calculation. This analysis confirms that the lack of data to determine a factor of dermal bioavailability is a major uncertainty.

Figure 34. Sensitivity analysis by tornado graph of the influence of the systemic bioavailability factor after dermal absorption.



The second exercise conducted consists in the test in the model of different values of systemic bioavailability factor after dermal absorption. The arbitrarily chosen tested values are the following: 5%, 10 %, 30 %, 50 % and 75%. For these five values, the respective internal doses' distributions were calculated, the other parameters of the model remaining unchanged. The five internal dose distributions (the percentile 95 of each of them) are then compared to the four internal DNELs to calculate the risks characterization ratios and characterize the risk. The results are presented in the table below with the RCRs calculated for each internal DNEL.

According to the REACH regulation methodology for risk characterization, the risk is considered negligible if the RCRs are inferior to 1 and not negligible if the RCRs are superior to 1.

Table 47. Calculation of the RCRs for cashiers pregnant women with different factors of systemic bioavailability for the four types of effects

		Internal DNELs (µg/kg bw/d)			
		0.005	0.01	0.009	0.0025
Factors of systemic bioavailability	dose of exposure : P95 from the distribution (µg/kg bw/d)	Brain and behavior	Female reproductive system	Metabolism and obesity	Mammary gland
5%	0,02	4	2	2.22	8
10%	0,04	8	4	4.44	16
30%	0,13	26	13	14.44	52
50%	0,22	44	22	24.44	88
75%	0,33	66	33	36.67	132

This table shows that for all five tested values of systemic bioavailability factor after dermal absorption and whatever the effect seen, the risk characterization leads to the conclusion that there are situations at risk.

Finally, it was determined what should be the systemic bioavailability factor after cutaneous absorption with the aim of not observing any more risk situations, for each of the reactions considered, the other model parameters remaining unchanged.

Considering the exposure model applied where the factor of systemic bioavailability after dermal absorption appears as a multiplicative factor, to find the value of this parameter allowing to consider the risk as negligible returns, based on the results of calculation of internal dose (see results presented in the report and outlined below) to find the value for which the P95 of the distribution is equal to each of the toxicological endpoints.

$$P95(DI) \times F_{biodisponibilité-cutanée} = RT$$

$$F_{biodisponibilité-cutanée} = \frac{RT}{P95(DI)}$$

For cachier scenario, the P95 of the distribution is 0.43 µg/kg bw/day. Considering this value, the factors of systemic bioavailability after subcutaneous absorption leading to qualify the risk of significant for each of the four effects considered are shown below.

Table 48. Calculation of the systemic bioavailability factors after subcutaneous absorption leading to qualify the risk of significant for each of the four critical effects considered.

	Internal DNELs (µg/kg bw/d) calculated for the four critical effects considered	Factor of systemic bioavailability after skin absorption leading to qualify the risk of insignificant
Brain and behavior	0,005	1,16 %
Female reproductive system	0,01	2,33 %
Metabolism and obesity	0,009	2,09 %
Mammary gland	0,0025	0,58 %

Scenario for pregnant female handling thermic tickets containing BPA during one day, as a consumer:

The same exercises as those developed for the "professional" scenario are led for the "consumer" scenario based on the model of "exposure using the rate of absorption percutaneously. Only the results are presented.

1) Parametric uncertainty

As a reminder, the model used to model the exposure dose through handling tickets for a consumer is the following:

$$DI_{ticket\_CT} = \frac{T_{abs} \times Q_{subs} \times N \times D_{abs}}{2 \times PC}$$

With :

- *DI_ticket CT*: Internal daily dose by contact with thermal tickets for consumers with a rate [µg/kg pc/j]

- *T abs :* Rate of absorption (established for an absorption duration of 2 hours) [%]

Q- subs : Amount of substance deposited by contact [µg/finger]

- *N* : Number of fingers in contact with the receipt [finger]

- *abs D* : Duration of absorption [h/day]

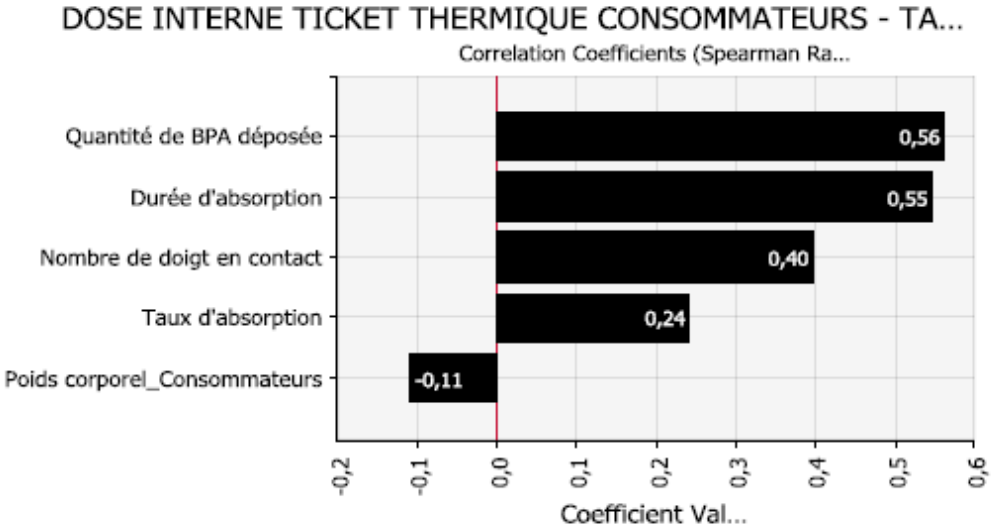
- *PC* : body weight [kg bw]

As described in the report, this model does not involve any factor of systemic bioavailability after dermal absorption. In fact, no data for determining this factor in the literature, a value of 100% was considered by default, meaning that all BPA absorbed through the skin was then bioavailable in the body. The probability distributions of the model parameters are as follows:

Parameters:	
Absorption rate	Triangular distribution 10%-27%-60%
Amount of substance deposited by contact	Uniform distribution [0.035 – 3.75]
Number of fingers in contact with the ticket	Uniform distribution [1 cm2 - 12 cm2]
Duration of absorption	Uniform distribution [2h]
Body weight	Discrete distribution

The graph below shows the sensitivity analysis by tornado graph of internal dose modeled for pregnant consumers handling thermal receipt containing BPA by application of the model mentioned above:

Figure 35. Sensitivity analysis by tornado graph of the modelled internal dose for pregnant women consumers handling thermal paper containing BPA.



This graph shows that the amount of BPA deposited on the fingers and the duration of absorption are the most influential parameters on the internal dose calculated, taking into account the variability of different probability of distributions in the model.

2) Uncertainty about the value of systemic bioavailability factor after dermal absorption:

Exercise 1: if we do not consider by default a factor of systemic bioavailability after dermal absorption of 100%, and if a uniform distribution of probabilities ranging from 0.01% to 100% for dermal bioavailability factor is introduced into the model, it allows seeing if the dermal bioavailability factor is an influential parameter.

$$DI_{ticket\_CT} = \frac{T_{abs} \times Q_{subs} \times N \times D_{abs}}{2 \times PC} \times F_{biodisponibilité-cutanée}$$

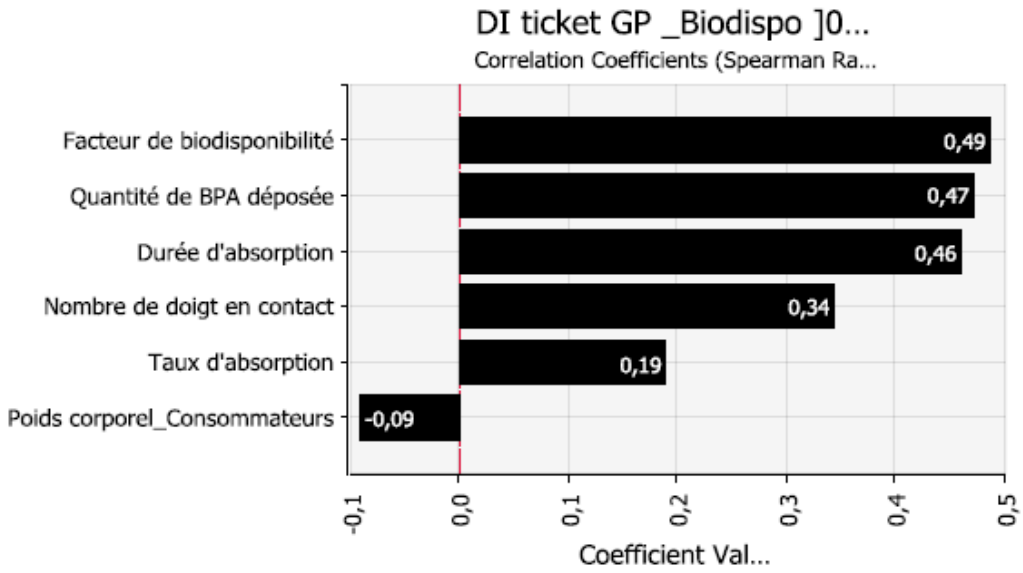
This exercise is a theoretical exercise conducted only to see through a sensitivity analysis by tornado graph on the obtained results, the extent to which factor systemic bioavailability after dermal absorption is an influential parameter.

The sensitivity analysis shows that the factor of systemic bioavailability after dermal absorption is the most influential parameter on the result of the calculation, given the high variability attributed to this factor.

However, the influence of the parameters "amount of BPA deposited" and "duration of the absorption" are of almost equal influence. The sensitivity of these parameters, the documentation of which is based on limited data in the model, tends to give more uncertainties

to the scenario of "consumer exposure handling thermal receipt" than to the scenario of "cashiers/workers exposure".

Figure 36. Sensitivity analysis by tornado graph of the systemic bioavailability factor after dermal absorption compared to the other parameters.



Exercise 2: In the same way as the "professional scenario", different values of systemic bioavailability factors are tested here in the model.

The tested values are the same as above: 5%, 10%, 30 %, 50 % and 75%.

For these five values, respective internal doses' distributions were calculated, the other parameters of the model remaining unchanged.

Then, the five internal doses' distributions (the percentile 95 of each of them) are compared with the four internal DNELs to calculate the risks characterization ratios and charactize the risk.The results are presented in the table below with the RCRs calculated for each internal DNEL.

According to the REACH regulation methodology for risk characterization, the risk is considered negligible if the RCRs are inferior to 1 and not negligible if the RCRs are superior to 1.

Table 49. Calculation of the RCRs according to the REACH regulation for consumers' pregnant women

Factors of systemic bioavailability	P95 of the distribution of the exposure dose (µg/kg bw/d)	Risk Characterisation Ratios (RCRs)			
		Internal DNELs (µg/kg bw/d)			
		Brain and behaviour 0.005	Female reproductive system 0.01	Metabolism and obesity 0.009	Mammary gland 0.0025
5%	0,004	0.8	0.4	0.44	1.6
10%	0,008	1.6	0.8	0.89	3.2
30%	0,03	6	3	3.33	12
50%	0,04	8	4	4.44	16
75%	0,06	12	6	6.67	24

This table shows that:

There is a risk on the mammary gland for all the five tested values of systemic bioavailability factor after dermal absorption;

The risk can be described as negligible for a systemic bioavailability of 5% for the three other types of critical effect, as well as for a systemic bioavailability of 10% for the critical effects on the female reproductive system and on metabolism and obesity;

For the three values of systemic bioavailability of 30, 50 and 75%, there are situations at risk for the four critical effects considered.

Finally, it was determined what should be the systemic bioavailability factor after cutaneous absorption with the aim of not observing any more risk situations, for each of the reactions considered, the other model parameters remaining unchanged.

The P95 of the distribution is 0.08 µg/kg bw/day for pregnant consumers' women. Considering this value, the factors of systemic bioavailability after subcutaneous absorption leading to qualify the risk of significant for each of the four effects considered are shown below.

Critical effect on:	Internal DNELs (µg/kg bw/d)	Systemic biodisponibility factor bringing to consider the risk as negligible

Brain and behaviour	0.005	6.25%
Female reproductive system	0.01	12.50%
Metabolism and obesity	0.009	11.25%
Mammary gland	0.0025	3.13%

B.10.1.1.3 Indirect exposure of humans via the environment

The exposure of the general public to BPA through their environment has been analysed, considering:

The air compartment (internal air and external air) – exposure through inhalation;

Sedimented dust – exposure through ingestion;

Food and drinks (including drinking water) – exposure through ingestion.

A distribution of internal exposure doses (IED) was modelled for each of the target populations considered and compared to each internal DNELs.

Figure 37. Characterisation of the risks associate with BPA contained in the air

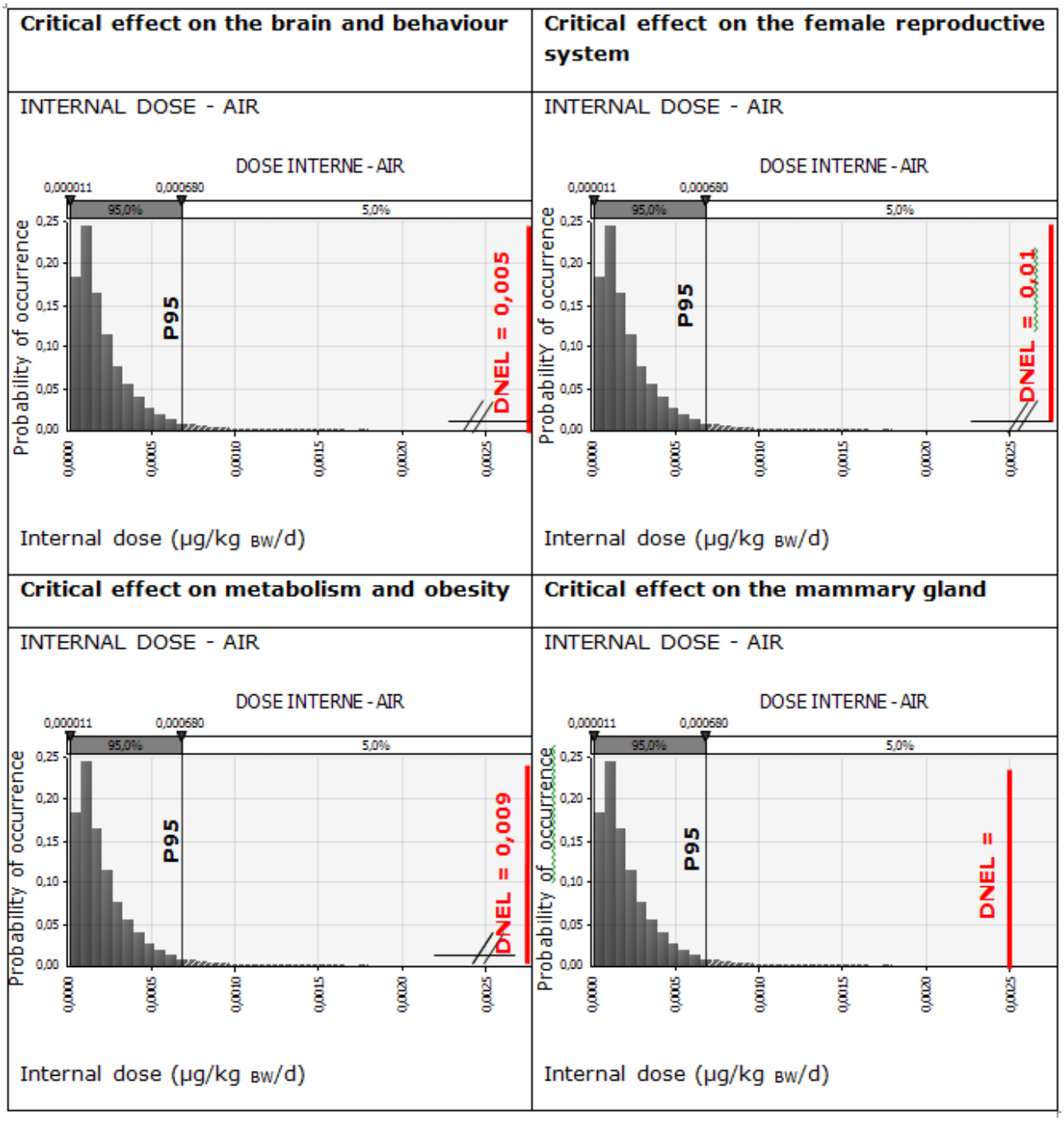


Figure 38. Characterisation of the risks related to BPA contained in sedimented dust

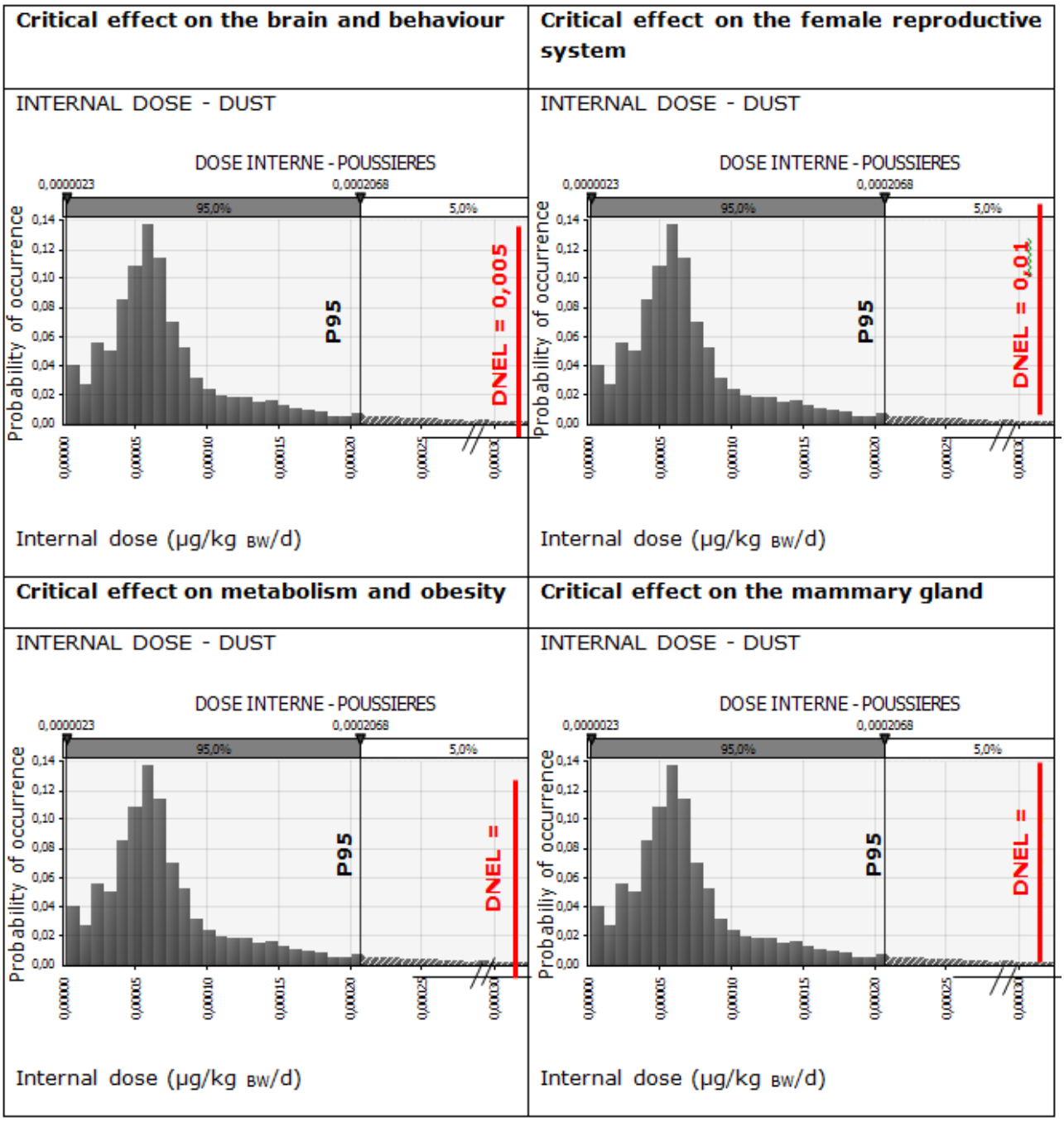
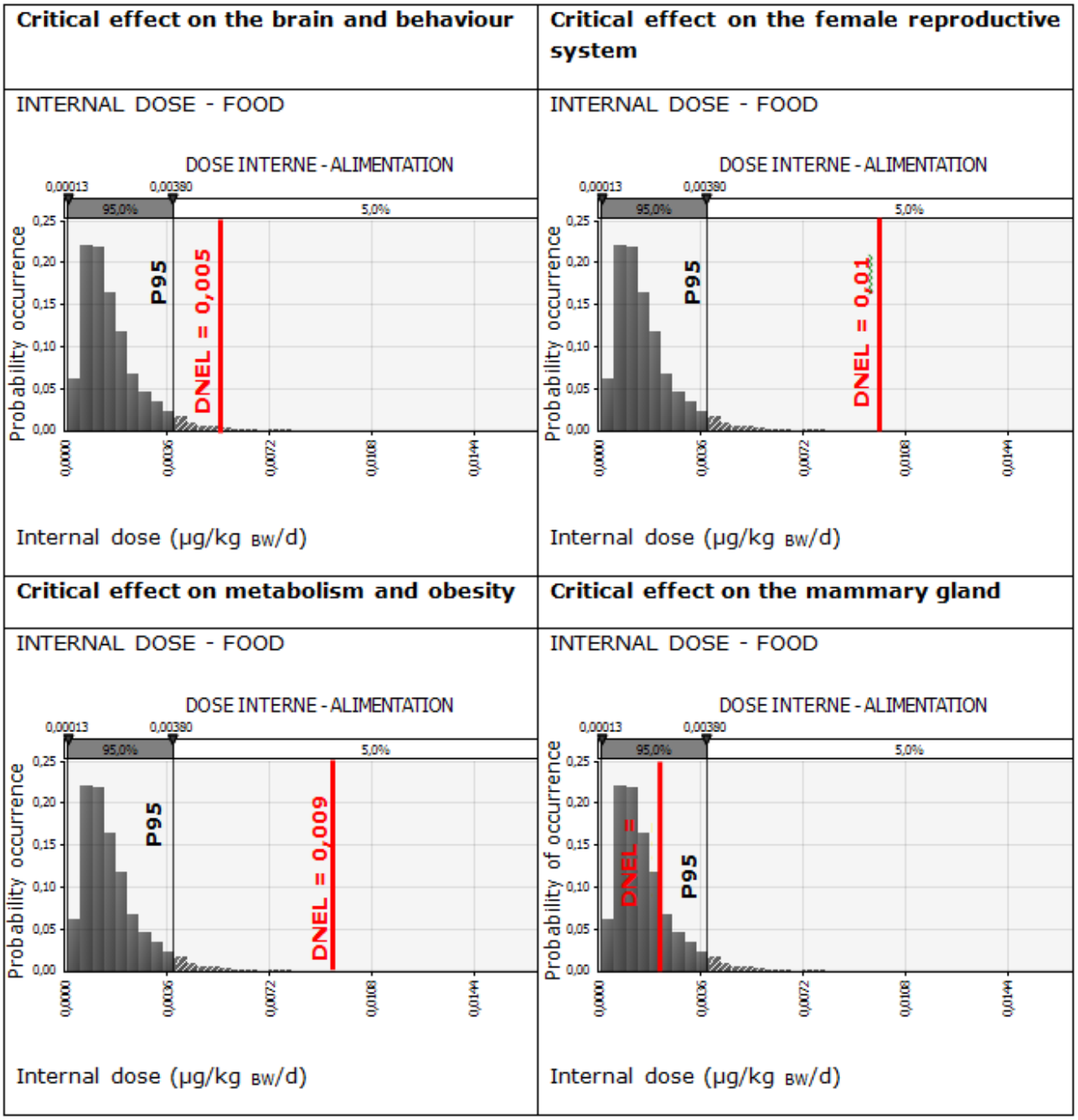


Figure 39. Characterisation of the risks related to BPA in food



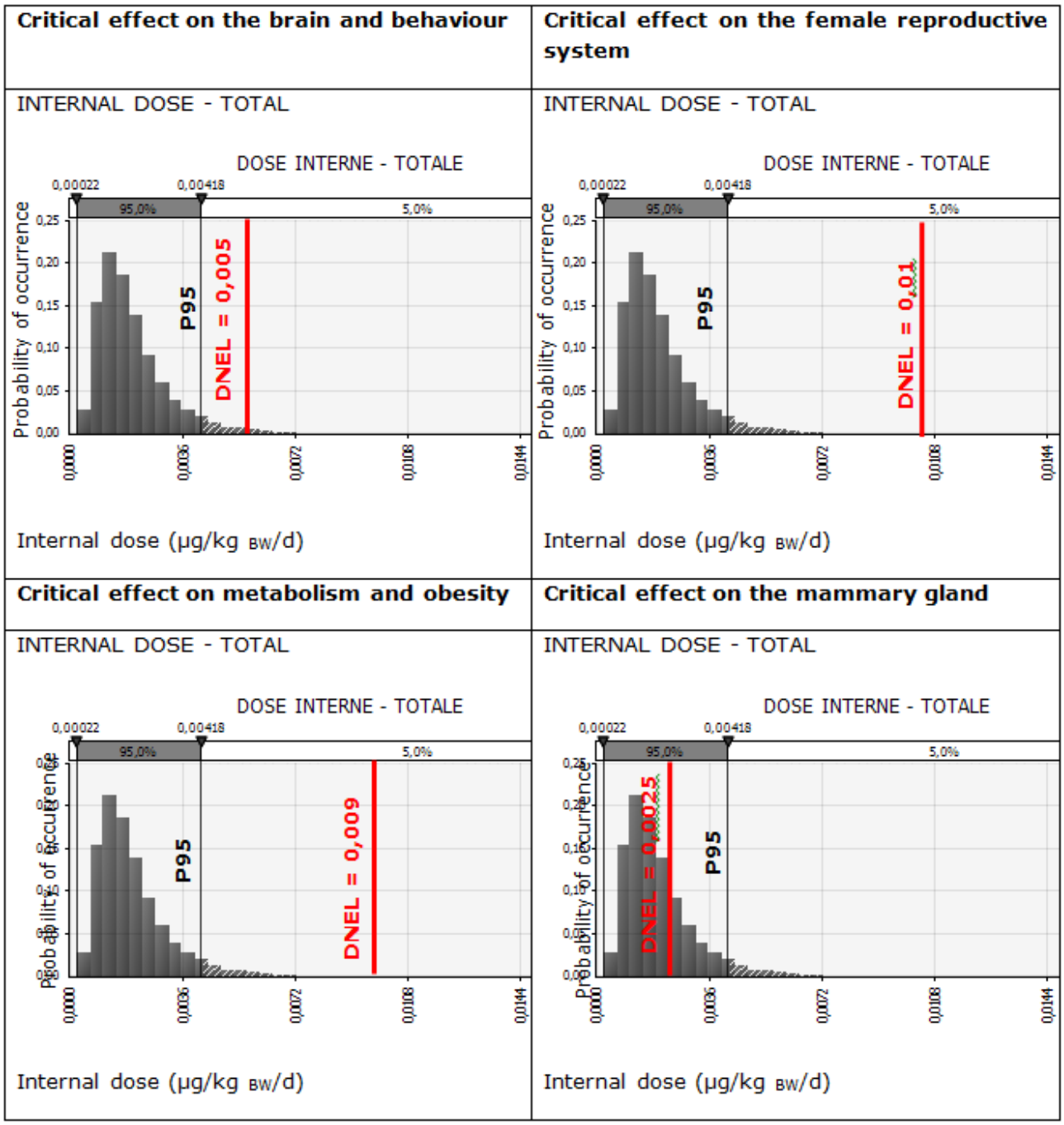
B.10.1.1.4 Combined exposure

In accordance with this approach, and by using distributions of internal exposure doses and internal DNELs, the results of the risk assessment carried out are summarised in the table below.

Table 50. Environmental and food exposure: health risks for the offspring of the human species assessed using critical effects observed in animals

Population exposed: **pregnant women** **Target population: offspring**	**Critical effect on:**			
	Brain and behaviour	**Female reproductive system**	**Metabolism and obesity**	**Mammary gland**
Air	Negligible risk	Negligible risk	Negligible risk	Negligible risk
Sedimented dust	Negligible risk	Negligible risk	Negligible risk	Negligible risk
Food	Negligible risk	Negligible risk	Negligible risk	Situations of exposure to risk exist
All means (except thermal papers)	Negligible risk	Negligible risk	Negligible risk	Situations of exposure to risk exist

Figure 40. Characterisation of the risks related to BPA through all the means – Air, sedimented dust and food



On average, the major contribution of the internal exposure dose is derived from food (84% for pregnant women). The ingestion of dust or inhalation of air contaminated by BPA contributes a small amount to the internal dose (4% and 12% respectively).

Lastly, following the methodology used, the results of the HRA for a total exposure through air, sedimented dust and food show that some exposure to BPA situations presents a risk to the mammary gland of the embryo and the foetus through maternal exposure.

For the critical effect on the mammary gland, and on the basis of the simulations of internal exposure doses carried out (which taken into account the variability of the parameters used in the calculation of the exposures as much as possible), we cannot exclude the appearance of this critical effect in 23% of the exposure situations (exceeding the toxicological benchmark).

It should be noted that exposure through food only led, for this same effect, to the observation of potential situations of risk (a probability of around 16%). For the other 3 types of effects, the 95thpercentile of the distribution of internal exposure doses is less than the respective toxicological benchmarks, which, according to the methodology used, leads to the classification of the negligible risk.

The risk associated to food is out of the scope of the REACH regulation.

B.10.1.2 Environnement

Not relevant

B.11 Summary on hazard and risk

Four types of effects observed in animals at different periods of life were used to assess the health risks for humans:

Effects on brain development

Effects on the mammary gland

Effects on the female reproductive system

Effects on the metabolism and obesity

Given the lack of good quality studies describing the effects of BPA on animals exposed exclusively as adults, young or during the pre-puberty period, health risks is only assessed for a single target population: **pregnant women and their unborn child**.

For the risk characterisation, the approach adopted is to use the critical doses selected and thereby derive the corresponding internal DNELs for each effect considered.

Table 51. Effects and related internal DNELs selected for the HRA.

Critical effects	**Study reference**	**Route of exposure**	**LOAEL**	**NOAEL***	**Internal NOAEL by application of a bioavailability factor of 3%**	**Internal DNEL by application of an Assessment Factor of 300 on the internal NOAEL**
			(µg/kg/d)	**(µg/kg/d)**	**(µg/kg/d)**	**(µg/kg/d)**

Brain and behaviour	**Xu, 2010**	oral	**/**	**50**	**1.5**	**0.005**
Female reproductive system	**Rubin, 2001**	oral	**/**	**100**	**3**	**0.01**
Metabolism and obesity	**Miyawaki, 2007**	oral	**260**	**87**	**2.6**	**0.009**
Mammary gland	**Moral, 2008**	oral	**/**	**25**	**0.75**	**0.0025**

*: NOAEL calculated from the LOAEL.

According to the results of the exposure calculations based on a probabilistic approach, the **handling of thermal receipts** leads to **risk situations** for **the four types of effects** considered, **both for pregnant women working as cashiers and tellers as well as for pregnant woman consumers handling thermal receipts**. Although the probabilistic approach used enables the maximum possible consideration of the variability of the exposure parameters, the models considered do not take into account the distribution and elimination of BPA by the body and assume that 100% of the dose absorbed by the skin is then bioavailable.

C. Available information on alternatives

Since risks are identified and demonstrated for exposures to BPA in thermal paper, the question of possible substitution is then crucial. Because the use of BPA in many sectors is becoming increasingly controversial, research on substitutes and substitution itself is underway. The literature provides a rather abundant review of 'potential' alternative substances for this particular use. The alternatives are considered as 'potential' regarding their similar technical properties than BPA and are thus deemed as possible alternative dye developers. However, when it comes to thermal paper, dye developers are chemicals that have to meet very specific technical requirements at low concentrations in order to make thermal paper efficient and appropriate for the targeted end-uses. Therefore, in terms of feasibility, any dye developer cannot meet those requirements and be effectively used in the manufacturing of thermal paper. Furthermore, between the theoretical 'potentiality' of alternatives, grounded on their physicochemical profile, and the desirability of those alternatives, based on other considerations such as human and environmental health or economic arguments, there might be a considerable gap.

As a result, in this section, the approach proposed is stepwise. The first step consists in a broad identification of all 'potential' alternative dye developers available from the public literature and other data sources and from the information collected from the consultations carried out during the elaboration of that proposal (MSCA consultation and INERIS, 2013). Then, the second step consists in a refinement and a selection among those identified alternatives in order to draw up a list of 'realistic' substitutes.

As recommended in the Guidance for Annexe XV Restrictions and the related template, all the alternative chemicals to BPA identified and selected are herein assessed as regards the criteria of availability, risks for human health and environment and technical and economic feasibility. A particular attention is paid to the uncertainties surrounding them. They are then summarized and compared to each other in order to get a global picture and assessment of the substitution of BPA in thermal paper, and finally to examine the possibility of making some recommendations.

Additionally to the identification and assessment of alternative dye developers, the analysis of alternatives herein also includes an analysis of alternative techniques. In other words, alternatives to thermal paper *per se* are scrutinized and assessed as regards the same criteria as required in the abovementioned Guidance.

C.1 Identification of potential alternative substances and techniques

C.1.1 Alternative chemical dye developers

As indicated in the introduction, the first step of the analysis of alternative dye developers in thermal paper carried out herein consists in a broad identification of all 'potential' substitutes.

C.1.1.1. Identification of potential alternative chemical dye developers

The identification of potential alternatives to BPA in thermal paper has been carried out based on the review of the available literature and the data gathered from the consulted market actors. These two channels of information have led to the preliminary conclusion that many other chemicals can in principle be used in thermal papers in replacement for BPA.

- As regards the review publicly available literature, the analysis has focused on 6 reports, considered as the most consistent and complete: the reports from RPA, 2003, US EPA, 2012 (updated from 2010), INERIS, 2010, ANSES, 2013, Danish E.P.A., 2013, Kemi, 2013.

From these 6 reports, 30 potential alternatives have been identified, based preliminarily on their physical and chemical properties and/or their commercial use:

- RPA, 2003 identified **5 potential substitutes**
- US EPA, 2012 identified **17 other chemicals** as potential functional substitutes to BPA. A 18th chemical is listed in the report but named as 'confidential' (proprietary), it cannot thus be properly identified. This work has been done through the program « Design for the Environment (Dfe) » initiated by the US EPA since 2010 about the substitution of toxic chemicals such as BPA in thermal paper. As to BPA specifically, the action plan includes a multi-stakeholder alternatives assessment in the framework of a partnership with coatings and paper industry.
- INERIS (2010) analysed 21 alternatives (including 16 alternatives listed in US EPA (2010) among which **7 chemicals** have been identified in the US EPA 2010 initial report then removed in the 2012 updated report, and the 5 chemicals identified in RPA, 2003)
- ANSES, 2013 listed the same alternatives identified by RPA, 2003 and US EPA, 2012 and identified **1 additional potential chemical.** This chemical was detected during the French study carried out by the SCL of Lyon on thermal tickets and receipts, as described above in section B.2. (DGCCRF, 2011)
- Danish E.P.A., 2013 provides a list of 30 potential substitutes (17 from US EPA, 2012 , 7 from US EPA (2010), 1 from ANSES, 2013 and 5 from RPA, 2003)
- Kemi, 2013 confirms 17 potential substitutes already listed in US EPA, 2012

- As regards the other data taken into account for the identification of alternative dye developers, the analysis has been based on the surveys carried out during the elaboration of this proposal, one carried out by Anses and one by INERIS, both in 2013 (MSCAs consultation; INERIS, 2013).

  From these two data sources, 28 chemicals have been identified:

- The MSCA survey (2013) mentioned **21 chemicals,** including 4 already identified through the literature review and 17 chemicals never mentioned before for that particular use in thermal paper. After some research on those, some seem to be pigments and not strictly dye developers and some others are unknown (maybe due to a formal indexation under another name or chemical number).
- INERIS survey (2013) brought up **11 alternatives**, all included in US EPA, 2012 and in the chemicals quoted in the MSCA survey.

Overall, the read-across of the different data available from the literature review and from the latest market surveys, allowed compiling 30 'potential' substitutes to BPA in thermal paper. These 30 chemicals are listed in the table below. This table presents the chemicals name as well as their CAS and EC numbers and the respective sources from which they have been got.

Table 52. Potential alternative dye developers identified

Common name (Chemical name)	**CAS number**	**EC number**	**Sources**							
			RPA, 2003	**INERIS, 2010**	**US EPA, 2012**	**ANSES, 2013**	**Danish E.P.A., 2013**	**Kemi, 2013**	**MSCA 2013 (see section G)**	**INERIS, 2013**
Bisphenol S (BPS) (4,4'-sulphonyldiphenol)	80-09-1	201-250-5		x	x	x	x	x	x	x
Bisphenol F (BPF) (4,4'-methylenediphenol)	620-92-8 (para)	210-658-2		x	x	x	x	x		x
(2,2'-methylenediphenol)	2467-02-9 (ortho)	219-578-2					x			
Bisphenol AP (BPAP or BAISTER or P-1) (1,1-bis(4-hydroxyphenyl)-1-phenylethane)	1571-75-1	433-130-5		x	x	x	x	x		x
TGSA (2,2'-diallyl-4,4'-	41481-66-7	411-570-9		x	x		x	x		x

Common name (Chemical name)	CAS number	EC number	Sources							
			RPA, 2003	INERIS, 2010	US EPA, 2012	ANSES, 2013	Danish E.P.A., 2013	Kemi, 2013	MSCA 2013 (see section G)	INERIS, 2013
sulfonyldiphenol)										
D-8 (or DD8 or ALD-2000) (4-(4-isopropoxyphenylsulfonyl) phenol)	95235-30-6	405-520-5		x	x		x	x	x	x
BPS-MAE (4-[[4-(2-Propenyloxy)phenyl]sulfonyl]phenol)	97042-18-7			x	x		x	x		x
DD-70 (4-4'-methylenebis(oxyethylenethio)diphenol)	93589-69-6	407-480-4		x	x		x	x		x
D90 (Phenol, 4,4'-sulfonylbis-, polymer with 1,1'-oxybis[2-chloroethane])	191680-83-8			x	x		x	x		x

Common name (Chemical name)	**CAS number**	**EC number**	**Sources**							
			RPA, 2003	**INERIS, 2010**	**US EPA, 2012**	**ANSES, 2013**	**Danish E.P.A., 2013**	**Kemi, 2013**	**MSCA 2013 (see section G)**	**INERIS, 2013**
biphenyl-4-ol (*p*-phénylphénol)	92-69-3	202-179-2		x			x			
4,4'-thiobisphenol	2664-63-3	220-197-9		x			x			
4-tert-butylphenol	98-54-4	202-679-0		x			x			
PHBB (benzyl 4-hydroxybenzoate)	94-18-8	202-311-9		x	x		x	x		
ethyl 4-hydroxybenzoate	120-47-8	204-399-4		x			x			
DMP-OH (dimethyl 4-hydroxyphthalate)	22479-95-4	245-023-9		x			x			
3,5-bis-tert-butylsalicylic acid	19715-19-6	243-246-6		x			x			
zinc 3,5-bis(α-methylbenzyl)salicylate	53770-52-8	258-753-8		x						

Common name (Chemical name)	CAS number	EC number	Sources							
			RPA, 2003	INERIS, 2010	US EPA, 2012	ANSES, 2013	Danish E.P.A., 2013	Kemi, 2013	MSCA 2013 (see section G)	INERIS, 2013
Pergafast 201 (or Pergafast or DP-201) (N-(p-toluenesulfonyl)-N'-(3-(p-toluenesulfonyloxy)phenyl) urea 3-([(4-methylphenyl)sulfonyl]carbamoylamino)phenyl 4-methylbenzenesulfonate) (not-phenol compound)	232938-43-1	432-520-2			x		x	x	x	x
BPS-MPE (p-[[p-benzyloxyphenyl]sulphonyl]phenol)	63134-33- 8	263-920-3			x		x	x		
UU (Urea Urethane Compound)	321860-75-7				x		x	x		x

Common name **(Chemical name)**	**CAS number**	**EC number**	**Sources**							
			RPA, 2003	**INERIS, 2010**	**US EPA, 2012**	**ANSES, 2013**	**Danish E.P.A., 2013**	**Kemi, 2013**	**MSCA 2013 (see section G)**	**INERIS, 2013**
B-Tum (4,4'-bis(N-carbamoyl-4-methylbenzenesulfonamide)diphenylmethane)	151882-81-4	418-770-5			x		x	x	x	
2,4-BPS (o-[(4-hydroxyphenyl)sulphonyl]phenol)	5397-34-2	226-421-1			x		x	x		
Bisphenol C (BPC) (4,4'-isopropylidenedi-o-cresol)	79-97-0	201-240-0			x		x	x		
MBHA (or Pyrene) (methyl bis(4-hydroxyphenyl)acetate)	5129-00-0	225-870-0			x		x	x		
BisOPP-A (4,4'-Isopropyllidenebis(2-phenylphenol))	24038-68-4				x		x	x		

Common name (Chemical name)	CAS number	EC number	Sources							
			RPA, 2003	INERIS, 2010	US EPA, 2012	ANSES, 2013	Danish E.P.A., 2013	Kemi, 2013	MSCA 2013 (see section G)	INERIS, 2013
6,6'-di-tert-butyl-4,4'-butylidenedi-m-cresol	85-60-9	201-618-5	x	x			x			
2,6-di-tert-butyl-p-cresol	128-37-0	204-881-4	x	x			x			
octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	2082-79-3	218-216-0	x	x			x			
pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate)	6683-19-8	229-722-6	x	x			x			
4,4',4''-(1-methylpropanyl-3-ylidene)tris[6-tert-butyl-m-cresol]	1843-03-4	217-420-7	x	x			x			
1,2-diphenoxyethane	104-66-5	203-224-9				x	x			x

C.1.1.2. Selection of alternative substances to be further assessed

As described in the introduction of Chapter C, the list of the alternative dye developers identified has been then refined in order to proceed to the selection of the most 'realistic' substitutes.

Criteria for selection

The refinement has been made regarding the following exclusion and inclusion criteria:

- **Exclusion criterion**: unknown or very unlikely use of the chemical in thermal paper
- **Inclusion criterion 1**: actual and known commercial use of the chemical in thermal paper
- **Inclusion criterion 2**: possible (very similar properties) or alternative newly placed or about to be placed on the market as a dye developer in thermal paper

The exclusion criteria led first to exclude 3 chemicals indicated as "unknown to be used in thermal paper" (US EPA, 2012): these chemicals are Bisphenol C, BisOPP-A and MBHA. It has to be noted that the use of DD-70 in thermal paper is also indicated as "unknown" in US EPA, 2012 but the latest consultation from INERIS, 2013 suggests this chemical as a possible substitute. It has thus been decided to keep it on the list.

Under the same criteria, 8 additional potential alternatives were also discarded because they are considered as "unlikely" since their physical or chemical properties may render them incompatible as a functional replacement developer to BPA in thermal paper. These chemicals are: p-tert-butylphenol, p-phenylphenol, 4,4'-thiobisphenol, 3,5-bis-tert-butylsalicylic acid, 4-hydroxybenzoate ethyl, 4-hydroxyphtalate de dimethyle (DMP-OH), PHBB and zinc 3,5-bis(α-methylbenzyl)salicylate (CAS 53770-52-8). This information is based on the feedback from the consulted industry and MSCAs as reported in the literature reviewed. It can be deemed reliable since these chemicals have never been mentioned in the surveys carried out during the elaboration of this proposal, what tends to confirm that they might not be realistic candidates for the substitution of BPA in thermal paper. That was also the reason why 6 of 8 of these chemicals (all except PHBB and and zinc 3,5-bis(α-methylbenzyl)salicylate (CAS 53770-52-8)) have been removed from any further assessment in the updated US EPA, 2012 report compared to the 2010 version. As to PHBB and and zinc 3,5-bis(α-methylbenzyl)salicylate (CAS 53770-52-8) are concerned, they are considered as possible substitutes to BPA in thermal paper by US EPA (2010) but as "unlikely" by INERIS (INERIS, 2010), due to their low efficacy. Further, no mention has been made about them in both the 2013 MSCA consultation and the INERIS survey (INERIS, 2013) . It has thus been decided to exclude them as well.

Concerning the 5 chemicals identified by RPA, 2003, they are already used today as antioxidants but the report specifies then that these substances show a poorer performance compared to BPA as developers and indicates that *"none of the five substances (…) would be used in thermal paper. The analysis of the five potential substances is given for indicative purposes only"* (RPA, 2003). These 5 chemicals are thus not selected for further assessment as they do not seem to be realistic substitutes. As indicated in Table 47 above, these substances are 6,6'-di-tert-butyl-4,4'-butylidenedi-m-cresol (CAS 85-60-9), 2,6-di-tert-butyl-p-cresol (CAS 128-37-0), octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (CAS 2082-79-3), pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) (CAS 6683-19-8) and 4,4',4''-(1-methylpropanyl-3-ylidene)tris[6-tert-butyl-m-cresol] (CAS 1843-03-4).

As a whole, 16 'potential' but not 'realistic' substitutes have been discarded under the exclusion criterion.

The consideration of the 1st inclusion criteria resulted in the selection of 7 chemicals as they are already used as dye developers in thermal paper in the EU. This is the case of Bisphenol S, Bisphenol F, 1,2-diphenoxyethane, Bisphenol AP, D8, D-90 and Pergafast 201. Some of them have been used for several years now and stands already for a non negligible market share (such as BPS which stands for the main drop-in developer in thermal paper, as also shown further in section C.2) and other are rather innovative and have been lately marketplaced (such as Pergafast 201). Those actual and known commercial uses are confirmed by the industry consultation (MSCAs consultation and INERIS, 2013). Kemi, 2013 also confirms that BPS and Pergafast 201 are present on the Swedish market. The study carried out by DGCCRF, 2011 shows as well that some thermal receipts tested contained neither BPA nor BPS as well but "other developers". However, the producers of thermal paper remain generally rather vague about the specific substances they use themselves and often claim as classified information. They may state that they have BPA-free, bisphenol-free and/or phenol-free thermal papers in their range of products (most of the time available on their website) and mention which developers are already in place on the market without specifying whether they use them themselves or not (MSCAs consultation and INERIS, 2013). Another proof that substitution of BPA is underway comes from the direct observation of the market: any people who do some shopping today can also notice that there are more and more tickets and receipts labelled (often on the back) with "BPA-free" or "bisphenol-free" or even "phenol-free". The use of D-8 and D-90 is mentioned in the thermal paper produced for labels (ETPA consultation). However, these might be not used in till receipts (from personal communication with industry from Danish E.P.A., 2013.

The introduction and assessment of each of these chemicals are provided further in this report, in the section C.2.

Then, under the 2nd inclusion criteria, 3 additional alternative chemicals have been selected: TGSA, DD-70 and UU on the ground that they are all quoted in the literature (although DD-70 was indicated as "unknown to be used" in US EPA, 2012 as explained above) and have been raised during the 2013 consultations. In particular, Kemi, 2013 reports that the BPA-free paper may contain urea-based materials instead of phenols such as UU (or Pergafast, already selected above) (Kemi, 2013). They thus seem to be realistic dye developers alternatively to BPA in thermal paper.

As far as the BPS-MPE and the 2,4 BPS are concerned, they are substances similar to BPS and given the important discrepancies of the available toxicological data on these substances, they have been discarded from further assessment. Besides, they have not been mentioned by any stakeholders surveyed. Likewise, B-Tum is discarded from further assessment because it is classified Carc 2 H351 under CLP Regulation.

Finally, although they have been found in the literature or quoted during the consultation, one chemical remains unknown and cannot be found in the usual chemicals databases (ECHA, ESIS, etc.): BPS-MAE. It has thus been removed from the selection.

Overall, **10 potential realistic alternative dye developers to BPA in thermal paper are selected to be further assessed.** These developers are considered as so-called 'drop in' substances and are listed in the table below.

Table 53. The selected potential realistic alternative dye developers to BPA in thermal paper

Common name **(Chemical name)**	**CAS number**	**EC number**
Bisphenol S (BPS)	80-09-1	201-250-5
Bisphenol F (BPF)	620-92-8	210-658-2
	2467-02-9	219-578-2
Bisphenol AP (BPAP or BAISTER or P-1)	1571-75-1	433-130-5
TGSA	41481-66-7	411-570-9
D-8 (or DD8 or ALD-2000)	95235-30-6	405-520-5
D90	191680-83-8	
Pergafast 201 (or DP-201)	232938-43-1	432-520-2
UU	321860-75-7	
DD-70	93589-69-6	407-480-4
1,2-diphenoxyethane	104-66-5	203-224-9

From the 10 alternative developers to BPA in thermal paper finally selected, 3 are bisphenols (BPS, BPF, BPAP), 5 are phenolic substances and 2 are urea-based (UU and Pergafast). Each of these chemicals is further assessed in the section C.2 as regards their availability, risks for human health and environment and technical and economic feasibility.

It has to be noted that, from the public consutation, information was provided about other (novel) alternatives to BPA:

1. Two innovative formulations, based on the use of organic acids such as Aspirine or Vitamin C, instead of phenols or urea-based compounds have been engineered by a US leading manufacturer and a pioneering company in direct thermal technology (Alpha Free technology).
    - The thermal material formulated with an organic acid is claimed to offer the customers a good imaging, ideal for POS receipts
    - The thermal material formulated with Vitamin C in particular is claimed to

provide a natural yellow color on front side, a white backside suitable for printing promotional messages and a good imaging ideal for POS receipts[23].

No further information is available regarding the potential hazards of such materials. This technology would cost 10-20% more than conventional thermal paper but prices would drop, with economies of scale[24].

2. It has also been reported that a substitute based on corn (biofuel) might be a possible option. However no further data is available and this alternative might cause sustainability issues[25].

3. Upon request of RIVM, experts from Wageningen University and Research –Section: Food and Biobased Research (WUR-FBR) have overviewed additional potential alternatives to BPA under the criteria of their classifications, availability, technical feasibility and economic feasibility[26]:

Alternative	**Classification** (CLP Annex VI or self classified)	**Availability**	**Technical feasibility**	**Economic feasibility**
Lauryl gallate	Annex VI Skin Sens Cat 1	confirmed	confirmed	unknown
Other gallic acid derivates	Not reviewed	-	-	-
Diphenolic acid	Self classified as Skin Irr Cat 2 and STOT-SE Cat 3	confirmed	feasible	unknown
Diphenolic derivates	Not reviewed	-	-	-
p-coumaric acid	Self classified as Skin Irr Cat 2 and STOT-SE Cat 3	unknown	unknown	unknown
Tocopherol (E 307)	Not Classified (by all c&l notifiers)	confirmed	unknown	unknown

[23] http://www.appvion.com/en-us/products/thermal/Pages/pos_alpha_free.aspx

http://appvion.com/en-us/products/thermal/Documents/Thermal/Alpha_Free_2.1.pdf

[24] Qualitative information provided by Appvion, quoted in Chemical & Engineering News (vol 32, issue 35, p22)

[25] http://www.fastcompany.com/1682423/coming-soon-corn-based-bpa-replacement

[26] *Analysis of alternatives for bpa in thermal paper*, report 1515, Dec 2014

Vanillin (huge market, many notifiers)	Self classified Eye Irr Cat 2, Skin Sens Cat 1, Acute Oral Cat 4	confirmed	unknown	feasible?

The gallic acid derivatives and diphenolic acid (and deirvatives) are claimed to be the most promising alternatives but no further information is available.

C.1.2. Alternative techniques/processes

Alternatively to the replacement of BPA as a dye developer in thermal paper, other ways for substitution can be considered. Indeed, on the one hand, second-best substitution could consist in adopting another printing technique, based on paper but not using thermal paper. On the other hand, it may alternatively imply to radically switch to free-paper techniques, based on electronic and IT technologies.

C.1.2.1. Identification of printing alternatives techniques/processes

For decades, other printing systems do exist such as matrix printing, inkjet printing, laser printing and thermal transfer printing. Those systems may also constitute in principle potential alternatives to BPA-based thermal paper printing.

C.1.2.1.1 Matrix printing technique

Matrix printing technique dramatically grew during the development of computer sciences and the adoption of IT technologies by businesses and companies, especially within the second half of 20th century, due in particular to their reliability and solidity. Matrix printing consists in dot matrix printing or impact matrix printing. This is a type of computer printing which uses a print head that runs back and forth, or in an up and down motion, on the page and prints by impact, striking pins over an ink-soaked cloth ribbon against the paper, much like the print mechanism on a typewriter. However, unlike a typewriter, letters are drawn out of a dot matrix, and thus, varied fonts and arbitrary graphics can be produced. The printhead is composed of 9 to 32 needles operated by several electromagnets (ANSES, 2013).

As far as their technical characteristics are concerned (speed, printing quality and noise), the speed of matrix printers can vary from 50 to over 500 characters per second (cps) and their print quality (namely resolution) may vary from 9 to 24 pins. Some dot matrix printers achieve 240 dots per inch by making repeated passes over the same printed area, though documents produced this way take much more time than with other printing technology. Compared to other printing techniques such as laser or inkjet printers**,** matrix printers are known to be rather slow and noisy. Since the development of faster, cheaper and quieter non-impact printing techniques, such as inkjet, laser or thermal transfer printing, matrix printers have lost significant market shares and have been generally replaced, considered to some extent to be outdated technology.

The table below summarizes the advantages and disadvantages of matrix printing technique.

Table 54. Advantages and disadvantages of matrix printing technique

Advantages	**Disadvantages**
Reliability and longevity (simplicity and robustness) Printing of several copies in one single shot High volume of printing capacity	Noise Average quality/resolution (up to 240 dpi) Generally monochromic printing Relatively high purchasing price Less efficient Requires ink ribbon Rather slow (50 to over 500 cps)

C.1.2.1.2 Inkjet printing technique

Inkjet printing technique consists in a computer non-impact printing that creates a digital image thanks to magnetized plates which propel droplets of ink onto paper (or plastics, or other substrates) and direct the ink in the desired shapes. The dots are extremely small and the ink dries more quickly than matrix printers. This system requires the use of ink cartridge. Inkjet printers are the most commonly used type of printer today and range from small inexpensive consumer models to very large professional machines that might cost tens of thousands of euros. The inkjet technology originated in the 19th century and was first extensively developed in the early 1950s. Starting in the late 1970s, inkjet printers that could reproduce digital images generated by computers were developed, mainly by Epson, Hewlett-Packard, and Canon.

Those printers are widely used for their attractive technical characteristics: they offer a high quality printing, approaching that produced by laser printers, with a typical resolution of 300 dots per inch, although some photo-quality inkjet printers have dpi resolution in the thousands (1200 to 4800 dpi), and they are especially popular as portable printers since they can be rather compact. In addition, color ink-jet printers provide an inexpensive way to print full-color documents. Inkjet printers can perform between 10 to 40 pages per minute (ppm).

In general, the price of ink-jet printers is lower than that of laser printers. However, they are also considerably slower. Other drawbacks of ink-jet printers is that they require a special type of ink that is apt to smudge on inexpensive copier paper and they are not designed for high-volume print job[27]. Furthermore, paper designed especially for inkjet printers is heavier than the paper used with laser printers or photocopiers (24 pound vs 20 pound), has higher brilliance and is somewhat more expensive.

The table below summarizes the advantages and disadvantages of inkjet printing technique.

Table 55. Advantages and disadvantages of inkjet printing technique

[27] http://whatis.techtarget.com

Advantages	Disadvantages
Silent printing Average to high resolution (300 up to 1200/4800 dpi) Relatively low purchasing price Little maintenance Color printing	Average printing speed (10 to 40 ppm) Requires (specific) ink cartridges Costly ink cartridges Risk of nozzles blockage when unused Not designed for high-volume print job

C.1.2.1.3 Laser printing technique

The laser printer was invented at Xerox in 1969 and the first commercial implementation of a laser printer was made in 1976. Laser printing is an electrostatic digital non-impact printing process that rapidly produces high quality text and graphics by passing a laser beam over a charged drum to define a differentially charged image. The drum then selectively collects charged toner and transfers the image to paper, which is then heated to permanently fix the image.

Laser printer speed can vary widely, and depends on many factors, including the graphic intensity of the job being processed. Personal low-end laser printers may print out around 8 ppm. and the fastest models can print over 200 monochrome pages per minute (12,000 pages per hour) or over 100 color pages per minute (6000 pages per hour). Very high-speed laser printers are used for mass mailings of personalized documents, such as credit card or utility bills. Production printers are needed for printing 50,000 or more pages per week. These are quite expensive and are used by commercial publishers[28]. The faster the printing, the higher the cost. The cost of this technology depends on a combination of factors, including the cost of paper, toner, drum replacement, as well as the replacement of other items such as the fuser assembly and transfer assembly. Often printers with soft plastic drums can have a very high cost of ownership that does not become apparent until the drum requires replacement.

The table below summarizes the advantages and disadvantages of laser printing technique.

Table 56. Advantages and disadvantages of laser printing technique

Advantages	Disadvantages
High resolution (300 to 1,200 dpi – standard: 600 dpi) Average to very fast printing (from 8 ppm up to 700 ppm)	Requires toners (up to 4 toners for full color printing) Need for maintenance Expensive

[28] http://whatis.techtarget.com

Silent printing Color printing Better designed for high-volume print job	

C.1.2.1.4 Thermal transfer printing technique

Invented by SATO Corporation in the late 1940s, this digital printing technique seems to be the main competitor to direct thermal printing for labels, especially bar codes. The thermal transfer system consists in the adhesion of a wax-based ink onto paper. A thermal printhead melts wax-based ink from the transfer ribbon onto the paper, so that it stays glued to the material on which the print is applied (Armor, 2012; ANSES, 2013; Truffi, 2000. When cool, the wax is permanent. This type of thermal printer uses an equivalent panel of ink for each page to be printed, no matter if a full page or only one line of print is transferred. Monochrome printers have a black page for each page to be printed, while color printers have either three or four colored panels for each page. A typical thermal transfer ribbon consists of three layers: the base material, the heat melting ink, and the coating on the print side of the base material. The coating and base material help keep ink from adhering to the printhead which can cause poor print quality. The standard resolution of thermal transfer printers ranges from 200 to 600 dpi, which ie the number of resistive heating elements per linear inch of printhead[29]. Monochrome and color thermal transfer ribbons are available. It is recommended that the printhead be cleaned between each ribbon change. Although acceptable in quality, the printouts from these printers cannot compare with modern inkjet printers and color laser printers. Currently, this type of printer is rarely used for full-page printing, and is now employed for industrial label printing due to its water fastness and speed. These printers are considered highly reliable due to their small number of moving parts. Printouts from color thermal printers are sensitive to abrasion, as the wax ink can be scraped, rubbed off, or smeared.

The thermal transfer printing contrasts with direct thermal printing where no ribbon is needed. The use of a heated ribbon aims to produce durable, long-lasting images on a wide variety of materials. Direct thermal media is more sensitive to light, heat and abrasion, which reduces the life of the printed material (Zebra technologies[30]).

The table below summarizes the advantages and disadvantages of thermal transfer printing technique.

Table 57. Advantages and disadvantages of thermal transfer printing technique

Advantages	**Disadvantages**
Reliability Speed (up to 300 inches per minute)	Requires inked ribbon Need for cleaning and maintenance

[29] http://www.barcode-solutions.com

[30] http://www.zebra.com

Average resolution (200 -600 dpi) especially for barcodes Better longevity of print-outs than direct thermal printing	Sensitive to abrasion

For the sake of understanding and representation, the table below shows some standard models of the printers presented above.

Table 58. Standard models of alternative printing techniques to direct thermal printing

Matrix printer	
Inkjet printer	
Laser printer	
Thermal transfer printer	

C.1.2.2. Free-paper alternatives

Additionally to the analysis of substitutable printing systems, the analysis of alternatives to BPA in thermal paper includes herein the possibility to switch to free-paper techniques, based on electronic and IT technologies. This other way of understanding substitution is broader and more radical since these alternatives no longer imply the use of (thermal or traditional) paper.

These free-paper alternatives can be sorted in three categories: electronic tickets (e-tickets or e-receipts), contactless payments (mobile or smart card payments) and receipt handling options.

Electronic tickets and receipts

As reported in Danish E.P.A., 2013 and US EPA, 2012, digital or electronic receipts (e-receipts) have gained a wide acceptance since Apple introduced the concept in its retail stores in 2005 and the market is increasing. E-receipts are basically electronic receipts sent from the store directly to a customer's e-mail address or to a password-protected web-site. The technology can be managed by a merchant himself simply by asking the customer for an e-mail address. This is basically already what happens when people purchase items online but more and more "real" stores now also propose this free-paper solution. This is particularly the case of large retailers such as E. Leclerc[31] in France. This French large retailer announced in March 2013 the implementation of an electronic till ticket sent by e-mail. This option is only provided to customers having the Leclerc loyalty card. For the time being, the company has decided to keep available the possibility for customers to ask for a paper ticket. Other French big groups such as Darty (households electrical equipments and appliances) and Decathlon (sportswear and sports equipments) still print out paper till receipts but provide e-tickets to their clients, via their loyalty card, and register them on their web account. The clients thus keep a record of their purchases offline and online and have access to other functionalities such as: printing out their tills, adding one ticket in case they would have forgotten their loyalty card for one purchase in shop, or comments and valuations on the goods purchased. The very last step would be to totally remove paper tickets.

Additionally, e-receipts specialized companies (with point of sale partners and payment solution partners) are also emerging all over the world that offer to manage the system for shops and customers that sign up. When a customer signs up, he/she adds his/her debit and credit card details and when purchasing from stores signed in for the service, the receipt is automatically sent to the customer in digital form. As reported in Danish E.P.A., 2013 and on different internet websites, the table below provides some examples of companies which have set on that emerging market.

Table 59. Some e-receipts companies in the world

Country	**Company**	**Date of creation / key-figures (when available)**
USA	Xpenser	2008 More than 50,000 clients
	MyReceipts/Third solution	2009
	Sailthru/Seamless Receipts	2008 More than 6 million receipts

[31] http://www.webdeveloppementdurable.com/blog/2013/04/10/e-leclerc-lance-son-ticket-de-caisse-electronique/

	Proximiant	2011
UK	eReceipts	2010 More than 9 million receipts
Norway	dSafe.no	2300 merchants
Denmark	kvittering.dk eKvittering.dk	1999 2007 17 different shops or chain stores

Some of these companies set forth environmental considerations and even the non use of BPA as marketing arguments.

This technology is thus about to be well established and shows many advantages. First, it does not require any change for the consumer as the payment is based on the traditional payment card. Then, tailored packages are supplied by the e-receipts webplatforms with different convenient functionalities for the customers: receipts sent on their mailbox, view of their digital receipts via the company web and mobile environments, provision of an analytics and reporting dashboard to allow data driven decision making, loyalty schemes, ability to send time and/or location specific promotions and/or transaction history for the customers (where they can find information related to their purchase such as the date, the amount, the place, and some other optional information). One of the most important assets for consumers is indeed that e-receipts allow keeping searchable records of purchases on the custormers' computer and allow reducing paper waste.

Mobile/sms payment

As reported in Danish E.P.A., 2013, mobile banking and payment is a fast growing area and the number of users globally is expected to double to one billion by the end of 2015 from almost 500 million by the end of 2012.These numbers correspond to an estimated global mobile payment volume reaching one trillion USD by end of 2015 from 200 billion USD in 2012[32].

Mobile banking and payment is a broad category of money transfer applications made using a mobile device such as a mobile phone or a smartphone. When using mobile payment, a consumer can pay for a wide range of services and digital or hard goods by a range of technologies that includes SMS based transactional payments, Apps, mobile web payments and contactless near field communication. For mobile payments, the proof of purchase is also received electronically and an increase in mobile payment options is therefore expected to lead to a reduction in the use of thermal paper for receipts and tickets Danish E.P.A., 2013.

SMS payment is the most well-known and is mostly used for buying music, ring tones, games and for charity donations. The option of SMS payment has e.g. also been introduced as means

[32] http://www.portioresearch.com/en/major-reports/current-portfolio/mobile-payments-2013-2017.aspx

for paying for parking in some areas in the EU such as reported for Copenhagen in Danish E.P.A., 2013. The payment is made via the phone bill and proof of purchase is in this case typically in the form of an SMS confirming the purchase.

Custom applications (apps) for smartphones are also emerging, such as for transportation. Other types of tickets such as movie tickets are also starting to become available for mobile purchase. Via these apps, the phone number and payment card details are registered prior to the very first purchase and subsequent payments require the use of a personal code only. So far the mobile app payments have primarily included purchases of small value but the amounts of transactions are expected to increase in the future (Danish E.P.A., 2013).

Danish E.P.A., 2013 also refers to mobile web payments as another approach to mobile payment. It means using the Wireless Application Protocol (WAP) facility on a smartphone to connect to the internet and then pay by entering credit card details on the company website or pay using an online payment method such as PayPal or an electronic wallet.

As to Near field communication (NFC), it employs a set of standards for smartphones and similar devices to establish radio communication between two endpoints by bringing them into close proximity. Today, the technology allows two-way communication between the devices and the technology can be used for contactless transactions and data exchange, including mobile payment (Danish E.P.A., 2013).

Contactless smart card payment

As reported again in Danish E.P.A., 2013, contactless smart card payment is based on the radio-frequency identification (RFID) technology, which is a wireless non-contact use of radio-frequency electromagnetic fields to transfer data.

A smart card includes an embedded chip, which enables connection to a contactless radio frequency interface/rader. The RFID technology incorporated into contactless smart cards is used globally as payment technology for 'ticketless travel'. The ticketless travel technology was initially introduced in South Korea in 1995 (Upass), followed by Hong Kong in 1997 (Octopus card), and in Europe, the Oyster Card was introduced in London in 2003, the OV-chipkaart in the Netherlands in 2005 and more recently the Rejsekortet in Denmark. Today, the Octopus card can – in addition to payment of transportation – also be used for payment of parking, at retail outlets, self-service machines, leisure facilities and schools as well as for online purchases[33] (Danish E.P.A., 2013). The technology is well-established and considered an off-the-shelf product.

Receipt handling options

As explained in Danish E.P.A., 2013, a number of means to minimize the handling of receipts have been implemented (or are being tested) in various shops, in particular with the aim of reducing the exposure to BPA of the employee working at the cash register. For instance, a costumer is regularly faced with the question if he/she wants a receipt. Often the receipt is

[33] http://www.octopus.com.hk/get-your-octopus/en/index.html

printed regardless of the answer, but solutions are available where receipts are only printed if desired by the customer.

C.2 Assessment of alternative "drop in" substances

As explained in section B.2.4, BPA in thermal paper shows several advantages such as efficacy, availability and low cost.

C.2.1 Assessment of BPS

Table 60. Identity of BPS

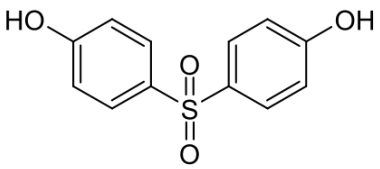
Public name	4,4'-sulfonyldiphenol
EC name	4,4'-sulphonyldiphenol
IUPAC name	4,4'-sulfonyldiphenol
EC number	201-250-5
CAS number	80-09-1
Annex VI Index number	Not assigned
Molecular formula	C12H10O4S
Chemical structure	

Table 61. Physico-chemical properties of BPS

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 1013 hPa	4,4'-sulphonyldiphenol is a white odourless solid powder	CSR lead	Value used for CSA: solid
Melting / freezing point	Meltingpoint = 245 - 248°C	CSR lead	Range of 8 values
Boiling point	not applicable	CSR lead	decomposition at 315°C The boiling point of the test item could not be determined,

			because at a temperature of 315°C a continuously increasing pressure was observed. This is presumably caused by a limited stability and a thermal change of the test item.
Relative density	density = 1.4 g/cm3	CSR lead	value out of 2 different sources
Vapour pressure	negligible	CSR lead	The melting point of the substance is between 200 °C and 300°C. The calculated value of vapour pressure at 25°C is quite low as expected (6.29E-10 hPa at 25°C).
Surface tension	not applicable	CSR lead	Based on chemical structure, no surface activity is predicted.
Water solubility	Water solubility = 1.1g/l at 20°C	CSR lead	Value used for CSA: 1.1 g/L at 20 °C
Partition coefficient n-octanol/water (log value)	1.2 at 23°C	CSR lead	
Flash point	Not applicable	CSR lead	The substance is a solid.
Flammability	not highly flammable The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	CSR lead	solid: not highly flammable. Non flammable solid. Flammability derived from screening test. Based on chemical structure pyrophoric properties and flammability in contact with water are predicted.
Explosive properties	non explosive	CSR lead	Value used for CSA: non explosive There are no chemical groups

			associated with explosive properties present in the molecule.
Self-ignition temperature	not applicable	CSR lead	The substance is a solid and self-heating of the substance up to 400° C is excluded.
Oxidising properties	no oxidising properties	CSR lead	Value used for CSA: Oxidising: no *The test substance is not considered an* *oxidising substanc*e because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture.
Granulometry	particles <100µm approximate 55%, particles <10µm approximate 1.8%, particles <4µm approximate 0.4%	CSR lead	
Stability in organic solvents and identity of relevant degradation products	not applicable	CSR lead	The stability of the substance is not considered as critical.
Dissociation constant	8.0 at 20 °C	CSR lead	Value used for CSA: pKa at 20°C: 8
Viscosity	not applicable	CSR lead	Substance is a solid at 20° C and atm. pressure.

C.2.1.1. Availability of BPS

The manufacturers of thermal paper consulted in INERIS, 2013 claim that BPS is already used in thermal paper as a dye developer in Europe and worldwide. The review of available literature as well as the measurements carried out on tickets in different countries (presented in section B.2) also confirms this use (US EPA, 2012, Jeffs, 2011).

According to the registration dossiers of BPS, BPS is produced at a level above 1000 tons per year.

The Danish E.P.A. 2013 report indicates that between 85-95% of the receipts on the Danish market are made of thermal paper and nearly all thermal paper on the Danish market contains BPA. New products are however coming up where the more expensive bisphenol S (BPS) is used in-stead of BPA because the producers try to meet an increasing demand for BPA-free products. Several different qualities but only few qualities are used in Denmark most is standard quality. Receipts with a 'top coat' are used in cash point machines, at some petrol stations, but also in furniture chains that have a long guarantee on many products and shops where you sell long-lasting consumer goods. Primarily BPS is used in qualities where long durability is required. The technical reason why BPS is used in these quality papers has not been investigated.

As a whole, BPS is considered as an available alternative.

C.2.1.2 Human health risks related to BPS

Currently, there is no harmonised classification for BPS. However, the self-classification given by the industrials for BPS are the following one: (with 7 different aggregated notifications; website of ECHA[34]).

Classification		Labelling			Number of Notifiers	Joint Entries
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)		
Not Classified					209	
Aquatic Chronic 3	H412	H412			94	
Eye Irrit. 2	H319	H319		GHS07 Wng	35	

[34] http://echa.europa.eu

					33	
					9	✔
		H319		GHS07 Wng	3	
		H315				
		H335				
Aquatic Chronic 3	H412	H412			1	

Toxicity on reproduction and development:

Animal data on BPS:

Two studies were listed in the ANSES report on "Compounds of the family of bisphenols: bisphenols M, B, AP, AF, F and BADGE" (ANSES, 2012): A study from 2001 (European Commission, 2012) but only a summary of this study is available with discrepancies in the available report. An OECD 421 guideline study was performed in rats (12 animals / sex / dose) exposed by oral route (incorporation in a vehicle consisted in 0,5 % of carboxyméthylcellulose sodic and 0,1 % of Tween 80, then gavage) to doses of 0, 10, 60 or 300 mg/kg/d of BPS. The BPS was administered to females 14 days before mating, during the gestation and until the 3rd postnatal day (PND 3). The males were administered BPS during 45 days (dates of the beginning and of the end of administration not specified). Males and females were coupled.

- No effect was observed at the dose of 10 mg/kg/d.

- The effects observed at the dose of 60 mg/kg/d are:

  - parental toxicity: distension of the caecum with a diffuse hyperplasia of the epithelial mucous membrane,
  - no toxicity on the development.

- The effects observed at 300 mg/kg/d are:

  - parental toxicity: decrease of the weight and the food consumption, hypertrophy of hepatocytes and distension of the caecum (with diffuse hyperplasia of the epithelial mucous membrane) to males and females. Increase of the size of the liver to males. Severe systematic toxicity observed but not specified in the summary of the study.
  - toxicity on the reproduction: increase of the duration of the oestrous cycle and decrease of the index of fertility in the mothers. Decrease of the number of alive births and the number of alive newborn pups at PND4. In dams, no modification of the mating, gestation and birth, the number of corpus luteum, the duration of gestation, the parturition and the behavior during the lactation were reported. Effects were noted with regard to number of implantations and implantation index. In pups, no modification of

the "sex-ratio", the weight in the birth, the ano-genital distance. No anomaly was found after external examination and autopsy. Contradictory results were reported regarding the number of alive pups at birth and PND4, the number of corpus luteum and the number of implantations and implantation index.

This study (OECD 421) was judged of reliability of 2 (reliable with limitations), although carried out in GLP compliance but because of a minimal description of the method and the results and discrepancies in the available report.

From these data, the authors propose the following NOAEL:

· NOAEL for parental toxicity of 10 mg/kg/d (critical effect: hyperplasia and caecale distension)

· NOAEL for reprotoxicity of 60 mg/kg/d (critical effects: decrease of the index of fertility, the number of alive births, the number of alive newborn children to PND4, increase of the oestrous cycle). It is however necessary to note that the detail of the study is not available.

An uterotrophic assay on young Sprague-Dawley rats of 20 days (6 animals / doses) was performed according to the OECD guideline 440 (Yamasaki K, 2004). Animals were exposed by subcutaneous injection (vehicle consisted of olive oil) to doses of 0, 20, 100 and 500 mg / kg / day of BPS during 3 days +/- added of 0,6 µg / kg / day of ethinyl estradiol (EE) and sacrificed 24 h after the last administration, and their uterus was weighed.

The results are the following:

- A significant increase of the absolute and relative uterine weight (wet and blotted) in the groups treated at 20 mg / kg / d (30 % on average) and 500 mg / kg / d (67 % on average) compared with the control group (0 mg / kg / d of BPS), but not in the group of 100 mg / kg / d.

- A significant increase of the absolute and relative uterine weight in the groups 20 mg / kg / d of BPS + ethinyl estradiol (20 % on average for the wet weights, 13 % for the mopped weights) compared with) the control group (0 mg / kg / d of BPS + ethinyl estradiol).

- No significant modification of the absolute and relative uterine weights in the groups 100 mg / kg / d of BPS ± ethinyl estradiol compared with their respective control group (0 mg / kg / d of BPS ± ethinyl estradiol).

- A significant decrease of the absolute and relative uterine weights in the groups 500 mg / kg / d of BPS + ethinyl estradiol (40 % on average) compared with the control group (0 mg / kg / d of BPS + ethinyl estradiol). This decrease of the weights of the uterus is similar to that observed during the co-administration of tamoxifene at 1 mg/kg/d.

Table 62. Summary table of the uterotrophic assay for BPS (Yamasaki K, 2004)

Dosages (mg/kg per day)	Body weight (g)	Uterine wet weight		Uterine blotted weight	
		Absolute (mg)	Relative (mg/100 g)	Absolute (mg)	Relative (mg/100 g)
Vehicle control	58.3 ± 3.4	37.4 ± 6.9	64.0 ± 9.3	36.8 ± 7.0	63.0 ± 9.4
20	59.7 ± 3.8	48.8 ± 9.0[a]	81.6 ± 13.3[a]	48.2 ± 8.8[a]	80.7 ± 12.8[a]
100	57.6 ± 1.9	42.6 ± 6.2	73.8 ± 8.5	41.9 ± 6.0	72.6 ± 8.3
500	58.0 ± 2.8	62.1 ± 13.3[b]	107.5 ± 25.0[b]	61.0 ± 12.6[b]	105.6 ± 23.8[b]
Vehicle + EE	60.6 ± 2.3	148.7 ± 9.4	245.3 ± 13.8	117.5 ± 8.8	193.8 ± 13.2
20 + EE	58.9 ± 4.0	181.1 ± 23.9[c]	307.5 ± 38.8[d]	128.7 ± 10.7	218.4 ± 10.6[d]
100 + EE	59.0 ± 2.8	151.6 ± 43.3	257.0 ± 73.7	118.6 ± 12.2	201.4 ± 21.2
500 + EE	58.2 ± 3.8	79.9 ± 10.5[d]	137.7 ± 20.8[d]	78.5 ± 10.4[d]	135.4 ± 20.3[d]
TMX + EE	57.5 ± 3.6	91.1 ± 9.8[d]	158.5 ± 15.2[d]	90.0 ± 9.7[d]	156.6 ± 15.0[d]

E2, 17β-estradiol; EE, ethynyl estradiol; TMX, tamoxifen.
[a] Significantly different from vehicle control at $P < 0.05$.
[b] Significantly different from vehicle control at $P < 0.01$.
[c] Significantly different from vehicle control plus EE at $P < 0.05$.
[d] Significantly different from vehicle control plus EE at $P < 0.01$.

Human data on BPS:

No data in humans are currently available.

Toxicity by repeated doses: subacute or subchronic

Animal data

A study dating from 1999 (website of ECHA, study " Repeated measures toxicity ") realized on rats (6 animals/sex/dose) at 0, 40, 200 or 1000 mg/kg/d of BPS by oral route during 28 days did not show any effects on the reproductive organs. Two males of the group of 1000 mg/kg/d died during the period of administration of a digestive bleeding located in the caecum. Among the observed effects, was described an increase of the size of the adrenal glands with hypertrophy of the cortical cells of the *zona fasciculata* of males having received 1000 mg/kg/d from BPS.

The observed effects are:

- Decrease of food consumption and a decrease of the weight gain of females treated by 200 and 1000 mg/kg/d of BPS and males treated by 1000 mg/kg/d of BPS.
- An anaemia in both sexes in the group 1000 mg/kg/d.
- A decrease of the total cholesterol in both sexes, an increase of the activity of the alkaline phosphatase of males and a hyperalbuminémie of females in the group 1000 mg/kg/d.
- An increase of the incidence of proteinuria in both sexes and the presence of urobilinogen in urines of the males in the groups 200 and 1000 mg/kg/d, as well as an acidification of urines of the males in the groups 200 and 1000 mg/kg/d and of the females in the group 1000 mg/kg/d.
- An increased weight (absolute or relative? not specified) of the thymus and the liver in both sexes and the adrenal glands of males in the group 1000 mg/kg/d, and an increased weight of kidneys of the males in the groups 200 and 1000 mg/kg/d.
- An abdominal distension in females and a dilation caecale in both sexes in the group 1000 mg/kg/d.

After histological analysis, non-neoplastic anomalies were observed:

- An hyperplasia of the mucous membrane caecale and a cellular necrosis in the epithelial mucous membrane in both sexes of the groups 200 and 1000 mg/kg/d.
- An hypertrophy of the cortical cells of the *zona fasciculata* of the adrenal glands of the males of the group 1000 mg/kg/d.
- An atrophy of the thymus in both sexes of the group 1000 mg/kg/d. This result is however contradictory to the increase of the weight of the thymus observed.
- A centrilobular hypertrophy of hepatocytes and extra-medullary hemopoiesis located in the liver in both sexes in the group 1000 mg/kg/d.
- An increase of the hemopoiesis observed in the thighbone and in the spleen in the males of the group 1000 mg/kg/d.

The authors propose a NOAEL at 40 mg/kg/d (critical effects: loss of weight gain, effects on kidneys, increase of the renal weight, proteinuria, acidification and presence of urobilinogen in urines, hyperplasia and caecale distension). No effects on the organs of the reproduction wereobserved. This GLP compliant study has a degree of reliability of 2 (reliable with limitations) because of a minimal description of the method and the results. The methodology of this study is similar to that of the OECD guideline 407.

Toxicity by repeated doses: subacute or subchronic

Data in human: No human data were identified upto this day.

Chronic toxicity:

No animal or human data identified to this day.

Carcinogenicity:

No data identified to this day.

Sensitization:

No data identified to this day.

Genotoxicity:

Several *in vivo* and *in vitro* genotoxicity studies were realized (cf hereabove: Synthesis of the data on the genotoxicity of the BPS). No genotoxic effect was observed *in vitro* with the BPS. A micronucleus test realized *in vivo* did not demonstrate any genotoxicity of the BPS. **The *in vitro* chromosomal aberration tests indicate a clastogen effect of the BPS without metabolic activation on CHO and CHL / IU cells.**

Table 63 Synthesis of data on genotoxicity of BPS

genotoxicity studies *in vitro*		
Test of gene mutation on prokaryote (OECD Guideline 471)	**Negative**: *S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 with and without metabolic activation. Gene : operon histidine Doses : 0,32-1,6-8-40-200-1000 µg/plate	European Chemicals Agency, 1989
	Negative : *S. typhimurium* TA1535, TA1537, TA98 et TA100 with and without metabolic activation. Gene : operon histidine Doses : 30-60-120-240-480-960 µg/plate	European Chemicals Agency, 1991
	Negative : *S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 with and without metabolic activation. Gene and without metabo Dosesistidineout metabolic activation.o	Seifried HE, 2006
	Negative: *S. typhimurium* TA1535, TA1537, TA98 et TA100 (Doses : 0, 78.1, 156, 313, 625, 1250, 2500, 5000 µg/plate), *E. coli* WP2 uvr A (Doses : 0-156-313-625-1250-2500-5000 µg/plate), with and without metabolic activation.	Office of Environmental Chemicals Safety Environmental Health B, 1999
	Negative : *S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 with and without metabolic activation. Doses : 20-80-320-1280-5120 µg/plate	European Chemicals Agency, 1987
	Negative: *S. typhimurium* TA1535, TA1537, TA98 et TA100, *E. coli* WP2 uvr A	European Chemicals Agency, 1996

	with and without metabolic activation. Doses : 20-39-78-156-313-625-1250-2500-5000 µg/plate	
Test of gene mutation on cells of mammals	**Negative**: ovarian cells of Chinese hamster (CHO) with and without metabolic activation. Gene : HGPRT Doses : 62,5-125-250-500-750-1000 µg/mL	European Chemicals Agency, 1990
Test of chromosomal aberration (OECD Guideline 473)	CHO cells with and without metabolic activation. **Positive without metabolic activation at** 500 et 600 µg/ml. Cytotoxicity at 700 µg/ml. **Negative without metabolic activation** at 125, 250, 500, 750 and 1000 µg/ml. Cytotoxicity at 750 and 1000 µg/ml.	European Chemicals Agency, 1991
Test of chromosomal aberration	Lung cells of Chinese hamster (CHL/IU) with and without metabolic activation. **Slightly positive without metabolic activation at** 400 µg/ml in continuous treatment of 24 hours.	Office of Environmental Chemicals Safety Environmental Health B, 1999
Inhibition of the polymerization of microtubules	**Negative**: System without cells, without metabolic activation. Doses : 50-200 µM	Pfeiffer E, 1997
Study of genotoxicity *in vivo*		
Micronucleus test	**Negative** : Male mice NMRI exposed by gavage (500, 1000, 2000 mg/kg), then sacrificed 24h after (and 48h after in the group at 2000 mg/kg). Test realized on bone marrow.	European Chemicals Agency, 2010

BACKGROUND DOCUMENT TO RAC AND SEAC OPINIONS ON
4.4’-ISOPROPYLIDENEDIPHENOL (BISPHENOL A; BPA)

Mechanism of action - Interaction with receptors:

Data *in vitro*

Several *in vitro* studies were realized to estimate the endocrine activity of the BPS (See hereabove: synthesis of the data on the *in vitro* endocrine activity of the BPS). Three studies (Chen MY, 2002; Hashimoto Y, 2000; Hashimoto Y, 2001) measured the oestrogenic activity of the BPS on hybrid yeasts. Two studies of Hashimoto showed an absence of oestrogenic activity of the BPS without metabolic activation, but a light oestrogenic activity after metabolic activation. However, this activity remains very low. Furthermore, no metabolite of the BPB was studied to support this hypothesis.

The study of Chen shows a light oestrogenic activity of the BPS obtained with a high concentration (200 mg /L, or 8.10-4 M). In the same concentration, the oestrogenic activity of the BPA is approximately 7 - 9 times as important.

Several studies carried out on mammal cells highlight an oestrogenic activity of the BPS. It is shown that the BPS is an agonist of the oestrogens receptors (ER) leading to the proliferation of MCF-7 cells (breast cancer cell line). Two studies from 2005 of Kitamura (Kitamura S, 2005) and Kuruto-Niwa (Kuruto-Niwa R, 2005) showed an oestrogenic activity of the BPS similar to the BPA's one (EC_{50} of the BPS: 1,75 and $1,1.10^{-6}$ M, EC_{50} of the BPA: 1,09 and $0,63.10^{-6}$ M). However, another study (Blair Rm FAU, 2014) showed an affinity of the BPS approximately 10 times as low as the BPA during tests of competition with the estradiol (IC_{50} of the BPS: $1,05.10^{-4}$ M and IC_{50} of the BPA: $1,17.10^{-5}$ M).This latest study was performed using a validated estrogen receptor competitive-binding assay from ER-reach supernatant coming from uteri from oariectomised SD rats.

IC_{50}s and Relative Binding Affinities (RBA) for Diphenyl Derivatives

Chemical name	Source	Purity (%)	Mean IC_{50} (M) ± SEM	RBA (%)	Log RBA
Diphenyl methane derivatives (bisphenol A's)					
2,2-Bis-(4-hydroxyphenyl)-butane (*bisphenol B*)	Aldrich	NA	$1.05 \times 10^{-6} \pm 0.46 \times 10^{-6}$	0.086	−1.07
Bisphenol A	Aldrich	99	$1.17 \times 10^{-5} \pm 0.64 \times 10^{-5}$	0.008	−2.11
2,2′-Methylenebis (4-chlorophenol)	Aldrich	90	$2.55 \times 10^{-5} \pm 0.15 \times 10^{-5}$	0.004	−2.45
BIS (4-hydroxyphenyl)-methane	Aldrich	98	$9.50 \times 10^{-5} \pm 0.50 \times 10^{-5}$	0.0009	−3.02
4,4′-Sulfonyldiphenol	Sigma	99	$1.05 \times 10^{-4} \pm 0.35 \times 10^{-4}$	0.0009	−3.07
Diphenolic acid	Aldrich	95	$1.20 \times 10^{-4} \pm 0.30 \times 10^{-4}$	0.0007	−3.13
4,4′-Methylenebis (2,6-di-*tert*-butylphenol)	Aldrich	98	$>1.00 \times 10^{-4}$	—	—
BIS (2-hydroxyphenyl)-methane	Aldrich	98	$>1.00 \times 10^{-4}$	—	—
Diphenyl ethane derivatives					
4,4′-Dihydroxystilbene[a]	NCI	NA	$3.20 \times 10^{-7} \pm 0.90 \times 10^{-7}$	0.281	−0.55
2,2′,4,4′-Tetrahydroxybenzil	NCI	NA	$4.30 \times 10^{-7} \pm 0.00$	0.209	−0.68
4,4′-Ethylene diphenol	NCI	NA	$2.45 \times 10^{-6} \pm 0.35 \times 10^{-6}$	0.037	−1.44
4-Phenethylphenol	NCI	NA	$4.40 \times 10^{-5} \pm 0.60 \times 10^{-5}$	0.002	−2.69
4-Stilbenol	NCI	NA	$>1.00 \times 10^{-4}$	—	—
Biphenyl derivatives					
4-Phenylphenol	Aldrich	90	$9.80 \times 10^{-5} \pm 5.20 \times 10^{-5}$	0.001	−3.04
3-Phenylphenol	Aldrich	90	$2.45 \times 10^{-4} \pm 0.45 \times 10^{-4}$	0.0004	−3.44
2-Phenylphenol	Aldrich	99	$>1.00 \times 10^{-4}$	—	—

[a] Chemical exhibited a U-shaped binding curve.

Source: Blair Rm, Fang and al., 2000 (Blair Rm FAU, 2014)

The RBA [Relative Binding Affinity = (IC_{50} E2 / IC_{50} BPS) 100] value was determined as 0,0009 % .

In another study performed on human ER recombinants, expressed in E Coli dispayed an RBA of 0,0055 % (Yamasaki K, 2004).

Moreover, there is a low anti-androgenic activity of the BPS (CI50: 17 µM) relatively close to the BPA's one (IC_{50}: 4,3 µM) (Kitamura S, 2005).

Data *in silico*

A study (Klopman G, 2003) uses an IT model (Multicompartment) to estimate the oestrogenic activity of a molecule according to its structure. A relative binding affinity (RBA) of 0,0006 % was estimated, with a probability of correct result of 87 %. This RBA is close to the study of Blair and al (Blair Rm FAU, 2014) who was 0,0009%. For comparison, the RBA of the BPA is estimated at 0,0014 % with the same probability of correct result. According to the studies *in vitro* and *in silico*, the BPS is an agonist of the receptors in oestrogens. The BPS would also have an anti-androgenic activity.

2013/2014 studies on BPS (not included at the time of the submission of the proposal)

- Recent animal studies report that BPS appears to be just as potent as BPA in altering brain development and causing hyperactive behaviour (Endocrine Society, 2014a[35]) or to cause heart damages (Endocrine Society 2014[36]).
- Other studies report a concern for BPS both for the human health and environment:
    - Ji K, Hong S, Kho Y, Choi K. Effects of bisphenol s exposure on endocrine functions and reproduction of zebrafish. Environ Sci Technol. 2013 Aug 6;47(15):8793-800
    - Naderi M, Wong MY, Gholami F. Developmental exposure of zebrafish (Danio rerio) to bisphenol-S impairs subsequent reproduction potential and hormonal balance in adults. Aquat Toxicol. 2014 Mar;148:195-203.
- Rosenmai et al 2014 study also reports concern about BPS effects on 17alpha-OH progesterone): Rosenmai AK1, Dybdahl M, Pedersen M, Alice van Vugt-Lussenburg BM, Wedebye EB, Taxvig C, Vinggaard AM. Are structural analogues to bisphenol a safe alternatives? Toxicol Sci. 2014 May;139(1):35-47. Epub 2014 Feb 22.

Summary of the BPS toxicological profile:

[35] http://www.sciencedaily.com/releases/2014/06/140623103933.htm

[36] http://www.sciencedaily.com/releases/2014/06/140623103935.htm

BPS possesses oestrogenic properties *in vitro*. It leads to the proliferation of the mammary cancerous human cells MCF-7 and possesses an affinity for the oestrogens receptors, depending on the model used. BPS is little (even not at all) oestrogenic in the test of yeasts associated with a gene reporter. However, after metabolic activation with S9mix, the oestrogenic activity of BPS increases, what seems to indicate that its metabolites possess oestrogenic properties. In vitro, the oestrogenic activity of the BPS is slightly lower than that of the BPA (of a factor from 2 to 10). An anti-androgenic activity is also observed in a study.

In vivo, an uterotrophic effect of the BPS is observed on the immature rats. The same study shows a decrease of the uterotrophic effect of the ethinyl estradiol when the BPS is coadministered at high dose (500 mg/kg/d).

A study on the reproduction and the development realized in rat show an increased duration of the oestrous cycle, the decrease of the index of fertility and a decrease of the number of the alive births and the alive newborn children to PND4 after maternal exposure of 300 mg/kg/d of BPS. This dose is however toxic for the mother. No effects were observed on the organs of the reproduction for fertility and development at non-toxic doses in the mother.

A study of subacute toxicity of 28 days does not show any effects of the reproductive functions, nor an endocrine disruption for doses of BPS until 1000 mg/kg/d.

Full study reports were not examined, because being of industrial property. The results described in this toxicological profile result from summaries of studies put at the disposal on ECHA website. At the time of the evaluation, the dataset for repeated and reproductive endpoints were incomplete (study ongoing mentioned).

The tests of genotoxicity *in vitro* are negative, except 2 tests of chromosomal aberration which are positive without metabolic activation. The mammalian erythrocyte micronucleus test realized *in vivo* in the mouse is negative.

Finally, NTP has developed a BPS Research Concept[37], which was recently approved by NTP's Board of Scientific Counselors. NTP concludes that: "Currently, there is insufficient in vivo toxicological data to adequately characterize the possible human health effects of BPS (data are limited in scope and power and has not been peer reviewed); however, of the limited toxicological data (primarily available from industry), there is evidence that BPS has effects on select organs and hematological factors".

C.2.1.3 Environment risks related to BPS

According to the disseminated data published on the website of ECHA on the registration dossier:

Environmental fate and pathways:

Stability: phototransformation in air: QSAR:

[37] http://ntp.niehs.nih.gov/ntp/about_ntp/bsc/2014/june/bisphenols_concept_508.pdf

The rate of photochemical degardation was calculated using the model Epiwin SRC AOP v1.92. The calculation is based on an overall OH rate constant of 14.5305 E^{-12} cm^3/molecule*sec and a OH-radical concentartion of 5.0E^{+05} molecules/cm^3

The substance is relatively fast photochemically degraded once released to air with a DT50 value of 26.5 hours.

Biodegradation in water: screening tests:

A Modified MITI Test (I) was conducted according to OECD Guideline 301 C over a period of 28 days.

No biodegradation of test substance was observed during a 28 d incubation period. The test item is

therefore considered to be not ready biodegradable.

Biodegradation in water and sediment: simulation tests

The study examined the biodegradability of 4,4 -sulphonyldiphenol in aerobic water and anaerobic sediment. No biodegradation of the substance was oberserved under arobic conditions in river water. Under anaerobic conditions, the biodegradation of 4,4-sulphonyldiphenol reached a level of about 60% at ca. day 80 after a lag phase of ca. 60 days.

Bioaccumulation aquatic/sediment:

A Bioaccumulation study to fish was conducted according to OECD Guideline 305 C. The species Cyprinus carpio were exposed to the test substance.over a period of 6 weeks at test concentrations of 50 and 500 µg/L in a flow through system.

The BCF for the test substance was measured to be very low (< 0.2 and < 2.2 at test concentrations of 500 and 50 µg/L). Thus, a bioaccumulation of the test substance to aquatic organisms is not expected.

Adsorption/distribution: QSAR:

The Koc value was calculated using the model Epiwin SRC PCKOC v1.66.

The Koc was calculated to be 7615. Therefore, a strong adsoprtion to soil can be assumed once released.

Henry's Law constant: QSAR

The Henry constant was calculated using the model Epiwin SRC Henry v3.10.

The Henry constant for the substance was calculated to be 2.73 Pa m^3/mol.

Distribution modelling calculated:

Over time, the substance will preferentially distribute into water.

Persistency:

Studies imply that BPS is more persistent in the environment in comparison to BPA (Ike et al., 2006; Danzl et al., 2009).

Ecotoxicological information

Aquatic toxicity:

Short term toxicity to fish:

The study examined the toxicity of 4,4 -sulphonyldiphenol on fish for 96 hours.The study was conducted in accordance to the OECD 203 guideline. No further study details are presented. The 96 hour LC50 value was determined to be above the limit dose of 100 mg/L.

Short term toxicity to aquatic invertebrates:

The study examined the toxicity of 4,4 -sulphonyldiphenol to Daphnia for 48 hours.The study was conducted in accordance to the OECD 202 guideline. No further study details are presented. The 48 hour EC50 value was determined to be 55 mg/L

Long term toxicity to aquatic invertebrates:

The study examined the effect of 4,4 -sulphonyldiphenol on the reproduction output of Daphnia for 21 days.The study was conducted accordance to the OECD 211 guideline. No further study details are presented. The 21 days NOEC and 21 days EC50 values were determined to be 2.7 and 14 mg/L.

Toxicity to aquatic algae and cyanobacteria

The study examined the effect of 4,4 -sulphonyldiphenol on green alga (Desmodesmus subspicatus) for 72 hours in a growth inhibition test. The cultures were exposed to nominal concentrations of 0, 1.02, 3.2, 10.2, 32, 102, 320 mg/L. The study was conducted under static conditions and in accordance with the OECD 201 guideline. The 72 hour NOEC value was determined to be 10.2 mg/L. The ErC50 was determined to be 106 mg/L. The NOEC and ErC50 values were based on cell concentration measurements. Since the analytically determined concentrations of the test substance in the test solutions were within ±20% of the nominal concentrations, the effect concentration was expressed relative to the nominal concentration. All validity criteria were fulfilled.

Toxicity to microorganisms

4,4-sulphonyldiphenol was tested in the 3 -hour activated sludge test according to OECD test guideline 209 and EU-method C.11.

In comparison to the inoculum controls the respiration rate of the activated sludge was inhibited dose-dependently in the concentration range from 62.5 to 1000 mg/L, displaying inhibition rates from -17 up to 96 %. The respiration rate of the activated sludge was inhibited by 22 %, at 250 mg/L and by 70 % at 500 mg/L.

Based on measured inhibition rates the 3 -hour EC10, EC20, EC50 and EC80 values were calculated by Probit analysis.

EC10: 200 mg/L

EC20: 250 mg/L

EC50: 390 mg/L

EC80: 590 mg/L

C.2.1.4 Technical and economic feasibility of BPS

From INERIS, 2013, ETPA indicated that BPS is the first alternative used in thermal paper because it has more or less similar properties as BPA, although it raises the same questions about potential harmful impacts on health, and even less studies than for BPA are available. Concerning the costs aspects, it would be also the first choice. BPS is currently used in Japan and USA instead of BPA. So this alternative could be the most likely to be widely used instead of BPA in the case of a regulation in terms of its technical and economic feasibility. A representative of a Japanese institution (AIST[38]) confirmed that in Japan, BPA has been replaced by BPS in thermal papers since 2003, but he added that the quality of the products obtained was not as good as with BPA. According to INERIS, 2013, BPS price ranges from 2,920€/t to 4,200€/t with an average price of 3,583€/t. It is thus higher than BPA price.

Thermal paper containing BPS as developer seems to have a longer persistence compared to BPA-based paper – the print will last at least 10 years. There seems to be no problem to adjust production, using BPS instead of BPA. **As a whole, BPS is claimed to be efficient and technically feasible by industry.**

C.2.1.5 Other information on BPS

Concerning the endocrine disruption, the BPS is classified in category 3b (absence or insufficiency of collected data) by the report of the DHI (DHI, 2007) and in a group 3 (absence or insufficiency of data to be listed) by the report of the European Commission (report BKH) (CE, 2002).

Moreover, an impregnation campaign is in progress (2013), realized by the national institute of scientific research (INRS, 2013). This evaluation consists in the urinary dosage by biometrology in BPA and BPS on cashiers exposed by percutaneous route.

BPS was detected in human urine samples from general populations of the United States, China, India, Japan, Korea, Kuwait, Malaysia and Vietnam (Liao C LF., 2012). This chemical was not included in the NHANES biomonitoring report (CDC, 2011).

Finally, BPS is currently being evaluated by Belgium. Depending on the conclusion of their Substance Evaluation under REACH, BPS might then be also regulated sooner or later.

To this respect, information provided by large retailiers in particular during the consultation carried out by the DS indicates that, although BPS is technically and economically feasible and is already used as an alternative, it still may be expected that industry would not necessarily switch to BPS since it is expected that BPS might be regulated in the near future (INERIS, 2013).

[38] http://www.aist.go.jp/index_en.html

C.2.2 Assessment of BPF

There is no indication that BPF is actually used in thermal paper.

Table 64. Identity of BPF

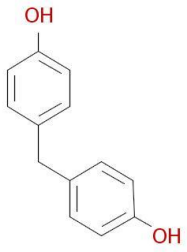
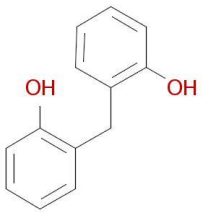
Public name	4,4'-methylenediphenol	2,2'-methylenediphenol
EC name	-	
IUPAC name	-	
EC number	210-658-2	219-578-2
CAS number	620-92-8	2467-02-9
Annex VI Index number	NA	
Molecular formula	$C_{13}H_{12}O_2$	
Chemical structure		

Table 65. Physico-chemical properties of BPF

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Molecular weight (g/mol)	200,23		
Boiling Point (°C)	390°C à 760 mmHg	[1]	
Melting Point (°C)	162-164	[2]	
Pression de vapeur saturante (Pa)	$1,22X10^{-6}$ mmHg à 25°C	[3]	
Relative Density	1,208	[1]	
Water solubility ($g.L^{-1}$)	1.1	[4]	

Log Kow	2,91	Hansch, 1995	
Koc (L/kg)	Non documenté		
Flash point(°C)	192,9	[1]	

[1]ChemNet : http://www.chemnet.com/Products/supplier.cgi?f=pclist;lang=en;site=chemnet;region=;skey=620-92-8%20bis%28phydroxyphenyl%29methane;use_cas=1;rand_id=

[2] Chemical Book http://www.chemicalbook.com/Search_EN.aspx?keyword=620-92-8

3) http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=12111

C.2.2.1. Availability of BPF

The data on the tonnage of BPF produced, used or placed on the EU market are not publicly available. BPF has not been registered under REACH so far.

As a result, it is difficult to conclude about the availability of BPF.

C.2.2.2 Human health risks related to BPF

Classification		**Labelling**			**Number of Notifiers**
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Supplementary Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**	
Skin Irrit. 2	H315	H315		GHS07 Wng	33
Eye Irrit. 2	H319	H319			
STOT SE 3	H335	H335			
Aquatic Chronic 3	H412	H412			
Skin Irrit. 2	H315	H315		GHS07 Wng	23
Eye Irrit. 2	H319	H319			
STOT SE 3	H335	H335			
Aquatic Chronic 3	H412	H412			6

Not Classified					5
		H319		GHS07 Wng	2
		H315			
		H335			
Skin Irrit. 2	H315	H315		GHS07 Wng	1
Skin Sens. 1	H317	H317			
Eye Irrit. 2	H319	H319			
Aquatic Chronic 3	H412	H412			

Number of Aggregated Notifications: 6

Toxicokinetic:

Absorption:

After a unique administration by gavage of 3H-BPF at the doses of 7 mg/kg or 100 mg/kg to Sprague-Dawley female rats, a systemic passage is observed. The levels of radioactivity found in the urine and the feces during 4 days following the administration of the dose suggest that the almost totality of the BPF is absorbed by oral route (Cabaton N, 2006). No other data on the absorption of the BPF by oral, cutaneous or respiratory route was found in the literature.

Distribution:

A study was realized on gravid/ non gravid Sprague-Dawley females, force-fed with radiolabelled BPF at the doses of 7 mg/kg or 100 mg/kg (Cabaton N, 2006). It was found 96 hours after administration:

-In the non-gravid female rats: 0,5 % of the initial dose of BPF in tissues (0,4 % in the liver, less than 0,05 % in the other tissues) and 6-8 % in the carcass.

-In the gravid female rats: 0,8 % of the initial dose of BPF in tissues (0,5-0,7 % in the liver, 0,1-0,2 % in the uterus, less than 0,05 % in the other tissues) and 6,7-8,4 % in the carcass.

The dose does not seem to have an incidence on the tissular distribution. The BPF crosses the foeto-placentaire barrier. Approximately 1 % of the total BPF is found in the foetus, distributed in fair quantities in the liver, the head and the rest of the body.

Metabolism:

In the study of Cabaton and al. (Cabaton N, 2006) realized in Sprague-Dawley females, 6 metabolites of the BPF were detected in urines after 96 hours. The main metabolite (> 50 %) corresponds to a sulfoconjugate of the BPF. A glucuronoconjugate of the BPF is also detected.

In an in vitro study (Cabaton N, 2008) realized with cellular fractions (S9 and microsomes) of human and rats hepatocytes, the hydroxylation via cytochromes P450 is the main way of metabolisation, with formation of BPF ortho-or meta - hydroxyled (main metabolite), of BPF di-hydroxyled, and dimers of BPF. Conjugated by-products BPF-glucuronide and BPF-sulfate are also detected.

Another in vitro study (Audebert, Dolo and al. 2011) studied the metabolism of the BPF on intestinal cellular lines (LS174T), hepatic (HepG2) and renal (ACHN). No metabolite was detected in cells ACHN. In cells HepG2, the majority of the BPF is metabolized to sulfoconjugate. In cells LS174T, the BPF is totally metabolized, mainly to glucuronoconjugate, but also to sulfoconjugate and to another not identified metabolite.

In the study of Dumont and al. (Dumont C., 2011) the BPF is metabolized to sulfoconjugate in cells of human hepatoma HepG2, as well as glucuronoconjugate in isolated human hepatocytes of 3 different individuals. It is shown that the metabolism of phase II of the BPF differs between the individuals as well as between 2 cellular studied models (more important sulfatation in the line of human hepatoma HepG2 than in the isolated human hepatocytes). After 24 hours of incubation, the metabolism is total in the human hepatocytes isolated for concentrations of BPF at 5, 10 and 25 µM, but is partial in HepG2 cells for concentrations of BPF from 5 to 100 µM.

In summary, if the BPF is capable of undergoing a hydroxylation via cytochromes P450 to form mainly mono-and di-hydroxyled by-products and dimers *in vitro*, it is mainly glucuro-and sulfo-conjugated *in vivo*.

Elimination:

In the study of Cabaton and al. (Cabaton N, 2006) realized in Sprague-Dawley females, 96 hours after administration by gavage of BPF radiolabelled at the doses of 7 mg/kg/d or 100 mg/kg/d, approximately 44 % of the radioactivity is found in urines of the not gravid female rats, against 14 % to 18 % in feces. In the same article, an analysis of the biliary elimination was realized during the 6 hours after gavage administration of 1,5 mg/kg of 3H-BPF. The accumulated values indicate a biliary excretion close to 50 % of the administered dose. This percentage, compared with the values found in faeces, suggests the existence of an enterohepatic cycle for the BPF.

Genotoxicity

In a report of the European Commission (EC, 2003), it is specified that "the BPF is not mutagen in procaryotes and is not clastogen on mammalian cells *in vitro*. Ambiguous results are obtained with the *in vitro* mammalian mutation test at high concentrations (cytotoxic doses). The *in vitro/in vivo* UDS test performed on rat liver shows negative results. In conclusion, the BPF is not genotoxic".

An Ames test shows no mutagen potential of the BPF (Cabaton N, 2009). A test of umu (Chen MY, 2002), evaluating its acute toxicity against Daphnia magna, mutagenicity, and estrogenic activity using the Daphtoxkit was also negative. They found no mutagen effect with or without metabolic activation. The study of Tsutsui, (Tsutsui T, 2014), did not find chromosome aberration nor mutagen effect (Na1 / K1 ATPase or hprt loci) of the BPF on SHE cells (Syrian hamster embryo). Another study of Cabaton et al; (Cabaton N, 2009) did not find genotoxic

effect of the BPF *in vitro* test on micronucleus. However, in the same study, the test of comets was positive on HepG2 cells (cells of hepatoblastoma).

Another study also finds an effect of BPF on DNA damage (test γH2AX, not-validated by the OECD) (Audebert, Dolo and al. 2011). This genotoxic effect is found with concentrations of 10, 50 and 100 µM on HepG2 cells, and with concentrations of 50 and 100 µM on LS174T cells. However, the BPF is cytotoxic on HepG2 cells at the concentration of 100 µM, and on ACHN cells and LS174T at the concentrations of 50 and 100 µM. In this study, the BPF is not metabolized in ACHN cells, but it is metabolized in HepG2 cells and LS174T. This suggests a genotoxic effect of the metabolites of BPF by DNA damage, therefore dependent on metabolic capacities of cells.

No genotoxic assay in vivo was identified.

Effects on the toxicity of the reproduction and/or the effects of endocrine disruption

Animal data

A Hershberger bioassay was realized on castrated rats Brl Han: WIST Jcl (GALAS) of 56 days old (Yamasaki K, 2003) by daily oral administration via a stomach tube of BPF diluted in olive oil. Rats were treated at various concentrations: 0, 50, 200 or 1000 mg/kg/d for 10 days. The administration of BPF was associated or not with a subcutaneous injection of 0.2 mg/ kg/d of propionate of testosterone.

Observations are the following:

-A decreased weight gain (7,3 %) and spontaneous locomotion in the group BPF 1000 mg/kg/d.

-No modification of the relative organ weight (ventral prostate with fluid, seminal vesicle with fluid, bulbocavernosus/levator ani muscle (BC/LA), glans penis, and Cowper's gland) in the groups BPF with or without co-administration of propionate of testosterone (except an isolated increase of the weight of the glands of Cowper in the group BPF 200 mg/kg/d + propionate of testosterone). The absolute weights from these organs are not specified. This study shows that BPF does not seem to possess any androgenic or anti-androgenic properties at doses from 50 to 1000 mg/kg/d.

An uterotrophic and vaginal cornification assays was realized in 2 types of Wistar female rats by gavage with BPF (diluted in PEG) and/or with 17 ß-estradiol (17 ß-E2) (diluted in corn oil) for 4 days (Stroheker T, 2014):

- Old immature female rats of 22 days, were treated by 17 ß-E2 (from 15 to 200 µg/kg/d)., or BPF (0, 25, 50, 100 or 200 mg/kg/d), or by 45 µg/kg/d of 17 ß-E2 + 100 mg/kg/d of BPF.

- Ovariectomized rats (6 weeks old) were treated by 17 ß-E2 (from 75 to 400 µg/kg/d), or by BPF at 100 mg/kg/d, or by 17 ß-E2 100 µg/kg/d + BPF at 100 mg/kg/d.

In the immature female rats, a dose-dependent increase of the relative wet uterine weight in the groups of BPF 100 and 200 mg/kg/j and an increase of the relative dry uterine weight in the group of BPF 200 mg/kg/d were observed.There was no effect of BPF 100 mg/kg/d co-administration on17 ß-E2 increased relative uterus weight.

An increase of the vaginal cornification in the group BPF 100 mg/kg/d (test not made in the other groups) and an increase of the effect of 17 ß-E2 on the rate of vaginal cornification in case of co-administration with the BPF 100 mg/kg/d was also observed in immature female rats.

In the ovariectomized rats, no significant increase of the vaginal cornification was observed in the group BPF 100 mg/kg/d (or as a co-administration with 17 ß-E2). In the end, the BPF seems to possess uterotrophic properties at 100 mg/kg/d, but only in the immature female rats.

A report from the OECD (2007) presents the results of an uterotrophic test realized in 2002 (unknown exact reference). In this study, BPF shows uterotrophic effect at 100, 300 and 1000 mg/kg/d, with a dose-dependent increase of the relative and absolute wet and dry uterine weight (with or without uterine secretions).

A report of the European Commission (EC, 2003), resumes the study of Perez and al (Perez P, 1998) which shows that the BPF possesses an *in vitro* estrogenic low activity on human mammary cancer cells.

There is no data available in humans.

Repeated dose toxicity: subacute or subchronic

Animal data

A study was realized in SD rats Crj:CD (8 weeks old) by gavage (0, 20 of BPF diluted in olive oil, 100 or 500 mg/kg/d) daily during 28 days (Higashihara N, 2007).

After 28d of treatment with 500 mg/kg/d, decreased body weight was observed in both sexes (-13 % on average), increase of certain biochemical parameters (rate of γGT and of alkaline phosphatase, total bilirubinemia) together with anaemia in the females at this dose. A decrease of the rate of thyroid hormone T3 (17 % on average) and an increase of the rates of T4 (20 % on average) was observed at this dose level without modification of the rate of TSH. An alteration of the conversion of hormones T4 in T3 is therefore suspected.

Certain biochemical parameters (cholesterol level, glycemia, albuminemia, uremia, rate of cholinesterase and glutamic-oxaloacetic transaminase) were decreased in a dose and sex-dependant manner. The relative weight of certain organs (liver, testicles (+16%), brain, loins and thyroid) increased in a dose and the sex-dependant manner.

No abnomaly of the sperm (morphology, numeration, resistance in a solution of NaCl 0.9 % + Triton-X100) and the oestrous cycle was observed. A LOAEL of 500 mg/kg/d for the reprotoxic effects (increase of the relative testicles weight) or of endocrine disruption (modification of the rates of hormones T3 and T4) can be estimated.

There is no human data identified to this day.

Carcinogenicity:

No data identified.

Other effects:

Cutaneous sensitization

A study carried out on guinea pig (Bruze M, 1986); (only summary is available) did not show a capacity of sensitization of the BPF.

Mechanism of action - Interactions with receptors:

Eight studies estimating the *in vitro* oestrogenic potential of BPF were found. All find an oestrogenic effect of BPF because of its affinity to the estrogens receptors (ER), leading to a proliferation on the mammary cancer cells MCF-7. Among these studies, 5 studies (Chen MY, 2002; Hashimoto Y, 2000; Hashimoto Y, 2001; (Kitamura S, 2005) ; Stroheker T, 2014) find an oestrogenic activity more or less similar to the BPA's one. Another study (Okada H, 2008) finds an affinity of the BPF for the receptor ERRγ (Estrogen-Related Receptor gamma).

In another study (Hashimoto Y, 2001), the oestrogenic activity (correlated to the activity of her ß-galactosidase associated with the estrogens receptors) is more important after metabolic activation, which suggests a role of BPFmetabolites in its oestrogenic potential. Four studies (Cabaton N, 2009); (Kitamura S, 2005); (Satoh K, 2004; Stroheker T, 2014) estimated the androgenic potential of the BPF. All these studies find an anti-androgenic effect of BPF. A study (Kitamura S, 2005) did not find activity of the BPF on the thyroid function.

Summary of the toxicologic profile:

In the rat, the data of distribution and elimination after administration of a unique dose of 7 mg/kg/d or 100 mg/kg/d suggest that almost all of the BPF is absorbed by oral route. The BPF crosses the foeto-placentar barrier (1 % of the initial dose is found in the foetus). The BPF is capable of undergoing a hydroxylation via cytochromes P450 *in vitro* to form mainly mono-and di-hydroxyled and dimers by-products. However, a study in the rat observes mainly glucuro- and sulfo-conjugations. Ninety six hours after oral administration in rat, approximately 44 % of the initial dose is found in urines, against 14 in 18 % in faeces.

In vitro, the BPF is an agonist of the estrogens receptors. It leads to the proliferation of human mammary cancer cells and competes with 17 ß-œstradiol on its receptors. Its estrogenic activity is close to the BPA's one. The BPF is also a ligand of the receptors ERRγ, with an affinity approximately 13 times lower than the BPA's one. According to a study (Hashimoto Y, 2001) the metabolites of the BPF also seems to possess an estrogenic activity. An anti-androgenic activity was also found.

In vivo, an uterotrophic effect of the BPF is observed on the immature female rats for doses superior to 100 mg/kg/d. An essay of Hershberger did not highlight androgenic or anti-androgenic property in doses until 1000 mg/kg/d.

A sub acute study does not show an alteration of the reproductive functions for doses of 20 and 100 mg/kg/d. An increase of the relative weight of testicles is observed in 500 mg/kg/d. At the level of the endocrine disruption, a change of the conversion of hormones T4 in T3 (increase of the rate of T4, decrease of the rate of T3, no modification of the rate of TSH) is observed with doses of BPF of 500 mg/kg/d.

Two recent in vitro studies of genotoxicity on the BPF turn out positive with metabolic activation: test of comets and test of detection of the breaks of double stalks of DNA (test

γH2AX). The tests of mutagenicity (test of Ames) are negative. This suggests a direct genotoxic effect of the BPF by DNA break.

The table below presents a summary of the observed NOAELs of BPF.

Table 66. Summary table of the NOAELs - toxicity on the reproduction Experimental data on rodents

	NOAEL or LOAEL/ exposure route / species	**Observed effects, type of study**
NOAEL dvpt *in-utero*	ND-Not determined	ND
NOAEL post-natal early	ND	ND
NOAEL perinatal	ND	ND
NOAEL post-natal late	ND	ND
NOAEL pre-juvenile	ND	ND
NOAEL reprotox (adult)	100 mg/kg/d during 28 days po/rat	Increase of relative weight of testicles (Higashihara, Shiraishi *et al.* 2007)
NOAEL markers of endocrine disruption	50 mg/kg/d during 4 days po/rat	Increase of the relative weight of the wet and mopped uterus (Yamasaki, Takeyoshi *et al.* 2003a)
	100 mg/kg/d during 28 days po/rat	Decrease of the T3 rate, increase of the T4 rate (Higashihara, Shiraishi *et al.* 2007)

Conclusion:

With little *in vivo* studies and in the absence of human data, it is difficult to state on the reprotoxicity for the organs of the reproduction of the BPF. The studies i*n vitro* studies together with *in vivo* reprotoxicity studies tend to demonstrate that the BPF possesses an activity of endocrine disruption via the estrogens receptors.

Two recent studies of genotoxicity of the *in vitro* BPF turn out positive without metabolic activation (performed on cell lines able to metabolise BPF). This suggests a direct genotoxic effect of the BPF by break of the DNA. However, no test of *in vivo* genotoxicity was realized.

In vivo studies of toxicity on the reproduction are essential to complete this toxicological profile and estimate its hazard for Human. These studies will have to include a large dose-range to take into account possible non-monotonic dose-effect relations, with observable

effects in small doses. Data on exposure would also help evaluating how thermal paper might contribute to it.

C.2.2.3 Environment risks related to BPF

There is no data available on BPF hazards for the environment and ecotoxicity.

C.2.2.4 Technical and economic feasibility of BPF

It is unknown whether BPF is actually used in thermal paper but there is no indication that it is not. Given that BPF has similar properties to BPA, it can be considered as (at least) theoretically usable as a dye developer in thermal paper and thus technically feasible.

As regards its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.3 Assessment of BPAP

There is no indication that BPAP is actually used in thermal paper.

Table 67. Identity of BPAP

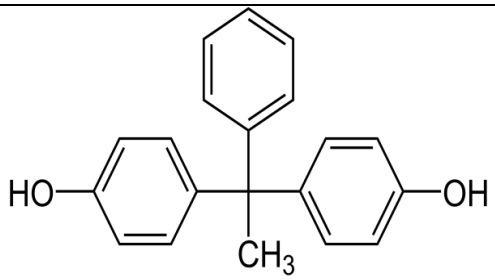
Public name	Bisphenol AP Synonyms: 1,1-bis(4-hydroxyphenyl)-1-phenylethane 4,4'-(1-Phenylethylidene)bisphenol 1,1-bis(4-hydroxyphenyl)-1-phenylethane 4,4'-(1-α-Methyl-benzylidene)bisphenol
EC name	NA
IUPAC name	NA
EC number	433-130-5
CAS number	1571-75-1
Annex VI Index number	NA
Molecular formula	$C_{20}H_{18}O_2$
Chemical structure	

Table 68. Physico-chemical properties of BPAP

Property	**Value**	**Comment (e.g. measured or estimated)**
Physical form (at ambiant temperature)	Not documented	
Molecular weight (g/mol)	290,36	Base de données GESTIS http://gestis-en.itrust.de/nxt/gateway.dll?f=templates&fn=default.htm&vid=gestiseng:sdbeng
Boiling point (°C)	Not documented	
Melting point (°C)	188-191	Site internet ChemBlink http://www.chemblink.com/products/1571-75-1.htm
Flash point in an open cup (°C)	Not documented	
Flash point in a closed cup (°C)	Not documented	
Lower Explosive Limit (LEL)	Not documented	
Upper Explosive Limit (UEL)	Not documented	
Saturated vapour pressure (Pa)	Not documented	
Density	Not documented	
Conversion factor	Not documented	
Solubility in water (g/L)	Not documented	
Log Kow	Not documented	
Koc (L/kg)	Not documented	
Self-ignition temperature	Not	

	documented	

C.2.3.1. Availability of BPAP

The data on the tonnage of BPAP produced, used or placed on the EU market are not publicly available. BPAP has not been registered under REACH so far.

As a result, it is difficult to conclude about the availability of BPAP.

C.2.3.2 Human health risks related to BPAP

BPAP is not classified for human health concern.

Concerning the endocrine disruption, the BPAP is not classified by the report of the DHI (DHI, 2007). However, the BPAP is classified in the group 3 (absence or insufficiency of data to be listed) by the report of the European Commission (European Commission 2002). The BPAP is pre-registered in the REACH regulation but not registered yet.

There is no toxicokinetic (absorption, distribution, metabolism and elimination) data on the BPAP identified. There is no *in vitro* data, in animal, in human, by repeated or chronic doses, on toxicology of the reproduction or on carcinogenicity, on genotoxicity or on sensitization of BPAP. No ecotoxicological data or relative to the observed effects on the wildlife were identified at the time of the dossier.

Mechanism of action - Interaction with the receptors:

A recombinants yeast assay (study of the activity of the ß-galactosidase associated with the expression of the estrogens receptors) (Zhang, Chen and al. 2009) compares the oestrogenic activity of 4 bisphenols (BPA, BPF, BPAF and BPAP). The results are the following: estrogenic activity: BPAF (EC_{50}: 7,44.10-7 M) > BPA (EC_{50}: 6,81.10-6 M) > BPF (EC_{50}: 7,52.10-6 M) > BPAP (EC_{50}: 1,43.10-5 M).

Summary of the toxicological profile:

In vitro, the BPAP is an agonist of the estrogens receptors. The estrogenic potential is approximately twice weaker than the BPA's one.

Conclusion on the toxicological profile:

A mecanistic study seems to show that the BPAP possesses an oestrogenic activity. However, in front of the absence of human and animal data, it is not possible to conclude on the activity of endocrine disruption of the BPAP.

Additional mecanistic studies, toxicokinetic studies and *in vivo* studies on the reprotoxicity are essential to complete this toxicological profile and estimate its hazard for the human. Furthermore, general exposure data would be helpful.

C.2.3.3 Environment risks related to BPAP

The BPAP is classified N; R50-53 (Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment) according to the directive 67/548/CEE and Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 according to the regulation CLP n°1272/2008.

These data (or other) related to the ecotoxicological and environment risks of BPAP are not publically available.

C.2.3.4 Technical and economic feasibility of BPAP

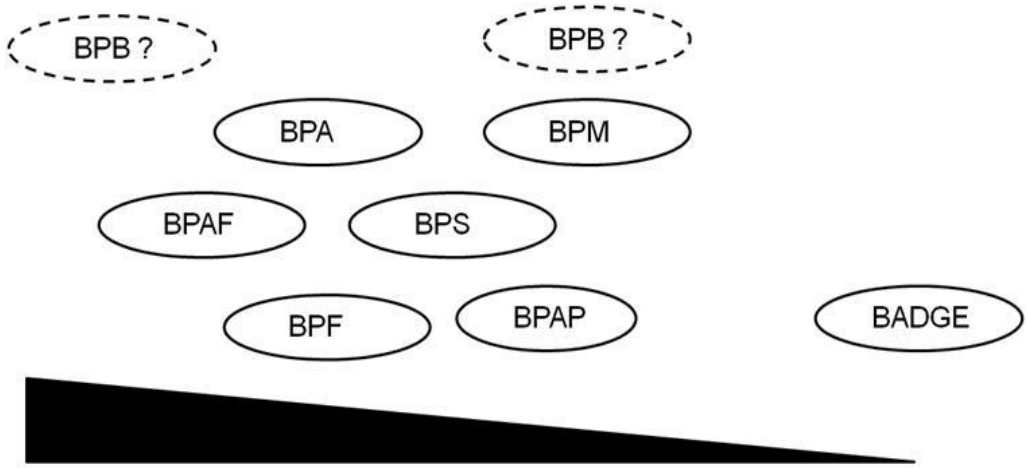
It is unknown whether BPAP is actually used in thermal paper but there is no indication that it is not. Given that BPAP has similar properties to BPA, it can be considered as (at least) theoretically usable as a dye developer in thermal paper and thus technically feasible.

As regards its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.3.5 Other information on BPAP

Analysis of the available data shows that the chemical structure common to compounds of the class of bisphenols gives them oestrogenic properties. Figure below proposes a ranking of the oestrogenic activity that takes into account available *in vitro* and *in vivo* data. It should be noted that this does not presuppose the toxicity of these substances (ANSES, 2012).

Figure 41. Illustration of the oestrogenic activity of various compounds of the class of bisphenols.

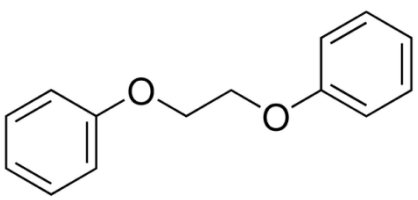


Of the seven compounds, according to ANSES, 2013 report on alternatives to BPA, BPS, BPF and BPAP are used as substitutes for BPA as developers in thermal paper. BPS is used as a polyethersulfone synthesiser that can also serve as a polycarbonate base, and is specifically used for the manufacture of baby bottles and children's tableware.

The other four compounds (BPB, BPM, BPAF and BADGE) were not identified as substitutes for BPA. However, the evidence gathered so far suggests that BPB, BPM and BPAF are used for the synthesis of polymers. For its part, BADGE is employed in the synthesis of certain epoxy resins that may be used in the internal coating of food containers (food and beverage cans).

C.2.4 Assessment of 1,2-diphenoxyethane

Table 69. Identity of 1,2-diphenoxyethane

Public name	KS-235
EC name	1,2-diphenoxyethane
IUPAC name	1,1'-[ethane-1,2-diylbis(oxy)]dibenzene
EC number	203-224-9
CAS number	104-66-5
Annex VI Index number	Not assigned
Molecular formula	$C_{14}H_{14}O_2$
Chemical structure	

There is not a harmonised classification for this substance. However, the self classification proposed is the following: Aquatic Chronic 2, H411.

C.2.4.1. Availability of 1,2-diphenoxyethane

According to the ANSES report (ANSES, 2013), 1,2-diphenoxyethane seems to be used in thermal paper. However, it is unknown to what extent since during the stakeholders consultation, no indication of its use was gathered.

Nevertheless, some data could be obtained from the REACH registration dossier of 1,2-diphenoxyethane. They indicate that 1,2-diphenoxyethane is produced at a level above 100 tons per year. As a result, 1,2-diphenoxyethane can be considered as a rather available alternative.

C.2.4.2 Human health risks related to 1,2-diphenoxyethane

The only available data are the non-confidential disseminated data from the registration dossier from the ECHA website. These data have not been evaluated by ANSES but are summarized below. There is no data on toxicity of 1,2-diphenoxyethane on pubmed on the 6 December 2013.

Acute toxicity:

A GLP and guideline (OECD 401) study on acute toxicity on rats by oral gavage shows that the LD50 is superior to 5000 mg/kg bw.

Studies on acute toxicity by dermal route and by inhalation are waived.

Skin irritation:

There is no data on skin irritation.

Eye irritation:

As reported on the ECHA website but not evaluated, the test item KS-235 (Batch No.: 91014), applied to rabbits' eye mucosa, caused significant conjunctival irritant effects which were reduced at 1 week after application. The effects were fully reversible within 2 weeks.

Skin sensitization:

As reported on the ECHA website but not evaluated, a LLNA (OECD 429) on females' mice shows that the substance is not a skin sensitizer.

Repeated toxicity:

As reported on the ECHA website but not evaluated, no toxic change was found in the 28-day repeated oral dose study of 1, 2-diphenoxyethane in rats. However, considering mild effects on the liver in animals of the 1,000 mg/kg group, the no-observed-adverse-effect level (NOEL) was concluded to be 100 mg/kg. As reported on the ECHA website but not evaluated, a combined repeated dose and reproduction / developmental screening study is available and shows only transient salivation and body weight reduction at 1000 mg/kg bw/d which is drives the NOAEL for systemic toxicity.

Genotoxicity:

As reported on the ECHA website but not evaluated, the substance is not mutagenic in all strains tested (S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2) in a bacterial reverse mutation assay (Ames test), in the absence or presence of metabolic activation.

An *in vitro* mammalian chromosome aberration test on Chinese hamster lung fibroblasts (V79) showed the induction of chromosome aberrations with metabolic activation. Therefore, the clastogenicity of 1,2-diphenoxyethane to mammalian cells was judged to be positive in the conditions of this study.

As reported on the ECHA website but not evaluated, the substance is reported to be non mutagenic in the Micronucleus test *in vivo* (OECD Guideline 474 Mammalian Erythrocyte Micronucleus Test).

Toxicity to reproduction:

As reported on the ECHA website but not evaluated, the effects of KS-235 on reproduction and/or development when administered through oral gavage to male and female Wistar rats prior to mating, during mating and gestation periods, and until post-partum day 3 was evaluated according to OECD TG421. Transient salivation and body weight reduction was seen at 1000 mg/kg bw/day. There was no adverse effect on reproduction and development up to the dose level of 1000 mg/kg bw/day.

Develomental toxicity / teratogenicity:

No additional study.

C.2.4.3 Environment risks related to 1,2-diphenoxyethane

Short term toxicity to fish:

As reported on the ECHA website but not evaluated, the acute toxicity to KS-235 to the freshwater fish rainbow trout (Oncorhynchus mykiss) has been investigated and gave a 96-hour LC_{50} of greater than 0.40 mg/I. Correspondingly the No Observed Effect Concentration was greater than or equal to 0.40 mg/I.

Long term toxicity to fish: waived

Short term toxicity to aquatic invertebrates:

As reported on the ECHA website but not evaluated, the acute toxicity of KS-235 to the freshwater invertebrate Daphnia magna has been investigated and gave a 48-Hour EC_{50} of greater than 0.40 mg/I. Correspondingly the No Observed Effect Concentration was greater than or equal to 0.40 mg/I.

Long-term toxicity to aquatic invertebrates:

The NOEC survival value is 0.54 mg/L WAF, the NOEC reproduction value is less than 0.54 mg/L WAF KS-235. Statistically-significant sub-lethal (reproductive) effects on D. magna were observed.

Toxicity to aquatic algae and cyanobacteria:

The effects of KS-235 on the growth of the green alga Selenastrum capricornutum (OECD Guideline No. 201) of the key study are:

ErC_{10} = 0.68 $mg.l^{-1}$

$ErC5_0$ = 1.9 (1.5 - 2.4) $mg.l^{-1}$

ErC_{90} = 5.1 $mg.l^{-1}$

EbC_{10} = 0.32 $mg.l^{-1}$ (95% confidence limits = 0.315 - 0.561 $mg.l^{-1}$*)

EbC_{50} = 0.82 $mg.l^{-1}$ (95% confidence limits = 0.561 - 1.00 $mg.l^{-1}$*)

EbC_{90} >1.89 $mg.l^{-1}$

* range between tested concentrations

In a supporting study, the effect of KS-235 on the growth of Scenedesmus subspicatus has been investigated and gave EC_{50} values (and corresponding NOAEL) greater than 0.40 mg/I.

Toxicity to microorganisms:

KS-235 exhibited no respiration inhibition and no dose response was observed in an Activated Sludge Respiration Inhibition Test (OECD Guideline 209). The definitive test was not required and was therefore not performed. KS-235 also demonstrated no abiotic response.

Terrestrial toxicity is not evaluated; it is waived because of exposure considerations.

C.2.4.4 Technical and economic feasibility of 1,2-diphenoxyethane

Given that 1,2-diphenoxyethane seems to be used in thermal paper, it could be deemed as technically feasible. However, information collected from the public consultation indicates that 1,2-diphenoxyethane should be excluded as a dye developer due to inappropriate chemical properties. Indeed, it is claimed that in case of phenolic substances (such as 1,2-diphenoxyethane), a number of prerequisites have to be met with regard to physical-chemical properties in order to consider an alternative as feasible: at least one phenolic hydroxyl group; pKa range and steric hindrance of hydroxyl group comparable to BPA and a melting point comparable to that of BPA. Under these prerequisites, 1,2-diphenoxyethane cannot be considered as technically feasible since the absence of acid functionalities in the molecule excludes it from being properly used as a dye developer for leuco dyes.

As regards to its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.5 Assessment of Pergafast 201

Table 70. Identity of Pergafast 201

Public name	Pergafast 201
EC name	-

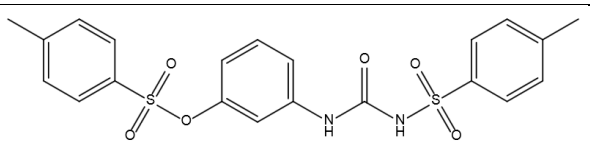
IUPAC name	N-(p-toluenesulfonyl)-N'-(3-(p-toluenesulfonyloxy)phenyl)urea 3-([(4-methylphenyl)sulfonyl]carbamoylamino)phenyl 4-methylbenzenesulfonate
EC number	432-520-2
CAS number	232938-43-1
Annex VI Index number	006-099-00-7
Molecular formula	C21 H20 N2 O6 S2
Chemical structure	

Table 71. Physico-chemical properties of Pergafast 201

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Appearance/physical state/colour	The test substance is a solid white powder at 20°C and 1013 hPa	Study report 1999 (disseminated data on ECHA website)	Measured
Melting point	157.7°C	Study report 1999 (disseminated data on ECHA website)	OECD Guideline 102 (Melting point / Melting Range)
Boiling point	>= 250 °C	Study report 1999 (disseminated data on ECHA website)	OECD Guideline 103 (Boiling point/boiling range)
Density	1.412 g/cm³ at 20.9 °C	Study report 1999 (disseminated data on ECHA website)	OECD Guideline 109 (Density of Liquids and Solids)
Particule size distribution (granulometry)	< 100 µm: 13.5%, < 10.2 µm: 11.4%, < 5.4 µm: 2.07%	Study report 2004 (disseminated data on ECHA website)	"Particle Size Distribution, Fibre Length and Diameter Distribution", June 1996, European Commission technical guidance document

Vapour pressure	1.35 x 10-12 Pa at 25°C	Study report 1999 (disseminated data on ECHA website)	OECD Guideline 104 (Vapour Pressure Curve)
Partition coefficient	Log Pow = 2.6 at 20°C	Study report 2010 (disseminated data on ECHA website)	OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method), HPLC method
Water solubility	slightly soluble (0.1-100 mg/L) 34.7 mg/L at 20°C	Study report 1999 (disseminated data on ECHA website)	OECD Guideline 105 (Water Solubility)
Surface tension	71.21 mN/m at 20°C (90% of saturation concentration in water)	Study report 1999 (disseminated data on ECHA website)	OECD Guideline 115 (Surface Tension of Aqueous Solutions)
Flash point	study scientifically unjustified	BASF	
Auto-flammability	not auto-flammable up to its melting point (at about 158°C). The self-ignition temperature is > 158°C.	Study report 1999 (disseminated data on ECHA website)	EU Method A.16 (Relative Self-Ignition Temperature for Solids)
Flammability	not flammable upon ignition	Study report 1999 (disseminated data on ECHA website)	EU Method A.10 (Flammability (Solids))
Explosiveness	non explosive	Study report 1999 (disseminated data on ECHA website)	EU Method A.14 (Explosive properties)
Oxidizing properties	no oxidising properties	(disseminated data on ECHA website)	No GLP compliance
Dissociation constant	The pKa of test substance is not detectable: the test substance does not have any dissociation constant.	Study report 2010 (disseminated data on ECHA website)	OECD Guideline 112 (Dissociation Constants in Water)
Dissociation	pKa1 = 12.5	SPARC	-

constant	pKa2 = 5.3 pKa3 = -3.8 pKa4 = -13.6 (Estimated)		
Viscosity	study technically not feasible	-	-

Pergafast or *Pergafast ® 201* (brand name) is a non-phenolic color developer in thermal paper manufacture.

Pergafast has a harmonised classification: Aquatic Chronic 2, H411. According to the Substitution Support Portal[39] Pergafast 201 "carries none of the classifications associated with health hazards to humans; it could however be dangerous if released into the aquatic environment. Due to how receipts are handled, most of them will probably not reach the aquatic environment and this is therefore considered an acceptable risk".

C.2.5.1. Availability of Pergafast 201

According to suppliers in Sweden, Pergafast 201 is the most used alternative developer on the Swedish market. Most retailers have chosen not to switch to BPS-based paper, due to the suspicion of hormone-disrupting properties (NICNAS Report Pergafast).

Pergafast 201 as color developer produces images that are more stable compared to the corresponding BPA-containing thermal papers. In particular, the images are more stable towards the effects of oils, fats and plasticizers. This is advantageous when printed thermal papers have to be archived, or when they are used under harsh environmental conditions.

Pergafast is only produced by one producer in Europe, based on a patent protection, marketed under the brand name Pergafast®201. This implies that this monopoly holds the whole market and this means that there is no possibility for flexibility regarding delivery from multiple suppliers. Pergafast is manufactured and sold as a color-developer for thermal paper applications since 2011 INERIS, 2013. This product can be used as an alternative to BPA and the manufacturer pointed out that it is a candidate in the ongoing US EPA dfE program "BPA Alternatives in Thermal Paper Partnership".

The manufacturers of thermal paper consulted INERIS, 2013 confirmed that Pergafast is already used in thermal paper. The large retailer Carrefour in France phased out BPA and uses now thermal tickets containing Pergafast (ETPA 2013 consultation). However, to what exact extent Pergafast is generally used is unknown.

Disseminated data on the registration dossier of pergafast can be found on the website of ECHA. However, the tonnage is confidential.

[39] www.subsport.eu/

As a whole, given that Pergafast is already used in thermal paper in several countries in the EU and produced by a big company, it can be deemed as available.

C.2.5.2 Human health risks related to Pergafast 201

A PubMed search for Pergafast 201 yielded no results on 6 December 2013.

Pergafast 201 has also been evaluated by the US EPA Thermal Papers project. The draft assessment draws its conclusion from confidential studies submitted to the US EPA and "professional judgement" of likely toxicity by comparison with similar molecules. For some environmental health end-points, there is no data at all. Based on information provided by BASF, most of the toxicological endpoints for Pergafast®201 are covered by experimental data on the product itself and determined in OECD guideline studies. Expert judgment was only used in part for toxicokinetics. Conclusions or suggestions on carcinogenicity, immunology and endocrine activity on the other hand were drawn by external toxicologists in absence of experimental data or based on secondary sources.

For human health hazards, 3 DNELs were derived according to the lead registrant:

- for **workers** via **dermal route** and for **long term** exposure: DNEL = 1.25 mg/kg bw/d
- for **general population** via **dermal route** and for **long term** exposure: DNEL = 0.625 mg/kg bw/d
- for **general population** via **oral route** and for **long term** exposure: DNEL = 0.625 mg/kg bw/d

*Toxicokinetic***:** According to the US EPA, 2012, Pergafast 201 is not estimated to be absorbed through the skin as dry material nor if it is in solution. It is not expected to be absorbed by inhalation because of the particle size distribution of the test substance. Absorption is more likely expected by gastro intestinal tract after oral exposure. Moreover, BASF indicates that a study was conducted according to OECD Guideline for Testing of Chemicals No. 428 ("Skin Absorption: in vitro Method") and OECD Guidance Document No. 28 for the conduct of skin absorption studies. Results of these studies indicate that absorption via the skin is negligible.

Acute toxicity: According to US EPA and the disseminated data (website of ECHA), Pergafast is estimated to be of low toxicity by oral route (LD50 > 2000 mg/kg bw), and by dermal route (no mortality was observed in both sexes after dermal application of 2000 mg/kg bw for 24h under semi occlusive conditions, OECD test guideline 402). No acute toxicity by inhalation is provided because the substance is not expected to be absorbed by this way.

Skin irritation/corrosion: after dermal application of 0.5 mg test substance according to OECD guideline 404 for 4 hours, skin was flushed with water and the skin was scored for erythema and edema after 24, 48 and 72 h. Since neither erythema nor edema were seen, the test substance is considered to be "not irritating" to rabbit skin according to US EPA, 2012.

Eye irritation: the test substance is not irritating on rabbits according to a study from BASF (2011) and slightly irritating on rabbits according to NICNAS (2004). So the substance is of low toxicity for eye irritation according to US EPA, 2012. However, it has to be noted that only one eye irritation test in rabbits is available.

Skin sensitization: the substance is considered of low toxicity for skin sensitization according to US EPA, 2012 in guinea pigs (BASF 2010). Skin irritation was observed (NICNAS, 2004) in

1/10 guinea pigs at 24 hours (but not at 48 hours) following induction and subsequent challenge. The severity of the response was not described in the available source.

Repeated dose toxicity via oral route is considered moderate by US EPA, 2012. A sub chronic study (study report, 2002) by oral gavage (OECD Guideline 408: Repeated Dose 90-Day Oral Toxicity in Rodents) established a NOEL of 25 mg/kg body weight/day and a NOAEL of 50 mg/kg body weight/day (the substance caused centrolobular hypertrophy in the liver). A 28-day oral toxicity study in rats identified a NOAEL of 30 mg/kg bw/d and a LOAEL of 150 mg/kg/d for clinical signs, organ weight changes and histopathologic liver and kidney lesions. Indeed, the lead registrant reports liver toxicity at high doses, in a document submitted to the US EPA. Depending on the severity of the findings, it should be noted that the available data could lead to a classification as STOT-RE2.

Reproductive toxicity: According to the US EPA evaluation, no effect was seen on fertility at the highest dose tested at 200 mg/kg/d. Thus the NOAEL is considered to be superior to 200 mg/kg/d. Consequently, the hazard of pergafast on the reproductive organs is estimated as moderate by US EPA.

Developmental effects: Decreased body weight (LDs 1 and 4) were observed in pups from dams exposed to 200 mg/kg bw-day. It should be noted that developmental effects occurred in the presence of maternal toxicity, although toxicity to dams (liver and kidney toxicity) does not appear to be the cause of developmental effects. Developmental toxicity effects are evaluated as high by US EPA. According to BASF, animal studies gave no indication of a developmental toxic effect at doses that were not toxic to the parental animals. The results were determined in a reproduction and developmental screening test (OECD 421) (information provided during the public consultation).

Genotoxicity: The test substance was not genotoxic when tested in the Ames test with different strains of Salmonella Typhimurium and the Escherichia coli strain WP2 uvrA, according to the OECD 471. An *in vitro* Mammalian Cell Gene Mutation Test (OECD Guideline 476) shows that the test substance is not mutagenic in the HPRT locus assay under *in vitro* conditions in CHO cells in the absence and the presence of metabolic activation. An *in vitro* mammalian chromosome aberration test (OECD Guideline 473 *In vitro* Mammalian Chromosome Aberration Test) shows induced structural chromosome aberrations in V79 cells (Chinese hamster cell line). However, strong increases were observed only in the presence of strong toxicity at cytotoxic concentrations. Nevertheless, US EPA estimated the genotoxicity of the substance as low. According to BASF, a study was also conducted according to OECD Guideline for Testing of Chemicals No. 474, Mammalian Erythrocyte Micronucleus Test. The frequency of detected micronuclei was not enhanced at any dose level and it is concluded that the test substance did not induce micronuclei in vivo (mouse) (information provided during the public consultation).

Carcinogenicity: There is no carcinogenicity study on pergafast and therefore an uncertainty exists due to the lack of data on carcinogenicity for this substance. BASF indicates during the public consultation that since from experimental data of several in vitro and in vivo assays Pergafast 201 is not genotoxic, it has therefore a low potential for genotoxic carcinogenicity.

Neurotoxicity: Pergafast is not expected to be neurotoxic because no structural alerts or mechanistic pathways associated with neurotoxic effect were identified (US EPA, 2012).

Mechanistic Estrogen Receptor (ER) alpha agonist study

In terms of endocrine activity, one confidential study "similar to OECD 455" (which tests the affinity of a compound to the principle nuclear oestrogen receptor) shows Pergafast 201 to have very low potency in comparison to 17-beta-estradiol. There is no study evaluating the affinity of Pergafast 201 for other hormone receptors.

An increase in luciferase activity was measured as marker for ER-alpha induction (OECD 455) but very low compared to 17 beta-estradiol. Relative potency of this sample was calculated to be approximately 10^7-fold less potent than 17 beta-estradiol. Thus it is considered negative for estrogenic activity.

BASF indicated during the public consultation that a H295R Steroidogenesis Assay according to OECD 456 is in progress.

Immunotoxicity: There is uncertain concern for immunotoxicity based on effects to the spleen and adrenal glands. According to BASF, microscopic changes of the adrenal glands are suggested to be an unspecific high dose effect after subacute and subchronic treatment rather than a symptom for immunotoxicity (information provided during the public consultation).

C.2.5.3 Environment risks related to Pergafast 201

Pergafast 201 has been evaluated under the Australian National Industrial Chemicals Notification And Assessment Scheme (NICNAS 2004). There are indicators that it may be toxic to aquatic organisms and persistent in the environment, though not bioaccumulative. It has also been evaluated by US EPA and presented here below and the analysys is consistent with the disseminated data of the registration dossier detailed on the ECHA website.

Ecotoxicity: US EPA estimates the acute toxicity as high based on a measured 72h EC50 of 0.77 mg/L for biomass in Scenedesmus subspicatus. Chronic aquatic toxicity is estimated as high based on an estimated ChV of 0.013 mg/L for green algae.

The transport evaluation for Pergafast 201 is based on available experimental and estimated physical and chemical properties. Based on the Level III fugacity models incorporating the available experimental property data, Pergafast 201 is expected to partition primarily to soil. Pergafast 201 is expected to have slight mobility in soil based on its estimated Koc. However, leaching of Pergafast 201 through soil to groundwater is not expected to be an important transport mechanism. Estimated volatilization half-lives indicate that it will be non-volatile from surface water. In the atmosphere, Pergafast 201 is expected to exist in the particulate phase, based on its estimated vapor pressure. Particulates will be removed from air by wet or dry deposition.

Persistence: US EPA estimates the persistency of the substance as very high. Experimental guideline studies indicate that little or no biodegradation was observed under aerobic conditions.

Bioaccumulation is considered as low because the measured BCF in fish is inferior to 100.

According to KEMI, there is a lack of data on pergafast to compare its performance with BPA.

C.2.5.4 Technical and economic feasibility of Pergafast 201

The manufacturer of Pergafast indicated that the process of coating is similar to the one using BPA. He added that the percentage in weight of Pergafast® 201 used in thermal paper is similar to that of BPA, between 1.1% and 1.3% in weight. Pergafast is already used by some large retailers and as already said above, in several countries in the EU. The use of Pergafast as a dye developer in thermal paper is thus technically feasible.

Pergafast is produced by only one company for the time being, which means there is no competition regarding price. The only producer of Pergafast, did not disclose information about the price of Pergafast but indicated that as a specialty chemical, its price is higher than BPA (Danish E.P.A., 2013). ETPA indicated that the Pergafast is around 10 times more expensive than BPA (that is around 15,000€/ton), but that the global impact on the paper's cost would be lower than that. The other quantitative data got from the consultation carried out INERIS, 2013 is that Pergafast 201 would price up to 30,000€/ton (one claim). **It has to be underlined that this high price might be overestimated since it is based only on one single declaration gathered during the consultation. This price couldn't be checked during the elaboration of this proposal.** To this respect, other information collected from the public consultation indicates that thermal paper with Pergafast would be around 10-25% more expensive than thermal paper with BPA (Danish EPA 2014, quoted in FBR-WUR report[40]). This information thus qualifies the information initially gathered and Pergafast might have a less high price than expected. This additional information confirms the assumption that the price of Pergafast might be overestimated.

Additional information collected late in the process from several stakeholders indicates that Pergafast-containing thermal paper is about 15%-20% more expensive than BPA-containing thermal paper (see Annex 9).This information is pretty close to the cost increase indicated in the public consultation (10-25%) (see section F.2) and is used further below to refine the substitution cost calculation (section F.2).

According to the sole manufacturer, Pergafast®201-containing thermal paper offers higher image stability compared to bisphenol A containing thermal paper. Furthermore, this alternative is labeled "non phenolic". The manufacturer expects a growth of demand from the market concerning this product. The price of Pergafast could thus decrease in the near future.

Therefore, although the use of Pergafast is technically feasible, its price might stand for a limit for its wide use, at least at short-term. It is considered as economically less feasible compared to other substitutes

C.2.6 Assessment of D8

Table 72. Identity of D8

[40] *Analysis of alternatives for bpa in thermal paper*, report 1515, Dec 2014

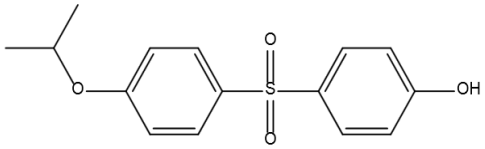
Public name	4-hydroxyphenyl 4-isoprooxyphenylsulfone
EC name	4-(4-isopropoxyphenylsulfonyl)phenol
IUPAC name	4-(4-isopropoxyphenylsulfonyl)phenol
EC number	405-520-5
CAS number	95235-30-6
Annex VI Index number	604-046-00-8
Molecular formula	C15H16O4S
Chemical structure	

Table 73. Physico-chemical properties of D8

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Melting Point (°C)	129	Submitted confidential study	Measured with adequate data quality
Boiling Point (°C)	>300	EPI; U.S. EPA, 1999	Estimated. Decomposition may occur before the boiling point is reached based on the experimental decomposition temperature of 315 degrees C for an analogous structure, bisphenol S. Cut-off value for high boiling point compounds according to HPV assessment guidance.
Vapor Pressure (mm Hg)	$<1x10^{-8}$	EPI; U.S. EPA, 2011	Estimated. Cut-off value for non volatile compounds according to SF assessment guidance.
Water Solubility (mg/L)	21	Submitted confidential study	Measured according to adequate data

Log Kow	3.1	EPI	Estimated
Flammability (Flash Point)			No data located.
Explosivity			No data located.
pH			No data located.
pKa	8.2	SPARC	Estimated

C.2.6.1. Availability D8

ETPA confirmed that some special applications of thermal papers required the use of alternatives such as D8 which is currently used in applications requiring highly sensitive paper (e.g. mobile printers needing less energy when the paper is more sensitive, queuing ticket printers...) (Danish E.P.A., 2013).

The data on the tonnage of D8 produced, used or placed on the EU market are indicated as 'confidential' in the REACH registration dossier of D8 and data on tonnage are not publicly available from other sources.

As a result, D8 may be available but it is difficult to conclude clearly about its availability.

C.2.6.2 Human health risks related to D8

For human health hazards, several DNELs or DMELs were derived according to the lead registrant:

- for **workers** via **inhalation route:** for **short-term exposure** DNEL = 400 mg/m^3 and for **long term** exposure: DNEL = 1.76 mg/m^3
- for **workers** via **dermal route** for short-term exposure DNEL = 40 mg/kg bw/day and for **long term** exposure: DNEL = 0.5 mg/kg bw/d
- for **general population** via **inhalation route** for **short-term exposure** DNEL = 200 mg/m^3 and for **long term** exposure: DNEL = 0.38 mg/m^3
- for **general population** via **dermal route** for **short-term exposure** DNEL = 20 mg/kg bw/d and for **long term** exposure: DNEL = 0.25 mg/kg bw/d
- for **general population** via **oral route** for **short-term exposure** DNEL = 50 mg/kg bw/d and for **long term** exposure: DNEL = 0.25 mg/kg bw/d

Toxicokinetics: As stated in US EPA, 2012 D-8 is estimated not to be absorbed through the skin as dry material and have poor skin absorption when in solution. D-8 is estimated to have good absorption via the lungs and gastrointestinal tract based on data for the analog bisphenol A.

Acute toxicity: No data exists for acute mammalian toxicity, therefore based on a read-across with BPS-MPE a low toxicity is estimated for this endpoint.

Carcinogenicity: Since no data has been located, there is an uncertain potential for carcinogenicity. Carcinogenic effects cannot be ruled out.

Genotoxicity: Concerning its mutagenicity, based on a negative adequate confidential study for chromosomal aberrations *in vitro* from an analog US-EPA estimated that a low concern exists. This is mainly due to a lack of data.

Reprotoxicity: About reproductive effects, an adequate Reproduction/Developmental toxicity screening study (OECD guideline 421) using oral exposure exists performed with an analog (BPS). In this study marked systemic effects were observed (parental NOAEL = 10 mg/kg bw/day) as well as reproductive effects such as prolonged estrous cycle and decreased fertility Index; NOAEL = 60 mg/kg bw/day). Then, US-EPA estimated that a moderate concern exists regarding the reprotoxicity of D-8. And the same designation was allocated to the concern for developmental toxicity based on the same study performed with BPS in which a decreased number of live pups (PND4) has been observed at the highest dose tested (300 mg/kg bw/day) the NOAEL chosen was then 60 mg/kg bw/day regarding developmental effects (See paragraph C.2.1.2 Human health risks related to BPS for details).

Neurotoxicity: The potential hazard concern for neurotoxicity is also moderate based on the presence of the phenol structural alert.

Repeated dose toxicity: However, a moderate hazard concern is reported for repeated dose effects, based on analogy to bisphenol S. In a 28-day guideline study performed in SD rats, systemic effects were reported and then a NOAEL = 40 mg/kg bw/day was chosen. In another study, mentioned above, performed according to the OECD guideline 421, based on the systemic effects observed a NOAEL of 10 mg/kg bw/day was chosen. A potential for liver and kidney toxicity was identified and then based on uncertainty as to the potential systemic toxicity in the range of 40 to 60 mg/kg-day, a High hazard concern is selected.

Sensitization and Irritation: Low hazard concern was identified for skin sensitization (negative results in an adequate quality study on the analog BPS-MPE) for eye irritation (slight irritant in rabbits in a study performed with analog BPS-MPE but clearing within 24 hours. Data were judged as adequate quality) and for dermal irritation (slight irritant at 24hours recovering within 2 weeks. Data were judged as adequate quality) (US EPA, 2012).

No data were located for respiratory sensitization or immunotoxicity.

Endocrine activity: Finally, concerning the endocrine activity of D-8, several *in vitro* studies were identified in which there is a limited evidence of endocrine activity. Indeed, negative results were reported in two ER binding assays and one competitive ER binding assay. The study was positive for anti-estrogenicity in a competitive binding assay in the presence of 17β-estradiol. All studies were judged by US-EPA as adequate data. However, this discrepancy of D8 toward ER binding affinity does not support US-EPA approach of read-accrossing its hazard with BPS. Moreover, it should be noted that different statement from US-EPA are diverging with the data from the registration dossier (eg. Low dose DNEL for dermal exposure while US-EPA reports poor skin absorption).

According to DK report (2013) D8 represents a moderate hazard for human health but an important hazard for aquatic life (a harmonised classification exists, however exposure of aquatic life is lower than for humans). Based on US EPA, 2012 a moderate risk exists for carcinogenicity, genotoxicity, reprotoxicity, development and neurotoxicity. A high risk exists for repeated dose toxicity.

C.2.6.3 Environment risks related to D8

An harmonised classification exists for D-8: Aquatic Chronic 2 - H411

Acute ecotoxicity: D-8 belongs to the ECOSAR phenols class. A fish 96-hour LC_{50} of 6.64 mg/L has been estimated. In addition a Daphnid 48-hour LC_{50} of 3.56 mg/L and Green algae 96-hour EC_{50} of 14.70 mg/L were calculated using ECOSAR. D8 is identified as a high concern for acute ecotoxicity based on these estimated LC_{50}, which are in the range of 1-10 mg/L.

Chronic ecotoxicity: Similarly, using ECOSAR phenols class, a fish 30-day ChV_{50} of 0.69 mg/L and a Daphnid 21-day ChV of 0.68 mg/L were estimated. Since these values were in the range of 0.1-1 mg/L a high concern for chronic aquatic toxicity was identified.

Environmental fate: Concerning the environmental fate, evaluation of D-8 transport (US EPA, 2012) has been based entirely on estimations on QSARs for fugacity (level III), disassociation constant (pKa), adsorption coefficient (Koc), volatilization, and vapor pressure. If released to air, an estimated vapor pressure of <1x10-8 mmHg at 25 °C indicates that D-8 will exist in the particulate phase in the atmosphere. Particulate-phase D-8 will be removed from the atmosphere by wet or dry deposition. If released to soil, D-8 is expected to have moderate mobility based upon an estimated Koc of 2,500. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant.

Persistence and Bioaccumulation: According to the same report the persistence of D-8 was estimated as moderate by analogy with bisphenol S and its bioaccumulation was estimated as low based on an estimated fish BCF of 53 and a BAF of 83. No data were located concerning the metabolism in fish.

No data located for environmental monitoring or ecological biomonitoring. And finally about human biomonitoring, D-8 was not included in the NHANES biomonitoring report (CDC, 2011).

C.2.6.4 Technical and economic feasibility of D8

It is unknown to what extent D8 is actually used in thermal paper but some specific applications exist such as labels. D8 can thus be considered as technically feasible.

As regards its economic feasibility, some quantitative data could be found on its price from INERIS, 2013: D8 price seems to range from 11,390€/ton to 15,104€/ton with an average price of 12,938€/ton. Moreover, ETPA indicated that D8 cost is around 5 times higher than BPA's cost (INERIS, 2013).

In conclusion, D8 is considered as economically feasible but more expensive than BPA.

C.2.6.5 Other information on D8

MSCA survey 2013

One leading manufacturer of thermal paper did not want to share detailed information regarding their formulations, but claimed to use urea based compounds in their BPA-free paper. Another manufacturer claims that the alternative developers used are included in the list compiled by US-EPA. Another has not provided detailed information regarding their products.

C.2.7 Assessment of D90

Table 74. Identity of D90

Public name	Phenol, 4,4'-sulfonylbis-, polymer with 1,1'-oxybis(2-chloroethane)
EC name	4-[4'-[(1'-methylethyloxy) phenyl]sulfonyl]phenol
EC number	427-620-8
CAS number	191680-83-8
Molecular formula	$C_{28}H_{26}O_9S_2$ (n = 1); $C_{44}H_{42}O_{14}S_3$ (n = 2)
Chemical structure	

Table 75. Physico-chemical properties of D90

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Melting Point (°C)	>361 < 431	Registration dossier	Assessed using differential Scanning Calorimetry (DSC)
Boiling Point (°C)	>300 (for n=1 and n=2)	EPI; U.S. EPA, 1999	Estimated. Estimates were performed on representative components of the polymer that have a MW <1,000; those with n = 1 or 2. Higher oligomers are expected to have a similar value. Cutoff value for high boiling point compounds according to HPV assessment guidance.
Relative density	1.46	Registration dossier	Measured using air comparison pycometer (for solids)
Vapor Pressure (Pa)	$<1.3x10^{-4}$ @ 25°C	Registration dossier	Measured using effusion method: vapour pressure balance
Water Solubility (mg/L)	$1.47x10^{-5}$	Registration dossier	Measured using column elution method
Log Kow	≥ 0.629 ≤ 5.67	Registration dossier	Measured using HPLC

Flammability (Flash Point)	None	Registration dossier	measured
Explosiveness	No	Registration dossier	measured
pH			No data located.
pKa	6.9-7.5 (identical values for both n=1 and n=2)	ACD/Labs, 2010	Estimated. SMILES notation was too long for SPARC estimations, which were used for the other chemicals assessed, and an alternative estimation method was used.

D-90 is a NONs which is therefore already registered under REACH. Some information are available on the dissemination site. This substance is not classified according to ESIS.

C.2.7.1. Availability of D90

According to Danish E.P.A., 2013, D90 is known to be used in thermal paper. The manufacturers of thermal paper consulted by INERIS, 2013 confirmed that D90 is already used in thermal paper. However, according to ETPA, as a printing stabilizer it cannot be used to reduce the amount of BPA in the paper, but only to improve the stabilization of the image, and cannot really be considered as an alternative to BPA. Nevertheless, one individual manufacturer of thermal paper claimed that D90 (as well as UU which is also a printing stabilizer) is a potential alternative.

Moreover, the data on the tonnage of D90 produced, used or placed on the EU market are not publicly available as it is claimed confidential on the registration dossier.

As a result, D90 may be available but it is difficult to conclude clearly about its availability.

C.2.7.2 Human health risks related to D90

For human health hazards, several DNELs were derived according to the lead registrant:

- for **workers** via **inhalation route** for **long term** exposure: DNEL = 29.4 mg/m^3
- for **workers** via **dermal route** for **long term** exposure: DNEL = 8.33 mg/kg bw/d
- for **general population** via inhalation **route long term** exposure: DNEL = 6.25 mg/m^3
- for **general population** via **dermal route** for **long term** exposure: DNEL = 4.17 mg/kg bw/d
- for **general population** via **oral route** for **long term** exposure: DNEL = 0.25 mg/kg bw/d

Toxicokinetics: As stated in US EPA, 2012 no data were located concerning dermal absorption or ADME for D-90.

Acute toxicity: Concerning acute mammalian toxicity, according to good guideline study provided in the registration dossier, a low concern exists since oral and dermal LD_{50} are >

2000 mg/kg bw/day. The studies were performed on Sragues-Dawley rats and no lethality or signs of systemic toxicity were observed in both studies.

Carcinogenicity: Since no data has been located, there is an uncertain potential for carcinogenicity. Carcinogenic effects cannot be ruled out. It is concluded that a moderate concern exists for this endpoint (US EPA, 2012).

Genotoxicity: Concerning the genotoxicity, based on a negative reverse mutation assay and on two negative adequate confidential study for chromosomal aberrations *in vitro* (OECD 473) US-EPA estimated that a low concern exists. No data were located for others genotoxicity endpoints.

Reprotoxicity: A recent (2010) one-generation toxicity study using oral exposure (gavage) in Wistar rat showed no effect and NOEL of 1000 mg/kg bw/day was derived for both Parental and F1 generation. US-EPA in their report (2012) estimated that a low concern exists for fertility based on the lack of effects in repeated dose studies. In the registration dossier, no study for developmental toxicity was provided. A low concern was assessed by US-EPA based on limited predicted absorption, low predicted metabolism, and lack of significant toxicological concerns from repeated dose testing suggests low potential for developmental effects, with lower confidence.

Neurotoxicity: The potential hazard concern allocated to neurotoxicity is moderate based on the presence of the phenol structural alert.

Repeated dose toxicity: A low hazard concern is reported by US-EPA for repeated dose effects, based on an adequate study in which no adverse effects (e.g., mortality; clinical signs; and changes in body weights, food consumption, urinalysis data, hematology data, gross pathology, organ weights, organ-to-body weight ratios or histopathology) were observed in a 28-day oral (gavage) study in male and female Fischer 344 rats. Increases in y-glutamyl transpeptidase were observed in females exposed to 300 and 1,000 mg/kg-bw-day, which did not correspond to histopathological effects. The NOEL chosen was 1,000 mg/kg-bw-day (highest dose tested).

Sensitization: Low hazard concern was identified for skin sensitization (negative results in an adequate quality guideline study).

Irritation: About eye irritation, D-90 was found as irritant in New Zealand White rabbits with iridial inflammation and moderate conjunctival irritation in a guideline study (OECD 405). Treated eyes appeared normal at the 48- or 72-hour observation. Then a moderate concern was identified. Conversely, D-90 was found non-irritant for dermal irritation in New-Zealand white rabbits. And then a very low hazard concern has been allocated for this endpoint.

No data were located for respiratory sensitization or immunotoxicity.

C.2.7.3 Environment risks related to D90

Acute ecotoxicity: D-90 belongs to the ECOSAR poly phenols class. A fish 96-hour LC_{50} of 4.76 (n=1) or 0.31 (n=2) mg/L has been estimated. In the registration dossier a 96h LC_{50} > 0.025 mg/L has been reported for a study performed in Oncorhynchus mykiss (1997). In addition, a Daphnid 48-hour LC_{50} of 9.46 (n=1) or 0.29 (n=2) mg/L and Green algae 96-hour EC_{50} of 3.36 (n=1) or 0.63 (n=2) mg/L were calculated using ECOSAR. In the registration dossier 48h-EC_{50}

> 0.025 mg/L and a 72h algae EC_{50} > 0.025 mg/L are reported. D-90 is identified as a low concern for acute ecotoxicity (US EPA, 2012) based on these estimated LC_{50} and EC_{50} that result in no effects at saturation, as obtained for representative components of the polymer that have a MW <1,000. Higher MW components of the polymer are expected to have similar behavior.

Chronic ecotoxicity: Similarly, using ECOSAR poly phenols class, a fish 30-day ChV of 1.08 (n=1) or 0.027 (n=2) mg/L, a Daphnid 21-day ChV of 1.20 (n=1) or 0.054 (n=2) mg/L and Green algae ChV of 0.51 (n=1) or 0.206 (n=2) mg/L were estimated. Based on these ChV values for fish, Daphnid, and green algae that result in no effects at saturation, as obtained for representative components of the polymer that have a MW < 1,000, a low hazard concern has been chosen. Higher MW components of the polymer are expected to have similar behavior.

Environmental fate: Concerning the environmental fate reported by US-EPA, evaluation of D-90 transport is based entirely on estimations on QSARs that were performed on two representative components of the polymer (n = 1 and n = 2) that are a MW <1,000, although the higher MW oligomers are anticipated to behave similarly. These representative structures are anticipated to be the predominate components of the polymeric mixture. D-90 is expected to have low mobility in soil based on its expected strong absorption to soil. If released to the atmosphere, D-90 is likely to exist solely as particulate. As a particulate, atmospheric oxidation is not expected to be a significant route of environmental removal. Level III fugacity models indicate that D-90 will partition predominantly to the soil and sediment.

Persistence and bioaccumulation: According to the same report by US EPA, 2012 the persistence of D-90 was estimated as very high. Evaluation of D-90 persistence was based entirely on estimations that were performed on two representative components of the polymer (n = 1 and n = 2) that have a MW <1,000 and are anticipated to be the predominant component of the polymeric mixture. Primary aerobic degradation was estimated to be in the order of weeks for both representative structures. Ultimate biodegradation was estimated to be in the order of months for the n = 1 polymer, and the n = 2 polymer was estimated to be recalcitrant. Estimated volatilization half-lives of >1 year for both representative structures indicate that volatilization is not expected to occur. D-90 does not contain functional groups that absorb light at environmentally-relevant wavelengths, and is not expected to be susceptible to direct photolysis. Atmospheric hydroxyl-radical photo-oxidation half-lives were estimated to be 2.5 and 1.4 hours, respectively. However, this is not expected to be an important removal process since D-90 is expected to exist in the particulate phase in the atmosphere. Higher MW components of the polymer are expected to have similar persistence behavior.

Similarly, the bioaccumulation potential of D-90 is estimated as high based on estimated on representative components of the polymer indicated, since the estimated BAF value for the low MW oligomers with n=2 is > 1,000. No data on metabolism in fish was located.

No data was located for environmental monitoring or ecological biomonitoring. And finally about human biomonitoring, D-8 was not included in the NHANES biomonitoring report (CDC, 2011).

C.2.7.4 Technical and economic feasibility of D90

It is unknown to what extent D90 is used in thermal paper but there is some indication it is. Based on that information, D90 can be considered technically feasible. However, as indicated above, as a printing stabilizer, D90 might not be used to reduce the amount of BPA in the paper, but only to improve the stabilization of the image. D90 might not be considered as an alternative to BPA. As a whole, this is thus difficult to conclude about the technical feasibility of D90.

As regards its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.8 Assessment of TGSA

Table 76. Identity of TG-SA

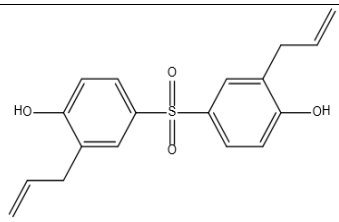
Public name	2,2'-diallyl-4,4'-sulfonyldiphenol or TG-SA
EC name	2,2'-diallyl-4,4'-sulfonyldiphenol
IUPAC name	2,2'-diallyl-4,4'-sulfonyldiphenol
EC number	411-570-9
CAS number	41481-66-7
Annex VI Index number	016-075-00-8
Molecular formula	$C_{18}H_{18}O_4S$
Chemical structure	

Table 77. Physico-chemical properties of TGSA

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Melting Point (°C)	151-155 ± 1	Nippon Kayaku Co., 1992b US EPA, 2012	Measured. Adequate data from a guideline study

	144	Submitted confidential study	Adequate data
Boiling Point (°C)	Decomposed prior to boiling	Nippon Kayaku Co., 1992b ; US EPA, 2012	Measured. Adequate data; decomposition occurs before the boiling point is reached.
Vapor Pressure (mm Hg)	$9.5x10^{-10}$	Nippon Kayaku Co., 1992b; US EPA, 2012	Measured. Adequate data; guideline study.
Water Solubility (g/L)	4.79 at 20.3°C ±0.5	Nippon Kayaku Co., 1992b; US EPA, 2012	Measured. Adequate data ; guideline study.
Log Kow	3.22	Nippon Kayaku Co., 1992b ; US EPA, 2012	Measured. Adequate data; guideline study.
Flammability (Flash Point)	Not highly flammable	Nippon Kayaku Co., 1992b; US EPA, 2012	Measured. Adequate data; guideline study.
Explosivity	Not explosive	Nippon Kayaku Co., 1992b ; US EPA, 2012	Measured. Adequate data; guideline study
pH			No data located.
pKa	8.3-8.5	SPARC	Estimated.

C.2.8.1. Availability of TGSA

TGSA has been quoted as an alternative to BPA by the stakeholders consulted by INERIS, 2013 and in the literature (US EPA, 2012) but there is no indication about its actual use in thermal paper.

The data on the tonnage of TGSA produced, used or placed on the EU market are not publicly available. There is a registration dossier of TGSA but tonnages are confidential.

C.2.8.2 Human health risks related to TGSA

Toxicokinetics: According to US EPA, 2012, TGSA as a neat material is not estimated to be absorbed through the skin and is expected to have poor skin absorption when in solution. It is estimated to be absorbed via the lungs and gastrointestinal tract based on data for bisphenol A, because of the analogy between the two substances. No data were located on dermal absorption.

Acute toxicity: The hazard concern of TGSA for acute toxicity is considered as low based on an oral LD_{50} > 2,000 mg/kg in Sprague-Dawley rats (Adequate study following OECD 401 guideline, Nippon Kayaku Co., 1991a; US EPA, 2012) and dermal LD_{50} > 2,000 mg/kg (Nippon

Kayaku Co., 1991b, US EPA, 2012 OECD guideline 402). No data were located for inhalation route.

Carcinogenicity: A moderate concern for carcinogenicity was allocated to TGSA by US-EPA (US EPA, 2012) based on data reported for the epoxide oxidation product. In addition, there is uncertainty due to the lack of data located for this substance. Carcinogenic effects cannot be ruled out.

Genotoxicity: TGSA is a potential cross-linking agent because it has two terminal double bonds that are expected to be oxidized in the body via an epoxide intermediate. About genotoxicity, a negative Ames assay (Nippon Kayaku Co., 1991c; US EPA, 2012) is available. Two negative assays assessing chromosomal aberrations were reported (one for chromosomal aberrations in human lymphocytes, the other one was for chromatid exchanges). I*n vivo,* no gene mutation study was available. A study (Nippon Kayaku Co., 1991d; US EPA, 2012) conducted according to OECD guideline 474 (mammalian erythrocyte micronucleus test in mice) showed negative results. Then a low concern exists for genotoxicity potential of TG-SA.

Reprotoxicity: No data on TGSA available. Using the results of a screening study available for analog Bisphenol S, in which oral exposure of parental rats resulted in marked systemic effects and the NOAEL for reproductive effects is 60 mg/kg-day (prolonged estrous cycle, decreased fertility index and decreased number of live offspring), a moderate hazard designation is selected. Similarly, based on reported data for the epoxide oxidation product a concern for male reproductive toxicity has been estimated.

Likewise, a moderate hazard concern has been identified for developmental effects based on data existing for analog Bisphenol S.

Neurotoxicity: Concerning neurotoxicity concern for TGSA, a moderate hazard designation was selected based on structural alert, since no data were located.

Repeated dose toxicity: A 28-day study (performed according to OECD guideline 474; Nippon Kayaku Co., 1991e; US EPA, 2012) is described by US-EPA. Sprague-Dawley rats were exposed orally and there was no mortality and no clinical signs of toxicity; increased salivation with wet fur and red/brown staining of body surface were observed at doses of 150 mg/kg-day and higher. A decreased body weight gain in females administered 1,000 mg/kg-day was reported; But there was no treatment related effects on hematology, serum chemistry, necropsy, or organ weights; increased incidence of basophilic tubules and interstitial mononuclear cell infiltrates in kidneys of males in the 1,000 mg/kg-day group; similar but less pronounce effect occurred at 150 mg/kg-day in males. Then a NOAEL = 15 mg/kg-day was derived leading to a high hazard concern for repeated dose effects endpoint. It should be noted that depending on the severity of the effects described by US-EPA, criteria for classification as STOT-RE2 might be met.

Sensitization and irritation: the US-EPA evaluation reports that TGSA was found strong sensitizer in a Magnusson & Klingman maximization test with a 70% sensitization rate in guinea pig. Nevertheless the substance was classified as non-sensitizer in a LLNA assay in female CBAJN mice. It should be noted that TGSA is classified as Skin Sens. 1, May cause an allergic skin reaction H317.

US-EPA evaluation states that a moderate concern exists for respiratory sensitization based on the epoxide oxidation product.

Similarly, it is stated that a low concern exists for eye irritation based on experimental data suggesting that TGSA is a minimal irritant to rabbit eyes and a very low concern for dermal irritation based on experimental data indicating that TGSA is not an irritant to rabbit skin.

Endocrine activity: The US-EPA evaluation states that endocrine activity of TGSA was assessed in an adequate study. The substance did not cause significant estrogenic activity in a recombinant yeast screen assay in *Saccharomyces cerevisiae:* it did not bind to estrogen receptor in recombinant yeast and the estrogenic response was 4 orders of magnitude less than 17β-estradiol and 1 order of magnitude less than bisphenol A. In an uterothrophic assay in immature rats, there was no evidence of estrogenic effects on uterus at oral doses up to 100 mg/kg bw. There was then no evidence that TGSA elicits estrogenic activity.

C.2.8.3 Environment risks related to TGSA

Acute ecotoxicity: TGSA belongs to the ECOSAR poly phenols class. A fish 96-hour LC_{50} of 4.0 mg/L has been reported for a study performed in Oncorhynchus mykiss (Nippon Kayaku Co., 1991; US EPA, 2012). The LC_{50} for medake was > 9.8 mg/L (Nippon Kayaku Co., 2011; US EPA, 2012). In addition a Daphnid 24-hour LC_{50} and 24-hour LC_{50} > 12 mg/L (immobilization) were determined in a recent study performed in Daphnia (Nippon Kayaku Co., 2011; US EPA, 2012). Green algae 72-hour EC_{50} of > 100 mg/L is reported in a study (Nippon Kayaku Co., 2000; US EPA, 2012) performed with a solution containing 50% of TGSA. Using ECOSAR for neutral organics a 96-h EC_{50} of 2.01 mg/L was estimated, lowered to 1.71 mg/L when using the poly phenols class. Then a high concern exists for acute toxicity based on experimental acute aquatic toxicity values for fish and Daphnia which are in the range of 1-10 mg/L.

Chronic ecotoxicity: Similarly, in US EPA, 2012, based on estimated ChV values for fish and algae that are in the range of 0.1-1.0 mg/L. Experimental chronic toxicity values were located for daphnia, but not for fish or algae. Experimental values for daphnia are in the Moderate hazard range of 1-10 mg/L. But, without experimental values for fish or algae, a conservative approach using estimated values will be the basis for the hazard designation, and a moderate concern was allocated. Nevertheless in the dissemination site, data from the registration dossier were found for long-term toxicity to fish. In a 28-day study (2011) performed in juvenile Oryzias latipes, a NOEC > 8 mg/L was derived based on effects on behaviour.

Environmental fate: Concerning the environmental fate, according to the US EPA report TGSA is expected to exist in both the neutral and anionic forms at environmentally-relevant pH. TGSA is expected to have moderate mobility in soil. Anionic TGSA may have higher mobility due to enhanced water solubility. However, leaching through soil to groundwater is not expected to be an important transport mechanism. In the atmosphere, TGSA is expected to exist in the particulate phase, which will be deposited back to the soil and water surfaces through wet or dry deposition. The Level III fugacity model indicates that TGSA will partition primarily to soil.

Persistence and bioaccumulation: According to the same report the persistence of TGSA was estimated as high based on an estimated half-life of 75 days in soil. TGSA is expected to partition primarily to soil. Experimental biodegradation data for TGSA were not located. Evaluation of the biodegradation potential for TGSA is based entirely on QSARs of aerobic and anaerobic biodegradation. Results from these models estimate ultimate biodegradation in weeks-months and primary degradation in days-week. Biodegradation under anaerobic methanogenic conditions is not probable based on results from estimation models. TGSA does

not contain functional groups that absorb light at environmentally-relevant wavelengths. Therefore, it is not expected to be susceptible to direct photolysis. It is not expected to undergo hydrolysis as it does not contain hydrolysable functional groups. The atmospheric half-life of TGSA is estimated at 1.8 hours, although it is expected to exist primarily as a particulate in air. Therefore, biodegradation is expected to be the main degradation pathway for TGSA.

Similarly, the bioaccumulation potential of TGSA is estimated as low based on estimate performed using experimental log Kow. A fish BCF was estimated at 62 and BAF at 18.

C.2.8.4 Technical and economic feasibility of TGSA

It is unknown whether TGSA is actually used in thermal paper. Moreover, although TGSA has a bisphenol structure, the presence of the pendant allyl groups can have various physico-chemical effects that would preclude its use as a dye developer. Without additional data, it is thus difficult to conclude on its technical feasibility.

Likewise, as regards its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.8.5 Other information on TGSA

A harmonised classification exists for TGSA, which is classified as Skin Sens. 1 – H317 and Aquatic Chronic 2 – H411.

No data located for environmental monitoring or ecological biomonitoring. And finally about human biomonitoring, D-8 was not included in the NHANES biomonitoring report (CDC, 2011).

C.2.9 Assessment of UU

Table 78. Identity of UU

Public name	Urea Urethane Compound
EC name	Not assigned
IUPAC name	Not assigned
EC number	Not assigned
CAS number	321860-75-7
Annex VI Index number	Not classified
Molecular formula	$C_{42}H_{36}N_6O_8S$

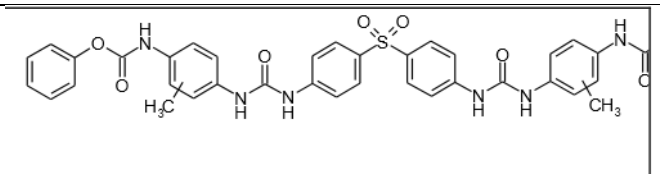
Chemical structure	

Table 79. Physico-chemical properties of UU

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Melting Point (°C)			No data located
Boiling Point (°C)	> 300	EPI. US EPA, 1999	Estimated. Estimates were performed on a representative component of the polymer shown above. This representative structure is anticipated to be the predominant component of the polymeric mixture. Cutoff value for high boiling point compounds according to HPV assessment guidance.
Vapor Pressure (mm Hg)	$<1x10^{-8}$	EPI. US EPA, 2011	Estimated. Estimates were performed on a representative component of the polymer shown above. This representative structure is anticipated to be the predominant component of the polymeric mixture. Cutoff value for high boiling point compounds according to SF assessment guidance.
Water Solubility (g/L)	$< 1x10^{-3}$	EPI. US EPA, 1999	Estimated. Estimates were performed on a representative component of the polymer shown above. This representative structure is anticipated to be the predominant component of the polymeric mixture. Cutoff value for high boiling point compounds according to HPV assessment guidance.
Log Kow	6.5	EPI	Estimated. Estimates were performed on a representative component of the polymer shown above. This representative structure is anticipated to be the predominant component of the polymeric mixture.
Flammability (Flash Point)			No data located.
Explosivity			No data located.
pH			No data located.

pKa	10.3	SPARC	Estimated. Estimates were performed on a representative component of the polymer shown above. This representative structure is anticipated to be the predominant component of the polymeric mixture.

C.2.9.1. Availability of UU

The manufacturers of thermal paper consulted by INERIS, 2013 confirmed that UU is already used in thermal paper. However, as mentioned for D90 above, according to ETPA, UU is a printing stabilizer and cannot be used to reduce the amount of BPA in the paper, but only to improve the stabilization of the image. It cannot thus really be considered as an alternative to BPA. Nevertheless, one individual manufacturer of thermal paper claimed that UU (like D90) is a potential alternative, based on the existence of patents assigned to some manufacturers on the use of UU in thermal printing.

It is however unknown to what extent UU is actually used today in thermal paper in the EU.

Moreover, the data on the tonnage of UU produced, used or placed on the EU market are not publicly available and UU has not been registered under REACH so far.

As a conclusion, it is difficult to conclude about the availability of UU.

C.2.9.2 Human health risks related to UU

Toxicokinetics: According to the US EPA, 2012 UU is not absorbed by skin, poorly absorbed by the lung and can be absorbed by the gastrointestinal tract, based on closely related analog with similar structure, functional group and physical/chemical properties.

Acute toxicity: The hazard concern of UU for acute toxicity is considered as low based on an oral LD_{50} = 2,000 mg/kg in rats (confidential study) and dermal LC_{050} = 3,161 mg/kg (confidential study). No data ware located for inhalation route.

Carcinogenicity: A moderate concern for carcinogenicity was allocated to UU by US-EPA (2012) based on the uncertainty due to a lack of data for this substance. Carcinogenic effects cannot be ruled out.

Genotoxicity: Two negative Ames assays with and without metabolic activation (confidential study) are available. No gene mutation *in vivo* was available. One negative assay assessing chromosomal aberrations *in vitro* in CHL cells has been submitted according to US EPA, 2012. No data were located for DNA damage. Then a low concern exists for genotoxicity potential of UU.

Reprotoxicity: A low hazard designation has been selected by US EPA based on a professional judgement since no data has been located but a combination of limited predicted absorption, low predicted metabolism, and lack of significant toxicological concerns from repeated dose testing on a close analog suggests low potential hazard, with lower confidence.

Likewise, a low hazard concern has been identified for developmental effects based on previous statements for reproductive effects.

Neurotoxicity: Concerning neurotoxicity concern for UU, a low hazard designation was selected since no structural alerts or mechanistic pathways associated with neurotoxic effect have been identified.

Repeated dose toxicity: A 28-day study (confidential study) is reported by US-EPA. Rats were exposed orally (gavage) and there was no clinical signs, no macroscopic or histopathological abnormalities. Then a NOAEL = 1000 mg/kg-day was derived leading to a low hazard concern for repeated dose effects endpoint.

Sensitization and irritation: US-EPA reports that UU was found as non-sensitizing in a confidential study in guinea pig. No data were located for respiratory sensitization and then a low hazard concern was allocated for the sensitization endpoint.

A low concern exists for dermal and eye irritation based on experimental data. A slight irritation was observed in rabbits following an exposure to UU, and UU was non-irritating in rabbits via dermal route.

Endocrine activity: No data were located for endocrine activity or immunotoxicity.

C.2.9.3 Environment risks related to UU

Acute ecotoxicity: UU belongs to the ECOSAR substituted ureas; amides and carbamate esters classes. A fish 96-hour study has been submitted, and a LC_{50} of > 250 mg/L was reported. Based on this measured 96-hour LC_{50} for fish and on estimated 96-hour LC_{50} for fish, 48-hour LC_{50} for Daphnid, and 96-hour EC_{50} for green algae that result in no effects at saturation (NES), as obtained for a representative component of the polymer that has a MW <1,000, a low hazard concern for this endpoint has been allocated by US EPA, 2012.

Chronic ecotoxicity: Similarly, based on estimated ChV values for fish, Daphnid, and green algae that result in no effects at saturation (NES), as obtained for a representative component of the polymer that has a MW <1,000, a low hazard concern was chosen for chronic ecotoxicity of UU.

Environmental fate: Concerning the environmental fate, according to the US EPA report evaluation of UU transport is based entirely on QSAR estimations that were performed on a representative component of the polymer that has a MW <1,000. This representative structure is anticipated to be the predominant component of the polymeric mixture. UU is expected to have low mobility in soil based on its expected strong absorption to soil. If released to the atmosphere, UU is likely to exist solely as particulate. As a particulate, atmospheric oxidation is not expected to be a significant route of environmental removal. Based on the Henry"s Law constant, volatilization from water or moist soil is not expected to occur at an appreciable rate. Level III fugacity models indicate that UU will partition predominantly to the soil and sediment.

Persistence and bioaccumulation: According to the same report the persistence of UU was estimated as very high since it is not ready biodegradable based on a Japanese MITI test (OECD 301C). 1% (by BOD) and 2% (by HPLC) biodegradation in 28 days were measured. Further evaluation of the persistence of UU is based on predictive QSAR models for the representative component estimates UU to be recalcitrant to ultimate biodegradation, and suggest a biodegradation half-life of >180 days. In addition, the larger oligomers in the polymeric mixture with a MW>1,000 are expected to have Very High persistence potential

based on DfE assessment guidance as they are likely too large and too water insoluble to be bioavailable.

The bioaccumulation potential of UU is estimated as low since the measured BCF for UU is <100 (4.6). The estimated BAF for the representative component of the polymer is <100 (7.9). Although the BCF model results in a higher hazard concern, the BAF model is anticipated to better account for metabolism for this class of compounds. In addition, the polymeric components of the mixture that have a MW >1,000 are not expected to be bioaccumulative because, in general, substances with a MW >1,000 are not bioaccumulative due to their large size.

C.2.9.4 Technical and economic feasibility of UU

It is unknown to what extent UU is actually used in thermal paper but there is some indication that it is. UU derivatives have no acidic protons, but are suggested to promote ring cleavage of fluoran dyes, and stabilize the dye/developer complexes (FBR-WUR, 2015). Therefore, UU can be considered in principle as technically feasible.

However, regarding its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.10 Assessment of DD-70

Table 80. Identity of DD-70

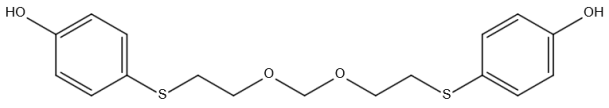
Public name	DD-70 or 1,7-bis(4- Hydroxyphenylthio)-3,5- dioxaheptane
EC name	4-4'-methylenebis(oxyethylenethio)diphenol
IUPAC name	4-4'-methylenebis(oxyethylenethio)diphenol
EC number	407-480-4
CAS number	93589-69-6
Annex VI Index number	604-049-00-4
Molecular formula	$C_{17}H_{20}O_4S_2$
Chemical structure	

Table 81. Physico-chemical properties of DD-70

Property	Value	Reference	Comment (e.g. measured or estimated)
Melting Point (easu	108	Submitted confidential study	Measured with adequate data quality
Boiling Point (dequ	>350	EPI; U.S. EPA, 1999	Estimated. Cut-off value for high boiling point compounds according to HPV assessment guidance.
Vapor Pressure (mm Hg)	$<1x10^{-8}$	EPI; U.S. EPA, 2011	Estimated. Cut-off value for non volatile compounds according to HPV assessment guidance.
Water Solubility (g/L)	0.13	EPI	Estimated
Log Kow	3.4	EPI	Estimated
Flammability (Flash Point)			No data located.
Explosivity			No data located.
pH			No data located.
pKa	9.6	SPARC	Estimated

C.2.10.1. Availability DD-70

DD70 is a commercial product. It is unknown to what extent DD-70 is actually used in thermal paper. The data on the tonnage of DD-70 produced, used or placed on the EU market are not publicly available. There is a registration dossier of DD-70 but tonnages are confidential.

C.2.10.2 Human health risks related to DD-70

Toxicokinetics: As stated in US EPA, 2012 DD-70, as a neat material, is estimated not to be absorbed through the skin and have poor skin absorption when in solution. DD-70 is expected to be poorly absorbed via the lungs and gastrointestinal tract as estimated by analogy with a confidential substance with similar structure, functional groups, and physical/chemical properties.

Acute toxicity: No data exists for acute mammalian toxicity. Based on expert judgment (high molecular weight, lack of absorption and absence of structural alerts) a low toxicity is estimated in the US-EPA report.

Carcinogenicity: Since no data has been located, US-EPA reports that an estimate has been performed using OncoLogic expert system. Using the "phenols and phenolic coumpounds" class the model describes a concern for this compound as a potential carcinogen or tumorigenesis promoter arising from its structural similarity to estrogenic/androgenic compounds.

Genotoxicity: Concerning the genotoxicity, since no data have been located, based on the absence of structural alerts, US-EPA estimated that a low concern exists. No other data were located.

Reprotoxicity: About reproductive effects, again no data were located. A moderate hazard concern was allocated by US-EPA since there is no appropriate analog however an analog for DD-70 is toxicologically active in repeated dose and developmental toxicity studies, such that potential reproductive toxicity cannot be ruled out.

And the same designation was allocated to the hazard concern for developmental toxicity based on professional judgement on available test for a confidential analog. Then by analogy a NOAEL = 300 mg/kg bw/day (in rabbits, oral route) and a LOAEL = 100 mg/kg bw/day (in rats, oral route) were estimated in the US-EPA report.

Neurotoxicity: The potential hazard concern for neurotoxicity is also moderate based on the presence of the phenol structural alert.

Repeated dose toxicity: No data was identified; the assessment from US-EPA was based on analogy to a confidential substance. In a 13-weeks study by oral route performed in rats, blood toxicity, severe gastro-intestinal irritation and histological changes in the glandular stomach were reported and then a NOAEL = 50 mg/kg bw/day was chosen. Because no LOAEL was identified, there is uncertainty as to the dose at which these effects occur. Using a conservative approach in the absence of a specified LOAEL, a moderate hazard concern is selected because it is possible that effects can occur at doses between 50 and 100 mg/kg bw/day.

Sensitization and Irritation: Moderate hazard concern was identified for skin sensitization and dermal irritation since positive results for skin sensitization in guinea pigs were reported for a confidential analog, and in addition a concern exists for dermal irritation. Concerning eye irritation, since a concern exists for potential corrosion to mucous membranes and eyes, a high hazard concern was chosen by US-EPA.

No data were located for respiratory sensitization or immunotoxicity.

Endocrine activity: No data located.

C.2.10.3 Environment risks related to DD-70

Acute ecotoxicity: DD-70 belongs to the ECOSAR poly phenols class and then a fish 96-hour LC_{50} of 5.39 mg/L has been estimated. In addition a Daphnid 48-hour LC_{50} of 13.6 mg/L and Green algae 96-hour EC_{50} of 2.28 mg/L were calculated using ECOSAR. DD-70 is identified as having a high concern for acute ecotoxicity based on these estimated LC_{50} for fish and algae, which are in the range of 1-10 mg/L.

Chronic ecotoxicity: Similarly, using ECOSAR poly phenols class again a fish 30-day ChV_{50} of 1.33 mg/L, a Daphnid 21-day ChV of 4.68 mg/L and a Green algae ChV of 0.422 mg/L were estimated. Because of this ChV value for green algae, a high concern for chronic aquatic toxicity for DD-70 was identified.

Environmental fate: Based on the Level III fugacity models incorporating the available experimental property data, DD-70 is expected to partition primarily to soil. DD-70 is expected

to exist in both neutral and anionic forms at environmentally-relevant pH, based on its estimated pKa. The neutral form of DD-70 is expected to be immobile in soil based on its estimated Koc. The anionic form may be more mobile, as anions do not bind as strongly to organic carbon and clay as their neutral counterparts. However, leaching of DD-70 through soil to groundwater is not expected to be an important transport mechanism. Estimated volatilization half-lives indicate that it will be non-volatile from surface water. Volatilization from dry surface is also not expected based on its estimated vapor pressure. In the atmosphere, DD-70 is expected to exist solely in the particulate phase, based on its estimated vapor pressure. Particulates may be removed from air by wet or dry deposition.

Persistence and Bioaccumulation: According to the same report (US EPA, 2012) the persistence of DD-70 was estimated as high based entirely on QSARs for aerobic and anaerobic biodegradation. Results from these models estimate primary biodegradation in days-weeks and ultimate degradation in weeks-months. DD-70 is expected to partition primarily to soil; the half-life is estimated as 75 days. Biodegradation under anaerobic methanogenic conditions is not probable. DD-70 is not expected to undergo hydrolysis since it does not contain hydrolysable functional groups. DD-70 does not contain chromophores that absorb at wavelengths >290 nm, and therefore, it is not expected to be susceptible to direct photolysis by sunlight. The vapor phase reaction of DD-70 with atmospheric hydroxyl radicals is estimated at 1.2 hours, although it is expected to exist primarily in the particulate phase in air. Concerning its bioaccumulation the estimated BCF for fish is less than the low criteria cutoff of 100 (75). In addition, the estimated BAF of 35, which accounts for metabolism, suggests that DD-70 will not bioaccumulate in higher trophic levels. And then there is a low concern for this endpoint.

C.2.10.4 Technical and economic feasibility of DD-70

It is unknown to what extent DD-70 is actually used in thermal paper. It is thus hard to draw a conclusion concerning its technical feasibility. However, given the apparent application of D90 in thermal paper (see above), also the use of DD70 could be feasible.

As regards its economic feasibility, it is impossible to conclude since no data could be found on its price.

C.2.10.5 Other information on DD-70

An harmonised classification exists for DD-70: Aquatic Chronic 2 - H411.

No data was located for environmental monitoring or ecological biomonitoring (nor human biomonitoring), as DD-70 was not included in the NHANES biomonitoring report (CDC, 2011).

C.3 Assessment of alternative techniques/processes

As explained above in section B.2., direct thermal printing shows many competitive advantages compared to alternative printing techniques. As a reminder, those advantages are fast printing/sensitivity, high image resolution, reliability and durability, small and compact printers, flexibility of paper size, low running and ownership costs, low energy consumption, no additional consumables, silent system, etc.

From INERIS, 2013, some thermal paper manufacturers indicated that they are not planning to replace direct thermal printing by another technology because thermal printing was far more efficient and advantageous in terms of quality.

Overall, according to Vehmas, 2011, printing industry has crossed a serious structural change in the past ten years. Consolidation has started and some overcapacity has been closed down. The whole European print market is expected to drop by some 9.5% between 2005 and 2015 due to decreasing print pricing, reduced pricing pratices, tough competition and innovation. The sector is under mutation and the e-technologies are growing.

C.3.1 Assessment of matrix printing technique

C.3.1.1. Availability of matrix printing technique

As explained above, since the development of faster, cheaper and quieter non-impact printing techniques, such as inkjet, laser or thermal transfer printing, matrix printers have lost significant market shares and have been generally replaced, considered to some extent to be outdated technology. This type of printers is available and still largely used worldwide and in the EU but is progressively replaced by inkjet or laser printers.

C.3.1.2 Human health risks related to matrix printing technique

There is no available data on human health risks related to matrix printing technique (arising from the use of ink ribbons e.g.).

C.3.1.3 Environment risks related to matrix printing technique

There is no available data on environment risks related to matrix printing technique (related to the generated wastes e.g.).

C.3.1.4 Technical and economic feasibility of matrix printing technique

In INERIS, 2013, it is indicated that dot matrix is slower and less reliable than direct thermal printers, and presents higher costs than direct thermal printing. No data on prices are provided herein because the prices of matrix printers may vary a lot from one machine to another. In general, these printers show a rather high purchasing price. Replacing direct thermal printers by matrix printers in retailers, shops, baks, etc. might be in principle technically feasible (requiring however major adjustments of cashiers workstations due to their big size in particular) but not economically feasible (due to the prices of these printers, the frequency of ink ribbons to be changed to the associated major equipment adjustments).

C.3.2 Assessment of inkjet printing technique

C.3.2.1. Availability of inkjet printing technique

As explained above, inkjet printers are widely used for their attractive technical characteristics that are close to laser printers. They are used for professional or personal uses. This type of printers is available and can be purchased in most of large retailers or specialised shops. Alternative printing technologies such as Dye Diffusion Thermal Transfer (DDTT or D2T2), inkjet and electrophotographic printing are continuing to develop and are predicted to grow,

particularly for professional and desktop printing. Inkjet is perhaps the closest rival to thermal printing. However due to the low cost of direct thermal printing relative to inkjet and the simple printing technology which avoids expensive peripherals, thermal printing still seems to be the preferred choice (Jeffs, 2011).

C.3.2.2 Human health risks related to inkjet printing technique

There is no available data on human health risks related to inkjet printing technique (arising from the use of ink cartridges e.g.).

C.3.2.3 Environment risks related to inkjet printing technique

There is no available data on environment risks related to inkjet printing technique (related to the generated wastes e.g.).

C.3.2.4 Technical and economic feasibility of inkjet printing technique

The prices of inkjet printers have been decreasing since many years while their use has been growing. No data on prices are however provided herein because their prices may vary a lot from one machine to another; the range of inkjet printers being large. In general, these printers show a competitive price but their ink cartridges are relatively expensive.

As regards the replacement of direct thermal printers by inkjet printers in retailers, shops, baks, etc., it might be in principle technically feasible (requiring however here also major adjustments of cashiers workstations due to their big size) but not economically feasible, due to the associated major equipment adjustments and the frequency of ink cartridges to be changed.

C.3.3 Assessment of laser printing technique

C.3.3.1. Availability of laser printing technique

Laser printers show similar advantages as inkjet printers and are largely and increasingly used for professional (offices, commercial publishers, etc.) uses mainly but also for personal uses. They have the competitive asset to be capable of printing a larger number of pages in a reduced amount of time. This technology is available and innovative.

C.3.3.2 Human health risks related to laser printing technique

There is no available data on human health risks related to laser printing technique (arising from the use of ink cartridges e.g.).

C.3.3.3 Environment risks related to laser printing technique

There is no available data on environment risks related to laser printing technique (related to the generated wastes e.g.).

C.3.3.4 Technical and economic feasibility of laser printing technique

As explained above, the cost of this technology depends on a combination of factors, including the cost of paper, toner, drum replacement, as well as the replacement of other items such as the fuser assembly and transfer assembly. The prices of laser printers may vary a lot from one machine to another. They are however generally quite expensive, depending on their speed and technical properties. For indicative purposes, some of them may cost from $1000 to $6000 and the fastest may cost $100,000 and up[41]. As a consequence, in order to meet the speed and frequency of printing required from the present use of direct thermal printers in retailers, shops, etc. the equipment cost might be very high. Therefore, the replacement of direct thermal printers by laser printers might be in principle technically feasible (requiring however many equipment and cashiers workstations adjustments) but not economically feasible.

C.3.4 Assessment of thermal transfer printing technique

C.3.4.1. Availability of thermal transfer printing technique

Thermal transfer printers seem to be the main competitor to direct thermal printing for labels, especially for bar codes. However, according to (INERIS, 2013), thermal transfer printing is restricted to a small number of devices available today.

C.3.4.2 Human health risks related to thermal transfer printing technique

There is no available data on human health risks related to thermal transfer printing technique (arising from the use of ink ribbons e.g.).

C.3.4.3 Environment risks related to thermal transfer printing technique

There is no available data on environment risks related to thermal transfer printing technique (related to the generated wastes e.g.).

C.3.4.4 Technical and economic feasibility of thermal transfer printing technique

In INERIS, 2013, it is pointed out that the technology of thermal transfer printing could be a valuable alternative solution to thermal printing. It is considered technically feasible and the close size of printers makes it a more suitable substitute than the other alternative printing techniques. However, the main disadvantage of thermal transfer printing compared to direct

[41] http://whatis.techtarget.com/

thermal printing, is an additional cost due to transfer ribbons to be purchased and often to be changed. As a consequence, the stakeholders consulted predict that direct thermal printing will still be used even if BPA is restricted.

C.3.5 Assessment of paper-free alternatives

C.3.5.1. Availability of paper-free techniques

As already shown above, these techniques have been implemented since a few years by some EU retailers and big stores. They are thus to some extent available but they are not supplied by all shops and services and are not developed at large-scale yet.

Moreover, these technologies present some shortcomings and weaknesses which might prevent them from being widely used. First of all, as regards e-tickets, there might be a lag time from purchase to receiving the e-receipt, which may be between 5 min and 12 hours depending on the store and the system employed (Danish E.P.A., 2013), which in addition to the cost of subscription to the service, may limit the spread of use. The e-mail address might also be (mis)used by merchants to send promotional e-mails and the system demands continuous updating when customers e-mail addresses change. The system cannot be used for cash payment and the need for sharing credit card details on registration with some companies may also be of high concern for the user. Likewise, the contactless payment technologies might suffer from unacceptability from some users. Indeed, their use requires some change in habit. For the elderly or people without a technological outlook, the use of smart card payments may thus be difficult. Finally, for everyday necessities or small purchases, a receipt may not be required but for many types of purchases, the receipt functions as a warranty that is required for later return or complaints (Danish E.P.A., 2013).

As a whole, additionnally to (for now) limited availability, there may also be some concern about the acceptability of paper-free techniques.

C.3.5.2 Human health risks related to paper-free techniques

No human health risks related to paper-free techniques is expected. However, Danish E.P.A., 2013 underlines that while free-paper techniques eliminate the issue of BPA-containing receipts, it may transfer the problem to BPA-containing labels used for tracking and shipping of the purchased items. Some of the labeling may be performed automatically and the exposure to BPA may be reduced.

C.3.5.3 Environment risks related to paper-free techniques

As environment is concerned, the paper-free techniques could be one possible solution to exposure to BPA or other toxic dye developers and an eco-friendly solution as well. As reported in US EPA, 2012, every year, an estimated 9.6 million trees are cut down in the United States for receipts (Clifford, 2011, although many companies strive for sustainability through stewardship and management programs. This figure might also be high as far as EU is concerned. As a whole, these techniques allow reducing paper waste.

C.3.5.4 Technical and economic feasibility of paper-free techniques

These technologies are secured and innovative and show the competitive advantage to be cheaper since no paper or consumables (ink ribbons, cartridges, etc.) are needed. They stand

for emerging solutions worldwide, supplying many services for the users. They are thus considered as technically feasible alternatives to thermal paper printing. As regards their economic feasibility, they would require from the retailers, shops, banks, etc. some costs associated to the development and the implementation of electronic terminals and appropriate softwares, as well as information to clients about this new solution. Some costs are then expected but they shouldot be significant compared to the other alternative techniques assessed above.

C.4 Analysis of alternatives to BPA in thermal paper: summary and comparison

C.4.1 Comparison of alternative substances

The table below provides an overview of the chemical alternatives assessed above.

Table 82. Comparison of alternative dye developers selected and assessed

Alternative chemicals	CAS number	EC number	Hazards HH/ENV	CLP	Registered	Availability	Technical feasibility	Economic feasibility	EU regulation
BPS	80-09-1	201-250-5	Oestrogenic properties, Anti-androgenic activity, Uterotrophic effect, Effects on the reproduction and the development at maternal toxic doses (300 mg/kg/d), Discrepancies between genotoxicity studies. (ANSES; study report on the bisphenol family compounds, 2012)	No harmonised classification; 209 registrants do not classify; Number of different aggregated notifications: 7; Proposed notifications: Aquatic chronic 3, H412; Eye irrit. 2, H319; Skin irrit 2, H315; STOT SE 3 resp irrit, H335;	yes >1000t	+++	+++	+ 2,920-4,200€/t	Will be evaluated in 2014 by Belgium for the following concern: Human health/Suspected CMR; Suspected endocrine disruptor; Exposure/Aggregated tonnage TPE in 2011: 90 days oral and pre natal dvptal toxicity

BPF	620-92-8	210-658-2	Difficult to state on the reprotoxicity for the organs of the reproduction; Activity of endocrine disruption via the estrogens receptors; Direct genotoxic effect by break of the DNA. (ANSES; study report on the bisphenol family compounds, 2012)	No harmonised classification; 5 registrants do not classify; Number of different aggregated notifications: 6; Proposed notifications: Aquatic chronic 3, H412; Eye irrit. 2, H319; Skin irrit 2, H315; STOT SE 3 resp irrit, H335; Skin sens 1, H317;	no	+?	++	?	
BPAP	1571-75-1	433-130-5	Oestrogenic activity, Not possible to conclude on the activity of endocrine disruption. No toxicokinetic data, no data on toxicity for the organ of reproduction. (ANSES; study report on the bisphenol family compounds, 2012).	Yes, harmonised classification: Aquatic acute 1 H400; Aquatic chronic 1 H410;	no	+?	++	?	

1,2-diphenoxyethane	104-66-5	203-224-9	Not evaluated by ANSES.	No harmonised classification Nb of aggregated notifications: 1 Proposed notification: Aquatic chronic 2 H411;	yes >100t	+	++	?	
Pergafast (DP 201) (N-(p-Toluènesulfonyl)-N'-(3-p-toluènesulfonyloxyphényl) urea)	232938-43-1	432-520-2	Not evaluated by ANSES.	Yes, harmonised classification: Aquatic chronic 2 H411;	yes confid.	+?	++	- 15,000€-30,000€/t	
D8 (ou DD8 ou ALD-2000) (4-(4-isopropoxyphénylsulfonyl)phenol)	95235-30-6	405-520-5	Not evaluated by ANSES.	Yes, harmonised classification: Aquatic chronic 2 H411;	yes (NONS) confid.		+	- 11,390-15,104€/t	
D90 (Phénol, 4,4'-sulfonylbis	191680-83-8	Not assigned	Not evaluated by ANSES.	No	no	+?	+?		

-, polymer with 1,1'-oxybis[2-chloroethane])									
UU (Urea Urethane Compound)	321860-75-7	Not assigned	Not evaluated by ANSES.	No	No	+?	+?		
TGSA (2,2'-diallyl-4,4'-sulfonyldiphénol)/notified substance subject to transitional measures	41481-66-7	411-570-9	Not evaluated by ANSES.	Harmonised classification: index nb: 016-075-00-8 Skin Sens. 1; H317 Aquatic Chronic 2; H411 seveso substance: 9ii (toxic to aquatic org and long term effects)	Yes?	+?	+?	?	
DD-70	93589-69-6	407-480-4	-Not evaluated by ANSES.	Yes. Harmonized classification: Aquatic chronic 2 – H411	Yes (NONS)	+?	+?	?	

BACKGROUND DOCUMENT TO RAC AND SEAC OPINIONS ON
4.4’-ISOPROPYLIDENEDIPHENOL (BISPHENOL A; BPA)

Incentives to substitute: the industry perspective (Jeffs, 2011)

Price pressures in recent years have resulted in low profitability and a high investment in automation. Manufacturers have a well-oiled machine in respect of their manufacturing and distribution supply chain and are unwilling to disrupt this.

It was agreed that client and regulatory pressure are two key levers to force the industry to act. Regulation does not currently restrict the use of bisphenol in thermal paper. In respect of client demand, there is currently only a very small demand from convertors for the bisphenol-free alternatives as most customers still demand the lowest priced products. Manufacturers have therefore not made a significant effort to market these papers for fear of cannibalizing their market share in what is a fiercely competitive market.

The current client and product profile of the manufacturers will to a certain extent determine how quickly they act in response to regulatory pressure/client pressure. Where manufacturers rely heavily on sales of non-top coated paper they are more exposed to regulatory/client demands for bisphenol-free paper as top-coated paper is already largely bisphenol-free. Overall it is expected that the market will take many years to move away from bisphenol, especially in developing markets.

INERIS, 2013 also indicates that in general, thermal paper manufacturers intent to keep BPA in some products (POS receipts for example) as far as there is no regulation. INERIS, 2013 indicates several incentives to substitute BPA in thermal paper (by decreasing order of claiming): a demand from customers, a marketing interest, a preoccupation concerning public health issues, an anticipation of regulatory aspects and an economic interest. Overall, marketing issues and a customers' demand seem to be currently the most important motivations to substitute BPA in thermal papers. Moreover, if the doubts concerning the compounds of the bisphenols family were confirmed, or if the regulation imposed a restriction, some manufacturers consulted would envisage a substitution solution towards non-bisphenols alternatives since they are available. According to them, these alternatives are currently partially already used because of quality aspects concerning some special applications.This is e.g. the case of D8 such as explained in section C.2 above.

Furthermore, according to an interview with the French Federation of retail companies (FCD), all the members of this federation use BPA-free POS receipts at least since 2011 (INERIS, 2013).

As regards the reluctancies to substitute, the higher cost of alternatives seems to be the major constraint to BPA substitution. Some manufacturers claimed that alternatives are more expensive, their availability is not sufficient, and that less information and fewer studies about their impacts are available compared to BPA. One also indicated that he has currently "no plan to replace BPA because this substance is the cheapest available raw material with sufficient quality for targeted usage". The second constraint to BPA substitution could be quality aspects.

From the industry perspective, ETPA's point of view provided in from the consultation (see section G) is that substitution of BPA would be currently difficult because:

- The 3 most 'obvious' alternatives (BPS, D8, Pergafast) are currently not available in sufficient quantities on the market and cannot replace completely BPA;
- It is impossible to replace directly BPA by one of these alternatives, which means that formulations have to be reviewed;

- If they have to produce thermal paper with different alternatives depending on the final application, they will have to change of chemicals all the time during the paper production process, which implies a loss of time and money; but as far as we understood from was communicated in the survey, this seems to be already the case given the variety of thermal paper qualities required by customers.
- For Pergafast there is only one producer which implies that the prices are high, and that he holds the whole market;
- Some printers cannot work with Pergafast because of quality issues.

ETPA concluded with the fact that BPS would be the easiest substance to be used to replace BPA if the regulation of thermal paper is changed. However, other information provided by large retailiers in particular during the consultation carried out by the DS indicates that, although BPS is technically and economically feasible and is already used as an alternative, it still may be expected that industry would not necessarily switch to BPS if it is expected that BPS will be regulated in the near future (INERIS, 2013).

C.4.2. Comparison of alternative techniques

The table below provides an overview of the alternative techniques assessed above.

Table 83. Comparison of alternative techniques selected and assessed

Alternative techniques		**Risks HH/ENV**	**Availability**	**Technical feasibility**	**Economic feasibility**	**Consumers acceptability**
Alternative printing techniques	matrix printing	No data	+↓	+	- -	+
	inkjet printing	No data	++	+	- -	+
	Laser printing	No data	++	+	- -	+
	thermal transfer printing	No data	++	++	-	+
Paper-Free techniques		No risks expected	+↑	+↑	++	-

The arrows express trends (↓: expected to decrease; ↑: expected to increase)

Although technical substitution with alternative printing techniques or free-paper alternatives may be in principle possible substitution solutions, it is deemed unlikely that direct thermal printing will be replaced, fast, largely and/or at an affordable cost by these solutions. In order to keep some proportionality of the analysis, it has thus been decided to only assess qualitatively the related impacts on these solutions expected on the supply chain further below.

D. Justification for action on a Community-wide basis

D.1 Considerations related to human health and environmental risks

It has been demonstrated that BPA might cause multiple effects on the health of unborn children due to their mother exposure such as effects on their reproductive system (for girls), their cholesterol (metabolism) and body weight, their spatial memory and learning functions and finally on effects on their developing mammary gland. These different health outcomes may express through very variable forms, from slight inconvenience due to more frequent menstruations to endometriosis, obesity or breast cancer and might affect the population targeted over their whole lifetime.

As the exposed population is female cashiers in the EU and as the population likely to develop the adverse health effects are their descendants, in principle, every EU country is concern by the risk generated by BPA in thermal paper.

Analysis of several hundreds of tickets and stakeholders consultation have demonstrated that BPA is still largely used as a dye developer in thermal paper, in particular in ecopaper used for point-of-sale receipts such as tickets and credit card receipts. From stakeholders and MSCA consultations, the share of BPA-containing thermal paper compared to the whole thermal paper is claimed to be between 70% and 100%. Moreover, it has been shown that BPA concentration in thermal paper is around 1-2% per weight. It seems to be the case for all EU countries. Further, it has been demonstrated that the BPA containing in thermal paper does migrate from the paper, especially from non topcoated and non protected ecopaper and may migrate on the cashiers and consumers' fingers while handling it. It has also been shown that BPA from thermal tickets or receipts might also be found on other objects in contact with them such as banknotes or wallets. Given all these data, the exposure of BPA from the handling of thermal paper by cashiers and consumers has been demonstrated.

D.2 Considerations related to internal market

The proposed restriction covers thermal paper that is extensively manufactured, traded and used among all EU countries. As shown in section B.2, thermal paper is also imported in the EU. None of the EU country has implemented yet any national legislation related to that product, although Sweden and Belgium have proposed a regulation for that purpose, however not adopted yet (see E.).

Beyond the considerations related to the risks for human health, the justification for acting on a Community-wide basis also originates from the need to prevent the EU Member States from adopting different legislative requirements which could be potentially in conflict and/or could create unequal market conditions. The proposed restriction under REACH would thus remove any distorting effect that national restrictions might have on the free circulation of goods on the common market. This equal treatment would create a level playing field for all EU manufacturers of thermal paper and all importers of thermal paper into the EU. A union-wide restriction would also give a clear message on the status of the requirements and would make

easier the communication of the different actors over the supply chain, especially to the suppliers outside the EU.

D.3 Other considerations

No other considerations.

D.4 Summary

The main reasons for acting on a Community-wide basis are related to the health extended risks it would remove and the equal treatment among producers and importers of thermal paper it would create on the common market.

E. Justification why the proposed restriction is the most appropriate Community-wide measure

This section provides justification for the reasoning that the proposed restriction is the most appropriate Community-wide measure. It gives an assessment of the effectiveness, practicality and monitorability of the proposed restriction as well as of other risk management options.

E.1 Identification and description of potential risk management options

E.1.1 Risk to be addressed – the baseline

The 'baseline' is the 'business as usual situation', that is, the situation in the absence of the proposed restriction or any further RMO, taking into account potential downward or upward trends.

In order to determine the baseline for that restriction proposal, it has to be clarified the 'business as usual' situation taking into account the different possible trends observed and expected on the thermal paper market, on the use of BPA in thermal paper, and more generally on the use of BPA.

Trends for the thermal paper market (Jeffs, 2011)

As shown above in section B.2, the thermal paper market is globally growing and as described in Jeffs, 2011, it is driven by increasing and decreasing forces.

On the one hand, a driving factor behind the success of thermal paper is the growth in global retail commerce. The increased use of bank cards over cash also increases the need for proof of purchase receipts, often a regulatory requirement. The low cost of direct thermal technology makes it especially attractive to developing markets. Furthermore, the technical advantages (reliability, low maintenance demands and non-dependence on peripherals) of thermal printing make it particularly attractive. Then, there is an exponential growth in the amount of information that is being printed. More and more receipts are being used as a vehicle for advertising. POS receipts are now often double sided, allowing advertising to be placed on the reverse side, with the front side containing much more than just the details of the items purchased. These printing processes create extra demands on the thermal paper and thus the quality of the thermal paper is of increasing importance. Travel is a growth market for thermal paper. Self-service terminals are increasingly being installed at airports in particular, but also at other central arrival and departure terminals for rail, ferry and bus. Most of these devices are equipped with thermal printers for tickets and baggage tags. Morevoer, there is an increasing trend towards printed 2D barcodes as information carriers, away from magnetic strips. Direct thermal printing is becoming increasingly popular for portable, mobile applications thanks to the compact technology used. The increased use of portable POS terminals, in restaurants e.g., makes the demand for thermal paper increasing. The higher use of laptops and smart phones which can connect to these devices also provides a further market opportunity for thermal paper. Finally, an increasing driver for thermal paper is technological. One of the early problems with direct thermal printing was that the paper would fade and curl with time or when exposed to heat, light, moisture or chemicals. Recent developments in coatings, both front and back, have meant that thermal paper can now be bought with a guaranteed 'non-fade' lifetime of upto 25 years. Thermal paper with resistance to chemical,

moisture and temperature extremes is also widely available on the market. This durability has increased the range of uses for thermal paper to include guarantees, proof of purchase, legal documents, expense reports, tax records and medical records. Many types of tickets, especially travel tickets for public commuter traffic, are prone to counterfeiting. The thermal paper manufacturers have responded with products which contain a range of security options including watermarks in the paper, colour inlays, UV-fluorescent fibres and UV-fluorescent security features under the topcoat. More recently, Ricoh have developed the first rewriteable thermal technology[42]. This allows images to be created and deleted through the controlled application of heat. Ticketing is a major potential application of this technology as the users travel period, zones, etc., can be changed without the need for the issuance of a new card.

On the other hand, the thermal paper market is also affected by decreasing driven forces. First, the thermal paper market endures tough competition (particularly from Asian countries whose the market grows faster) and (consequent) depressed prices. Then, this market is highly dependant on techological evolutions. A worldwide drop off in the 1990s for fax paper had significantly decreased the demand for thermal paper at that time. However, due to technological innovations such as described above and thanks to a high demand for other applications for thermal paper (such as POS ticketing), this drop off has been mitigated. Moreover, mobile payment technology, enabled through smart phone technology, is increasingly being used for all types of transactions, including payments and mobile transaction volumes are expected to grow in the future (Jeffs, 2011). This trend has been described in section C. Mobile banking is also set to become more mainstream which will have an impact on the number of ATM transactions undertaken and more paperless transactions in the future are expected, thereby reducing the demand for thermal paper. As a whole, resulting from these different technological evolutions, ETPA foresees a low sales increase by 3-6% per year during the 3 future years and then an interrogation remains about the evolution of new technologies that could have an impact on the thermal paper market development (payment by mobile phone, etc.), particularly on the thermal paper for POS applications. On the contrary, the thermal labels production is expected to increase because of e-market development (packages, etc.) (INERIS, 2013).

Overall, in spite of some defavourable factors, the thermal paper market continues to be a resilient, diversified and growing industry, pushing by positive and powerful drivers.

Trends in the use of BPA in thermal paper

The hazards of BPA and the risks caused by its use in thermal paper for the unborn child of women exposed (workers as well as consumers) are described and argumented above in section B. Without any restriction or further risk management measure to remove these risks, it can be expected that this population will keep on being exposed to BPA-containing thermal paper and thus being at risk. However, it can be expected that these exposures might still be reduced in the future due to the already ongoing substitution of BPA in thermal paper. To that respect, it has been shown above in section C that several 'drop in' dye developers are available, technically and economically feasible and some of them are already used in thermal paper in Europe and worldwide. This is particularly the case of BPS. BPA is still the widest used developer in thermal paper (around 70%) but given its toxicity and the repetitive attacks on

[42] http://www.ricoh.com/about/company/technology/tech/004.html

that ground from public opinion, medias and health and environment agencies, its substitution got started.

Moreover, some countries all over the world have already decided to restrict or ban BPA in thermal paper and some others are about to. Japan prohibited the use of BPA in thermal paper in 2001 and has phased out this use ever since. In Taiwan, BPA was banned in thermal papers in 2011. In the USA, some States have recently voted on Bills banning BPA in thermal receipts. Enacted in July 2011, Connecticut Senate Bill 210 prohibits the manufacture, sale or distribution of thermal receipt paper or cash register receipt paper containing BPA. The restrictions take effect by October 2013, unless the U.S. Environmental Protection Agency does not identify a safe alternative to BPA in these products, in which case the restrictions take effect by July 2015[43]. Other States such as Maine and Illinois are considering the adoption of the same position. Besides, the largest thermal paper producer in the USA (Appvion) substituted BPA from its thermal paper formulation with BPS in 2006, because of growing concern about the safety of BPA[44].

As regards the EU, three countries have already taken position and/or action on that particular use. In Belgium, a proposal for a law to ban BPA in receipts and credit card receipts was submitted to the Belgian Senate in 2010 but not achieved so far. In Sweden, the Government referred to the KEMI in an attempt to evaluate the risks of BPA in thermal receipts, to identify and evaluate the dangers of its alternatives, and to develop a proposal for a law to ban BPA in thermal paper (Kemi, 2013). Then, Kemi has prepared a national provision for banning this use, suggested to be implemented in the Swedish Environmental Code 1998:808. The proposal is currently on hold by the Swedish authorities which are awaiting this REACH Restriction proposal (Kemi, 2013). Finally, consequently to the risk assessment report on the risks caused by BPA-containing thermal paper published by ANSES in 2013 (ANSES, 2013), the French authorities decided to propose hereby a restriction under REACH.

Overall, several legislations are already in place or in the pipeline regarding the use of BPA in thermal paper all over the world and in the EU. BPA is being phased out in several countries and voluntarily by some manufacturers. As a consequence, the trend in use of BPA in thermal paper is thus globally decreasing. This ongoing decrease will surely make the exposures to BPA reduced in the near future. To what extent this decrease will be fast or significant without any regulatory obligation remains however uncertain.

Trends in the general use of BPA

Additionnally to the positions or actions already taken by some countries regarding specifically the use of BPA in thermal paper, BPA is also targeted by many other regulations as to its other uses worldwide (such as in food contact materials in particular), such as shown in section

[43] http://www.cga.ct.gov/2011/act/pa/2011PA-00222-R00SB-00210-PA.htm

[44]http://www.appvion.com/en-us/documents/historical%20news/appleton-bpa-free-news-release.pdf

B.9.1. Furthermore, several risk management processes are currently planned under REACH or CLP at the EU level: BPA has been evaluated under REACH by Germany in 2012-2013, based on hazards and environmental exposure and the French CLH proposal on BPA reprotoxicity (reprotox 1B) is currently being evaluated by the Risk Assessment committee of ECHA.

As a result of these actions and positions, BPA is more and more 'blacklisted'. Industry thus tends increasingly to show a turn towards general substitution of BPA. It has however to be emphasized and reminded that without any regulatory pressure, many market actors remain reluctant to switch to substitutes, mainly based on economic grounds (see section C).

Business as usual situation

In conclusion, the market of thermal paper is expected to keep on growing at a rate nonetheless dependent mostly on the evolution of other ticketing/payment technologies. This application widely uses BPA today as a dye developer but, due to upcoming legislations on BPA and already ongoing substitution, this use might tend to decrease in the future. As a result of those trends, the baseline for that restriction proposal is defined by the following characteristics:

- A growing market of thermal paper
- An expected 'spontaneous' decreasing in the use of BPA in thermal paper
- An already underway substitution of BPA by alternative developers in thermal paper
- A need for regulatory incentive to ensure a full phase out of BPA in thermal paper in spite of existing voluntary actions on the market

E.1.2 Options for restrictions

Two options for restriction are explored and further assessed in section E.2.

RMO 1 (the proposed restriction): limitation of the concentration of BPA in thermal paper

The proposed restriction will ban the use of BPA in thermal paper into the EU within the limit of the concentration set, as analysed with one of the currently available methods, such as listed en presented below in E.2. The transitional period proposed for the entry into force of the restriction is 3 years (36 months).

The restriction proposed covers the new thermal paper placed on the EU market after this sunset date. As shown in section B.2, thermal paper is used in many applications such as point-of-sales (POS) tickets and receipts, self-adhesive labels, lottery tickets or fax paper. In principle, all applications are likely to contain BPA although information collected during the elaboration of this proposal indicates that the POS applications mainly contain BPA. These applications stand for around 65% of the thermal tickets placed on the EU market and seem to represent the main source of BPA exposure from thermal paper for workers and consumers. Indeed, this type of tickets and receipts are made with relatively low quality thermal paper, namely 'ecopaper', without protective topcoating, so that the BPA contained in the thermal coating layer migrates easily to the fingers or any objects in contact with it. With respect to top coated thermal paper (or 'protected thermal papers') most often used for transportation tickets, cinema tickets and adhesive labels (food packaging, etc.), for example, BPA seems to not having been used since 2000 according to a communication from a French manufacturer of top coated thermal paper. However, this claiming is not supported by any available study.

Moreover, although topcoatings might reduce the migration of BPA from the tickets, it cannot thus be excluded that BPA still migrate from them and might generate some risk. For these reasons, the restriction proposed herein aim to cover all types of thermal paper, from point-of-sales applications (namely 'ecopaper') to topcoated 'protected' thermal applications. Nevertheless, due to a higher amount of information collected for POS receipts, the exposure and risk assessments as well as the socio-economic analysis have been carried out for these specific applications. Moreover, from a control and enforcement perspective, it would be difficult to distinguish between thermal papers produced for one application or another, especially because 'thermal paper' is not explicitely defined and categorized as such in the existing nomenclatures for products and articles (Prodcom and TARIC in particular). This information has been confirmed by the DGCCRF consulted (see section G.3).

The restriction proposed does not cover the 'second-hand' market for thermal paper since it cannot be really considered that a second-hand market exist for that kind of product. Thermal tickets and receipts are not strictly speaking 'articles'. After issuance, they are usually either rapidly thrown away by consumers, either filed in their personal documents. They are a support for information which can be subsequently used for a claiming or as a guarantee but they don't have any utility or market value *per se* (initial jumbo rolls do but not the final issued tickets). Therefore, they are not sold or exchanged on a second-hand market such as other consumption goods. However, it can be expected that old BPA-containing tickets and receipts which end up into homes files or pockets for a long period of time might still be a source of exposure for delayed handling. This exposure should only concern consumers since it is not expected that old tickets or receipts remain at workplaces such as cashier station after printing. These tickets and receipts are known to be degraded with time (characters and images fade), after several years or even less (especially for eco-paper), but it does not mean that BPA vanishes from them after a certain period of time. It has been shown that the BPA contained in thermal paper may contaminate other papers, objects or surfaces it may be in contact with (see section B), causing a so-called secondary contamination. The extent to which the BPA contained in one (eco-paper) ticket remains over the ticket or spreads around is highly dependent on its storage conditions. As a consequence, old tickets and receipts containing BPA which have been filed, forgot or lied around in homes might in principle still be a source of human contamination after the entry into force of the proposed restriction. Nevertheless, it can be also expected that the risk generated by this residual exposure might be rather unsignificant given the fact that the frequency of their handling might be rare. This residual exposure is expected to some lesser extent from thermal paper such as protected paper (labels, secured tickets, etc.) since they are topcoated with a protective layer and thus much less likely to migrate.

This restriction would impact primarily EU manufacturers of thermal paper or importers of thermal paper into the EU who would have the responsibility for making sure that their products do not contain BPA above the compliant concentration limit (or as shown in section E.1.2.1, do not contain BPA at all). It would also impact control authorities, importers and retailers.

This option is further assessed in section E.1.2.1. as regards its effectiveness, practicality and monitorability.

RMO 2: limitation of the migration of BPA in thermal paper

Another option for restriction examined hereunder consists in limiting the migration of BPA from thermal paper placed on the EU market by setting a migration limit.

The transitional period remains unchanged as for RMO 1. Equally, this option for restriction would cover the new thermal paper placed on the EU market after the sunset date and not any 'second-hand' market.

This restriction would also impact primarily EU manufacturers of thermal paper or importers of thermal paper into the EU who would have the responsibility for making sure that the BPA contained in their products does not migrate above the compliant migration limit. It would also impact control authorities, importers and retailers.

This option is further assessed in section E.1.2.2. as regards its effectiveness, practicality and monitorability.

A third option for restriction had initially been thought to be developed: a REACH restriction with a wider scope including a grouping of all bisphenols likely to be used in thermal paper. Given the fact that the other bisphenols identified and assessed in section C as possible alternatives may have the same adverse properties and effects on human health as BPA does, this option for restriction could have been of great interest and consistency. This even restriction proposal could have been scoped in that way. It would have guaranteed the non-replacement of BPA by other dye developers, such as BPS particularly, suspected to be as much as toxic. However, due to the current lack of toxicological data on some bisphenols' profile on the one hand (expected to be partially filled in by the 2014 BPS SEv by BE), and taken into account that risks from BPA in thermal paper have already been demonstrated, this option has been discarded and this proposal focuses on BPA only.

E.1.3 Other Community-wide risk management options than restriction

Other possible community-wide risk management measures than a REACH restriction are outlined in the table below. Two will be selected to be further assessed. For the other ones, it is concluded that none can be considered as realistic, effective or proportionate to address the risk targeted herein. They have not been then further assessed.

Table 84. Possible other community-wide options to address the risks targeted

Risk Management Option	**Content and Reasons for discarding/not discarding this RMO**

Risk Management Option	**Content and Reasons for discarding/not discarding this RMO**
SVHC Identification/REACH Authorisation	BPA is not yet identified as an SVHC. Authorisation under REACH would concern all uses of BPA and wouldn't be proportionate to address only the specific risks addressed herein. Moreover, BPA-containing thermal paper that is imported into the EU wouldn't be covered by the REACH authorisation procedure. The exposures and risks related to this residual thermal paper would thus remain into the EU. Conclusion: this RMO has been discarded and is not further assessed
Voluntary industry agreement	In spite of the repetitive attacks against BPA from public opinion, medias and health and environment agencies all over the world the last past years, the few emerging voluntary initiatives haven't lead to a significant reduction of the use of BPA in thermal paper. Although substitution of BPA is underway, the spontaneous incentives of the market to phase out might not be strong enough without regulation. Conclusion: this RMO has been discarded and is not further assessed

Risk Management Option	**Content and Reasons for discarding/not discarding this RMO**
Information to end-users/retailers, workers and consumers incl. labelling	The message could be: *To end-users/retailers – avoid handling/not to be handled by workers/cashiers.* This RMO does not seem to be sufficiently effective as it needs to be controlled by the competent authorities, it will be very expensive etc. *To workers - avoid handling/not to be handled without skin protection.* This RMO is highly dependent on voluntary labelling from thermal paper manufacturers/convertors and on information communicated by the endusers to their employees (assuming that the latter possesses the information). Unless the information is perfectly disclosed along the supply chain down to the workers, this RMO wouldn't fully address the risk. *To consumers – avoid handling/not to be handled without skin protection. Again, this RMO is highly dependent* on voluntary labelling from thermal paper manufacturers/convertors and on information communicated by the endusers to the consumers. Unless the information is perfectly disclosed along the supply chain down to the consumers, this RMO wouldn't fully address the risk. <u>Conclusion</u>: this RMO has been discarded and is not further assessed

Risk Management Option	**Content and Reasons for discarding/not discarding this RMO**
Regulatory requirement for pregnant workers to wear protective gloves	This requirement could be added under the Directive 98/24/EC on the protection of the health and safety of workers from the risk related to chemical agents at work and Directive 89/391/EC "Framework Directive" on the introduction of measures to encourage improvements in the safety and health of workers at work -discriminatory measure among workers -would not protect workers who ignore their pregnancy -would not protect workers who have not declared their pregnancy yet or who wouldn't like to -would not protect consumers <u>Conclusion</u>: this RMO has been discarded and is not further assessed
Regulatory requirement for workstation re-layout	Most of the time, the cashier's workstation is lay-out in such a way that the printing device is located on her right-hand-side and the consumer who then gets the ticket or receipt is located in front of the cashier on her left-hand side. As a result, in the most common situation, the cashier gets the ticket from the printing machine with her right hand, and then gives it to the consumer who is on her left-hand side. A re-layout of the workstation could consist in placing the printing device on the left-hand-side on the cashier, so that the consumer could get it back himself/herself without the cashier intermediary. -would not be economically suitable -would not protect the consumers <u>Conclusion</u>: this RMO has been discarded and is not further assessed

As a whole, 2 RMOs are further assessed in the following section E.2: the REACH restriction proposed (named RMO 1) and an alternative option for REACH restriction (RMO 2).

E.2 Assessment of risk management options

E.2.1 RMO 1: restriction option 1 – limitation of the concentration of BPA contained in thermal paper (the proposed restriction)

The RMO 1 corresponds to the restriction proposed. It consists in banning the use of BPA in thermal paper within the EU in the limit of the concentration set, as analysed in accordance with the existing methods (such as explained below in section E.2.1). This limit is proposed to be set at 0.02% by weight of thermal paper.

It has been shown above that the concentration of BPA in thermal paper is currently optimized and fully adjusted to the functional characteristics targeted for each specific end-use (printing durability, speed, printing device, etc.). This information has been got from industry consultation (INERIS, 2013). As a result, the BPA content currently present in thermal paper can be considered as the content which garantees the technical efficiency of the thermal paper. As a consequence, the low concentration limit proposed herein (0.02% by weight) can be considered as a technical hindrance to the manufacture of thermal paper which could no longer be produced efficiently.

The restriction is thus equivalent to a total ban of BPA in thermal paper.

E.2.1.1 Effectiveness

Effectiveness is defined such as the RMO must be targeted to the effects or exposures that cause the risks identified, capable of reducing these risks to an acceptable level within a reasonable period of time and proportional to the risk (ECHA, 2007).

E.2.1.1.1 Risk reduction capacity

The proposed restriction is considered to be the most appropriate measure from a risk reduction capacity perspective.

E.2.1.1.1.1 Changes in human health risks/impacts

The restriction proposed would significantly reduce the risks to human health demonstrated in section B. for the 4 critical effects described. The concentration limit is set very low for that purpose. Indeed, it has been shown that while handling BPA-containing thermal paper, pregnant workers and consumers expose their unborn child to adverse effects for their reproductive system (for females), metabolism and body weight, brain and behaviour and their mammary gland. It has also been demonstrated that any concentration of BPA in thermal paper, in particular in eco-paper receipts which are not topcoated, would in principle migrate from the paper and thus adversely expose the targeted population. This thus implies that there is no 'safe' concentration level of BPA for that particular use. As a consequence, for protective purposes, the choice has been made to propose the lowest limit as possible, in line with the detection limits of BPA. There is currently no standard analytical method to detect BPA in thermal paper. The limit has thus been set at the average of the detection limits of the

different existing methods. Referring to Table 11 above, the average of the LD is thus calculated at 0.02%, considered to be the lowest and the safest limit.

It has to be noted that a limit of BPA expressed in units of paper surface had been considered. However, for enforcement reasons, this possibility has been discarded.

Until the sunset date, exposure and risk will however continue to occur with notwithstanding a decreasing trend, as explained above in the baseline description (section E.1). After the entry into force of the restriction proposed, all kind of thermal paper containing BPA will be removed from the EU market. As a result, the risks for human health are expected to come down close to zero, taking into account the possible residual (but considered as low) source of exposure from the old tickets and receipts likely to remain in homes (see section E.1.2 for more details).

This risk and exposure reduction will occur for workers as well as for consumers and is deemed to be maximized with this RMO.

As regards the associated health benefits, they are assessed in section F.1. They correspond to the costs avoided due to the reduction in adverse effects and diseases such as described in section B. As a whole, **the total health benefits expected from the proposed restriction are estimated to range <u>at least</u> from €3.5 million to €5.2 million in 2019 (discounted) value, keeping in mind that <u>not all health benefits have been qualitatively assessed</u>. The total benefit of the restriction are thus expected to be higher** and these figures have to be interpreted as a minimum range of benefits. Moreover, some uncertainties surround the human heath impact assessment carried out and a sensitivity analysis has been developed in section F.1.1. in order to make the assessment qualify and transparent. Furthermore, not all health benefits have been quantified. Therefore, these figures have to be interpreted as

Nevertheless, when it comes to the consideration of the human health risks likely to be cause by the substitutes that might replace BPA after the restriction has been implemented, this conclusion could be jeopardized. Indeed, it has been shown in section C that several alternatives to BPA are available and technically and economically feasible for being used in thermal paper. Some are less hazardous than BPA. However, BPS could be one of the most expected candidate, being rather cheap and already used as a dye developer in thermal paper. To that respect, the conclusion above would not be that clear-cut if BPS was actually the alternative selected by the market and if it was proven as much as toxic. However, it has to be reminded that information provided by large retailiers in particular during the consultation carried out by the DS indicates that, although BPS is technically and economically feasible and is already used as an alternative, it still may be expected that industry would not necessarily switch to BPS if it is expected that BPS will be regulated in the near future (INERIS, 2013).

Finally, a collateral benefit from the restriction could also be due to the reduction in risks for workers exposed to BPA on the production chain of BPA-containing thermal paper (UK, 2008).

E.2.1.1.1.2 Changes in the environmental risks/impacts

Environmental exposure is not strictly at concern in that dossier but some indirect environmental impacts can still be expected from the restriction proposed.

Indeed, it has been shown above (section B.1) that BPA in thermal paper could be the source of secondary contamination of foodstuffs and objects in contact with tickets or receipts such as banknotes (EWG, 2010, Liao, 2011) and wallets. Moreover, thermal paper is currently recycled in the EU up to 50% (see section B.2) and is re-used to produce other paper-based products such as recycled paper, napkins, toilet paper, paper towels, newspapers or magazines (Gehring, 2004).. Those products might thus contain BPA traces. The secondary contamination and the BPA traces coming from paper recycling contribute to the general population exposure to BPA via the environment and would thus be avoided by the restriction proposed.

Moreover, as far as environment itself is concerned, it has been shown above that the recycling of thermal paper containing BPA is suspected to be one of the main sources of contamination via aqueous effluent recycling containing BPA-chlorinated derivatives or sludge from sewage purification plants (UBA, 2010). It is estimated that about 350-500 tons/year of BPA enter the recycling supply sector, which stands for 70% of total annual aquatic releases (EU RAR 2008, OECD, 2009 , INERIS, 2010) (see section B.9.3.2.4). These releases would also be avoided by the restriction proposed.

E.2.1.1.2 Proportionality

E.2.1.1.2.1 Economic feasibility

It has been shown above that the concentration of BPA in thermal paper is currently optimized and fully adjusted to the functional characteristics targeted for each specific end-use. As a consequence, the low concentration limit proposed herein (0.02% by weight) can be considered as a technical hindrance to the manufacture of thermal paper which could no longer be produced efficiently. As already said, the proposed restriction is thus equivalent to a total ban of BPA in thermal paper.

Raising the question of the economic feasibility of the restriction proposed comes to raising the question of the economic feasibility of substitution and compliance control.

Costs

As regards the economic feasibility of substitution, thermal paper manufacturing already uses other dye developers than BPA for quality paper that require specific thickness and/or security and protection features (self-adhesive labels, museum tickets, etc.) as well as for ecopaper (cash tickets or receipts). It has thus been shown that, on the one hand, manufacturers of thermal paper in the EU are already diversified as regards the range of developers they use in their formulations, and on the other hand, that 'drop-in' alternatives exist, are available and technically and economically feasible (section C). Some of them, such as BPS in particular, are even already increasingly used in replacement to BPA. This situation allows thus expecting the restriction proposed as economically feasible. Indeed, from a cost perspective, as section F.2 shows, the restriction proposed is expected to mainly cause economic impacts to the manufacturers of thermal paper. This segment of the supply chain is deemed to be the most affected since manufacturers will have to replace BPA in their coatings (formulated on site or purchased already prepared) with other dye developers. This substitution is costly given the fact that the prices of all alternatives are all higher than the price of BPA. However, the range of prices is rather large between alternatives and BPS e.g. is only slightly more expensive.

Moreover, it can be expected that following the adoption of this restriction, the demand of alternative dye developers will dramatically increase, pushing their prices downward. **As a whole, based on 3 scenarios (min, max and medium), and a share of BPA-containing thermal paper in the EU of 70%, the chemical substitution cost for the replacement of BPA by manufacturers of thermal paper is estimated to range from around €0.7 million (min) and €61 million (max, probably overestimated) with a (more realistic) average estimate between €1 million and €25 million per year over the period 2019-2030.** A sensitivity analysis has been carried out on several parameters in particular on the share of BPA-containing thermal paper placed on the market today. In any case, BPS is shown to be the most affordable alternative.

The impacts on the markets of alternative printing techniques and free-paper alternatives are only qualitatively analysed in section F.2. Based on the available information and from stakeholders consultation, the former would imply a much higher –probably prohibitive- cost and wouldnot meet all technical requirements expected by endusers. The latter is cheaper but not expected to be adopted at very large scale at short or medium-term and its evolution is deemed too uncertain to be considered as a realistic and fast-implemented alternative.

The costs impacts on the other segments of the supply chain (manufacturers of BPA upstream and end-users downstream) are qualitatively assessed and considered to be unsignificant.

As regards the economic feasibility of the compliance, it refers to the costs associated with compliance control, that is, the testing of BPA content in thermal paper after the entry into force of the restriction. Testing would be required primarily from the control authorities which will have to control the BPA content in the thermal paper produced and placed on the EU market as well as the thermal paper imported into the EU.

Then, testing could be required from EU manufacturers of thermal paper who would keep on using BPA in their product while being compliant to the concentration limit proposed. However and as already mentioned, at the very low level of the limit proposed, the thermal paper could no longer be efficient and the the concentration limit of 0.02% is thus considered to be equivalent as a total ban of BPA in thermal paper. As a consequence, if EU manufacturers no longer use BPA in their products after the entry into force of the restriction, they won't have in principle to test them. It cannot be excluded from the reasoning that at least theoretically, a mixture of 0.02% of BPA with another dye developer could be formulated by manufacturers of thermal paper in order to comply with the new restriction while keeping on using BPA. Nonetheless, there is no indication from the research carried out neither from the stakeholder's consultation that could make think that it is technically possible. Moreover, it appears doubtful that a so tiny quantity of BPA would be kept in the production process of thermal paper for economic reasons. In general, it is thus expected from the EU manufacturers of thermal paper that they will phase out from BPA and switch to substitutes after the entry into force of this restriction.

As regards convertors and traders (distributors) of thermal paper in the EU, they will have to be sure that the thermal paper they make entered the EU market and they distribute is compliant and may have to carry out some tests. **As a whole, the compliance control costs for manufacturers and convertors are estimated between €146,255 and €254,472 per year over 2019-2030, based on the existing methods possibly used to measure the content of BPA.** Those costs are likely to be split to some extent between convertors and traders.

However, given the concentrated (oligopolistic) structure of the production market in the EU, it can be expected that convertors, traders and manufacturers have trust and transparent relationships which may make the information disclosure on products (ecopaper and other types) easy along the supply chain. Taking this aspect into consideration, the compliance control costs assessed might be largely overestimated.

The compliance control costs made by the importers of thermal paper in the EU have not been assessed due to the lack of data on the imported volume of thermal paper. However, the assessment of the compliance costs provided in section F.2. gives some order of magnitude of these costs.

Overall, the costs of the restriction proposed for the thermal paper market (substitution and compliance control costs) are estimated to range from around €0.9 million (low range) to around €61.3 million (high range, probably overestimated) with a more realistic average estimate between €1.2 million and €25.3 million per year over 2019-2030. These average costs stand for between 0.18% and 4.60% of the total production value of thermal paper manufactured for POS applications over 2019-2030.

However, 42% of this production is exported outside the UE. Depending on whether the manufacturers will pass on the extra costs entirely on the non-EU customers or not, the substitution costs would be actually only 42% of the costs presented herein.

Timing

The restriction proposed includes a transition period enabling the market to adjust. The transition period has to take depletion of stocks into account. As for the length of this transition period, a balance must be found between the need for protecting human health and the possibility for the market to reach compliance. Given the fact that BPA is already being substituted in thermal paper and that 'drop-in' alternatives are available and technically and economically feasible, a transition period of 3 years is considered as being appropriate.

Cost and Benefits Ratio

The Costs and benefits ratio and the proportionality of the restriction proposed are closely linked to the choice of the substitute. BPS could be the most obvious alternative particularly in terms of its technical and economic feasibility.,If Industry replaces BPA with BPS after 2019 and assuming that BPS shows similar effects on human health, the expected benefits of the restriction would be compromised. However, although BPS is technically and economically feasible and is already used as an alternative to BPA in thermal paper, from the information collected from industry and market surveys (from large retailers in particular), it still may be expected that industry would not necessarily switch to BPS if it is expected that BPS will be regulated in the near future. Taking into account this situation, two "non-use scenarios" can be set:

1. Non-Use Scenario 1: Industry will switch to BPS to replace BPA after the entry into force of the restriction:
    - the human health benefits expected from the restriction might be **close to zero** (assuming that BPS is as much toxic as BPA and has similar effects on human health)
    - the total costs of the restriction are expected to be between €1.1 million and €1.9 million **with a medium and more realistic cost of €1.4 million (substitution costs of BPS + control costs)**

   Under scenario 1, the costs would outweigh the benefits and the restriction would be hardly considered as proportionate.

2. Non-Use Scenario 2: Industry will switch to non-bisphenol alternatives to replace BPA after the entry into force of the restriction:
    - the human health benefits expected from the restriction exceed zero and are estimated **between (at least) €3.5 million and €5.2 million** due to the non-use of BPA. The potential adverse effects for human health of these alternatives are not included and could affect downward these benefits but given that non-bisphenols seem to be largely safer for human health, the benefits are not expected to be significantly impacted. Moreover, given that not all health benefits expected from the restriction of BPA have been evalued, **the total benefits from the restriction are expected to be higher**.

    - the costs of the restriction are expected to be between €9.7 million and €61.2 million **with a medium and more realistic cost between €19.3 million and €25.3 million (substitution costs of D8 or Pergafast + control costs), with a probably overestimated upper bound due to the high uncertain maximum price of Pergafast (based on only one not-checked claim from Industry and qualified by information provided by the public consultation).**

   Under scenario 2, taking into account all these considerations, the benefits may outweigh the costs under reasonable assumptions for which the restriction may thus be deemed proportionate.

The table below summarized these two scenarios.

	Human health benefits (B)	Costs (substitution+control) (C)
Scenario 1 (BPS)	(likely) ≈ 0	medium cost = €1.4m
Scenario 2 (non-bisphenol alternatives – D8 and Pergafast)	> €3.5 million and €5.2 million (not all benefits quantified and valued)	medium cost= [€19.3m; €25.3m] (upper bound likely to be overestimated)

E.2.1.1.2.2 Technical feasibility

Taken as granted that the proposal is *de facto* a total ban of BPA in thermal paper, its technical feasibility has thus to be analysed as regards the technical feasibility of the substitution. As shown in section C and below in section F.2, there are available and technically and economically feasible chemical alternatives and some of them are already used as a dye developer in thermal paper. There might not thus be any significant changes needed in technical process or equipment except some adjustments related to reformulations of thermal layer coatings. This information has been confirmed by the stakeholders consultation. As a consequence, the restriction proposed, considered as a total ban of BPA in thermal paper and thus as a regulatory incentive to substitute, is deemed to be technically feasible.

E.2.1.2 Practicality

Practicality is defined such as the RMO must be implementable, enforceable and manageable (ECHA 2007).

E.2.1.2.1 Implementability

Implementability implies that the actors involved are capable in practice to comply with the RMO. To achieve this, the necessary technology, techniques and alternatives should be available and economically feasible within the timeframe set in the RMO (ECHA 2007).

As regards this criterion, industry actors concerned by the proposed restriction should be capable of complying with the requirements in practice since concentration tests (although no EU standard method exists) and alternatives are available and technically and economically feasible. As already described, the supply chain of thermal paper manufacturing is concentrated around a small number of actors in the EU. The producers in particular are few and large and are thus not expected to encounter major difficulties to comply with the new obligations. The only SMEs likely to be concerned by the new restriction are retailers such as corner shops who will have to buy BPA-free thermal paper rolls for their receipts and tills. However, it is considered that they shouldn't face major additional costs (due to higher prices of thermal paper) the cost of the rolls they buy from distributors is likely to be a very tiny share of their total operating costs and consumables (see section F.2).

RMO 1 is considered as implementable.

E.2.1.2.2 Enforceability

Enforceability means that the authorities responsible for enforcement need to be able to check the compliance of relevant actors with the RMO. The resources needed for enforcement have to be proportional to the avoided risks (ECHA 2007).

As explained in section F.2, there is no standard analytical method to measure the content of BPA in thermal paper today in the EU but several methods still exist to measure BPA in other materials and could be used for that purpose. Those methods are listed and presented in section F.2. The establishment of an EU standard method could make the routine implementation of these tests easier but it would also take time and money. Therefore, given that methods still exist, the absence of an EU standard analytical method is not considered as a hindrance to the enforceability of the proposed restriction.

The restriction proposed is thus deemed to be enforceable.

E.2.1.2.3 Manageability

Manageability supposes that the RMO should take into account the characteristics of the sectors concerned (for instance, the number of SMEs) and be understandable to affected parties. The means of its implementation should be clear to the actors involved and the enforcement authorities and access to the relevant information should be easy. Furthermore, the level of administrative burden for the actors concerned and for authorities should be proportional to the risk avoided (ECHA 2007).

The means of implementation of the proposed restriction (concentration tests, substitution of BPA, etc.) are clear and understandable to the actors involved, in particular because substitution of BPA in thermal paper is already underway and the information about the concerns of BPA seems to circulate smouthly along the supply chain, at least down to the distributors. As regards the endusers, in particular the SMEs such as corner and unipersonal shops, some effort may be needed to access to this information from their suppliers.

An issue dealing with the manageability of the restriction could however be related to the fact that there is no EU standard method to measure BPA content in thermal paper and the market actors would have thus to get some information and put additionnal training efforts in order to be able to carry out the compliance tests needed. This is mainly the case of manufacturers of thermal paper and SMEs shouldn't be affected.

E.2.1.3 Monitorability

Monitorability is defined such as it must be possible to monitor the results of the implementation of the RMO. Monitoring is understood widely and may cover any means to follow up the effect of the RMO in reducing the exposure. The most appropriate means of monitoring depend on the type of measure and on the related conditions. Such monitoring may include, for example, follow up of the amounts of substance manufactured and imported, follow up of the amounts of substance used for different uses, measuring of the concentration of the substance in preparations or articles, measuring of the relevant emission and/or exposure levels, etc (ECHA, 2007).

Stakeholders involved in the monitoriability activities are the authorities responsible for the enforcement of the REACH regulation in the different EU Member States (control authorities and customs services) and the laboratories in charge of performing the tests.

Given that several existing analytical methods could be used to measure BPA content in thermal paper (although no standard exists), the restriction proposed is considered to be monitorable by control authorities and customs services. However, as regards monitorability there might be some concern about the exact product to be monitored since no specific existing TARIC (or Prodcom) code is attributed to 'thermal paper'. This information has been confirmed by the DGCCRF consulted (see section G.3).

Several TARIC codes could in principle cover 'thermal paper'. There could be:

TARIC Code	**Description of corresponding goods**
481190	Other paper, paperboard, cellulose wadding and webs of cellulose fibres (under the code 4811: Paper, paperboard, cellulose wadding

	and webs of cellulose fibres, coated, impregnated, covered, surface-coloured, surface-decorated or printed, in rolls or rectangular (including square) sheets, of any size, other than goods of the kind described in heading 4803, 4809 or 4810)
4823	Other paper, paperboard, cellulose wadding and webs of cellulose fibres, cut to size or shape; other articles of paper pulp, paper, paperboard, cellulose wadding or webs of cellulose fibres
4821	Paper or paperboard labels of all kinds, whether or not printed
480220	Paper and paperboard of a kind used as a base for photosensitive, heat-sensitive or electrosensitive paper or paperboard (under the code 4802: Uncoated paper and paperboard, of a kind used for writing, printing or other graphic purposes, and non-perforated punchcards and punch-tape paper, in rolls or rectangular (including square) sheets, of any size, other than paper of heading 4801 or 4803; handmade paper and paperboard)

Costs of the monitoring consist in control costs. They are the same type of costs as borne by convertors, distributors in the EU as well as importers to test the thermal paper they will make entered the EU market, such as described and evaluated in section F.2.

E.2.1.4 Overall assessment of RMO 1

The overall assessment of RMO 1 for restriction is summarised at the end of this section. The restriction proposed is deemed to be proportional, considering the medium and more realistic scenario, the overestimated upper bound of costs and taking into account the comparison of costs with the total production value of thermal paper. Feedbacks from MSCAs and EU health and environment institutes consulted seem to recognise its effectiveness, its practicality and monitorability, with some interrogation regarding the definition of thermal paper to be monitored (MSCA consultation). Although some industry actors surveyed do not see any risk from thermal paper, they all confirm that alternatives are available and substitution already well engaged (INERIS, 2013).

E.2.2 RMO 2: restriction option 2 - limitation of the migration of BPA from thermal paper

This option has been considered quite early in the process of elaboration of this restriction dossier. Indeed, since the risk assessed herein comes from the exposure to BPA via the dermal contact with thermal paper, the BPA migration rate can be considered as the most relevant indicator to describe potential exposure from the thermal paper handling. However, such option does not seem to be the most appropriate for several reasons which are explained below.

E.2.2.1 Effectiveness

E.2.2.1.1 Risk reduction capacity

E.2.2.1.1.1 Changes in human health risks/impacts

No correlation could be determined between the quantity of BPA likely to end up onto the fingers and the quantity of BPA contained (and likely to migrate) into the thermal ticket or receipt handled. It is thus difficult to define a 'safe' level of BPA content that would allow no migration or 'safe' migration from the thermal paper. The only way to limit the migration of BPA and ensure the reduction of the risks addressed herein would be either to limit the content of BPA as much as possible (this is what RMO 1 proposes), either to create some technical 'barrier' to BPA migration onto or into the thermal paper itself. The technical and economic feasibility of this solution is analysed below.

If this technical 'barrier' was theoretically feasible, in principle, the exposures and risks would be reduced and the changes in human health risks (and the associated health benefits) could thus be considered as of the same order of magnitude of the ones assessed for RMO 1. A limit to that conclusion is however that the efficiency of that 'barrier' couldn't be checked by any available study so far. As a consequence, although topcoatings might reduce the migration of BPA from tickets, it cannot thus be excluded that BPA still migrate from them and might generate some risk.

E.2.2.1.1.2 Changes in the environmental risks/impacts

Depending on the level of the limit of BPA migration that would be set under such an option for restriction, the changes in environment would be expected to be from low up to (at best) the same as for RMO 1. However, as shown in the previous section, the only way to be sure that the risks for human health would be reduced would consist in implementing a technical 'barrier' to BPA migration onto or into the thermal paper itself. In that context, BPA would still remain in the matrix of the paper and releases of BPA to effluents from thermal paper recycling would not consequently be removed.

E.2.2.1.2 Proportionality

E.2.2.1.2.1 Economic feasibility

Costs

From ETPA consultation, it appears that there does not exist any manufacturing technique able to remove migration of BPA from thermal paper. They indicate however that a 'protected' thermal paper (such as used today for most of the self-adhesive labels or for tickets that

require security features agains counterfeiting such as museums or transport tickets), which is topcoated, could stand theoretically for a technical 'barrier' to migration of BPA.. Nevertheless, additionally to the uncertainty of the actual efficiency of this 'barrier' to reduce the exposure, applying such a protection on all types of thermal paper, especially on (cheap) ecopaper which is widely used for POS receipts would not be economically feasible. Keeping in mind that POS applications represent about 65% of thermal ecopaper end-uses in the EU today, such a constraint would probably imply a significant cost for industry. This cost has however not been quantified.

As regards compliance control costs associated with RMO 2, they refer to the testing of BPA migration in thermal paper after the entry into force of the restriction. These costs are formally similar to the compliance control costs associated with RMO 1, except that the migration of BPA would have to be tested instead of the content. The migration tests would be required from the control authorities, from manufacturers of thermal paper and from importers and retailers. Control authorities (national authorities and EU customs services) will have to control the quantity of BPA migrating from the thermal paper produced and placed on the EU market (including the imports into the EU). Likewise, if the migration limit set allows the manufacturers of thermal paper to keep on using (even a bit less) BPA in their products (while limiting technically its migration), they will have to test their paper to be sure to comply with the new requirements. Finally, the importers and retailers will have to get the guarantee that the thermal paper they make entered the EU market or they distribute is compliant and will have to carry out some tests.

The costs associated to those migration tests depend on the protocol and analytical method used to test the migration of BPA. According to the SCL consulted during the elaboration of this proposal (see section G.4), there is no standard method to measure such a migration but it still seems to be feasible, based on the standards existing for food contact materials, such as mentioned above for RMO 1.

Although no information could be got about the costs of the measurement of the migration of BPA from thermal paper, these costs are expected to be higher than the costs of testing BPA content. Indeed, the sample pre-treatment is likely to be more complex than a simple BPA content analysis. According to the SCL consulted, measuring the migration of BPA from one material needs one additional step (an extraction with a solvent) such as described in the protocols included in the abovementionned standards for food contact materials. As a consequence, if the same assumptions are applied for RMO 2 as for RMO 1 (see section F.2), the compliance control costs associated to RMO 2 would be expected to be higher than the compliance control costs of RMO 1.

As a whole, the costs associated to compliance in order to 'stop' the migration of BPA from thermal paper (especially ecopaper) might be significant, due to the general need for a 'technical topcoating barrier' on any type of thermal paper and due to control costs. The total costs of RMO 2 can be considered as higher than the costs of RMO 1.

Timing

As for RMO 1, the transition period of 3 years is considered as proportionate since it would enable the market to adjust, taking depletion of stocks into account, the availability and technically and economically feasibility of alternatives and the ongoing substitution of BPA in thermal paper.

E.2.2.1.2.2 Technical feasibility

If the implementation of the abovementioned technical 'barrier' was the guarantee to limit the migration of BPA under RMO 2 (which is, again, not sure, since this is not supported by any available study), then RMO 2 could be considered as technically feasible. Indeed, protective topcoatings are already largely used for a wide range of applications of thermal paper (cinema tickets, metro tickets, self-adhesive labels, etc.) and could be technically applied for thermal paper targeting to POS applications.

However, it could be expected some needs for adjustments related to changes in the base paper used or, more importantly, in the printing devices used by end-users since protected (higher quality) thermal paper is usually thicker. To what extent this increase in thickness might make significant changes in printing devices or systems necessary is yet uncertain.

E.2.2.2 Practicality

E.2.2.2.1 Implementability

As regards the implementability of RMO 2, industry actors concerned might encounter a bit more difficulties to comply with the requirements in practice compared to RMO 1 since migration tests of BPA are to some extent more complex to carry out due to samples pre-treatment, as mentioned above.

Therefore, the analytical methods could be less rapidly implementable for them and could require more efforts to be understood and practiced. As for RMO 1, no EU standard method exists to measure migration of BPA from thermal paper. Moreover, the way of achieving the reduction of the migration required might be difficult, especially as regards the actual efficiency of possible protective topcoatings added to every thermal tickets. This theoretical technical 'barrier' would be very costly for the manufacturers of thermal paper. To that respect, RMO 2 is not considered as implementable since hardly economically feasible. Additionnal costs passed on the supply chain downstream could be much higher than for RMO 1.

E.2.2.2.2 Enforceability

According to the SCL consulted during the elaboration of this proposal (see section G.4), although there is no EU standard analytical method to measure the migration of BPA from thermal paper, it still appears to be materially feasible to do it in line with what is already implemented to measure migration of BPA from materials in contact with food. The use of this type of method could need some time to be practiced routinely. As a whole, given that testing migration of BPA from thermal paper seems to be technically feasible, the absence of an EU standard analytical method is not considered as a hindrance to the enforceability of the proposed restriction.

E.2.2.2.3 Manageability

The means of implementation of RMO 2 (migration tests in particular) are clear and understandable to the actors involved, except that the analytical methods seem to be a bit more complex and more costly than for RMO 1. The market actors concerned would have thus to put some efforts in the access to information and training in order to be able to carry out the compliance tests needed. This is again mainly the case of manufacturers of thermal paper.

E.2.2.3 Monitorability

No major difference is expected to be observed between RMO 2 and RMO 1 regarding their monitorability. Given that it is possible to measure BPA migration from thermal paper (although no standard method exists), RMO 2 is considered to be monitorable by control authorities and customs services. As regards thermal paper to be monitored, there might be the same concern however concerning the definition of the specific product to be controlled since no specific existing TARIC code is attributed to this type of product. Several TARIC codes could in principle cover 'thermal paper'. Likewise, there could be: 481190, 4823, 4821 and 480220 such as described in section E.2.1.3.

E.2.2.4 Overall assessment of RMO2

As a whole, RMO 2 is considered to be enforceable, manageable and monitorable. It is to some extent effective to reduce the risk if the content of BPA in thermal paper was set the lowest as possible to reduce its migration. However, its capacity to reduce the risk would be only theoretical as regards the possibility to remove the migration of BPA thanks to protective topcoatings since this technical 'barrier' has not been proven by any study. Moreover, it would not be economically feasible according to industry.

E.3 Comparison of the risk management options

Table 85. Comparison of the RMOs assessed

Assessment Criteria			RMO 1: restriction proposed (0.02% BPA concentration limit and a transitional period of 3 years)	RMO 2: alternative restriction option (BPA migration limit and a transitional period of 3 years)
Effectiveness	Risk reduction capacity		++	+
	Proportionality	Economic feasibility	++	--
		Technical feasibility	++	+-
Practicality	Implementability		+	-

	Enforceability	+	+
	Manageability	+	+
Monitorability		+	+

F. Socio-economic Assessment of Proposed Restriction

This section aim at documenting and assessing the impacts expected from the restriction proposed namely the human health (and -if relevant- environmental) impacts and the economic impacts. In other words, this section includes an assessment of on the one hand, the health benefits (considered as avoided costs) and one the other hand, the costs for the supply chain and the society as a whole. The assessment carried out herein is semi-quantitative. Most of the expected impacts have been quantified and valued. Some others are qualitatively analysed.

F.1 Human health and environmental impacts

F.1.1 Human health impacts

As already demonstrated, the restriction proposed herein would result in a total ban of BPA in thermal paper. The purpose of this section is thus to estimate the health benefits from that total ban.

As a reminder, the risk assessment carried out in support of this restriction proposal has demonstrated risks for the unborn child exposed in utero to BPA contained in thermal paper handled by his/her mother for 4 critical effects:

- Effects on the female reproductive system
- Effects on metabolism and obesity.
- Effects on the mammary gland
- Effects on the brain and behaviour

These effects are described in section B above. Performing a human health impacts assessment of the restriction proposed implies in principle to valuate economically every health outcome associated with these 4 effects. This valuation allows attributing a quantitative and monetary value to each of them and doing so, assessing the health benefits (considered as avoided costs) expected from the proposed restriction. Such an exercise basically includes the following steps: identifying the health outcomes associated with the effects occurred after BPA exposure, estimating probabilities of experiencing such health outcomes for the population at risk (namely herein the unborn child), estimating monetary values for the avoidance of each of the adverse health outcomes and finally, integrating the outcomes, probabilities, and monetary values associated with a hypothetical reduction in exposure to calculate health benefits.

Given the numerous effects at stake and the uncertainties surrounding them (see section B), the approach chosen herein to carry out the human health impact assessment is step-wise and as much careful as possible. The human health impact assessment is based on the health impacts likely to be experienced by the unborn child of workers (F.1.1.1.) and consumers (F.1.1.7).

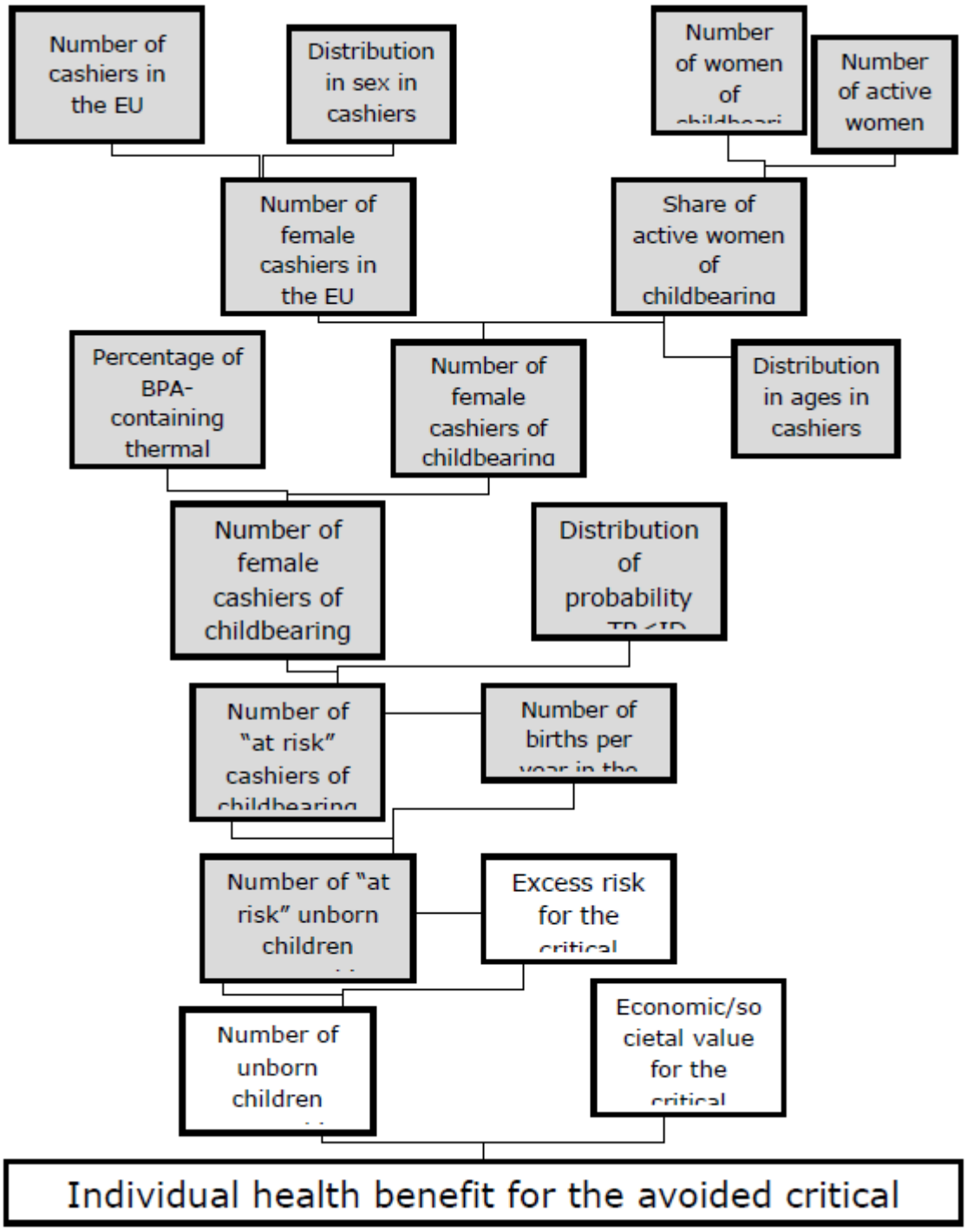
F.1.1.1. The human health impacts assessment for workers

The 'workers' population is approached herein by the workers in charge of the cashing namely the 'cashiers'. This is consistent with the population taken into consideration in the risk and exposure assessment ("cashiers") performed above (see section B.9). More precisely, the impact assessment targets the female cashiers exposed to BPA-containing thermal paper in the EU.

Moreover, to be consistent with the risk assessment carried out in section B, the HHIA is performed hereunder based on point-of-sale tickets and receipts only, made of ecopaper, which stand for around 65% of the whole thermal paper produced int the EU, as shown in section B.2.

For the purposes of the assessment of these health benefits, some assumptions have been made. The figure below presents the logigram which has been followed and the inputs data used for the assessment.

Figure 42. Logigram for the economic evaluation of the human health benefits or BPA restriction in thermal paper for workers



The boxes in grey stand for the assumptions and input data common to the evaluation of the human health benefits for the 4 effects. These assumptions and input data are developed in this section below. However, the boxes in white are effect-specific and are assessed further under each health impact subsection.

- ***Number of 'cashiers' in the EU***

It is hardly feasible to find data and occupations classifications and statistics exactly tailored to the population targeted herein.

The ESCO (European Classification of Skills/Competences, Qualifications and Occupations) classification provides a valuable classification which could be used as a reference. The ESCO classification is the European version of the ISCO (International Standard Classification of Occupations[45]) which is itself one of the main international classifications for which ILO (International Labour Organization) is responsible. Under ESCO, the occupation corresponding to 'cashiers' is classified under the code "5230 Cashiers and ticket clerks". This code is a subclassification of the following codes categories: 5. Service and sales workers / 52 Sales workers / 523 Cashiers and ticket clerks. ESCO illustrates the occupations related to the code 5230 as to be: car-park ticket seller, cash desk assistant (cafeteria), cash desk assistant (restaurant), cashier assistant, cash office worker, church cashier, cinema cashier, farming cashier, hotel cashier, office cashier, restaurant cashier, shop cashier, theatre cashier, ticket seller and union treasurer. The ESCO classification aims to stand for a reference for the national Member State labour and occupations statistics. ESCO provides a common European terminology for the European labour market and for education and training. ESCO was created very lately, mid-2013 and as such it does not intend to provide quantitative data.

The European statistics portal Eurostat does not provide such data either and is not linked to ESCO yet. The data on employment available are numerous but too large, too aggregated and not exactly targeting on the population concerned herein.

Different EU statistics databases offices have been contacted as well as the international and EU retailers and trade associations (such as Eurocommerce or Independant Retail Europe) without success. The data searched is not available at the EU level. As a consequence, the choice has been made to refer to the data collected for France since the French INSEE (*Institut national de la statistique et des études économiques*) provides some detail data on that particular occupation. This occupation is classified under the code 552a 'caissiers de magasin' (translated by 'shop cashiers') which is a subclassification of 5. Employees / 55. trade employees. According to INSEE, 274,320 people are referenced to practice this occupation in France in 2010[46]. This category is strict and only reference cashiers. However, it cannot be excluded that this category might not be totally representative and might to some extent exclude workers who might be in contact with thermal tickets, such as e.g. owners of unipersonal or small shops/ who are at the same time owners, salers and cashiers. If the larger group of occupations classified under the INSEE category "trade salers" is now taken into account (salers in food stores, in flower shops, clothes, cultural goods, services, etc.) the number exceeds 1.5 million people but is likely to include people never in contact with tills. Another source of information, the French FDC (*Federation du commerce et de la distribution*, French branch of the Eurocommerce mentioned above) also provides the figure of cashiers for food retailers and wholesale traders as to be 115,900. However this second data is more restrictive than the first one since it only concerned retailers and traders predominantly supplying food.

From this, and to get an order of magnitude for the EU number of cashiers, it has been inferred a correlation between the number of cashiers (all types of trades included) and the number of the general population. Indeed, even rough, this correlation sounds reasonable

[45] http://www.ilo.org/public/english/bureau/stat/isco/isco08/

[46]http://www.insee.fr/fr/themes/detail.asp?reg_id=0&ref_id=fd-dads2010&page=fichiers_detail/dads2010/telechargement.htm

since the number of cashiers reflect a certain number of shops and trades distributed over a territory, reflecting themselves a certain demand and distribution of population on that territory. The number of cashiers is to some extent deemed proportional to the general population although some differences between countries can be observed due e.g. to population density or consumption habits. Among the EU countries, although some (minor) gaps could be observed due e.g. to differences in consumption habits, given their cultural closeness and the similarity of their consumption needs, it can be expected that the number of trades and shops, and consequently the number of corresponding cashiers, may be evenly distributed over the whole Europe. For France, the total French population is 67,327,724 people in 2012 (Eurostat[47]) and there would thus be 0.42% cashiers compared to this population (based on 274,320 cashiers), that is to say, less than one cashier for 100 people or 2.2% "retail salers" (based on 1.5 million employees numbered under this larger group).

Data got from other countries are consistent with the French data. Norway statistics databases indicate that around 3.7% of the population work with in-person-sales in (2012 data, based on 183,000 *butikkemedarbeidere* -shop/store personnel- and a total population of 5 million inhabitants in 2012)[48]. Similarly, UK statistics indicate that the category '711 - Sales Assistants and Retail Cashiers" accounts for 1.5 million workers[49], that is around 2.3% of total population in 2014. Likewise, 2012 data from the US indicate that ca. 1.1% of the population work as cashiers (3.4 million people[50]) and ca. 1.5% as retail sales workers (4.7 million people[51]), standing for a total of 2.6%. Although these data are from non-EU countries and that it can be expected that some specificities could explain differences in numbers from one country to another, again there is no major reason to think that the situation for this particular occupation may be very different within the EU countries. However, like the INSEE category "trade salers", all these data might be overestimated to approach the number of workers strictly in contact with tickets and receipts since some proportion included in these data might never be exposed.

As a result, taking into account the likely underestimation of the strict (French) category of "cashiers" and the possible overestimation of the statistics related to the larger groups of occupations presented, it seems that a reasonable number of workers likely to handle tickets and receipts may be around **2% of the total population**.

Based on this assumption, the distribution of the number of cashiers (in the broad sense) within the EU has been established taking into account the EU population for each EU country. Eurostat provides the demographic data useful for this purpose. The results are presented in the table below.

Table 86. Number of cashiers compared to the general population in the EU countries

[47] http://epp.eurostat.ec.europa.eu

[48] https://www.ssb.no/statistikkbanken/SelectVarVal/Define.asp?MainTable=SyssKjYrk&KortNavnWeb=regsys&PLanguage=0&checked=true)

[49] http://www.ons.gov.uk/ons/publications/re-reference-tables.html?edition=tcm%3A77-373844

[50] http://www.bls.gov/ooh/sales/cashiers.htm

[51] http://www.bls.gov/ooh/sales/retail-sales-workers.htm

EU Country	**Population of the EU country in 2012**	**Number of cashiers estimated for all EU countries (based on a share of 2% of the general population)***
Belgium	11 094 850	221 897
Bulgaria	7 327 224	146 544
Czech Republic	10 505 445	210 109
Denmark	5 580 516	111 610
Germany	81 843 743	1 636 875
Estonia	1 333 788	26 676
Ireland	4 582 707	91 654
Greece	11 123 034	222 461
Spain	46 818 219	936 364
France	65 327 724	1 306 554
Croatia	4 275 984	85 520
Italy	60 820 696	1 216 414
Cyprus	862 011	17 240
Latvia	2 044 813	40 896
Lithuania	3 003 641	60 073
Luxembourg	524 853	10 497
Hungary	9 931 925	198 639
Malta	417 546	8 351
Netherlands	16 730 348	334 607
Austria	8 408 121	168 162
Poland	38 538 447	770 769
Portugal	10 542 398	210 848
Romania	20 095 996	401 920
Slovenia	2 055 496	41 110

Slovakia	5 404 322	108 086
Finland	5 401 267	108 025
Sweden	9 482 855	189 657
United Kingdom	63 495 351	1 269 907
TOTAL	**507 573 320**	**10 151 466**

Source: Eurostat; *estimated figures

The number of cashiers (broad sense), noted C, in the EU can thus be estimated at C=10,151,466.

- ***Number of female cashiers in the EU***

Given that aggregated statistics on professional data on cashiers in the EU are not available, the distribution in sex within cashiers is not available neither. However, the data provided by INSEE for France gives the distribution in sex in the cashiers population under the code 552a as to be the following:

	Total	Not declared	Men	**Women**
'Caissiers de magasin 552a' in 2010 (shops cashiers)	274,320	12	29460	**244,848**
Share over the total (%)*	100	0.004	11	**89**

Source: INSEE France; figures marked with an asterisk are calculated figures

Therefore, 89% of cashiers in France are women. This occupation is women-dominated in France. Although the lack of data on the distribution in gender in cashiers for other EU countries, it is assumed that this distribution is likely the same as the French one. As a consequence, it is considered that similarly to France, **89% of cashiers in the EU are women**.

Related to the number of cashiers estimated above, the number of female cashiers in the EU is thus N=Cx 89% = 9,034,805.

- ***Number of female cashiers of child-bearing age in the EU***

Statistically speaking, the number of women of child-bearing age may be defined as the number of women between 15 and 50 years old (INSEE France). This data is not available as such for EU cashiers. However, the data provided by INSEE for France gives the ages of cashiers under the code 552a as to be the following (for both sex):

BACKGROUND DOCUMENT TO RAC AND SEAC OPINIONS ON
4.4’-ISOPROPYLIDENEDIPHENOL (BISPHENOL A; BPA)

Table 87. Distribution in ages in French cashiers

	Total	Not declared	[0,15)	[15,20)	[20,25)	[25,30)	[30,35)	[35,40)	[40,45)	[45,50)	[50,55)	[55,60)	[60,65)	[65,70)	[70,75)	[75,80)	[80,85)	[85,90)	[90,)
'Caissiers de magasin 552a' in 2010 (shops cashiers)	274,320	132	24	20,544	76,896	44,868	29,568	28,200	24,720	22,104	14,736	10,032	2,136	300	60	-	-	-	-
Share over the total (%)*	100	0.05	**0.01**	**7.49**	**28**	**16.36**	**10.78**	**10.28**	**9.01**	**8.06**	5.37	3.66	0.78	0.11	0.02	-	-	-	-
TOTAL 15-50 years old (%)*	-	-	**-**	**90.00**							-	-	-	-	-	-	-	-	-

Source: INSEE France (figures marked with an asterisk are calculated figures)

Therefore, 90% of cashiers in France are between 15 and 50 years old.

As this kind of distribution in ages could not be obtained for the whole EU cashiers, the number of women of child-bearing age in the EU related to the EU female active population has also been investigated for comparison purposes with French data for cashiers. From the EU demographic statistics databases this number can be extracted. Eurostat provide such statistics and splits the general population by group of ages and by age. There is no aggregated figure reported for the class 15-50 years old but the number for the class 15-64 years old is given as being 168,832,608 (note that the figure for EU27 is not available for this class of age). As Eurostat also provides the number of women per age, the numbers of women between 51 and 64 years old could thus be summed and then subtracted to 168,832,608. This results in 121,672,696 women between 15-50 years old (childbearing age) in the EU 28 in 2012[52]. The table below summarizes the different data resulting in this number.

Table 88. Number of women of childbearing age in the EU 28 in 2012

Women age 51 years old	3,643,175
Women age 52 years old	3,599,304
Women age 53 years old	3,543,409
Women age 54 years old	3,519,435
Women class age 55-59 years old	16,863,516
Women class age 60-64 years old	15,990,800
TOTAL Women 51-64 years old	*47,159,639**
Women class age 15-64 years old	168,832,608
TOTAL Women childbearing age 15-50 years old	**121,672,696***

Source: Eurostat; figures marked with an asterisk are calculated figures

According to Eurostat, the active female population (defined from 15 to 64 years old) in EU 28 amounts to 109,018,400. Related to the number of women of childbearing age in the EU provided above, **the share of active women of childbearing age (over the total number of women –active and inactive- of childbearing age) can be inferred and equals 89.6%** (109,018,400/121,672,696). This figure is very close to the French data on the share of female cashiers of childbearing age. It can thus be reasonably assumed that there may be an even distribution in ages in French female cashiers as in the EU female active population. This sounds quite realistic since the cashiers population is generally rather young and more generally, active population is between 15 and 60 or 65. As there is no particular reason that distribution in ages in EU female cashiers would be different from the French one, the share of 90% can thus be reasonably considered as relevant and representative of EU cashiers of childbearing age (15-50 years old).

[52] Age 51= 3,643,175; age 52= 3,599,304; age 53= 3,543,409; age 54= 3,519,435; Class of age 55-59 years old= 16,863,516; class of age 60-64 years old= 15,990,800

Taking now into account this share, the number of female cashiers of childbearing age in the EU is thus F= 90%xN= 8,131,324.

- ***Number of female cashiers of childbearing age likely to be exposed to BPA-containing thermal paper in the EU***

This data is not available as such either. However, it has been shown above that the number of female cashiers of childbearing age can be estimated at F=1,707,578. Moreover, it has been indicated in section B.2.4.1 that the share of BPA-containing thermal paper compared to the total thermal paper placed on the EU market ranges from 75% (1 claim) and 100% (1 claim) with a central estimate between 90% and 99% (3 claims). ETPA indicates that 70-80% of thermal paper produced in Europe contains BPA (ETPA 2013 consultation, see section G). As a first approach, 70% is chosen herein, of which only 65% are taken into account for POS tickets and receipts. As a result, reported to the previous data, **the number of female cashiers of childbearing age likely to be exposed to BPA-containing thermal paper in the EU can be calculated to be equal to E= 70%x65%xF= 3,699,753.**

- ***Number of exposed pregnant female cashiers likely to be "at risk" (or number of unborn children exposed in utero likely to be "at risk")***

The section B above has shown that the risk assessment results in the construction of distributions of cumulated probabilities of internal exposure doses for the 4 critical effects. These distributions indicate all possible values that BPA internal doses may have and show the share among those values which exceed the toxicological benchmark. It has to be noted that when the exposure exceeds the toxicological benchmark, there is a risk that an effect will appear, but not all these exposure situations necessarily generate the effect associated with this toxicological benchmark ANSES, 2013 . As a result, these distributions may be interpreted as providing the share of the population exposed to be "at risk", that is to say, likely to develop an adverse effect, resulting from the fact that their internal dose might exceed the toxicological benchmark.

From these distributions and from Figure 33 above, it may thus be inferred the probability to develop an adverse effect. Depending on the effect, the whole distribution or most of the distribution of internal dose for workers exceed the value of the toxicological benchmark: 99.85% of the distribution exceed the toxicological benchmark for the effects on female reproductive system, 99.95% of the distribution exceed the toxicological benchmark for the effects on metabolism and obesity and 100% of the distribution exceed the toxicological benchmark for the effects on mammary gland. It is then considered that respectively 99.85%, 99.95% and 100% of the pregnant female cashiers exposed to BPA-containing thermal paper are "at risk". These probabilities are noted P, with P= (0.9985; 0.9995; 1). The corresponding number of cashiers at risk is thus A= PxE= (3,694,203; 3,697,903; 3,699,753) respectively.

This number of cashiers are strictly "at risk" for their descendants. Therefore, this number has to be related to the average annual number of births in the EU (both sex). From Eurostat data, this number is estimated to be 5,337,433 on average over 5 years from 2007 to 2011 for EU27 and for both sex, as shown in the table below; the number of births for more recent years or for EU28 have not been made available at the time of this proposal. This number stands for B=

4.4% of the childbearing age women population (which is as a reminder 121,672,696 as calculated above), B being the average annual EU birth rate.

Table 89. Number of births in the EU over 2007-2011

EU27/TIME	2007	2008	2009	2010	2011	**2007-2011 average***
Number of births (both sex)	5,283,841	5,429,210	5,372,512	5,372,527	5,229,077	**5,337,433***

Source: Eurostat; figures marked with an asterisk are calculated figures

As a result, the number of unborn children exposed in utero likely to be "at risk" annually can be estimated respectively for each critical effect to R= AxB= (162,054; 162,216; 162,298) per year.

The table below summarizes the input data collected and calculated for the human health impact assessment (HHIA) common to all critical adverse effects (corresponding to the grey boxes in the logigram presented above).

Table 90. Summary of the input data common to all critical adverse effects for the HHIA for workers

Input data	**Value**
Number of « cashiers » in the EU	C=10,151,466
Share of women in cashiers	89%
Number of female cashiers in the EU	N=0.89xC=9,034,805
Share of active women of childbearing age in the EU	90%
Number of female cashiers of childbearing age in the EU	F= 0.9xN= 8,131,324
Share of BPA-containing thermal ecopaper on the EU market	65%x70%
Number of female cashiers of childbearing age exposed to BPA-containing thermal paper in the EU	E= 0.7x0.65xF= 3,699,753

Probability to develop an adverse effect (on the female reproductive system; on the metabolism and obesity; on the mammary gland)	P= (0.9985; 0.9995; 1)
Number of female cashiers "at risk" (same effects respectively)	A= PxE= (3,694,203; 3,697,903; 3,699,753)
Average annual birth rate in the EU	B=4.4%
Number of unborn children exposed in utero in the EU likely to be "at risk" annually (same effects respectively)	R= AxB= (162,054; 162,216; 162,298)

From these input data, the human health impacts corresponding to each of the 4 critical adverse effects are assessed in the following subsections. The health benefits for workers are noted B^w_i, with i=e (for endometriosis), b (for body weight), c (for cholesterol) and g (for mammary gland).

F.1.1.2. The human health benefits for workers– female reproductive system

Regarding the effects of BPA on the female reproductive system, and as shown above in section B, the following critical effects (based on effects observed in animals and on the Rubin, 2001 and Signorile, 2010 key studies chosen for the human risk assessment) have been selected:

- Increase in the occurrence of ovarian cysts

- Increase in the frequency of the appearance of endometrial hyperplasia

- Disruption of ovarian cycles

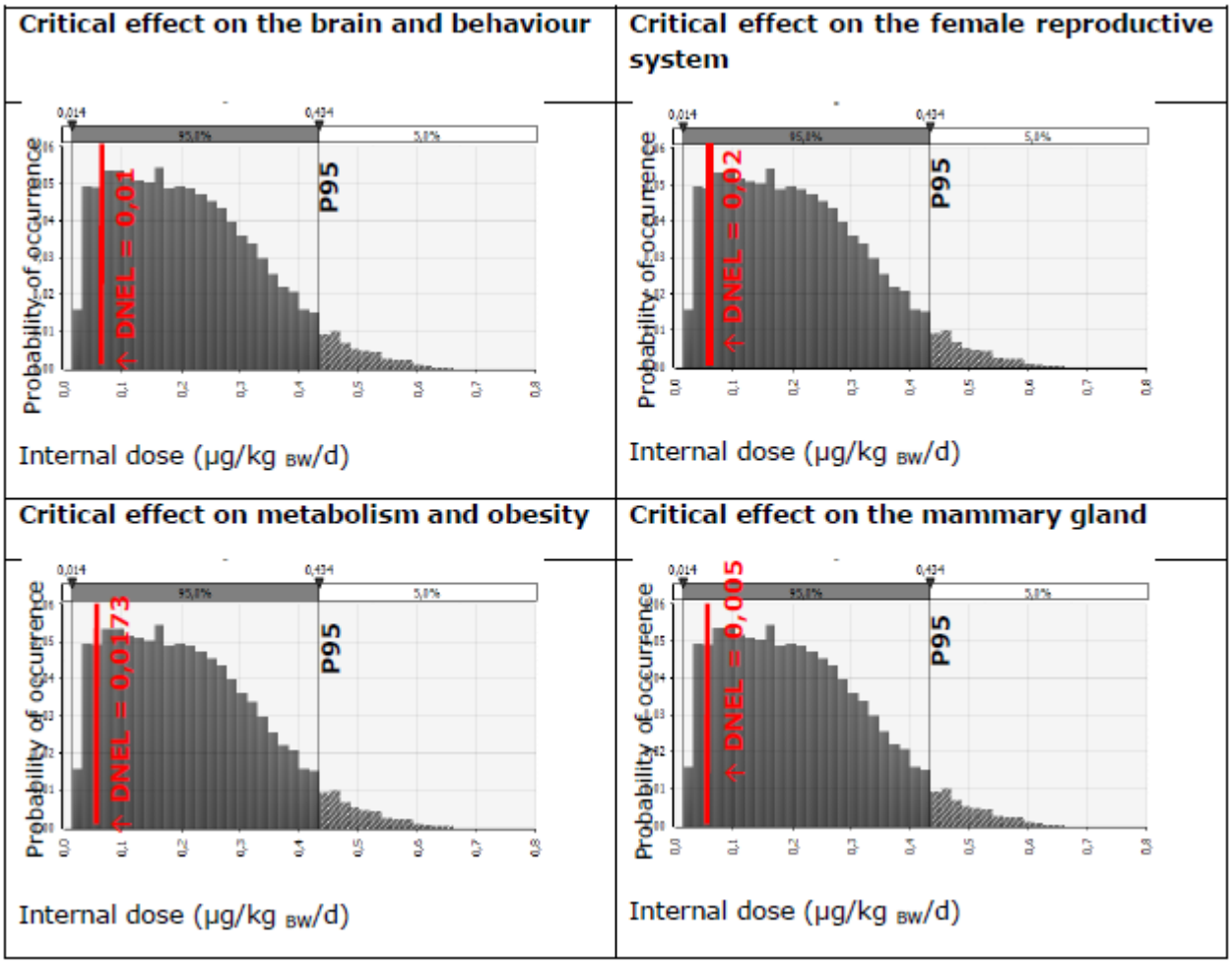
As a reminder, the DNELs and the RCRs for workers pregnant women are presented here below with Table 90 and Figure 45.

Table 91. Calculation of risk characterisation ratios for workers pregnant women with an intraspecies assessment factor of 5.

Critical effects	DNELs for workers pregnant	RCRs calculations with P95 =

	women with an intraspecies assessment factor of **5**	0.43 (toxicological benchmarks)
Brain and behaviour	0.01	43
Female reproductive system	0.02	21.5
Metabolism and obesity	0.0173	24.85
Mammary gland	0.005	86

Figure 43. Characterisation of the risks associated with handling thermal receipts containing BPA – "Cashier/Teller" scenario



F.1.1.2.1. Increase in the occurrence of ovarian cysts

An ovarian cyst is any collection of fluid, surrounded by a very thin wall, within an ovary. Any ovarian follicle that is larger than about two centimetres is termed an ovarian cyst. Such cysts range in size from as small as a pea to larger than an orange. Most ovarian cysts are functional in nature and harmless (benign, meaning they are not cancerous). Ovarian cysts affect women

of all ages (from puberty to menopause) but occur most often during a woman's childbearing years.

Some ovarian cysts cause problems, such as bleeding and pain and some might rupture. A rupture of an ovarian cyst is usually a self-limiting, and only requires expectant management and analgesics. The main symptom is abdominal pain, but can also be asymptomatic. The pain may last from a few days to several weeks.

For more serious cases where cysts are large and persisting, doctors may suggest surgery. This may involve removing the cyst, or one or both ovaries. Features that may indicate the need for surgery includes persistent complex ovarian cysts, persistent cysts that are causing symptom, simple ovarian cysts larger than 5-10 centimeters and women who are menopausal or perimenopausal.

Cysts that persist beyond two or three menstrual cycles, or occur in post-menopausal women, may indicate more serious disease and should be investigated through ultrasonography and laparoscopy, especially in cases where family members have had ovarian cancer. Such cysts may require surgical biopsy. Additionally, a blood test may be taken before surgery to check for tumor markers.

The increase in the occurrence of ovarian cysts may thus cause inconveniences and pain such as described above. Given that most of the ovarian cysts are benign and that the human risk assessment carried out in that dossier does not show in any way that the increase in the occurrence of ovarian cysts resulting from pre or postnatal exposures to BPA would cause particularly cancerous cysts, the tumorous cysts cannot be considered herein as a representative effect and is not included in the assessment of health benefits of the BPA restriction.

As a result, only symptoms of benign ovarian cysts (pain and bleeding) and the assessment of corresponding costs (treatment and indirect costs) are taken into account herein. Treatment for cysts depends on the size of the cyst and symptoms.

Pain and bleeding caused by ovarian cysts may be treated with:

- Pain relievers, nonsteroidal anti-inflammatory drugs, or narcotic pain medicine may help reduce pelvic pain.
- Informal care such as warm baths, or heating pads, or hot water bottles applied to the lower abdomen near the ovaries can relax tense muscles and relieve cramping, lessen discomfort, and stimulate circulation and healing in the ovaries.
- Combined methods of hormonal contraception such as the combined oral contraceptive pill – the hormones in the pills may regulate the menstrual cycle, prevent the formation of follicles that can turn into cysts, and possibly shrink an existing cyst.
- Limiting strenuous activity may reduce the risk of cyst rupture or torsion.

The costs of some of these treatments could be rather easily valuated, particularly the costs of medicines such as pain relievers, non-steroidal anti-inflammatory drugs and narcotic pain medicine as well as the costs of hormonal contraception. The "costs" of bleeding may also be approached through the frequent use of hygienic protections such as sanitary towels e.g. As to surgery, the cost of the surgical intervention and the related daycares and hospitalizations costs may be evaluated based on available data from the healthcare systems. Moreover, indirect costs can be associated with these symptoms and treatments such as the loss of

working days due to sick leaves as well as inconveniences due to potential secondary effects of medication and more 'moral' costs such as physical discomfort and pain.

However, although the economic valuation of the benefits of BPA restriction (understood as avoided costs) related to the increase in the occurrence of ovarian cysts could be carried out in principle without major difficulty, at least regarding the direct costs mentioned above, a sizeable problem arises when it comes to the calculation of an excess risk associated to that effect. Indeed, this calculation is not relevant because the NOAEL chosen in the health risk assessment above (see section B) doesn't match with this critical effect. There is only a LOAEL. As a consequence, an excess risk cannot be consistently modelled.

In conclusion, there may be health benefits due to the BPA restriction in thermal paper associated with the (avoided) increase in the occurrence of ovarian cysts. These benefits have however not been quantified but only qualitatively described for the reasons already invoked.

F.1.1.2.2. Increase in the frequency of the appearance of endometrial hyperplasia

The increase in the frequency of the appearance of endometrial hyperplasia corresponds to the risk of endometriosis.

Endometriosis is a gynecological condition in which cells from the lining of the uterus (endometrium) appear and flourish outside the uterine cavity, most commonly on the membrane which lines the abdominal cavity, the peritoneum. The uterine cavity is lined with endometrial cells, which are under the influence of female hormones. Endometrial cells in areas outside the uterus are also influenced by hormonal changes and respond in a way that is similar to the cells found inside the uterus. Most common symptoms of endometriosis are pelvic pain (dysmenorrhea, chronic pelvic pain, dyspareunia and dysuria), abnormal bleeding, chronic fatigue and infertility. The pain often is worse with the menstrual cycle and is the most common cause of secondary dysmenorrhea. Endometriosis is typically seen during the reproductive years; it has been estimated that endometriosis occurs in roughly 2–10% of women in general population (Simoens, 2012). Symptoms may depend on the site of active endometriosis. Its main but not universal symptom is pelvic pain in various manifestations.It is difficult to determine the prevalence of endometriosis because of the diversity of symptoms and their severity, and because endometriosis may be asymptomatic. Endometriosis is found almost exclusively in women of reproductive age, with diagnosis usually during a woman's 30s (UK data[53]). According to Abbas et al. (2012) the highest prevalence was observed in 2012 in women aged 35–44 with 12.8 per 1000 women based on a german cohort, which is consistent with UK and EU general data. Endometriosis is a common finding in women with infertility, with prevalence up to 30-50% (Simoens, 2012; Meuleman, 2009). Endometriosis is observed in women from the puberty to the end of life (after the menopause). Endometriosis has a significant social and psychological impact since it might disrupt quality of life and family life. There is no cure for endometriosis, but it can be treated in a variety of ways.

Pain and bleeding caused by endometriosis may be treated with:

- pain relievers, anti-inflammatory drugs or pain medicine
- hormonal and contraceptive pills e.g. to suppress the natural cycle

[53] Sources: http://www.patient.co.uk/doctor/endometriosis-pro; Abbas et al, 2012.

Infertility due to endometriosis in younger women can be treated by surgical intervention to remove endometrial tissue and preserving the ovaries without damaging normal tissue. After the surgery, the patients can be treated with fertility medication, or with IVF.

Chronic fatigue is generally mitigated with the mentioned medication or surgery.

From this, in order to assess the costs associated with this effect and thus the health benefits of a restriction of BPA in thermal paper associated to that effect, input data related to economic/societal values of endometriosis and the corresponding excess risk are needed.

- Regarding the excess risk of endometriosis for the targeted population of this human health impact assessment, an attempt has been carried out to compute the probability of occurrence of this effect from the data provided in Signorile, 2010 The authors have been contacted in order to get the raw data basing their study. From these data, it is proposed herein an approach, similar to the approach for cancer risk assessment (adjusting animal doses to equivalent human doses, deriving the point of departure by fitting a mathematical model to the data, and linearly extrapolating from the point of departure to lower doses). The method used is the same for all the critical effects assessed in this human health impact assessment and is presented in details in Annex 2.

From the regression curve established for endometriosis, the fraction of the targeted population likely to be affected by endometriosis can be inferred. As shown above, in section B.9.3.2.1., the average BPA internal dose corresponding to the cumulated probabilities distribution (Figure 22) is 0.21µg/kg bw/d for workers. This average dose is used for the computation of excess risks (considered as reasonably representative). It thus corresponds to an excess risk of 0.07%.

In conclusion, the excess risk of endometriosis, noted ER, for the targeted population is ER= 0.07%.

The excess of risk of endometriosis applies to the female unborn population only. Eurostat provides the annual number of live male and female births. The table below presents these numbers for 2007-2011 in EU28 (figures for 2012 are not available yet). The annual average numbers and the average share of female births over the total number of births have been calculated and inserted in the Table as well. On average, **the annual rate of female birth in the EU is G= 48.7%.**

Table 92. Number of live births in the EU over 2007-2011

EU28/TIME	**2007**	**2008**	**2009**	**2010**	**2011**	**Average***
female live births	2 591 144	2 664 911	2 636 226	2 636 425	2 565 903	**2618922**
male live births	2734607	2808052	2780863	2779463	2704371	**2761471**
TOTAL	*5 325 751*	*5 472 963*	*5 417 089*	*5 415 888*	*5 270 274*	***5380393***
share female/total*	**48,653%**	**48,692%**	**48,665%**	**48,679%**	**48,686%**	**48,7%**

Source: Eurostat; figures marked with an asterisk are calculated figures

The number of unborn (female) children exposed likely to be affected by endometriosis is thus estimated to be equal to ERxRxG= 0.0007x162,054x0.487= 55.

- Regarding now the economic valuation of endometriosis, several studies exist providing endometriosis-associated costs to society.

A literature review of economic studies has been carried out including all publications until November 2013. Around ten studies have been selected among which Simoens (2007), {Simoens, 2011 837 /id}), Simoens (2012), Luisi (2009), Nnoaham (2011), Klein (2013), Holoch (2010) considered as the most consistent. As mentioned above, the symptoms of endometriosis might have physical, social and psychological impacts. The assessment carried out herein is thus based on the Simoens' cost-of-illness analysis (Simoens, 2012) which is particularly relevant for the purposes of this impact assessment since it takes into account direct and indirect costs. Further, this is the only study assessing these costs at large-scale includingseveral EU countries. From a sample of 909 women with a laparoscopic and/or histological diagnosis of endometriosis from 10 countries (9 EU and the USA), the study results on an average total annual cost amounted to 9,579€ per woman, ranging from 8,559€ to 10,599€, including direct costs such as health care costs (surgery, monitoring tests, hospitalization, physician visits, medication, informal care and other treatments) and non-health care costs (transportation and support household activities) and indirect costs of productivity loss. This study is built on a multivariate regression model and QALYs (quality-adjusted life years) indicator. The value, noted V, provided in this study is thus used as a basis for the economic assessment of endometriosis.

From this, the benefit expected from the endometriosis avoided is then calculated taking 2019 as the year of reference (the year of the entry into force of the restriction). As a result, the value V has been inflation-adjusted to get its 2019 value, based on the inflation rate forecasted by ECB which is around 1.9% per year over 2014-2019 (5 years ahead)[54]. It yields **V= €10,524** per (woman) patient in 2019 value. Then, has been taken into account a decreasing discounting rate of 4% over 2019-2049 (first 30 years) and then 2% (for benefits occurring after 30 years) in order to take into consideration intergenerational equity. However, it is considered that the value of preventing a fatality has a constant utility value over time and it is therefore uprated in real terms each year by real GDP per capita growth. An uprating factor, usually based on GDP per capita growth and income elasticity, estimated around 2%, based on OECD forecasts[55] was used. Therefore, when combined with a 4% (2% for benefits accuing after 30 years) discount rate, it gives an 'effective' discount rate for health benefits of 2% over 2019-2049 and 0% for benefits accruing after 2049. Moreover, based on the information gathered on endometriosis prevalence presented above, it is assumed that endometriosis would occur in the future generation mostly at the age of 35. **The year 2054 is thus the expected year of occurrence for endometriosis (2019+35).**

The table below summarizes the input data calculated or selected to carry out this assessment.

[54] http://www.ecb.europa.eu/stats/prices/indic/forecast/html/table_hist_hicp.en.html

[55] OECD long-term forecast estimates a growth in GDP per capita between 1.92% in 2019 and 1.35% in 2060 (forecasts not available after 2060) (http://knoema.fr/iuacek/euro-area-gdp-growth-forecast-2013-2015-and-up-to-2060-data-and-charts) and the elasticity recommended to be used by OECD is 0.8 +-0.4.

Table 93. Summary of input data for the HHIA of endometriosis

Input data	**Value**
Excess risk for the "at risk" female cashiers/unborn children regarding endometriosis	ER= 0.07%
Economic/societal value of that human health outcome	V= **€10,524** in 2019 value

Given the other input data already collected or calculated above, the burden of endometriosis from a societal and economic perspective, noted B_e, due to in utero exposure to BPA from thermal paper of cashiers thus amounts to B_e= 55xV= €314,523 in 2019 value.

F.1.1.2.3. Disruption of ovarian cycles

The disruption of ovarian cycles may happen with different outcomes in women affected: elongation or shortening of the oestrous cycle, erratic periods cycles, irregularity of menstruation flows, etc. These outcomes may have various and more or less serious impacts on their everyday life such as abnormal bleeding (menstruation flow), disruption of their fertility (due e.g. to fewer ovarian cycles and thus a lower probability of getting pregnant in the case of elongation of cycles), disruption of their sexuality, discomfort and inconvenience, and generally a lower quality of life. This kind of disruption may occur from puberty to menopause. The magnitude of adverse impacts is dependent on the gravity of the effects likely to appear, from a slight elongation of ovarian cycles to complete amenorrhoea.

These different outcomes of the disruption of ovarian cycles are one of the main causes of gynaecologists consultations. They can be treated and mitigated through different ways such as medication like primarily the use of birth control pills which can help to regulate menstrual cycles and erratic flows. For more serious disruption, other medical treatment may be prescribed such as substitutive treatment based on ovarian stimulation.

Although these treatments are well known and that it would be rather easy to get the corresponding medicines prices and consultation costs, it has been decided herein to carry out a qualitative analysis of the expected benefit from the avoidance of that critical effect. The reason is twofold. Firstly, the risk assessment performed in section B above concludes on the risk of ovarian cycles disruption in a rather broad sense and the exact outcomes and gravity of symptoms of that disruption cannot be determined with sufficient accuracy. Therefore, the risk of overestimation of valuated benefits is considered to be too high. Secondly, beyond the exposure of BPA which is addressed herein, many other causes may result in such a disruption (other than pregnancy or breast-feeding): eating disorders, extreme weight loss or excessive exercising, polycystic ovary syndrome, premature ovarian failure (loss of normal ovarian function before age 40), pelvic inflammatory disease, or uterine fibroids. The attributability to BPA only is consequently hard to establish. To that respect, the corresponding benefits have not been valued.

In conclusion, there may be health benefits due to the BPA restriction in thermal paper associated with the (avoided) disruption of ovarian cycles. These benefits have however not been quantified but only qualitatively described for the reasons already invoked.

Conclusion

As a whole, the health benefits that can be expected from the restriction of BPA in thermal paper as regards the effect on the female reproductive system are summarized in the table below.

Table 94. Heath benefits from the avoided effects on the female reproductive system

Avoided adverse health outcomes	**TOTAL health benefits in 2019 values (discounted)**
Increase in the occurrence of ovarian cysts	>0 (Qualitatively described)
Increase in the frequency of the appearance of endometrial hyperplasia (endometriosis)	B_e= €**314,523**
Disruption of ovarian cycles	>0 (Qualitatively described)

F.1.1.3. The human health benefits for workers – metabolism and obesity

Regarding the effects of BPA on the metabolism and obesity, and as shown above in section B, the following critical effects (based on effects observed in animals and on the Miyawaki, 2007 key study chosen for the human risk assessment) have been selected:

- the increase in body weight
- the increase in plasma lipids (such as cholesterol and triglycerides)
- the increase in lipogenesis

In the framework of this HHIA, the increase in lipogenesis is approached through the increase in body weight and the increase in plasma lipids is approached through the increase of cholesterol.

F.1.1.3.1. The increase in body weight

The increase in body weight is a proxy to assess effect on overweight and obesity. According to WHO[56], overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Worldwide obesity has nearly doubled since 1980 and in 2008, more than 1.4 billion adults, 20 and older, were overweight. Of these over 200 million men and nearly 300 million women were obese. More than 40 million children under the age of five were overweight in 2011. This effect may occur at every age in principle and there seems to be no particular age distribution for it.

[56] http://www.who.int

At the EU level, according to country estimates for 2008, over 50% of both men and women in were overweight, and roughly 23% of women and 20% of men were obese. Estimates of the number of overweight infants and children in the WHO European Region rose steadily from 1990 to 2008. Over 60% of children who are overweight before puberty will be overweight in early adulthood. Childhood obesity is strongly associated with risk factors for cardiovascular disease, type 2 diabetes, orthopaedic problems, mental disorders, underachievement in school and lower self-esteem[57]. Currently about 20% of children and adolescents are overweight, and of these a third are obese.

Overweight and obesity are evaluated in relative terms, based on the Body mass index (BMI). The BMI is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height. The WHO definition is:

- a BMI greater than or equal to 25 is overweight
- a BMI greater than or equal to 30 is obesity

BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. However, it should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals.

BMI is used differently for children. It is calculated the same way as for adults, but then compared to typical values for other children of the same age. Instead of set thresholds for underweight and overweight, then, the BMI percentile allows comparison with children of the same sex and age. A BMI that is less than the 5th percentile is considered underweight and above the 95th percentile is considered obese for people 20 and under. People under 20 with a BMI between the 85th and 95th percentile are considered to be overweight[58]. From the information collected and the research made on BMI, no BMI distribution seems to exist for the EU population.

Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischaemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity.Overweight and obesity are linked to more deaths worldwide than underweight.

From this overview, in order to assess the costs associated with the increase in body weight and thus the health benefits of a restriction of BPA in thermal paper associated to that effect, input data related to economic/societal values of overweight and obesity and the corresponding excess risk are needed.

- Regarding the excess risk of the increase in BW for the targeted population, an attempt has been carried out to calculate the probability of occurrence of this effect from the data provided in Miyawaki, 2007 . The authors have been contacted in order to get the raw data basing their study. Then, the data obtained have been computed similarly to the excess risk of the other effects, such as described in Annex 2.

[57] http://www.euro.who.int/en/health-topics/noncommunicable-diseases/obesity/data-and-statistics

[58] http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html

From the regression straight line established, the fraction of the targeted population likely to be affected by an increase in BW at birth (increase of about 12%, defined for P90 of the control group) can be inferred. Like previously, the average BPA internal dose corresponding to the cumulated probabilities distribution (Figure 22) of 0.21 µg/kg bw/d for workers is used. This dose corresponds to an excess risk of 0.33%.

In conclusion, the excess risk of obesity, approached through the increase in BW, noted again ER, for the targeted population is ER= 0.33%.

The number of unborn children exposed likely to be affected by an increase in BW is thus estimated to be equal to 535 (0.0033xR). Both sexes are concerned here.

- Regarding now the economic valuation of overweight and obesity, many studies exist providing associated costs.

A literature review of economic studies (literature on 'obenomics') has been carried out including all publications until November 2013 in collaboration with INERIS (INERIS, 2013). The search identified 30 articles, 21 articles were excluded from the study due to absence of costs data. From the remaining 9 articles, 3 studies that quantitatively report the effects of the intervention in terms of % BMI (body mass index) variation have been selected. The studies which reported negative costs of overweight vs. normal weight or with not enough detailed data have been discarded. Finally, 2 studies based on social benefits and 1 study based on cost-of-intervention are used.

As regards costs of intervention, Moodie, 2010 assesses for Australia the cost and efficiency of an intervention program on children aged from 5 to 11, based on providing extra physical activities after school. Since the efficiency is reported in terms of % of BMI decreased by child per year, it has been possible with this study to derive a cost per % BMI decrease. After converting from AUS$ and correcting for inflation, a value of **1060 € per % BMI decrease per child per year** could be derived. All costs –except set-up and design costs - to the health sector, participants and families, and other sectors involved in the delivery of the intervention are included. However, a limit is that the intervention was not cost-effective given usually agreed thresholds, as acknowledged by the author, and also as mentioned in the review by John, 2012. Moreover, the cost/DALY (disability-adjusted life year) of the intervention studied in Moodie, 2010 is around 20 times higher than those of cost/efficient similar programs reported in John, 2012. Therefore, the economic value of the unit % BMI decrease derived from Moodie, 2010 cannot be used as such. Nevertheless, the 20eth of this value could be considered to provide a very crude estimate, in terms of order of magnitude only, of the value of the % of BMI decrease, that is **50 € per % BMI decrease per child per year**. It should also be noted that another source of uncertainty is that it is unclear to what extent the costs and health benefits calculations carried out in this article for Australia can be extrapolated to EU.

Regarding the social benefits of decreasing overweight in the population, two studies were used: Wang, 2010 and Brown III, 2007.

Wang, 2010 calculates yearly benefit of 21,075$ (in 2007), equivalent to 17,600€ 2013, per avoided (overweight or obesity) case in adolescents aged 12-19. Benefits are lifetime avoided healthcare costs, discounted to the age 17 of surveyed population. The estimation does not include the monetisation of QALYs (Quality Adjusted Life Year) gained. QALYs gained could be

monetised and added, but this probably could involve double counting and could be unreliable. It is also unclear whether medical costs can be extrapolated to EU (differences in economic costs and in the health systems).

Brown III, 2007 estimates benefits as 4500 $ (2004), equivalent to **3760 € 2013 per overweight avoided case**, for US children aged 8-11. Benefits include averted future medical costs (age 35 to death) and labour productivity gains (age 40 to 65). Likewise it is unclear whether US medical costs can be extrapolated to EU.

The benefits estimated in Wang, 2010 are higher than in Brown III, 2007. The reason might be that Wang includes in the study both overweight and obesity, whereas Brown studies only overweight, and for younger children. For that reason, it seems more adapted to the BPA context (smaller impacts and for children) to use the value derived from Brown III, 2007 as an upper bound for benefits of the restriction.

The difference between values derived from cost-of-intervention and from cost-of-illness is large and seems higher herein than for the case of cholesterol (see above). An explanation may be that intervention on obesity/overweight can be carried out on a single year and be efficient on the long-term, whereas drug-based interventions against cholesterol are generally on a longer-term and therefore more expensive. Furthermore, obesity being heavily involved in an array of health and societal impacts, benefits from the intervention may spread across a range of outcomes. It should be reminded also that, as reported in the review by John, 2012, the cost and cost/effectiveness of interventions against overweight is very variable and that sometimes even negative costs are reported. For that reason, deriving benefits from economic figures based on avoided cost of the intervention could be questionable. Moreover, the value derived from Moodie, 2010 is expressed in % per BMI decrease. In order to use this value, the distribution of BMI for the EU children population would be needed and more importantly would be to determine the impact of BPA exposure in utero on that distribution. From Miyawaki, 2007 , an increase in BW of 12% of the cohort has been inferred from the patterns and could be used for that purpose. However, to what extent this increase can be strictly and robustly extrapolable to humans and this case is highly uncertain. Further, it should be assumed that the EU distribution of BMI in children is affected evenly by this increase of 12%, their height remaining constant (so that BMI, which is the ratio between BW and square-height is also increased of 12%), which is also highly uncertain. As a consequence, it has been deemed preferable to use in that particular circumstance the social benefit estimate, better reflecting the overall impacts of reducing overweight, despite this approach is providing only an upper-bound estimate of benefits. The value derived from Brown III, 2007 is thus used for the following. It is thus noted **V= €3,760 per overweight avoided case per year (2013 value)**. To get the 2019 value of V, it has been inflation-adjusted with ECB projected inflation rate (5 years ahead, 1.9%) and it yields a benefit of **V=€4,131 per overweight case avoided**.

The table below summarizes the input data calculated or selected to carry out this assessment.

Table 95. Summary of input data for the HHIA of the increase in BW

Input data	**Value (in 2019)**
Excess risk for the "at risk" female cashiers/unborn	ER= 0.33%

children regarding an increase in BW	
Economic/societal value of an increase in BW	V= €4,131 per avoided case per year (social cost)

This benefit has then been discounted similarly to what has been done for the other effects, with a decreasing discount rate (2% then 0% after 30 years – see the section on endometriosis for further details). However, on the contrary to endometriosis above and breast cancer below, the benefit of the avoided increase in body weight has been discounted not based on one specific year of occurrence but over 2019-2069[59], given that it seems that there is no particular distribution in age for this disease, as explained above (neither there is a BMI distribution). The costs have thus been calculated and discounted over the period and expressed then in average annual value (taking again 2019 as the reference). As a result, the burden of the increase in body weight, approached through overweight and obesity, from a societal and economic perspective, noted Bc, is thus estimated to B_b=€1,512,655.

It has to be noted that this value might be overestimated since it implicitely assumes that any increase of body weight would lead to overweight. However, this outcome is not systematic and depends on the initial weight (or mass) of the person. A sensitivity analysis is carried out on that value below in F.1.

F.1.1.3.2. The increase in cholesterol

Cholesterol is a molecule found in cells. It is a type of lipid which is a fat or fat-like molecule. Cholesterols main function is as a structural component of cell membranes. It is also the starting material for bile acids that are made by the liver and used to digest fats, and for steroid hormones. High levels of cholesterol in the blood (hypercholesterolemia) can lead to atherosclerosis, an inflammatory disease of artery walls in which white blood cells invade the vessel wall and become engorged with cholesterol and other lipids. These areas can slowly close off a blood vessel or can suddenly rupture and trigger formation of a blood clot. Atherosclerosis is the main precursor of cardio-vascular diseases. According to the organ or tissue affected by atherosclerosis, more or less serious adverse effects may be occur, from legs arteritis and aneurysm to heart disease, myocardial infarction (heart attack) and stroke.

Cholesterol is measured in mmol/l (blood) and is of two types: HDL-cholesterol (high density lipoprotein) is sometimes called 'good cholesterol' compared to LDL-cholesterol (low density lipoprotein) which is considered as 'bad cholesterol' because people with high levels of LDL have more atherosclerosis and associated cardiovascular diseases.

Table 96. Cholesterol levels – LDL and HDL

Cholesterol	**Normal levels**

[59] 2069 being the furthest year of occurrence selected within all effects – for mammary gland, see further below

Cholesterol	**Normal levels**	
Total Cholesterol	**1.6-2.4 g/l**	**4.1-6.2 mmol/l**
LDL - Cholesterol ('bad' cholesterol)	0.8-1.55 g/l	2.05-3.95 mmol/l
HDL - Cholesterol ('good' cholesterol)	0.35-0.8 g/l	0-90-2.05 mmol/l

For a 'normal' person (without particular risks), the total cholesterol is below or equal to 2 g/l[60]. The higher this measure, the bigger the cardiovascular risks. Above 2g/l of cholesterol, treatment may be considered.

Excess in cholesterol can be diagnosed from the childhood to the end of life. Although hypercholesterolemia seems to increase with age, it may occur at every age and there is no particular age distribution for this disease. This excess can be remedied primarily by changes in everyday life habits (low-fat food, exercise, etc.) and can then be treated with medication. There exist a rather large range of medicines to treat hypercholesterolemia, namely statins. Statins are cholesterol-lowering drugs which aim to block the action of an enzyme (called HMG-CoA reductase) in the liver that is necessary for making cholesterol. Several types of statins exist such as atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin. Atorvastatin and rosuvastatin are the most potent, while fluvastatin is the least potent. These medicines are sold under several different brand names at different prices.

From this, in order to assess the costs associated with the increase in cholesterol and thus the health benefits of a restriction of BPA in thermal paper associated to that effect, input data related to economic/societal values of an increase in cholesterol and the corresponding excess risk are needed.

- The excess risk ER has been modelled with the same approach as above, based on the raw data got from Miyawaki, 2007 (see Annex 2). From the regression straight line established, the fraction of the targeted population likely to be affected by an increase in (total) cholesterol (increase of about 6%, defined for P90 of the control group) can be inferred, taking again into account the average BPA internal dose corresponding to the cumulated probabilities distribution (Figure 22) of 0.21 µg/kg bw/d for workers. This dose corresponds to an excess risk of 0.73%.

60

/www.nhlbi.nih.gov/health/public/heart/chol/wyntk.htm

In conclusion, the excess risk of the increase in (total) cholesterol for the targeted population is ER=0.73%.

However, it is of primary importance to note that an increase in cholesterol does not necessarily mean a need for medical treatment, provided that the total cholesterol remains at the "safe" level (around 2g/l blood). One difficulty herein is that the dose/response relationship modelled between BPA exposure and cholesterol relies on total cholesterol and not to LDL-C. Therefore, given the lack of data on the magnitude of the increase in cholesterol likely to be caused by BPA exposure in utero, especially in 'bad' LDL-cholesterol, and thus the lack of information about this increase compared to the "medication threshold", the health impact herein cannot be reasonably calculated for the whole 1,184 (0.0073x162,216) unborn children estimated as "at excess risk". Indeed, among them, some might incur an increase in cholesterol without medication needs (their total cholesterol remaining below the "safe" level). As a consequence, it has to be determined the EU population at risk from BPA exposure who has elevated cholesterol levels and *should be* treated. For that purpose, in order to get an idea, even roughly, of the proportion of these children who might suffer from a too high cholesterol level (above the "medication threshold"), it has been decided to take as a proxy, the prevalence of raised cholesterol in the EU general population who should be thus theoretically treated. According to the WHO, this prevalence is 54% for both sex[61].

The 'adjusted' number of unborn children exposed to BPA likely to be affected by an *adverse* increase in cholesterol (above the medication threshold) and thus needing medication is thus estimated to be equal to 639 (0.54x1,184).

- Regarding now the economic valuation of an increase of cholesterol, several studies exist providing cholesterol-associated costs.

The economic literature aiming to valuate such costs for raised cholesterol from a prevention perspective is abundant. The search included all publications until November 2013 and 43 articles have been identified (INERIS, 2013). 17 articles were excluded due to absence of costs data about the treatment of lowering cholesterol. The remaining 26 articles were studied in detail, and 19 articles were identified as not useful for the purposes (lack of data about the level of reduced cholesterol, cost-effectiveness calculated per QALY with no possibility to relate to cholesterol reduction, the aim of the study was only to document price differences between drugs; LDL value before treatment not reported; treatment costs not reported, etc.). Three studies have finally been selected out of the remaining 7, on the basis of the quality and date of the study. Among these 3 remaining studies, there are 2 articles on cost-of-intervention (Benner, 2005 and Lachaine, 2007) and one on cost-of-disease (Grabowski, 2012).

As a first approach, the health benefits of an avoided increase in cholesterol can be estimated from the avoided direct costs of medication (cost-of-intervention), based on the different statins medicines placed on the market and proven as efficient.

To that respect, the Benner, 2005 study provides the average cost of medication related to a decrease in LDL-cholesterol for 6 statins medicines, from the perspective of a managed care payer. The study evaluates the whole costs of lipid-lowering therapy, including the statin use, physician office visits and laboratory monitoring. The results of are presented in the table

[61] http://www.who.int/gho/ncd/risk_factors/cholesterol_text/en/index.html

below, converted in 2013 euros. The cost per unit of % LDL-cholesterol (LDL-C) and the average values have been calculated and added to the table.

Table 97. Data from Benner (2005) on the cost of medication of statins medicines

Drugs	**Average annual cost (US$ 2004 year converted in € 2013; US$1 = €0,7444)**	**Average % of decreased LDL-C**	**Annual Cost/unit % of decreased LDL-C (US$ 2004 year converted in € 2013; US$1 = €0,7444)**	**LDL-C mmol/L**
Lovastatin	€ 1,245.60	30	€ 41.52	4.16 mmol/L – 6.5 mmol/L
Fluvastatin	€ 1,208.62	30	€ 40.29	
Rosuvastatin	€ 1,290.36	46	€ 28.06	
Atorvastatin	€ 1,363.35	38	€ 35.88	
Pravastatin	€ 1,730.21	30	€ 57.68	
Simvastatin	€ 1,902.45	37	€ 51.42	
Average values*	***€ 1,456.77***	***35***	***€ 42.5***	

Source: data from Benner, 2005 , converted in euros

*calculated values not included in the original study

According to this study, the lipid-lowering therapy thus amounts to around V= 42.5€ per % of decreased LDL-cholesterol for one person treated per year. To get the 2019 value of V, it has been inflation-adjusted with ECB projected inflation rate (5 years ahead, 1.9%) and it yields a cost of around **€47 per % of decreased LDL-cholesterol for one person treated**.

As a comparison, the objective of Lachaine, 2007 was to compare the cost-effectiveness of atorvastatin and generic simvastatin in terms of annual drug cost per patient treated to Canadian LDL-C targets. The results of the study have been used to derive the following mean estimate (among 2 drugs and 4 treatment doses studied) of V= 11 € per % of decreased LDL-cholesterol for one person treated per year (converted and corrected for inflation original figures in CAN$ 2005 to present €). **In 2019 value, V=€12 per % of decreased LDL-cholesterol for one person treated.**

Differences in Benner, 2005 and Lachaine, 2007 (by a factor of 4) probably come from differences in the scope for cost: Lachaine, 2007 appears to only include the cost of drugs, whereas Benner, 2005 also includes physician and laboratory resource use.

These annual costs are incurred for 1 % of LDL-C decreased only. To be in line with the patterns established for the dose-response relationship presented above, these costs should be multiplied by 6 to reflect the 6% (avoided) increase in cholesterol due to BPA exposure.

For the whole number of unborn children exposed to BPA likely to be affected by an increase in cholesterol (above the medication threshold) calculated above (639), **the annual avoided cost or benefit thus equals to €46,369 or €179,152 per person in 2019 values.**

As a second approach, the health benefits of an avoided increase in cholesterol can be estimated from a societal perspective, that is to say, from the social cost of raised cholesterol. To that respect, the Grabowski 2012 study is consistent with the concern raised herein since it calculates survival benefits resulting from statin therapy for the period 1997-2008 through the consumer surplus approach, from a cost-of-disease perspective. The benefits of the therapy are avoided costs that can be transposed and interpreted as (one of) the benefits of the restriction of BPA in thermal paper. Financially speaking, the restriction (*ex ante*) and the therapy (*ex post*) can indeed be deemed as bringing the same health benefits, the former in a prevention purpose, the latter in a remediation purpose. Using combined population and clinical data, the study provides one value of consumer surplus computed as the social value from reduced LDL-cholesterol including fewer deaths, fewer hospitalizations for heart attacks and fewer hospitalizations for strokes less the costs of statins. The study includes a number of covariates, such as year indicators, sex, age, ethnicity, race, diabetes, prior stroke, obesity, coronary heart disease, family history of coronary heart disease, use of another lipid-lowering therapy, angina, average blood pressure reading, prior heart attack, physical physical fitness, and smoking but does not include possible side effects of statins. The calculation has been primarily carried out for the USA but as a sensitivity analysis, the authors used estimates of statin coverage in Europe relative to the USA, combined with 2008 population estimates from the OECD to calculate prevented health events (deaths, heart attacks, strokes) in 2008 across the European Union. To calculate the overall economic value of the survival gains among statin users, they then scaled the number of statin users by the ratio of statin users in Europe relative to the USA. As a result, the net social value for EU27 is estimated at $433 billion in consumer surplus in Europe for statins in 1988–2008.

In order to have more targeted and recent data, some adjustments of these values have been made. Using the figures for the period 2004-2008 only for the social benefits net of treatment costs provided by the author, the value of V'=123 € per unit % LDL-cholesterol decreased per person has been derived. It has to be noted that the VOLY (value of saved life year) value used in this study ($150,000) is close to the mean VOLY used in the EU Clean Air For Europe program, and therefore can be considered as a relatively high value (comparatively to the median value in the CAFÉ review[62]). For this reason, the value V' derived from this article is to be considered as an upper-bound of social benefits. **In 2019 value, V'=€135 per unit % LDL-cholesterol decreased per person.**

For the whole number of unborn children exposed to BPA likely to be affected by an increase in cholesterol (above the medication threshold) calculated above (639), this annual social net benefit equals to €518,488 per person in 2019 value.

It can be noticed that the ratio between values derived from the two approaches (cost-of-intervention and cost-of-illness) is roughly between 3 and 10, a ratio which is consistent with the finding by Grabowski, 2012 of a 4 to 8 benefit/cost ratio in its own assessment of statins.

[62] Clean Air For Europe, VOLY median value= 52,000€, http://ec.europa.eu/environment/archives/cafe/pdf/cba_methodology_vol2.pdf

The table below summarizes the input data calculated or selected to carry out this assessment.

Table 98. Summary of input data for the HHIA of the increase in cholesterol

Input data	**Value (in 2019)**
Excess risk for the "at risk" female cashiers/unborn children regarding an increase in cholesterol	ER= 0.73%
Fraction of people whose increase in cholesterol needs to be treated (inferred from the general population)	54%
Economic/societal value of an increase in cholesterol	V= €12 or €47 per % of decreased LDL-cholesterol for one person treated per year (based on medical cost) Or V'=€135 per % of decreased LDL-cholesterol for one person treated per year (based on social cost)

These benefits have then been discounted similarly to what has been done for the other effects, with a decreasing discount rate (2% then 0% after 30 years – see the section on endometriosis for further details). However, on the contrary to endometriosis above and breast cancer below, and similarly to the effect on the body weight, the benefits of the avoided increase in cholesterol have been discounted not based on one specific year of occurrence but over 2019-2069[63], given that it seems that there is no particular distribution in age for this disease, as explained above. The costs have thus been calculated and discounted over the period and expressed then in average annual value (taking again 2019 as the reference). As a result, the health benefits from an avoided increase in cholesterol **Bc and B'c thus amount respectively to €31,736 or €122,617 (corresponding to the two values of V selected for medical costs) and €354,867 per year (corresponding to V' for social cost).**

It has to be noted that the benefits B_c and B'_c cannot be summed since the latter includes already somehow the former.

Some limits of this assessment have to be pointed out in order to qualify the quantitative results. On the one hand, the benefits are expressed in % LDL avoided although the dose-response relationship between BPA doses and an increase in cholesterol presented above has

[63] 2069 being the furthest year of occurrence selected within all effects – for mammary gland, see further below

been modeled based on total cholesterol. As a consequence, the benefits might be to some extent overestimated. On the other hand, the calculation implicitly assumes that the marginal benefit (or avoided cost) of the reduction in LDL-C is constant, which makes the analysis simpler but which might also bring some overestimating or underestimating biases (INERIS, 2013).

Conclusion

As a whole, the health benefits that can be expected from the restriction of BPA in thermal paper as regards the effect on metabolism and obesity are summarized in the table below.

Table 99. Heath benefits from the avoided effects on the metabolism and obesity

Avoided adverse health outcomes	**TOTAL Health Benefits in 2019 values (discounted)**
Increase in body weight	B_b=€**1,512,655**
Increase in cholesterol	B_c= **€31,736 or €122,617** (medical cost) Or B'_c= €**354,867** (social cost)

F.1.1.4. The human health benefits for workers – mammary gland

Regarding the effects of BPA on the mammary gland, and as shown above in section B, the following critical effects (based on effects observed or suspected in animals and on the Moral, 2008 , Murray et al. (Murray, 2007), Jenkins et al. (Jenkins, 2009) key studies chosen for the human risk assessment) have been selected:

- The architectural changes to the mammary gland in adulthood such as:
  - the development of ductal hyperplastic lesions in connection to pre- or peri-natal exposure
  - the development of neoplastic-type lesions
- an increase in the likelihood of mammary glands subsequently developing mammary tumours during co-exposures to a carcinogenic agent (due to an increase in TEB, TD and HD, such as shown in section B)

These architectural changes (increase in the terminal end buds (TEB), terminal ducts (TD) and hyperplastic ducts (HD)) to the mammary gland may induce an increase of vulnerability (or susceptibility) of the developing mammary glands during puberty and may make it more sensitive to subsequent co-exposures to carcinogenic agents. As a result, this increased vulnerability of the mammary gland increases the risk of developing breast cancer. The health impacts associated to the effect of BPA on mammary glands are thus approached hereunder by the assessment of avoided increased occurrence of breast cancer; the structural changes being considered as precursors to breast cancer.

Breast cancer is an uncontrolled growth of breast cells leading to a malignant tumor. The risk factors for breast cancer are numerous. Among the most known, it can be found: the gender (more than 99% of breast cancer affect women), the age (the risk goes up when getting older), the genetics (about 5% to 10% of breast cancers are thought to be hereditary), some medication history (such as hormone replacement therapy), being overweight, smoking, drinking alcohol, exposure to chemicals. Within the EU, breast cancer is the most common form of cancer in women, accounting for 28%. With lung, stomach, liver and colon cancers, breast cancer causes the most cancer deaths each year. Breast cancer is responsible for the most cancer-related deaths in women (17.2% of the total) (WHO). Breast cancer is likely to occur from puberty to end of life. The incidence rate of breast cancer is reported to be around 90 per 100,000 in EU women (89.7 per 100,000 women in Western Europe by WHO[64] and 94.2 per 100,000 EU women by IARC (Ferley et al, 2013)). The life time risk[65] for women of getting breast cancer in EU28 is around 1 in 8 women, or approximately 13% (e.g. 12.5% in France, 12.90% in the UK[66], 14.3% in the Netherlands (Paap et al, 2008)).

Initially, breast cancer may not cause any symptoms. A lump into the breast may be too small to feel or to cause any unusual changes (swelling of all or part of the breast, breast pain, nipple turning inward, etc.). Often, an abnormal area turns up on a screening mammogram which leads to further testing. In some cases, however, the first signs of breast cancer are a new lump or mass in the breast or a skin irritation or dimpling that people can notice themselves. As screening and preventive tests, yearly mammograms are more and more given routinely to (even healthy) women especially older than 50, in order to find breast cancer early, before any symptoms can develop so that the cancer is usually easier to treat. Then, after screening, diagnostic tests (such as biopsy) are given to people who are suspected of having breast cancer, either because of symptoms they may be experiencing or a screening test result. These tests are used to confirm whether or not breast cancer is present and, if so, whether or not it has traveled outside the breast. Diagnostic tests also are used to gather more information about the cancer to guide decisions about treatment. Then, once breast cancer is diagnosed, many tests are used during and after treatment to monitor how well therapies are working. Monitoring tests also may be used to check for any signs of recurrence.

Additionnally to these preventive or control tests, breast cancer can be treated by surgery, medical therapies and/or medication:

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[64] http://www.who.int/cancer/detection/breastcancer/en/index1.html

[65] The lifetime risk of cancer is an estimation of the risk that a newborn child has of being diagnosed with cancer at some point during its life.

[66] http://www.cancerresearchuk.org/cancer-info/cancerstats/incidence/risk/statistics-on-the-risk-of-developing-cancer

- Surgery is usually the first line of attack against breast cancer from total removal of a breast (mastectomy) to a breast-conserving surgery (lumpectomy) followed by radiation
- Radiation (or radiotherapy) is a highly targeted way to destroy cancer cells in the breast and it reduces the risk of breast cancer recurrence
- Chemotherapy treatment uses medicine to weaken and destroy cancer cells in the body, including cells at the original cancer site and any cancer cells that may have spread to another part of the body. Chemotherapy is a systemic therapy, which means it affects the whole body by going through the bloodstream. In some cases, chemotherapy is given before surgery to shrink the cancer.
- Drugs for cancer treatment are prescribed complementarily to therapies (anti-estrogen, cytotoxic, and endocrine drugs)
- Medicines can also be prescribed to treated patients in order to mitigate side effects of radiotherapy such as (for the most common) skin effects (redness, with itching, burning, soreness, and possible peeling), armpit discomfort or chest pain or side effets of chemotherapy such as e.g. anemia, nausea, infection, diarrhea, vaginal dryness, temporary infertility issues, and more generally important fatigue and pain for both types of therapy. Pain relievers are most of the time taken by patients.

Some side effects of treatment, such as hair and nail changes or permanent infertility (due to some heavy treatments) cannot however be alleviated by medication.

To the tests and the treatments available today to prevent, cure or treat breast cancer, direct costs can be attributed, based on available data from healthcare systems and economic literature. Beyond direct costs, and similarly to other health effects assessed above, breast cancer can also be considered as costly to other -more indirect- respects. Indeed, next to treatments, breast cancer might cause many other adverse consequences for patients such as absenteeism at work, social isolation, psychological depression, anxiety, and more generally a lower quality of life; the worst adverse effect being the death.

Overall, taking into account all these direct and indirect costs of breast cancer might be significant in terms of impact. The assessment carried out hereunder attempts to valuate this impact by estimating more precisely the health benefits associated to the avoided increased occurrence of breast cancer resulting from BPA restriction in thermal paper. The approach adopted is similar to the one used for the other health benefits assessed above.

- The excess risk ER has been modelled based on the same approach as described in Annex 2, from the raw data got from Moral, 2008 for the increase in TEB and in TD and Murray, 2007 for the increase in hyperplastic ducts (95 days); Murray, 2007 for 50 days and Jenkins, 2009 couldn't be robustly modelled (no dose-response relationship because no difference between the control group and the tested group). The computation resulted in 3 values of excess risk.

From Moral, 2008 (TEB), the fraction of the targeted population likely to be affected ("at excess risk") by an increase in TEB (and thus an increase in mammary gland vulnerability to cancer) has been inferred, taking again into account the average BPA internal dose corresponding to the cumulated probabilities distribution (Figure 22) of 0.21 µg/kg bw/d for workers. **This dose corresponds to an excess risk of 0.61%.**

From Moral, 2008 (TD), the fraction of the targeted population likely to be affected ("at excess risk") by an increase in TD (and thus an increase in mammary gland vulnerability to cancer) has been inferred to be **0.55%.**

From Murray, 2007 (95 days), the fraction of the targeted population likely to be affected ("at excess risk") by an increase in HD (and thus an increase in mammary gland vulnerability to cancer),has been inferred to be **0.055%.**

The table below summarizes the values of excess risk related to this critical effect on mammary gland which are used hereunder for the valuation of the corresponding health benefit.

Table 100. Values of excess risks modeled for the effects on mammary gland

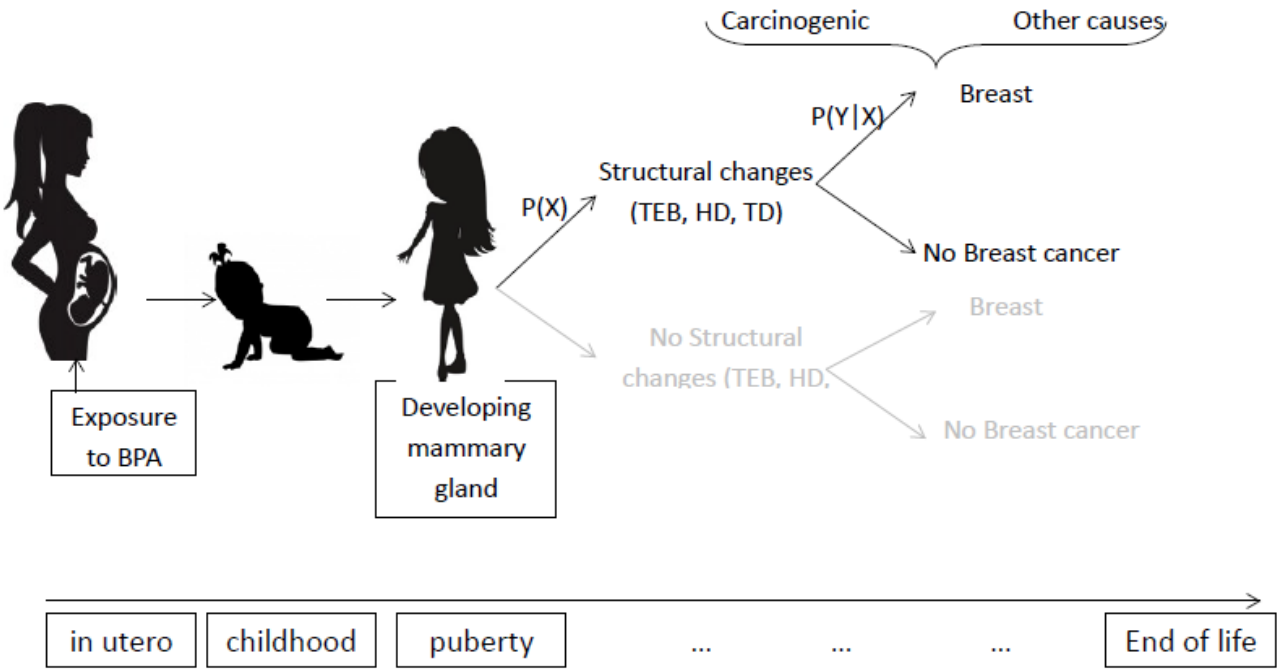
Study	**Values of Modeled excess risk (ER)**
Moral, 2008 (TEB)	0.61%
Moral, 2008 (TD)	0.55%
Murray, 2007 (HD, 95 days)	0.055%

In conclusion, the excess risks of suffering from mammary gland structural changes (and thus of an increase in mammary gland vulnerability to breast cancer subsequently to the co-exposures to carcinogenic agents) for the targeted population is respectively evalued to ER= 0.61%, 0.55% and 0.055%.

Moreover, given that more than 99% of breast cancers affect women and in order to be consistent with the economic studies selected further for the valuation of costs related to hits disease (only computed for women), the female unborn children are only taken into account. Like for endometriosis, the annual rate of female birth in the EU, G= 48.7%, is used.

The number of unborn female children exposed to BPA likely to be affected by an increase of TEB, TD and/or hyperplastic ducts thus respectively equals to ERxRxG= ER x 162,298 x 0.487= 482, 434 or 43. A worst-case can be calculated corresponding to the (though very unlikely) situation where, simultaneously the future child would suffer from an increase in TEB, TD and HD. In this case, the number of children concerned would be 960 (that is 482+434+43).

From this, only a portion of these female chidlren who are likely to suffer from mammary gland structural changes during puberty might then develop breast cancerous tumours subsequently to co-exposures with carcinogens agents. The sequence between the exposure to BPA in utero and the development of breast cancer is indirect and follows two steps: first of all, the mother's exposure to BPA likely to lead to structural changes to their future children's mammary gland which is defined as event X ; second of all, the development of breast cancer (event Y) due to the occurrence of these structural changes, corresponding to a conditional probability P (Y|X), as illustrated in the Figure below.



From the risk and exposure assessments, the strict probability of getting breast cancer after having suffered from an increase in TEB, TD and/or HD couldn't be determined. However, mammary hyperplasia (for which data on risk cancer exist) can be used as a proxy for the purpose of the human health impact assessment. Mammary hyperplasia is an increase in the number of mammary cells and can occur in the ducts (ductal hyperplasia) or the lobes (lobular hyperplasia). It can be graded as mild, moderate or florid (more extensive), according to how the cells look under the microscope. Hyperplasia may also be referred to as epithelial hyperplasia, usual epithelial hyperplasia, usual ductal hyperplasia or hyperplasia of usual type. Atypical hyperplasia is when the cells in the breast increase in number and also develop an unusual pattern or shape. It can occur in the ducts (atypical ductal hyperplasia or ADH) or the lobules (atypical lobular hyperplasia or ALH)[67].

The different types of hyperplasia affect breast cancer risk differently[68]:

- Mild hyperplasia of the usual type: This does not increase the risk for breast cancer
- Moderate or florid hyperplasia of the usual type (without atypia), also known as usual hyperplasia: The risk of breast cancer is about 1½ to 2 times that of a woman with no breast abnormalities.
- Atypical hyperplasia (either atypical ductal hyperplasia [ADH] or atypical lobular hyperplasia [ALH]): The risk of breast cancer is about 3½ to 5 times higher than that of a woman with no breast abnormalities

From this information, according to the American Cancer Society the risk of getting breast cancer given that the woman has hyperplasia (mild, moderate or atypical) is thus **0 - 5 times** higher than that of a woman with no breast abnormalities.

[67]http://www.breastcancercare.org.uk/breast-cancer-information/breast-awareness/benign-breast-conditions/hyperplasia-atypical-hyperplasia

[68]http://www.cancer.org/healthy/findcancerearly/womenshealth/non-cancerousbreastconditions/non-cancerous-breast-conditions-hyperplasia

According to probabilities rules:

P(Y | not X)= P(Y) - P(Y | X)

P(Y | X) = - P(Y | not X) + P(Y) (1)

P(Y) being the probability of getting breast cancer in general (all causes) with P(Y)= 13% in EU-28 as shown above.

Using the interval of increase factor risk {0, 5}, in (1) yields:

- For the lower increase factor risk of 0, P(Y | X)= 0;
- For the higher increase factor risk of 5, P(Y | X) = - 1/5 P(Y | X) + P(Y)
  ⇔ P(Y | X) = 0.83 x P(Y)= 0.11

As a result, the probability of getting breast cancer after having suffered from hyperplasia (used as a proxy of the increase of TEB, TD and/or HD) is comprised within the interval P(Y | X) = {0, 11%}, with a mid probability of 5.5%. Applying this mid value to the number of unborn female children exposed to BPA likely to be affected by an increase of TEB, TD and/or HD calculated just above, gives then an expected number of unborn female children exposed to BPA likely to develop breast cancer (after having suffered from mammary structural changes) respectively of 27, 24 and 2 (with a worst-case number of 53). For comparison, applying now the upper probability of 11% gives an expected number of unborn female children exposed to BPA likely to develop breast cancer (after having suffered from mammary structural changes) respectively of 53, 48 and 5 (with a worst-case number of 106).

- Regarding now the economic valuation of breast cancer, several studies exist providing breast cancer-associated costs.

The economic literature is abundant as regards the valuation of breast cancer from an individual or societal perspective. The assessment hereunder is based on a selection of economic studies aiming to be representative with a rather wide range of values. A literature review of economic studies has been carried out including all publications until November 2013. Some economic studies have been discarded because they have been considered to be either too much oriented towards specific treatments either too much targeted to particular stages of cancer (such as terminal stage). It has been finally decided to select 4 economic studies on the grounds that they are consistent with the purpose of this assessment by providing a cost per patient and including direct and/or indirect costs. The studies and the corresponding economic values are presented in the table below.

Table 101. Economic values of breast cancer direct and indirect costs

Economic Study	Economic value	Scope of the study	Assumptions/Limits
Gruber, 2012	Annual cost per patient (only direct costs): 9,000€ for patients between 30-55yo	German population	Data for 1999

	8,500€ in 57yo 5,000 in 69yo 3000€ for patients above 90 yo **TOTAL= €3,000-€9,000**		(inflated to 2010).
Campbell, 2009	Annual cost per patient (only direct costs): Initial care= $3,436-$41,000 Continuing care= $1,084-$11,844 Terminal care= $4,737-$57,000 **TOTAL**≈ $20,000-$100,000 (per study min – max) = $7,896-$34,500 (per study on average*) **≈ €5,800€-€25,365** (per study on average*)**	US women population Meta-analysis of 29 studies over 1984-2003	The treatment costs are for the USA Data for 1984-2003
Marino, 2003	Annual cost per patient (only direct costs): **TOTAL= €22,755-€32,284**	French population (95 patients)	Treatment considered mainly based on chemotherapy 1998 data
Radice, 2003	Annual cost per patient (direct and indirect): Initial care= 10,813$ Continuing care= 1,084$ Terminal care= 17,886$	US women population	The treatment costs are for the USA 1995 data

	TOTAL= 29,783$ **= 29,245€****		

*values calculated not included in the study

**costs converted from US$ to € with US$ 1 = 0.73540€ and inflation-adjusted

Campbell, 2009 is a meta-analysis carried out over 29 cost-of-illness studies (1984-2003) based on US data. Costs include only direct medical costs with three treatment stages: initial treatment, continuing care and terminal care. The treatment costs are higher in the USA than in other developed countries, therefore some overestimation is expected. However some of the data used are old (somehow outdated) from 1984 e.g., so to some other extent underestimation is also expected.

Gruber, 2012 provides age-specific estimates for breast cancer attributable costs in Germany, based on sickness funds data for 1999 (inflated to 2010). The costs assessed are only direct costs and include all costs for inpatient care, i.e. physician costs, medication costs, general costs for hospital stay and nursing care. Outpatient care costs or indirect costs are not included.The authors note that they might be underestimated.

In Radice, 2003, direct costs are estimated using the annual average number of physician visits, cost per physician visit, diagnostic costs, drug and radiation therapy costs, surgery costs, the number of patients receiving home healthcare visits and the number of average annual visits. Indirect costs for later stages of the disease are estimated including the loss of revenue for treatworkers due to their disability to work and days of missed work. As for Campbell, 2009, the treatment costs are higher in the USA than in other developed countries, therefore some overestimation is expected. However the data used are for 1995, so to some other extent underestimation is also expected.

In Marino, 2003, only direct costs are estimated, based on different modes of chemotherapy for inflammatory breast cancer in France. The cost combines an assessment of resource utilisation, in physical quantities, from the start of chemotherapy until radiotherapy. Monetary values are assigned for 95 patients.

As a whole, regarding these different studies, the direct costs of breast cancer range from 3,000€ to 32,284€ per patient per year. The Campbell, 2009 study seems to provide an average in between (€5,800€-€25,365).In order to be as representative of the possible range of costs as possible and in a sensitivity analysis perspective, **the range of values for the valuation is thus V=€3,000€-€30,000 per (woman) patient for 2013. As 2019 is taken as the year of reference, these values have been inflation-adjusted to get their 2019 values, based on the inflation rate forecasted by ECB which is around 1.9% per year over 2014-2019 (5 years ahead)[69]. As a result, V=€3,296€ and V'=€32,960 per (woman) patient in 2019 values.**

The table below summarizes the input data calculated or selected to carry out this assessment.

[69] http://www.ecb.europa.eu/stats/prices/indic/forecast/html/table_hist_hicp.en.html

Table 102. Summary of input data for the HHIA of the increase of breast cancer occurrence

Input data	**Value (in 2019)**
Excess risk for the "at risk" female cashiers/unborn children regarding an increase of mammary gland structural changes occurrence	ER=0.61% (TEB), 0.55% (TD) and 0.055% (HD)
Probability of getting breast cancer (Y) after having suffered from hyperplasia (X) (used as a proxy of the increase of TEB, TD and/or HD)	**P(Y | X) = {0, 11%};** **mid value = 5.5%**
Economic/societal value of breast cancer	(V; V')= (€3,296€-€32,960) per (woman) patient (2019 values)

Given the other input data already collected or calculated, the burden of breast cancer (resulting from an increased vulnerability of developing mammary glands to subsequent carcinogenic agents co-exposures) can be computed. This avoided burden thus takes 3 different values accordingly to the three values of excess risk calculated above and the probability P(Y | X)= {0, 11%}. The values of this burden are calculated for the lower bound V and the upper bound V' and for 2 values of P(Y | X), the mid value 5.5% and the upper value 11%. The benefits are calculated like the other health benefits taking 2019 as the year of reference with a decreasing discounting rate of 2% over 2019-2049 (first 30 years) and then 0% (for benefits accruing after 30 years) in order to account for intergenerational equity as well as adjustements for inflation (for further details on the approach, see section on endometriosis above). Moreover, it is assumed that the breast cancer would occur in the future generation mostly after the age of 50, consistently with the distribution in age of breast cancer occurrence[70]. **The year 2069 is thus the expected year of occurrence** (2019+50).

As presented in the tables below, the health benefits B^w_g and B'^w_g thus range from €4,263 (for HD) to €472,824 (for TEB) using the mid value of P(Y | X) (5.5%) and €8,526 (for HD) to €945,649 (for TEB) using the upper value of P(Y | X) (11%). The corresponding worst-case situations are respectively between €94,177 and €941,773 and between €188,355 and €1,883,547.

Table 103. Health benefits from avoided breast cancers with mid value of P(Y | X)

Values of Excess risk	0.61% (TEB)	0.55% (TD)	0.055% (HD)	*Worst case*

[70]http://www.cancerresearchuk.org/cancer-info/cancerstats/types/breast/incidence/uk-breast-cancer-incidence-statistics#age

				(TEB+TD+HD) *1.22%*
P(Y | X)= {0, 11%};	**mid value= 5.5%**			
Number of unborn female children likely to be affected in the EU per year	27	27	2	*53*
health benefit B^{w}_{g} lower bound (with V=€3,296€) for 2019	**47,282 €**	**42,632 €**	**4,263 €**	**94,177 €**
healt benefit B'^{w}_{g} upper bound (with V'=€32,960) for 2019	**472,824 €**	**426,317 €**	**42,632 €**	**941,773 €**

Table 104bis. Health benefits from avoided breast cancers with upper value of P(Y | X)

Values of Excess risk	0.61% (TEB)	0.55% (TD)	0.055% (HD)	*Worst case* *(TEB+TD+HD)* *1.22%*
P(Y | X)= {0, 11%};	**upper value= 11%**			
Number of unborn female children likely to be affected in the EU per year	53	48	5	*106*
health benefit B^{w}_{g} lower bound (with V=€3,296€) for 2019	**94,565 €**	**85,263 €**	**8,526 €**	188,355 €
healt benefit B'^{w}_{g} upper bound (with V'=€32,960) for 2019	**945,649 €**	**852,634 €**	**85,263 €**	1,883,547 €

As uncertainties or overestimating factors, one can note that:

- **Whether the probability inferred based on hyperplasia is representative for the mammary structural changes in questions herein (TEB, TD and HD) is uncertain. It has been used as a proxy since hyperplasia also corresponds to structural changes to mammary gland and exact information is missing about the conditional probability between the increase in TEB, TD and HD and the occurrence of breast cancer**
- **the computation of the values of excess risk and health benefits are uncertain since one the one hand, the number of animals tested basing the studies is low (4 per dose for the HD for Murray, 2007 and 8 for the TEB and TD in Moral, 2008), what makes the confidence in the result limited**
- **It has to be also pointed out that even though not all mammary gland changes may lead to breast cancer, this does not mean that the valuation factor for these changes is zero. If a benign tumor is developed as a consequence of these structural changes, this might still induce medical costs due to diagnostic, testing, treatment and/or interventions, as well as a reduction in life quality for the affected individual. However, these costs have not been included in the benefits. They would have increased the overall benefits related to the effects on mammary gland.**

F.1.1.5. The human health benefits for workers – brain and behaviour

Regarding the effects of BPA on the brain and behaviour, and as shown above in section B.2, the following critical effects (based on effects observed in animals and on the Xu et al. (**Xu, 2010)** key study chosen for the human risk assessment) have been selected:

- alteration of memory

- alteration of learning functions

As regards these effects, it seems that the main adverse effect in humans would be linked to difficulties encountered on learning functions. The physiological mechanism in the brain in rats and humans is similar; that is the reason why ANSES, 2013 expresses a rather high confidence level (about 50% to 100%) in the extrapolation from rats to humans. Nevertheless, it seems meanwhile impossible to quantitatively estimate the magnitude of these effects and the exact outcomes of them. They may occur through many various forms such as disorientation, weak memory, loss of IQ points, etc. Furthermore, the timescale over which the outcomes might occur is difficult to predict: although only the unborn children will be affected, the effects may be observed over their whole lifetime, from childhood to working life and even later on.

For all these reasons, given the high degree of uncertainty surrounding the valuation of the human health benefits associated to effects on brain and behaviour, it has

been decided to not quantity them. Still, these benefits may in principle concern every unborn child exposed in utero to BPA from thermal paper handling by their mother, both sex included. As a consequence, they necessarily exceed zero and weigh positively in the total benefits of the restriction proposed.

F.1.1.6. Summary of the human health benefits for workers

Table 105. Summary of the HHIA for workers

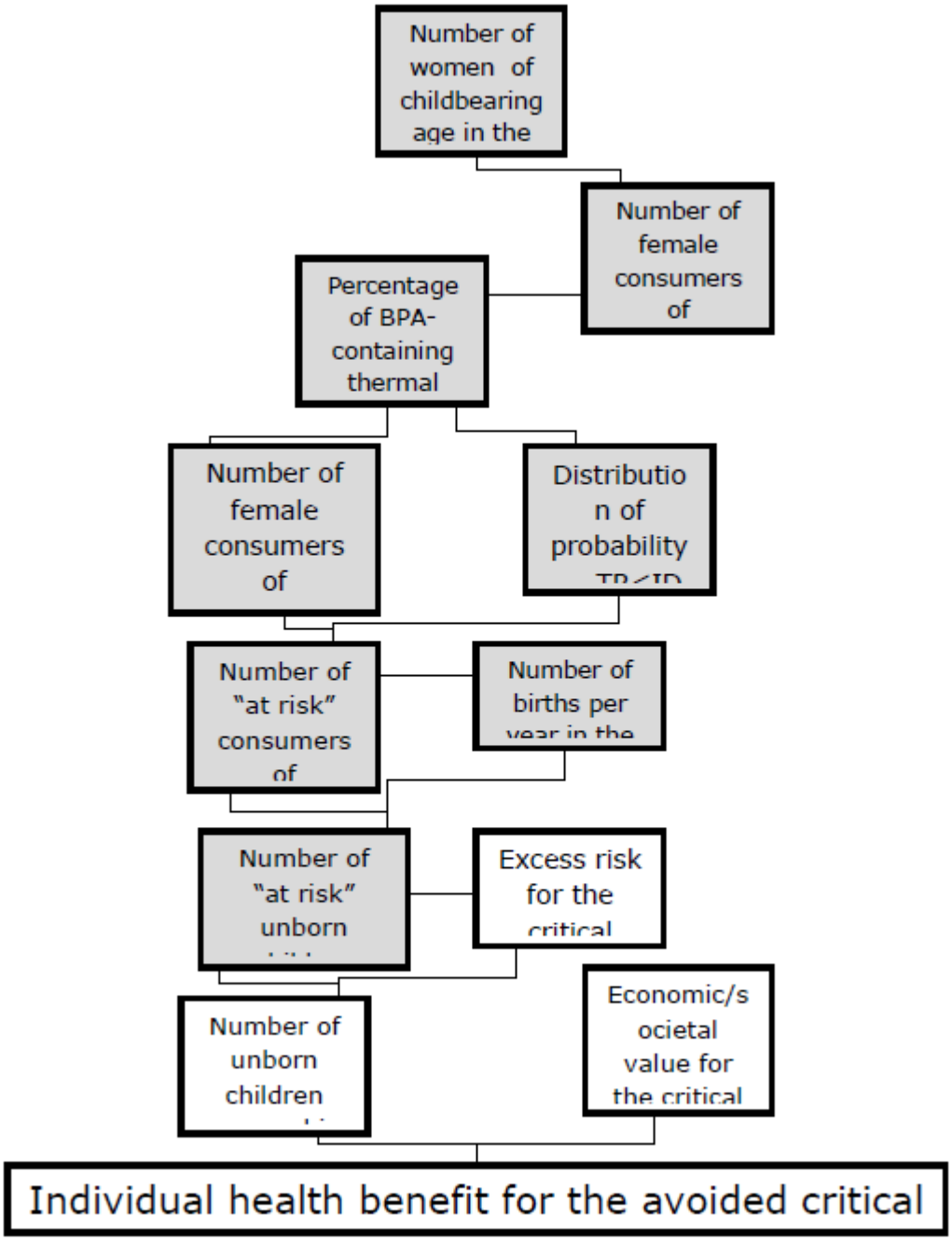
Critical Effect	**Health adverse outcomes**	**Excess Risk (ER)**	**Number of unborn children at 'excess risk'**	**Annual health benefit (2019 values) (B^w_i; i=e, g, bw, c)**
Female reproductive system	Increase in ovarian cysts	-	-	>0
	Endometriosis	0.07% (female only)	55	**B^w_e = 314,523 €**
	Disruption of ovarian cycles	-	-	>0
Mammary gland	Increase in vulnerability to breast cancer (due to increase of TEB, TD and/or HD)	0.61% (TEB) 0.55% (TD) 0.055% (HD) (female only) *Worst-case: ∑=1.22%*	• **P(Y/X)= 5.5%** 27 (TEB) 24 (TD) 2 (HD) (∑=*53*)	**[B^w_g ; B'^w_g] for each precursor** • **P(Y/X)= 5.5%** **[€47,282;€472,824] (TEB)** **[€42,632; €426,317€] (TD)** **[€4,263; €42,632] (HD)**
		Conditional probability of getting breast cancer after the discovery of mammary gland structural changes P(Y/X)={0; 11} -Mid value 5.5%	• **P(Y/X)= 11%** 53 (TEB)	Worst case: **[€94,177; €941,773]**

		-Upper value 11%	48 (TD) 5 (HD) (∑=*106*)	• **P(Y/X)= 11%** **[€94,565; €945,649] (TEB)** **[€85,263; €852,634] (TD)** **[€8,526; €85,263] (HD)** Worst case: **[€188,355; €1,883,547]**
Metabolism and obesity	Increase in BW	0.33%	535	**B^{w}_{bw}= €1,513,544**
	Increase in Cholesterol	0.73 % (then adjusted to the general population fraction of 54%)	639	**B^{w}_{c}= 34,868€** **B'^{w}_{c}= 389,885€**
Brain and behaviour	Spatial memory	-	-	>0
	learning functions	-	-	>0
TOTAL in 2019 values (discounted and inflation-adjusted)				**>[€1,863,178; €2,654,870] (except worst-case)**

F.1.1.7. Human health impact assessment for consumers

The valuation of health benefits has also been carried out for consumers. The assessment has followed the same kind of logigram as for workers. The logigram for consumers is presented below.

Figure 44. Logigram for the economic evaluation of the human health benefits or BPA restriction in thermal paper for consumers



The consumers exposed to BPA-containing thermal paper targeted herein are any woman of childbearing age who is likely to handle tickets or receipts made of thermal paper after a purchase or a cash withdrawal for example. It happens in everydaylife and may concern every woman in the EU in that class of age. As shown above, from Eurostat data, the number of

women of childbearing age (between 15-50 years old) in the EU has been calculated to be equal to 121,672,696 in the EU 28 in 2012, compared to the EU general women population which is 259,793,939. The former is thus also considered to be the number of consumers addressed herein, noted F'.

Similarly to the HHIA carried out for workers, the share of BPA-containing thermal paper present on the EU market is estimated to be 70%, of which 65% are used for POS tickets. The number of female consumers of childbearing age likely to be exposed to BPA-containing thermal paper in the EU is thus estimated at E'=0.7x0.65xF'= 55,361,077. From the risk assessment performed in section B, similarly to the workers, the probabilities to develop an adverse effect are inferred from the distribution of BPA internal doses due to the exposure to thermal paper likely to exceed the benchmark dose. For all critical effects , the distributions of internal doses for consumers do not entirely exceed the toxicological benchmark (Figure 25). Therefore, only one portion of the probabilities distributions between the toxicological benchmark and the P100 has to be taken into account for the estimate of the number of consumers at risk:

- For the effect on female reproductive system, this probability is P= 60.11% (between P39.89, corresponding to the toxicological benchmark of 0.01µg/kg bw/d, and P100)
- For the effect on metabolism and obesity, this probability is P= 63.01% (between P36.99, corresponding to the toxicological benchmark of 0.009µg/kg bw/d, and P100)
- For the effect on mammary gland, this probability is P= 85.92% (between P14.08, corresponding to the toxicological benchmark of 0.0025µg/kg bw/d, and P100)

Then, similarly to the HHIA carried out for workers, the number of consumers "at risk" is estimated at A'= PxE'= 33,277,543 for the effect on female reproductive system, 34,883,014 for the effect on metabolism and obesity and 47,566,237 for the effect on mammary gland.

From this, in order to get the number of unborn children to be at risk due to the exposure of these female consumers (their mother), the annual birth rate of B=4.4% is used here again and result in a **number of unborn children at risk of R'= 1,459,791 for the effect on female reproductive system, of 1,530,218 for the effect on metabolism and obesity and of 2,086,595 for the effect on mammary gland.**

The table below summarizes the input data collected and calculated for the human health impact assessment (HHIA) for consumers.

Table 106. Summary of the input data for the HHIA for consumers

Input data	**Value**
	C'=259,793,939

Number of women in the EU	
Number of women/consumers of childbearing age in the EU	F'=121,672,696
Share of BPA-containing thermal ecopaper on the EU market	65%x70%
Number of female consumers of childbearing age exposed to BPA-containing thermal paper in the EU	E'= 0.7x0.65xF'= 55,361,077
Probability to develop an adverse effect	P= 0,6011 (female reproductive system) P=0,6301 (metabolism and obesity) P=0.8592 (mammary gland)
Number of female consumers "at risk"	A'=33,277,543 (female reproductive system) A'=34,883,014 (metabolism and obesity) A'=47,566,237 (mammary gland)
Average annual birth rate in the EU	B=4.4%
Number of unborn children exposed in utero	

in the EU likely to be "at risk" annually	R'= A'xB= 1,459,791 (female reproductive system) R'= A'xB= 1,530,218 (metabolism and obesity) R'= A'xB= 2,086,595 (mammary gland)

The health benetifs are then quantified and valued for consumers, based on the same economic values and the same methodological approach (decreasing discount rates, uprating factor for adjustments for inflation, 2019 taken as the year of reference) as used for workers. The computation of excess risks has been however calculated specifically for consumers with the corresponding average internal dose of BPA: as shown in section B.10.1.1.2, this dose equals to 0.02 µg/kg bw/day. The excess risks have then been calculated based on the exact same method as described in Annex 2. The health benefits for consumers are noted B^{c}_{i}, with i=e (for endometriosis), b (for body weight), c (for cholesterol) and g (for mammary gland). The results are presented in the table below.

Table 107. Summary of the HHIA for consumers

Critical Effect	**Health adverse outcomes**	**Excess Risk (ER)**	**Number of unborn children at 'excess risk'**	**Annual health benefit (2019 values) (B^c_i; i=e, g, bw, c)**
Female reproductive system	Increase in ovarian cysts	-	-	>0
	Endometriosis	0.0064% (female only)	45	**B^c_e = €259,039**
	Disruption of ovarian cycles	-	-	>0
Mammary gland	Increase in vulnerability to breast cancer (due to increase of TEB, TD and/or HD)	0.059% (TEB) 0.053% (TD) 0.005% (HD) (female only) *Worst-case: Σ= 0.46%*	• **P(Y/X)= 5.5%** 33 (TEB) 30 (TD) 3 (HD) (Σ=*65*)	**[B^c_g ; B'^c_g] for each precursor** • **P(Y/X)= 5.5%** **[€58,796; €587,961] (TEB)** **[€52,817; €528,168] (TD)**

		Conditional probability of getting breast cancer after the discovery of mammary gland structural changes P(Y/X)={0; 11} -Mid value 5.5% -Upper value 11%	• **P(Y/X)= 11%** 66 (TEB) 59 (TD) 6 (HD) (∑=*131*)	**[€4,983; €49,827 (HD)** Worst case: **[€116,596; €1,165,956]** • **P(Y/X)= 11%** **[€117,592; €1,175,921] (TEB)** **[€105,634; €1,056,336] (TD)** **[€9,965; €99,654] (HD)** Worst case: **[€233,191; €2,331,911]**
Metabolism and obesity	Increase in BW	0.032%	490	**B^{c}_{bw}= 1,384,489€**
	Increase in Cholesterol	0.07% (then adjusted to 54%)	578	**B^{c}_{c}= 28,707€** **B'^{c}_{c}= 320,996€**
Brain and behaviour	Spatial memory	-	-	>0
	learning functions	-	-	>0

TOTAL in 2019 values (discounted and inflation-adjusted)	**>[€1,677,218; €2,552,485] (except worst-case)**

F.1.1.8. Summary of the total human health benefits

Table 108. Summary of the HHIA due to the BPA restriction in thermal paper for workers and consumers

Critical Effect	**Health adverse outcomes**	**Excess Risk (ER)**		**Economic values (V and V') (2019)**	**Annual health benefit (2019 values) B_i (i=e, g, bw, c) = B^w_i+B^c_i**
		workers	**consumers**		
Female reproductive system	Increase in ovarian cysts	-	-	-	>0 Qualitatively described
	Endometriosis	0.07% (female only)	0.0064%	V=10,524€ per woman per year	B^w_e + B^c_e = €573,562
	Disruption of ovarian cycles	-	-	-	>0 Qualitatively described
Mammary gland	Increase in vulnerability to breast cancer (due to increase of TEB, TD and/or HD)	0.61% (TEB) 0.55% (TD) 0.055% (HD) (female	0.059% (TEB) 0.053% (TD) 0.005% (HD) (female	[V=€3,296€; V'=€32.960] per woman per year	$[B^w_{g+} B^c_{g;}$; $[B'^c_{g+} B'^w_g]$ for each precursor [€106,079; €1,060,785] (TEB) [€95,449; €954,485] TD) [€9,246; €92,459] (HD) Worst case: $[\Sigma B^i_g ; \Sigma B'^i_g]$ = [€210,773; €2,107,729]

		only) *(Worst-case:* ∑ER= *1.22%)*	only) Worst-case: ∑ER= 0.117%		
Metabolism and obesity	Increase in BW	0.33%	0.032%	V= €4,131 per avoided case per year	$B^{w}_{bw} + B^{c}_{bw}$ = €2,897,144
	Increase in Cholesterol	0.73% (then adjusted to the general population fraction of 54%)	0.07% (then adjusted to the general population fraction of 54%)	[V=12€-47€; V'=135€] per % of decreased LDL-cholesterol for one person treated/year	$[B^{w}_{c}+ B^{c}_{c}; B'^{w}_{c} + B'^{c}_{c}]$ = [€60,443; €675,864]
Brain and behaviour	Spatial memory	-	-	-	>0 Qualitatively described
	learning functions	-	-	-	>0 Qualitatively described

TOTAL in 2019 values (discounted and inflation-adjusted)	**>[3,540,395; 5,207,355] (except worst-case)**

As a whole, the total health benefits expected from the proposed restriction are estimated to range at least from €3.5 million to €5.2 million in 2019 (discounted) value, keeping in mind that not all health benefits have been qualitatively assessed. The total benefit of the restriction are thus expected to be higher.

Sensitivity analysis (for illustrative purposes)

A sensitivity analysis has been carried out on several potentially sensitive parameters in order to judge about their influence on the total health benefits (workers and consumers):

the share of BPA-containing thermal paper in the EU: the initial share of 70% might be overestimated to some extent and has been made varied with 50% (considered as realistic given the already ongoing substitution) and 30% (considered as less likely especially for eco-paper) (and 85% for illustrative purposes). All other values have been remained unchanged. The results are presented below.

Table 109.Total health benefits - sensitivity analysis on the share of BPA-containing thermal paper

Share of BPA-TP	**Lower bound of total health benefits**	**Upper bound of total health benefits**
85% (for illustrative purposes)	3,974,911€	5,999,076€
70% (for comparison)	*3,540,395€*	*5,207,355€*
50%	2,961,041€	4,151,726€
30%	2,381,687€	3,096,098€

As expected, the higher the share of BPA-containing thermal paper on the market, the higher the health benefits. This conclusion is however only valid if it is assumed that there is not any other as much as toxic substitute totally replacing BPA.

The share of cashiers in the EU compared to the general population has been inferred from different data from some EU countries the share for France and was considered to be around 2%. This share is not directly available and couldn't be double-checked for the whole EU labour market, so it has been made varied to 3% and 4% in order to take into account the uncertainty related to the possible exclusion of some workers likely to handle BPA-containing thermal paper but not referenced strictly within 'cashiers' occupation. Indeed, there might be a certain amount of workers who should be included in the HHIA, e.g. the owners of unipersonal or small enterprises who are at the same time owners, salers and cashiers. They might concern many people such as craftsmen or the owners of corner shops. All other values have been remained unchanged. The results are presented below.

Table 110. Total health benefits - sensitivity analysis on the number of cashiers

Share of cashiers	**Lower bound of total health benefits**	**Upper bound of total health benefits**
2% (for comparison)	*3,540,395€*	*5,207,355€*
3%	*3,715,656€*	5,778,462€
4%	3,890,918€	6,349,570€

As expected, the higher the number of cashiers (or more generally workers likely to handle BPA-containing thermal paper) in the EU, the higher the health benefits.

As regards the economic valuation of the increase in body weight, the number of children likely to be affected by overweight or obesity has been estimated to be 535 for workers' children and to be 490 for consumers' children. It has been implicitely assumed that any increase of body weight would lead to overweight or obesity which might be highly overestimating. These numbers have thus been made varied to (arbitrarily since there is no other data available) 100 and 200 for the former and to 200 and 300 for the latter. This sensitivity analysis is all the more important than the health benefit related to the increase in BW stands for a large share of the total benefits. Moreover, as already mentioned above, according to WHO, 20% of children and adolescents are overweight today in Europe. This data can thus also be taken into account for the sensitivity analysis, assuming that these children would be overweight anyway independently from their potential exposure to BPA in utero from thermal paper handling. For the sake of completeness, additional values are then used: 428 (535 less 20% considered as overweight anyway) for workers' children and 392 (490 less 20% considered as overweight anyway) for consumers' children. All other values have been remained unchanged. The results are presented below.

Table 111. Total health benefits - sensitivity analysis on the number of children likely to suffer from an increase in BW

Number of unborn children likely to be affected (workers+consumers)	**Lower bound of total health benefits**	**Upper bound of total health benefits**
100+200	1,491,469€	3,158,469€
200 +300	2,056,947€	3,723,907€
428+392	2,691,713€	4,628,673€

535+490(for comparison)	*3,540,395€€*	*5,207,355€*

As expected, the lower the number of unborn children at excess risk, the lower the health benefits.

Finally, a collateral benefit from the restriction could also be mentioned, due to the reduction in risks for workers exposed to BPA on the production chain of BPA-containing thermal paper ({UK, 2008 816 /id}).

F.1.1.9. Uncertainty treatment on the human health impact assessment

F.1.1.9.1 Uncertainty and confidence in the HHIA

The uncertainties surrounding the HHIA can be summarised as below:

- The following uncertainties might be overestimating:

  - As regards the economic valuation of the increase in body weight, as already explained, it has been implicitely assumed that any increase of body weight would lead to overweight or obesity (at worst). A sensitivity analysis has been carried out on that parameter.
  - The impacts for human health of alternatives to BPA in thermal paper have not been assessed and the assumption has been made that the health benefits related to BPA restriction are 'absolute', that is to say that with the restriction the adverse effects will disappear. However, this might not be entirely valid if some substitutes (such as BPS) have similar effects on human health.

- The following uncertainties might be underestimating:

  - Some benefits are not quantified:
    - the health benefits related to critical effects on brain and behaviour (avoided alteration of memory and learning functions)
    - some health benefits related to critical effects on the female reproductive system such as the increase in ovarian cysts occurrence and the disruption of ovarian cycles

  - Some avoided disease costs are not taken into account:

    - The costs of breast diseases due to an increase in TEB, TD and/or HD but not leading to breast cancer such as benign tumors have not been taken into account. even though not all mammary gland changes may lead to breast cancer, this does not mean that the valuation factor for these changes is zero. If a benign tumor is developed as a consequence of these structural changes, this

might still induce medical costs due to diagnostic, testing, treatment and/or interventions, as well as a reduction in life quality for the affected individual. However, these costs have not been included in the benefits. They would have increased the overall benefits related to the effects on mammary gland

- no intangible costs have been taken into account (pain and suffering) for breast cancer

- The number of 'cashiers' estimated is only based on the professional 'cashiers', referenced as such but there may be many unipersonal enterprises or corner shops in which the owner is also the accountant, the saler and the cashier. As a consequence, not taking into account this number of potentially exposed workers to BPA-containing thermal paper might underestimate somehow the health benefits. A sensitivity analysis has been carried out on that parameter.

- The health benefits for other workers who may handle thermal paper are not taken into account in the evaluation. This might be the case of medical staff who may handle thermal paper from e.g. ECG or ultrasounds medical tests. Compared to cashiers, they might concern a relatively low number of persons but including them in the HHIA would still increase the health benefits.

- As already said, a collateral benefit from the restriction could be also the reduction in risks for workers exposed to BPA during the production of BPA-containing thermal paper. Including these avoided adverse exposures would increase the total health benefits of the restriction

➢ Other uncertainties can also be reported. It is not clear whether there would be overestimating or underestimating:

- It is unclear to what extent the health benefits calculations carried out based on US or Australian studies can be extrapolated to EU

- As regards the economic valuation of the increase in body weight, the body of literature on "obenomics" is huge, and due to time constraints and for the sake of analysis proportionality, the literature search and analysis has been restricted. Additional literature searches and more refined analysis might have provided a more complete assessment.

- As already underlined, as regards the computation of the values of excess risk and health benefits related to the increase of vulnerability of mammary gland (precursor of breast cancer) are highly uncertain due to the low number of animals tested basing the

studies selected and the lack of information on the caucal link between the increase in TEB, TD and HD and the occurrence of breast cancer

- The health benefits have been assessed assuming that one alternative would totally replace the BPA contained in thermal paper in the EU. It could be more realistic to consider that several alternatives may replace it, depending on the choice of the actors of the supply chain.

- Whether the probability inferred based on hyperplasia is representative for the mammary structural changes in questions herein (TEB, TD and HD) is uncertain. It has been used as a proxy since hyperplasia also corresponds to structural changes to mammary gland and exact information is missing about the conditional probability between the increase in TEB, TD and HD and the occurrence of breast cancer

- There is an inherent uncertainty related to the calculation of the excess risks computed as a basis for the HHIA carried out above. Indeed, the excess risks have been modelled based on studies from which a proportion of affected animals has been inferred. This proportion has been then extrapolated to humans. Although some risk factors have been applied in order to take into account this uncertainty, one has to be aware of that methodological limit.

F.1.1.9.2 Caveat about the human health impact assessment

As it has been shown above in section C and demonstrated below in section F.2, the substitution of BPA by BPS (or other bisphenols) is expected to be more than likely. Indeed, BPS in particular is already largely used in thermal paper worldwide and appears to be the most technically and economically feasible "drop-in" alternative. However, taken into account the toxicological profile of BPS, this substitute might cause similar adverse health effects as BPA. As a result of those expectations and hazards, it has to be pointed out that the health benefits estimated herein due to the restriction of BPA in thermal paper could be reduced and to some extent come down to zero if BPA was actually and totally replaced by BPS and if BPS was actually proven as much as toxic. Indeed, the avoided adverse effects of BPA would be compensated by the adverse effects of BPS. Although BPS is not targeted by this restriction proposal, it is of primary importance to make some warning and to draw special attention to that particular situation. The Substance Evaluation planned by Belgium in 2014 will help in mitigate some uncertainties on the toxicity of BPS.

F.1.1.10. Consideration about potential human health benefits based on kidney effects

It has to be noted that adverse effects on kidney are not part of the initial proposal submitted. Given the RAC position on the effects of BPA on the kidney from which the EFSA TDI is

derived, some consideration related to the potential human health benefits from the avoidance of these effects is thus added herein.

As presented in its 2014/2015 opinion on the risk of BPA to public health (EFSA, 2015), effects of BPA on the kidney were considered as "likely" adverse effects in animals (changes in the mean relative kidney weight in a two generation toxicity study in mice from Tyl 2002/2008 studies) and were used to identify the point of departure from which the TDI is derived. on It may be noted that "likely" adverse effects on mammary gland also underwent the benchmark dose response modelling in the EFSA assessment but no $BMDL_{10}$ could be calculated for mammary gland effects.

From this critical endpoint on kidney, 2 components would be needed in order to perform a quantitative human health impact assessment:

- First of all, a dose-response relationship is needed in order to infer the likelihood of developing the adverse effects, and from this, the number of cases expected to occur in the targeted population. However, the adverse effects on kidney weight were observed at quite high doses and at these levels of doses, it may be expected that no cases might occur. As a result, there might be no need for further exploration for a dose-response relationship and in terms of disease burden, there might be no benefits expected for human health regarding effects on kidney.
- Second of all, assuming that a dose-response relationship could be built, it might be then difficult to clearly identify the diseases resulting and attributable to an increase in kidney weight. EFSA specified in its opinion that the increased kidney weight was associated with nephropathy at the highest BPA dose but at the other (lower) doses, no indication of precise pathology is provided.

F.1.2 Environmental impacts

As already précised in E.2.1, environmental exposure is not strictly at concern in this dossier but some indirect environmental impacts can still be expected from the restriction proposed.

Indeed, it has been shown above (section B.1) that BPA in thermal paper could be the source of secondary contamination of foodstuffs and objects in contact with tickets or receipts such as banknotes (EWG, 2010 ; Liao, 2011) and wallets. Moreover, thermal paper is currently recycled in the EU up to 50% (see section B.2) and is re-used to produce other paper-based products such as recycled paper, napkins, toilet paper, paper towels, newspapers or magazines (Gehring, 2004). Those products might thus contain BPA traces. The secondary contamination and the BPA traces coming from paper recycling contribute to the general population exposure to BPA via the environment and would thus be avoided by the restriction proposed.

Moreover, as far as environment itself is concerned, it has been shown above that the recycling of thermal paper containing BPA is suspected to be one of the sources of contamination via aqueous effluent recycling containing BPA-chlorinated derivatives or sludge from sewage purification plants (UBA, 2010). It is estimated that about 350-500 tons/year of BPA enter the recycling supply sector, which stands for 70% of total annual aquatic releases (EC, 2008 , OECD, 2009 , INERIS, 2010) (see section B.9.3.2.4). These releases would also be avoided by the restriction proposed.

These impacts are not assessed further since they are out of the scope of this proposal. Assessing such impacts would increase the total benefits of the restriction.

F.2 Economic impacts

Economic impacts of the proposed restriction have been assessed for four interlinked markets as to the restricted use of BPA in thermal paper:

- economic impacts for the market of BPA

- economic impacts for the market of thermal paper itself

- economic impacts for the markets of alternative dye developers

- economic impacts for the markets of alternative printing techniques

F.2.1. Economic impacts for the market of BPA

If the use of BPA in thermal paper is restricted, the market of BPA will be obviously impacted. To what extent it will be is the question addressed in this section.

As shown in section B.2X above, the BPA market is global-oriented and supplies a high range of end markets from polycarbonates resins, to flame retardants, as well as thermal paper and epoxy resins. Within the EU, the BPA production is oligopolistic and stands for 25% of the worldwide production. Thermal paper accounts for 0.16% of total EU use of BPA be it about 1,890 tons in 2008 and 2,400 tons in 2012. Therefore, thermal paper is a very minor end-use of BPA. Moreover, and as already mentioned, given its toxicological and ecotoxicological profiles (see section B.1), the growing consumers demand for substitution solutions, the availability of alternatives dye developers on the market (see C.2) and the increasing regulatory actions BPA is being subjected to within the EU and in the rest of the world (see section E), this use has started to decline. The section C then analyses the substitutes to BPA as alternative dye developers in thermal paper and demonstrates that 'realistic' alternatives exist, that some of them are already used in thermal paper, and that they are available and technically and economically feasible. This analysis is first and foremost based on literature review and is corroborated by the consultations of industry carried out during the elaboration of this proposal.

As a result of these situation and trends, it can be reasonably expected that the BPA market might not be significantly impacted by the restriction proposed. The BPA market may concentrate its production capacity on its other (and major) uses without important foreseen difficulties or costs. Of course, given that BPA is also currently about to be regulated (or proposed to be so in some EU countries and worldwide) for other and more significant uses such as the use of BPA in food contact materials, the market of BPA could be much more affected downward in the future due to those upcoming additional regulations and risk management measures. However, the analysis and evaluation of the impacts of those are out of the scope of that proposal and will not be further developed. This response of the BPA market is considered as the most likely.

Another possible response from the BPA manufacturers to the restriction proposed would consist in keeping thermal paper as an end-use for their substance and doing so, in making the choice to diversify their production by incorporating one (or some) alternative dye

developer in their range of products. That response would allow them capturing some new demand and shares on the market of that alternative. It could also bring them some positive impact in terms of "safe and eco-friendly" image and make them compliant with the new restriction. Nevertheless, such a decision would also imply some (possibly high) extra costs.

These extra costs include firstly the purchasing costs of the alternative itself. As we shown above in section C.2, all the chemical alternatives to BPA are more expensive than this latter but their prices are expected to decrease significantly as a result of the restriction in the near future.

For comparison purposes, the table below provides the current prices of BPA and some of the alternative dye developers selected and assessed in section C. These prices are compiled from the data gathered from MSCAs consultation and the INERIS survey 2013 (INERIS, 2013) as well as ICIS, 2009 .

Table 112. Prices of BPA compared to alternatives (euros per ton)

Chemical Dye developer	**Minimum average price**	**Maximum average price**	**Average Price**
BPA	1,263	1,906	1,585
BPS	2,920	4,200	3,583
D8	11,390	15,104	12,938
Pergafast 201	15,000	30,000	22,500

Concerning the price of Pergafast, it has to be emphasized that the maximum price indicated might be overestimated since it is based only on one single declaration gathered during the consultation. This price couldn't be checked during the elaboration of this proposal. As already said in section C.2.5.4, other information collected from the public consultation indicates however that thermal paper with Pergafast would be around 10-25% more expensive than thermal paper with BPA (Danish EPA 2014, quoted in FBR-WUR report[71]). This information thus qualifies the information initially gathered and Pergafast might have a less high price than expected. This additional information confirms the assumption that the price of Pergafast might be overestimated.

The detailed data sources of these prices are quoted in the section F.2. It has to be noted that these prices are slightly different from the one indicated in INERIS, 2013 since the former are the results of the compilation of several data sources, such as explained in section B.2.

To date, BPA is the cheapest dye developer. Its relatively low price is explained by a comparatively higher demand for BPA on the market for developers. Further, the market of

[71] *Analysis of alternatives for bpa in thermal paper*, report 1515, Dec 2014

thermal paper is growing worldwide and in spite of the increasing use of alternative chemicals, BPA still dominates the market. However, according to the information provided by the supply chain consulted (INERIS, 201), the growing use of alternatives makes their prices progressively decrease: the substitution is ongoing and substitutes are available; the demand is increasing and the supply seems to follow the trend, allowing for scale economies. Based on this information, the expected continuous increase in demand for substitutes (speeded up by the restriction of BPA) is thus not expected to encounter any particular difficulty to be met in the near future. The prices for substitutes indicated in the previous table are indicative 2013 prices and don't take into account these market trends. One UK printer device distributor reported that his suppliers indicated that BPA substitutes should cost more than BPA, "at least initially". This could mean that he believes that the prices are likely to decrease in future years (INERIS, 2013). Moreover, those are whole sale prices and independent on their uses and their final applications. Therefore, they don't account for any specific thermal paper market-related price constraints. To some extent, they are likely to be somehow overestimated.

However, although the future prices of alternatives might be expected to rapidly get comparable to the today's BPA price, as regards operating costs, manufacturers of BPA who would decide to diversify their substances portfolio might not benefit from the same economies of scale and increasing returns for the new dye developer (likely to be produced in much lower quantity) as for BPA, produced today in high volume. As a consequence, producing an alternative dye developer might durably be more costly than producing BPA for BPA manufacturers. This cost gap could be nonetheless alleviated in case the new dye developer is highly demanded on the thermal paper market as an alternative to BPA, so that the new demand could somehow compensate the (expected low) loss of profit due to the BPA phase-out. That being said, to what extent the manufacturers would be able to make the 'right' strategic choice concerning the 'right' alternative to target is hardly foreseeable as well as is the future orientation of demand on one particular dye developer rather than another.

Secondly, the extra costs due to diversification could also include the cost of investments needed for technological changes in new equipments adapted to producing the alternatives and associated costs such as staff training.

Overall, this second possible response of the BPA market is based on highly uncertain grounds and appears to be costly compared to the possible gains. Strategically speaking and given that the BPA market has already many other significant end-markets to supply, there is no reliable information that could make think that it would occur. This response is thus considered as unlikely.

The table below summarizes the possible responses of BPA market to the restriction proposed. In order to make some sensitivity analysis on those responses, it has been attributed a qualitative likelihood to each (based on the whole information gathered and some conjectures).

Table 113. Likely responses and economic impacts for the market of BPA

Market	Likely responses and economic impacts
	Purpose: Phasing-out from thermal paper end-use

BPA manufacturers	Response to the restriction: Focus on other and major end-uses Impact: Loss of very little (0.16%) market share - No significant impact expected Likelihood: high
	Purpose: Maintain the end-use of thermal paper Response to the restriction: Diversification and production of one (or some) alternative dye developer Impact: Loss of profit from BPA non-use by thermal paper market Gain from new demand for the alternative developer Costs related to the production of the new alternative (purchasing cost, operating costs, equipment investments, staff training, etc.) Likelihood: low

F.2.2. Economic impacts for the market of thermal paper

Additionally to the BPA market, the restricted use of BPA in thermal paper would also impact, and even primarily, the market of thermal paper. Indeed, as shown above in section B.2, the market of thermal paper is directly dependent on the market of chemical dye developers since thermal paper basically has to contain a dye developer in order to efficiently operate.

The market of thermal paper is expected to be much more impacted by the restriction that the BPA market. The expected economic impacts for that market are addressed in this section. The

question is two-fold: it concerns on the one hand the likely responses of the market as regards substitution (chemicals vs techniques) and on the other hand, the distribution of impacts between the different segments of the supply chain.

As explained in section E.1 and as reported in Jeffs, 2011 , the market of thermal paper has been growing since the 1960s and benefits from strong favourable driving forces which flow into its expansion. Thermal paper is today used in a very wide range of applications and the market penetration of direct thermal printing is being maintained thanks to its inherent advantages over other alternative methods of printing, such as shown in section B.2. The assessment of the economic impacts borne by the market of thermal paper is carried out herein for each segment of the supply chain. As a reminder, the supply chain of thermal market is structured into 5 distinct segments namely supply of raw materials, manufacturing, converting, trade and consumption (see section B.2. for more details on each segment). The economic impacts of the restriction proposed on the BPA market has already been analysed in the previous section and the impacts on the market of alternative dye developers will be in the next section.

F.2.2.1. Economic impacts for thermal paper manufacturers

The assessment of the economic impacts for thermal paper manufacturers consists in the assessment of the compliance costs they will have to face after the entry into force of the proposed restriction. The compliance costs are defined such as the costs to the manufacturers (and the supply chain if relevant) complying with the "non-use" scenario (ECHA, 2010).

It has been shown that the thermal paper manufacturing is oligopolistic and dominated by global-oriented and diversified companies. In Europe there are in total 10 thermal paper manufacturers. How these manufacturers are expected to be affected by the proposed restriction?

It has been shown in section E.2. that with the very low concentration limit of BPA proposed herein, the thermal paper could no longer be produced efficiently. Indeed, as already mentioned above (section B.2) , the BPA content in thermal paper is optimised and fully tuned to the functional characteristics targeted for each specific end-use (printing durability, speed, printing device, etc.). From the manufacturers consulted, the claim has been made that any decrease of BPA concentration would degrade the thermal paper properties (INERIS, 2013). For example, a reduction in the concentration of BPA in thermal paper could impact the thickness of the thermal coating and subsequently its performance: the density of image on thermal paper relates directly to the concentration of BPA in the coating layer and the thickness of the layer is often dictated by the requirements of the client (RPA, 2003).

The restriction proposed is thus deemed as a total ban of BPA in thermal paper. Therefore, taking as granted that the restriction will undoubtedly push the manufacturers of thermal papers towards the substitution of BPA they have been using so far, the question to be addressed relates to the magnitude of the associated costs. Section C shows that alternative dye developers are available, technically and economically feasible. As a first step, the direct and indirect substitution costs have to be assessed. Direct substitution costs are basically related to the gap in purchasing prices between BPA and its substitutes. Indirect substitution costs include the costs collateral to substitution such as new equipment and investment costs, potentially some costs related to needs for re-formulations, staff training, employment changes, R&D expenses, and potentially extra costs to cope with due to new technical

compatibility requirements with other components of the printing system used downstream. The assessment of the substitution costs is done in section F.2.2.1.1 below. Then, after the restriction proposed would have entered into force, the manufacturers would have to ensure (and prove somehow) that they comply with it, by implementing control tests on their products before placing them on the market (to the convertors or directly to the traders). Consequently, as a second step, the cost of these compliance controls is also evaluated, in section F.2.2.1.2. It has to be noted that these costs would also have to be borne by importers of thermal paper within the EU. This impact is dealt with in the section F.2.4. The way these additional costs would be passed on the supply chain or absorbed by the manufacturer himself is rather uncertain. From the stakeholders consultation, it has been mentioned that the most likely responses would be either an entire absorption by the manufacturer, distributing the extra cost all over his whole range of products, either a (rather small) increase of sale price of jumbo rolls to the convertors having also an impact on the distributors' price and then the user's price, or something in between (INERIS, 2013). To what extent the profitability of the manufacturers would be affected by higher prices of inputs developers if they absorb entirely the substitution costs is hardly predictable.

As regards the other possible impacts related to substitution to alternative chemicals, some competitive advantage could occur favourably to EU vs. extra-EU BPA-free thermal paper manufacturers, depending on the price of the BPA-free thermal paper and depending on the potential new patents developed consecutively to the adoption of alternatives. BPA-free thermal paper producers within the EU could in that way be able to offer their products all over the world, which may currently be a profitable "green niche". The production of BPA-free thermal paper could also give some positive image to the manufacturer and could be an argument for him to charge a premium price on the new safer products. On the contrary, the opposite effect could also be observed with a competitive advantage in favour of extra-EU thermal paper manufacturers. This adverse economic effect has been raised by the stakeholders consulted: ETPA indicated that a ban of BPA for the use in thermal paper would have an "extremely negative impact on the European thermal paper producers, like serious competitive disadvantages towards the non-European competitors which would have unforeseeable consequences for the European producers, the market and its stakeholders" (INERIS, 2013). Finally, ETPA also indicated that some other non-EU markets in the world could require BPA containing papers, so European producers would still like to be able to produce BPA containing papers for exports. However, given the multiple factors at stake, the uncertainties surrounding them, and for analysis proportionality purposes, these potential impacts have not been quantitatively assessed. Moreover, no information has been provided during the different consultations carried out indicating that reaction. Moreover, if adopted, the proposal is expected to result in a total ban of the EU marketplacing but also in the stop of the manufacture of BPA-containing thermal paper. Indeed, it is not expected that the EU manufacturers will continue to produce BPA-containing thermal paper for exports only, since most of them have already started to substitute or at least to elaborate some plans to substitute. EU manfuacturers are thus expected to keep on producing thermal paper by switching to an alternative dye developer. Nevertheless, if some non-EU countries keep on using BPA-containing thermal paper, the manufacturers who used to produce for these non-EU markets could thus no longer meet their demand and these markets would be lost. The same goes for the EU convertors who used to carry out the finishing steps for exports (see below, section F.2.2.2).

The analysis of alternatives carried out in section C also examined alternative printing and free-paper techniques. As previously explained, it has been shown that they are available and widely used (mainly for other applications) but they seem to be much more expensive compared to direct thermal printing and not technically suitable for the specific applications targeted. As a result, it can be expected as very unlikely that these systems would replace direct thermal printers on a large scale. Even though some marginal end-users made the choice to switching to one of those alternative printing systems, thermal paper manufacturers and their profitability might thus not be significantly affected. Finally, regarding the possible switch of end-users to free-paper techniques, it has been considered to be probable but uncertain in the short-term and expected to expand in the long-term. Though unpredictable, the potential growth of the use of these IT techniques might proportionally affect the profitability and market shares of thermal paper manufacturers. Accounting for the unlikelihood or uncertainties surrounding these possible responses from end-users and for analysis proportionality purposes, these impacts have not been quantitatively assessed.

Similarly to the previous table, the table below summarizes the possible responses of the EU manufacturers of thermal paper to the restriction proposed and provides a list of the positive and negative economic impacts they are expected to face.

Table 114. Likely responses and economic impacts for the EU manufacturers of thermal paper

Segment of the supply chain	Likely responses and economic impacts
	Purpose: keep on producing thermal paper while being compliant Response to the restriction: Substitution to alternative dye developers Negative economic Impacts: -direct substitution cost due to the higher (but decreasing) prices of BPA alternatives -indirect substitution costs such as: (potentially) new equipment cost to adapt to alternatives, investment cost, potentially some costs related to re-formulation, staff training, R&D expenses (new developers and potential patents), potential new technical compatibility requirements with other components of the system

EU thermal paper manufacturers	-compliance control costs: costs of testing -additional costs incorporated in the (slightly higher) sale price of thermal paper and finally passed on the whole supply chain OR entirely absorbed by the manufacturers and not passed on the supply chain -loss of profitability due to higher prices of alternatives, at least in the short-term (then decreasing prices) except if the extra cost is passed on the supply chain -changes in the relationships with distributors, in the supply chain (which seems to be currently a well-oiled machine that manufacturers do not want to disturb) Likelihood: high/very high Positive economic impacts: -potential competitive advantage compared to non-EU BPA-free thermal paper producers (depending on the EU vs. extra-EU price of thermal paper) -there may be a possibility to charge a premium on the new BPA-free products -company "green" image -potential competitive advantage due to the development of new patents Likelihood: average/uncertain
	Impacts in case that customers/end-users switch to alternative printing techniques: -loss of profitability proportional to the magnitude of the switch -loss of market shares -even production stopped (in the extremely unlikely worst-case) Likelihood: low/very low
	Impacts in case that customers/end-users switch to free-paper

	alternatives: -loss of profitability proportional to the magnitude of the switch -loss of market shares Likelihood: positive but uncertain / increasing in the long-term

In conclusion, the costs expected to be borne by the manufacturers of thermal paper that are discussed and assessed further are compliance costs including:

- The costs related to the substitution of BPA in thermal paper
- The costs of compliance controls via analytical testing for BPA content in thermal paper

F.2.2.1.1 Substitution costs for the manufacturers of thermal paper

In principle, substitution costs include direct and indirect costs.

Direct costs of substitution

Direct costs of substitution are usually basically assessed based on the gap in purchasing prices between BPA and its drop-in substitutes. Within the manufacturing of thermal paper, chemical developers are raw materials and the purchasing price of raw materials stand for one of the components of the total cost of the final product manufactured from them. Therefore, the gap in purchasing prices between BPA and alternative developers is considered to be reflected in the total production cost of the final jumbo thermal paper rolls supplied and then in the final price of the jumbo roll.

Indirect substitution costs

The final production cost of the jumbo thermal paper rolls supplied may be also affected by changes in other costs due to indirect extra substitution costs. Those costs correspond to all the 'other' costs associated to substitution additionally to the purchasing price of the alternatives itself. They may include investment costs for new equipments adapted to the new developer, potentially some costs related to needs for re-formulations, staff training, R&D expenses to fit to the new developer, and extra costs to cope with potentially new technical compatibility characteristics with other components of the printing system used downstream, required by the new chemical used. All those costs are likely and might also imply changes in the total cost of the final jumbo thermal paper rolls produced by the manufacturers. It is however hard to precisely know the order of magnitude of these costs because the changes in production costs can be driven by opposite downward and upward forces which make difficult the final outcome to foreseen.

The driving forces which could push the indirect substitution costs upward (and thus the production costs of the thermal paper) are linked to the technological change and to the loss of products efficiency.

On the one hand, there are indeed technological challenges for the thermal paper manufacturers when switching from one substance to another since some adjustments may be needed in principle in the production of the thermal paper. Not only the BPA is replaced, but some adjustments may be necessary for the entire product, for instance modifying other parts of the chemistry of the product. Based on the consultation of manufacturers, it could take several years e.g. to complete the adjustments for BPS since BPS is less reactive than BPA (Danish E.P.A., 2013). Moreover, as shown in section E.1., price pressures in recent years have resulted in low profitability and a high investment in automation on the market of thermal paper and manufacturers are unwilling to disrupt their well-oiled machine in respect to their manufacturing and distribution supply chain (Jeffs, 2011). From their perspective, complying with a new regulation can thus be challenging when their production is based on path dependency and ease of fabrication and when their profitability is likely to decrease. As a consequence, switching to alternative developers in principle might not be costless.

On the other hand, additional costs associated to substitution could arise related to the loss of efficiency of the thermal paper produced with new developers due to differences in performance with BPA. For example, thermal paper produced with alternative substances may have lower sensitivity and impact the quality of the image or words printed out. It could also cause 'runability' problems on printers or deteriorate the condition of thermal printing heads. Yet, the performance of thermal paper is also appreciated through its compatibility with printing devices used by end-users. The manufacturers of thermal paper narrowly collaborates with various manufacturers of thermal printheads, printers, and printing mechanisms in order to tune the varieties of thermal paper as closely as possible to the equipment, and vice versa. Besides, approvals and certifications on different grades of thermal paper can be granted, and prior to them, comprehensive tests are usually performed to ensure the long life of thermal printers and their components, and constant printout quality (Koelher website[72]). As a consequence, any changes in the compatibility of the paper with the printing devices used might cause practical printing problems and thus needs for adjustments and finally extra costs.

Furthermore, the replacement of BPA with other chemicals could require some reformulation or the use of a further protective coating, which could entail extra cost and time to fit to the clients' requirements (RPA, 2003 and ETPA 2013 consultation).

Other driving forces could push the indirect substitution costs downward and make them more affordable. These drivers are mainly due to the diversification already in place in the manufacturers. Indeed, it has been collected from the consultation of manufacturers of thermal paper that they are doubly diversified: they usually produce a wide range of paper products in addition to thermal paper and within their thermal paper-related activities, most of them already use alternative developers additionally to BPA. A few of them even claim to have already phased-out BPA (MSCAs consultation and INERIS, 2013). As a result, it can be reasonably considered that the existing equipment may already be technically capable of fitting to the substitutes and that corresponding formulations are already known and experienced by the manufacturers, or at least most of them. The potential needs for changes in equipment can thus be reasonably expected to be low and rather marginal. So may be the related extra

[72]http://www.koehlerpaper.com/media/docs/en/produktinformationen/Thermo-Produktbroschuere_GB.pdf

operating costs. Nonetheless, the production capacity of the existing equipment using alternative developers might possibly be insufficient to insure a certain output necessary for the supply to meet the demand for thermal paper, at least in the short-term, and extra costs to expand this capacity (by investing in supplementary equipment units) might occur.

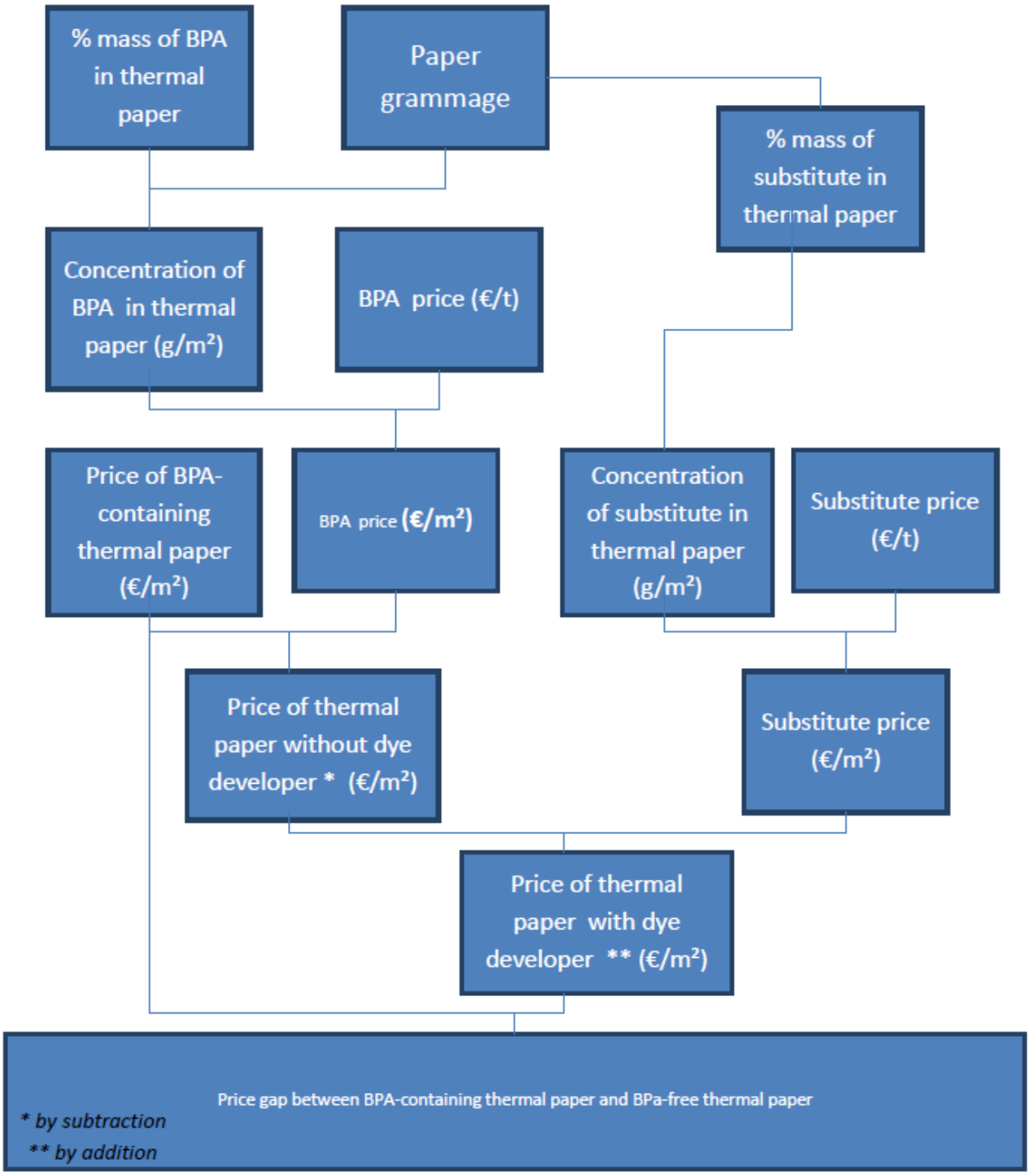
Overall, it is rather difficult to draw a clear-cut conclusion regarding the indirect substitution costs. In principle, the substitution might not be costless and could induce extra costs of different nature for the manufacturers of thermal paper, particularly due to technological changes and loss of technical efficiency of the thermal paper. However, there are tangible indications that the production process of thermal paper wouldn't actually be significantly altered to fit use of alternatives to BPA. To that respect, the replacement of BPA with drop-in chemical alternatives can be considered to be achievable without major changes in the design of production equipment, especially because most of the manufacturers already used other dye developers alternatively to BPA in some of their products. The additional costs could then likely be much more attributable to R&D or to the expansion of production capacity. It has to be noted that INERIS, 2013 consultation indicates that one manufacturer acknowledged that the main reason why he suspects higher production cost for BPA-free thermal paper is the higher cost of alternative chemicals. Therefore he recognized that process adaptation or research costs would be less significant than raw material costs. Due to the lack of data regarding these other costs and given the claiming that they might not be significant, they have not been quantitatively assessed.

The evaluation of the substitution costs

The evaluation of the substitution costs is thus focused on the evaluation of the direct costs of substitution. These direct costs are considered to be reflected in the total production cost of the final jumbo thermal paper rolls supplied and then, in the final price of the jumbo roll. As mentioned above, due to the important hindrances to access to available data regarding the prices of substitutes, the analysis and the quantification of the substitution costs cannot possibly be exhaustive herein. Section F.2.1 provides an overview of the differences in prices that could be collected for some alternatives: BPS, D8 and Pergafast. The quantitative evaluation of substitution costs is therefore preliminary based on those three alternatives. The results of this evaluation have been presented in section C.2, when addressing the economic feasibility of each of them.

For the purposes of the assessment of the substitution costs, some assumptions have been made. The figure below presents the logigram which has been followed and the inputs data used for the assessment.

Figure 45. Logigram for the economic evaluation of the chemical substitution of BPA in thermal paper



An increase in the cost of the input dye developer used in the thermal paper produced is considered to imply a proportional increase in the total cost of the thermal paper and then in its price. This assertion is built on the assumptions that, all things being equal, the structure of the production cost remains the same with and without BPA.

The approach adopted herein to assess the price gap in the final thermal paper resulting from the substitution of BPA with chemical substitutes has required the following key input data:

- price of BPA and its alternatives (when available)

- concentration of BPA and its alternatives in thermal paper

- grammage (basis weight) of thermal paper

- price of thermal paper

- Price of BPA and its alternatives

The prices of BPA and its alternatives have been collected from different sources such as websites on chemical products (ALIBABA[73], LOOKCHEM[74], CHEMICAL BOOK[75] and ICIS[76]), contacts with manufacturers/distributors of these chemicals (INERIS, 2013), the 2013 ANSES MSCA consultation already quoted (see section G) and the available recent literature ICIS, 2009 . On the specialised websites, the search on the substances has been carried out through both their name and/or their CAS number. In the case of the ALIBABA website, the large number of suppliers has been reduced by selecting only "Golden supplier"[77] and "On site check"[78]. The search on prices has consisted in requiring a quote from manufacturers and/or distributors of the substance of interest (BPA or one of its alternatives). The quote was based on the following criteria: Quantity = 1 ton; Purity≥ 99%; Use = thermal paper manufacture; Shipping included.

As presented above, compiling these different sources, **the price of BPA ranges from 1,263€/t to 1,906€/t, with an average at 1,585€/t.**

According to these data and taken into account the inflation, the price of BPA can be seen as relatively stable over time, at least since the 5 last years.

As far as the price of alternative developers are concerned, and as already presented:

- **the price of BPS ranges from 2,920€/t to 4,200€/t, with an average at 3,583€/t.**

- **the price of D8 ranges from 11,390€/t to 15,104€/t, with an average at 12,938€/t**

[73] http://french.alibaba.com/

[74] http://www.lookchem.com/

[75] http://www.chemicalbook.com/

[76] The website ICIS (www.icis.com) provides, for a list of given substances, more detailed market information (contracts prices, spot prices, import/export, and evolution of prices…).

[77] Gold Supplier is a paid membership on Alibaba.com. All Gold Suppliers in China must pass our Onsite Check while those from other countries and regions must pass our A&V Check.

[78] Premises of the supplier have been audited by the Alibaba com staff to ensure that on-site operations

- **the price of Pergafast may range from 15,000€/t to 30,000€/t (probably overestimated, as already mentionned) with an average at 22,500€/t**

Despite searches on all available sources, no data were found for the other alternative developers. Therefore, no cost calculation could be computed for them.

- Concentration of BPA and its alternatives in thermal paper

The concentration of BPA has been extensively studies in the section B.2 the conclusion is that the BPA concentrations range from 0.3% to 3.2% of the weight of the thermal receipts analysed with an average at 1.46%. These concentrations are consistent with the data collected from the INERIS, 2013 survey and US EPA, 2012 which indicate a concentration between 1% and 2% (% weight) (INERIS, 2013 ; US EPA, 2012).

As far as the concentration of alternative dye developers in thermal paper is concerned, during the INERIS, 2013 survey, a few manufacturers indicated that alternatives to BPA are generally contained between 1.3% and 2.5%, depending on the type of substance and the coating formulation developed. This concentration is slightly (barely) higher than the concentration of BPA. As a first approach, it is assumed that the quantity of alternative chemical developer to be used would equal the quantity of BPA. Consequently, for the calculation of the substitution cost, it is assumed that BPA and its alternatives are at equal concentration in thermal paper, and that this concentration ranges from 1% to 2% (weight %), standing for the min and the max cost and the average concentration of 1.5% (1.46% rounded up) is considered for the calculation of the mean cost. As a second approach, and to stick to the market reality, a sensitivity analysis has been carried on that input data further taking into account the claimed higher concentrations of dye developers.

- Grammage of thermal paper

The grammage is the weight of paper expressed as grams per square meter. The data for grammage have been taken from INERIS, 2013 which did some research on manufacturers' websites selling thermal paper and thermal paper reels for fax printers, weighing scales, cash registers, etc. the search revealed that thermal paper grammage is between 48 g/m² and more than 200g/m². The most widespread grammage for the thermal paper (e.g. for P.O.S applications) is 55 g/m².

As a result, the range of grammage used in the assessment of the substitution cost is 48-200g/m², and the average value taken into consideration for the mean cost is 55g/m² (considered as the most common grammage and thus likely to be representative).

- Price of thermal paper

The search on the price of thermal paper has been done based on quotes carried out on specialized websites similarly to the quotes made on the price of BPA and its alternatives. To fit to the actual products sold by the manufacturers of thermal paper to convertors, the quotes focused on "jumbo" format. The details of the quote are the following: Quantity: 20 tons; Grammage: 55g/m² (corresponding to ecopaper); Width: 70cm. In addition, it was asked whether the thermal paper contains BPA and 75% of answers were positive. The price taken into consideration is thus considered to be representative of the price of BPA-containing jumbo rolls. The prices collected are very close to each other and range from 0.066€/m² to 0.074€/m², with an average at 0.069€/m² (geometrical average).

Table 115. Overview of the assumptions made and the input data taken into consideration in the substitution cost calculation

Input data (2013 values)	**Min**	**Max**	**Medium**
Price of BPA (€/ton)	1,263€	1,906€	1,585€
Price of BPS (€/ton)	2,920€	4,200€	3,583€
Price of D8 (€/ton)	11,390€	15,104€	12,938€
Price of Pergafast (€/ton)	15,000€	30,000€	22,500€
Concentration of BPA in thermal paper	1%	2%	1.5%
Concentration of alternative developers in thermal paper	1%	2%	1.5%
Grammage of thermal paper	48g/m²	200g/m²	55g/m²
2013 Price of thermal paper	0.066€/m²	0.074€/m²	0.069€/m²

As a reminder, it has to be noted that the upper price might be overestimated since it is based only on one single declaration gathered during the consultation. This price couldn't be checked during the elaboration of this proposal. As already said in section C.2.5.4, other information collected from the public consultation indicates however that thermal paper with Pergafast would be around 10-25% more expensive than thermal paper with BPA (Danish EPA 2014, quoted in FBR-WUR report[79]). This information thus qualifies the information initially gathered and Pergafast might have a less high price than expected. This additional information confirms the assumption that the price of Pergafast might be overestimated.

As mentioned in section C.2.5, additional information collected late in the process from several stakeholders indicates that Pergafast-containing thermal paper is about 15%-20% more expensive than BPA-containing thermal paper (see Annex 9) which is pretty close to the cost increase indicated in the public consultation.This information is used below to refine the substitution cost calculation.

[79] *Analysis of alternatives for bpa in thermal paper*, report 1515, Dec 2014

From these data, the substitution costs have been calculated based on 3 scenarios: maximum, minimum and median cost. The logigram presented above thus allowed getting the following step-by-step results: first, the calculation of the price of thermal paper without dye developer; next, the calculation of the price of thermal paper containing the substitutes and then, the calculation of the difference in prices of thermal paper with and without BPA, reflecting the cost of substitution. The following tables present the results for the min, max and medium values.

Table 116. Price of BPA-free thermal paper with the medium values of input data in 2013 values

Paper grammage (g/m²)	% mass of BPA	Concentration of BPA (g/m²)	BPA price (€/t)	BPA price (€/m²)	**Price of BPA-containing thermal paper (€/m²)**	Price of thermal paper without dye developer (€/m²)	Substitute	% mass of substitute	Concentration of substitute (g/m^2)	Price of substitute (€/t)	Substitute (€/m²)	**Price of BPA-free thermal paper (containing the substitutes) (€/m²)**
55	1.5	0.83	1,585	0.00131	**0.06963**	0.06832	BPS	1.5	0.83	3,583	0.00296	**0.07128**
							D8	1.5	0.83	12,938	0.01067	**0.07900**
							Pergafast 201	1.5	0.83	22,500	0.01856	**0.08688**

Table 117. Price of BPA-free thermal paper with the min values of input data in 2013 values

Paper grammage (g/m²)	% mass of BPA	Concentration of BPA (g/m²)	BPA price (€/t)	BPA price (€/m²)	**Price of BPA-containing thermal paper (€/m²)**	Price of thermal paper without dye developer (€/m²)	Substitute	% mass of substitute	Concentration of substitute (g/m^2)	Price of substitute (€/t)	Substitute (€/m²)	**Price of BPA-free thermal paper (containing the substitutes) (€/m²)**
48	1	0.48	1,26	0.0006	**0.06583**	0.06522	BPS	1	0.48	2,920	0.00140	**0.06663**

			3	1			D8	1	0.48	11,390	0.00547	**0.07069**
							Pergafast 201	1	0.48	15,000	0.00720	**0.07242**

Table 118. Price of BPA-free thermal paper with the max values of input data in 2013 values

Paper grammage (g/m²)	% mass of BPA	Concentration of BPA (g/m²)	BPA price (€/t)	BPA price (€/m²)	**Price of BPA-containing thermal paper (€/m²)**	Price of thermal paper without dye developer (€/m²)	Substitute	% mass of substitute	Concentration of substitute (g/m^2)	Price of substitute (€/t)	Substitute (€/m²)	**Price of BPA-free thermal paper (containing the substitutes) (€/m²)**
200	2	4.00	1,906	0.00762	**0.07392**	0.06630	BPS	2	4.00	4,200	0.01680	**0.08310**
							D8	2	4.00	15,104	0.06042	**0.12671**
							Pergafast 201	2	4.00	30,000	0.12000	**0.18630**

These computations result in the following price gaps and cost of substitution.

Table 119. Calculation of the price gap of thermal paper with and without BPA in 2013 values

Alternative	Price gap BPA-containing vs. BPA-free thermal paper (min) (€/m²)	Price gap BPA-containing vs. BPA-free thermal paper (max) (€/m²)	Price gap BPA-containing vs. BPA-free thermal paper (medium) (€/m²)
BPS	0.00080	0.00918	0.00165
D8	0.00486	0.05279	0.00937
Pergafast 201	0.00659	0.11238	0.01725

Table 120. Extra cost of chemical substitution of BPA in thermal paper in 2013 values

Alternative	Substitution extra cost (min) (%)	Substitution extra cost (max) (%)	Substitution extra cost (medium) (%)
BPS	1.2%	12.4%	2.4%
D8	7.4%	71.4%	13.5%
Pergafast 201	10.0%	152.0%	24.8%

Overall, based on the 3 scenarios min, max and medium such as defined above, the chemical substitution cost of BPA in thermal paper in 2013 values would be on the wide range from 1.2% (whole substitution with BPS) to 152% (whole substitution with Pergafast which corresponds to a probably overestimated scenario), depending on the substitute adopted, with an average comprised between 2.4% and 24.8%. Each extra cost indicated in the table corresponds to the situation where only one substitute would be chosen to replace the whole BPA containing in thermal paper today. If substitution of BPA involves in reality the selection of several alternatives, the extra cost would be somewhere in between. As expected, given its much higher purchasing price, the substitution to Pergafast appears to be the most costly. However, it is important to highlight that this assessment is based on uncertain values of price for Pergafast obtained from the INERIS, 2013 survey, due to the lack of information on this substitute. The results for Pergafast are thus to be interpreted with cautiousness and might be overestimated. In general, these substitution costs have to be to some extent overestimated since they are based on the current prices of alternatives which might be reduced over time after the entry into force of the restriction proposed and the expected increase in demand for these alternatives.

The corresponding values of the substitution costs have been then calculated based on the computed total price (total value) of thermal paper produced in the EU for POS applications (eco-paper) containing BPA, be it €353.2 million, €308.2 million and €84.8 million respectively corresponding to min, medium and max scenarios (2013 data). These total prices (or values) have been got from the following data:

- the quantity of thermal paper produced in the EU: 540,000 tons in 2012 (from ETPA consultation; INERIS, 2013; 540,000 tons include 229,000 tons produced for the EU market and 311,000 tons exported)
- the quantity of thermal paper produced in the EU for POS applications (eco-paper): 65%= 351,000 tons (calculated)
- the share of eco-paper containing BPA produced today in the EU: 70%= 245,700 tons
- the average price of thermal eco-paper: 0.069€/m² (INERIS, 2013)
- the grammage values of thermal eco-paper: 48g/m²; 55 g/m²; 200g/m² (INERIS, 2013)

To these values for total price have been applied the extra costs due to substitution estimated in Table 118 (expressed in percentage) from 2019; 2019 being the proposed date of entry into force of the restriction. Moreover, due to the proposed restriction and given that substitution of BPA in thermal paper is already underway, the prices of alternatives are being reduced and are expected to keep on decreasing over time as a result of their (already) growing supply in response to their higher demand. This trend has been confirmed by Industry (but no figure has been provided). This evolution in prices have thus to be taken into account and the costs have been discounted accordingly.

This assessment over time is based on the following assumptions:

- The price of BPS, the currently cheapest alternative, is estimated to decrease over time, reaching the 2013 price of BPA within 10 years (for all the scenarios). This stands for a decrease of 8% per year over 2013-2023. From 2023, it will be then considered that the extra cost due to the use of BPS is zero.
- In the meantime, the prices of the other (initially more expensive) alternatives are considered to also decrease at the same rate as BPS over 2013-2023, all other things being equal, and then will decrease more slowly (set as -5%) over 2024-2030.
- The growth rate of thermal paper production is based on the information provided by the industry and ETPA in particular. Thermal paper market has grown around 10% per year the last ten years and is still a resilient and growing market (see section B.2 and E.1). However, it is suffering from tough competition (from Asia and to some lesser extent from free-paper alternatives and mobile payment) and decreasing profits, therefore it is expected to grow slower in the future. The annual growth of thermal paper over 2019-2030 is thus estimated to be between 5% (low range) and 7% (high range) per year.
- The substitution extra-costs are borne by the manufacturers of thermal paper not only the first year they substitute but to some extent also every next year, compared to the (lower) costs they previously faced. However, due to the decreasing prices of alternatives, the extra-cost is expected to overall decrease over time. Although the substitution is already underway and will probably accelerate before the entry into force of the restriction, it is considered to be fully achieved for 2019.

The results are presented in the tables below. The costs are discounted with a discount rate of 4% over 2019-2030, are rounded up in order to reflect inflation-adjustment between 2013 values and 2019 values and expressed in average annual value.

Table 121. Average annual chemical substitution cost of BPA over 2019-2030 - annual growth of thermal paper production of 7% (in 2019 values)

Alternative	**Substitution extra cost (min) (€)**	**Substitution extra cost (max) (€)**	**Substitution extra cost (medium) (€)**	**Period**
BPS	769,372 €	1,564,981€	**1,211,343€**	For 2019-2023 then zero
D8	11,218,590 €	25,208,958 €	**19,193,862 €**	For 2019-2030
Pergafast 201	16,026,640 €	60,545,438 €	**39,341,185 €**	For 2019-2030

Table 122. Average annual chemical substitution cost of BPA over 2019-2030 – annual growth of thermal paper production of 5% (in 2019 values)

Alternative	**Substitution extra cost (min) (€)**	**Substitution extra cost (max) (€)**	**Substitution extra cost (medium) (€)**	**Period**
BPS	674,360 €	1,375,313 €	**1,062,880 €**	For 2019-2023 then zero
D8	9,458,427 €	21,262,482 €	**15,691,408 €**	For 2019-2030
Pergafast 201	13,503,618 €	50,992,375 €	**32,066,838 €**	For 2019-2030

Depending on the annual growth rate of the production of thermal paper in the EU and the decreasing trend of the prices of alternatives, the annual substitution cost over 2019-2030 ranges from around €0.7 million to €61 million with a (probable more realistic) average between €1 million and €39 million in 2019 value. However, it has to be emphasized that the upper bound of the substitution costs might be overestimated since it is based on the maximum price of Pergafast, which is highly uncertain.

As already mentioned, the volume of 540,000 tons of thermal paper produced by ETPA in 2012 include 229,000 tons produced for the EU market and 311,000 tons exported. As a result, 42% of thermal paper produced only are dedicated for the EU marketplacing. From this, and from the expected restriction costs calculated above,

only a fraction of these costs could be relevant (up to 42%) if the boundary of the analysis is EU only. It is also dependent on whether the EU manufacturers will pass on the extra costs downstream the supply chain in the EU (and the way they will do it) and dependent on whether the extra costs for the 58% exported share are entirely passed on to non-EU consumers or not. The stakeholders consulted didn't provide any information on this point.

Refinement of the substitution cost calculation regarding Pergafast in the light of the additional information collected late in the process (see section C.2.5 and Annex 9: Substitution from BPA to Pergafast 201) on the price of Pergafast vs. BPA-containing thermal paper.

- The information collected from several stakeholders indicates that the Pergafast-containing thermal paper is about 15%-20% more expensive than BPA-containing thermal paper: the 2 values of 15% and 20% are then used for the refinement
- The refinement is applied on the medium scenario only and is based on the same assumptions as indicated above. The sole assumption which has been amended is the price of Pergafast which has been changed (compared to Table 114) in order to be in line with a 15% or 20% cost increase.
- The refined cost is also discounted over 2019-2030 and takes into account the (unchanged) alternative price developments such as described above.

The substitution costs for the medium scenario are thus changed as follows (for an annual growth of thermal paper production of 7%):

☐ For a cost increase of BPA vs Pergafast of 15% (the price of Pergafast is assumed to be 14,200€ instead of 22,500€):

Substitute	substitution cost medium	average annual discounted cost over 2019-2030
BPS	2.4% (unchanged see Table 118)	1 211 343 € (unchanged see Table 119)
D8	13.5% (unchanged see Table 118)	19 193 862 € (unchanged see Table 119)
Pergafast 201	**15%**	**21 852 921 €**

☐ For a cost increase of BPA vs Pergafast of 20% (the price of Pergafast is assumed to be 18,500€ instead of 22,500€):

Substitute	substitution cost medium	average annual discounted cost over 2019-2030
BPS	2.4% (unchanged see Table 118)	1 211 343 € (unchanged see Table 119)
D8	13.5% (unchanged see Table 118)	19 193 862 € (unchanged see Table 119)
Pergafast 201	**20%**	**30 913 106 €**

The substitution costs for the medium scenario are thus changed as follows (for an annual growth of thermal paper production of 5%):

☐ For a cost increase of BPA vs Pergafast of 15% (the price of Pergafast is assumed to be 14,200€ instead of 22,500€):

Substitute	substitution cost medium	average annual discounted cost over 2019-2030
BPS	2.4% (unchanged see Table 118)	1 062 880€ (unchanged see Table 120)
D8	13.5% (unchanged see Table 118)	15 691 408€ (unchanged see Table 120)
Pergafast 201	**15%**	**17 852 650 €**

☐ For a cost increase of BPA vs Pergafast of 20% (the price of Pergafast is assumed to be 18,500€ instead of 22,500€):

Substitute	substitution cost medium	average annual discounted cost over 2019-2030
BPS	2.4% (unchanged see Table 118)	1 062 880€ (unchanged see Table 120)
D8	13.5% (unchanged see	15 691 408€ (unchanged

	Table 118)	see Table 120)
Pergafast 201	**20%**	**25 216 627 €**

As a result, depending on the annual growth rate of the production of thermal paper in the EU, the refined medium annual substitution cost over 2019-2030 ranges from around €1 million to €25 million in 2019 value.

Sensitivity analysis

A sensitivity analysis has been carried out on several input data, considered as key data.

- The concentration of alternative developers, assumed as a first approach to be ranged from 1% to 2%, with an average value at 1.5%, such as BPA, is then made varied from 1% (min value) to 2.5% (max value), with an average value at 1.75% (all other input data unchanged).

Table 123. Average annual chemical substitution cost of BPA over 2019-2030 - sensitivity analysis on the alternatives concentration (exemple with the annual growth of thermal paper production of 7%) – 2019 values

Alternative	Substitution extra cost (min) (concentration unchanged= 1%)	Substitution extra cost (max) (concentration= 2.5%)	Substitution extra cost (medium) (concentration= 1.75%)	**Period**
BPS	769,372 €	1,956,226€	**1,413,233 €**	For 2019-2023 then zero
D8	11,218,590 €	31,511,198 €	**22,392,840 €**	For 2019-2030
Pergafast 201	16,026,640 €	75,681,798 €	**45,898,049 €**	For 2019-2030

For the max and medium values, the cost of substitution increases with the alternatives concentration.

- The medium scenario has been recomputed including the min and max BPA price, all other input data unchanged. The impact of these variations of the alternatives concentration for the 3 scenarios and the recalculation of the medium scenario with low and high BPA prices are respectively shown in the table below.

Table 124. Cost of chemical substitution of BPA in thermal paper - sensitivity analysis on the BPA (2013) price for the medium scenario (exemple with the annual growth of thermal paper production of 7%) – 2019 values

Alternative	Substitution extra cost (medium with **low** BPA price)	Substitution extra cost (medium with **high** BPA price)	Substitution extra cost (medium with **medium** BPA price) (unchanged)	**Period**
BPS	**2,376,388 €**	**420,088 €**	**1,211,343€**	For 2019-2021 then zero
D8	**20,832,661 €**	**17,560,153 €**	**19,193,862 €**	For 2019-2030
Pergafast 201	**40,979,984 €**	**37,707,476 €**	**39,341,185 €**	For 2019-2030

As expected, with the low price of BPA, the gap price between thermal paper with and without BPA gets wider and the substitution cost increases and with the high price of BPA, the gap varies in the opposite way since the price of alternative substances and BPA get closer. One can note that in that case, the price of BPS would reach more rapidly the 2013 price of BPA and the extra cost for BPS would be zero faster (in 2021).

- The evaluation of substitution costs is based on one uncertain data about the share of BPA-containing thermal paper compared to the total thermal paper placed on the EU market is uncertain. As shown above, the data gathered to that respect from the MSCAs consultation indicate an estimated share ranging from 75% (1 claim) to 100% (1 claim) with a central estimate between 90% and 99% (3 claims) and ETPA indicates that around 70-80% of thermal paper produced in Europe contains BPA (ETPA consultation). The share used in the calculation is 70% but it might be overestimated to some extent given that this data couldn't be double-checked and given that substitution of BPA is already underway. A sensitivity analysis is thus done on that input data in order to judge about its influence on the substitution costs. The share is made varied to 50% and 30%. For illustrative purposes, the share of 85% is also used. The results are presented below.

Table 125. Cost of chemical substitution of BPA in thermal paper – sensitivity analysis on the share of BPA-containing thermal paper (exemple with the annual growth of thermal paper production of 7%) – 2019 values

Alternative	Substitution extra cost (min)	Substitution extra cost (max)	Substitution extra cost (medium)

(share of BPA to be substituted: 85%)	(€)	(€)	(€)
BPS	934,237€	1,900,334 €	**1,470,916 €**
D8	13,622,574 €	30,610,878 €	**23,306,833 €**
Pergafast 201	19,460,919 €	73,519,460 €	**47,771,439 €**
Alternative (share of BPA to be substituted: 50%)	Substitution extra cost (min) (€)	Substitution extra cost (max) (€)	Substitution extra cost (medium) (€)
BPS	549,551 €	1,117,844 €	**865,245 €**
D8	8,013,279 €	18,006,399 €	**13,709,902 €**
Pergafast 201	11,447,600 €	43,246,741 €	**28,100,846 €**
Alternative (share of BPA to be substituted: 30%)	Substitution extra cost (min) (€)	Substitution extra cost (max) (€)	Substitution extra cost (medium) (€)
BPS	329,731 €	670,706 €	**519,147 €**
D8	4,807,967 €	10,803,839 €	**8,225,941 €**
Pergafast 201	6,868,560 €	25,948,045 €	**16,860,508 €**

As expected, the lower the share of BPA-containing thermal paper on the market today, the lower the quantity of BPA to substitute and the lower the substitution costs. Given that

substitution is already ongoing, 50% might be a more realistic share than 70% but no information is at hand to confirm or disconfirm this assumption.

- Price development of alternatives: an alternative (slower) trend has been attempted: -5% over 2014-2030 and -2% then. The other input values are unchanged.

Table 126. Cost of chemical substitution of BPA in thermal paper - sensitivity analysis on the price development of alternatives (exemple with the annual growth of thermal paper production of 7%) – 2019 values

Alternative	Substitution extra cost (min) (€)	Substitution extra cost (max) (€)	Substitution extra cost (medium) (€)	Period
BPS	769,821 €	1,802,997€	**1,286,480 €**	For 2019-2030
D8	17,477,277 €	39,991,305 €	**31,009,371 €**	For 2019-2030
Pergafast 201	24,268,984 €	89,906,560 €	**59,889,101 €**	For 2019-2030

One can note that the substitution cost is sensitive to the price development of alternatives. With this alternative trend, the price of BPS would only reach 2013 BPA price after 2030 (2031).

F.2.2.1.2 Compliance control costs for the thermal paper manufacturers

As regards the costs related to compliance control, it refers to the costs associated to testing, that is, the testing of BPA content in thermal paper after the entry into force of the restriction. Testing would be required primarily from the control authorities who will have to control the BPA content in the thermal paper produced and placed on the EU market as well as the thermal paper imported into the EU. This cost is addressed further in section F.2.2.5. Then, testing would be required from importers and convertors and traders (distributors) of thermal paper in the EU who will have to be sure that the thermal paper they make entered the EU market or they distribute is compliant. They are expected thus to carry out some tests. This cost is assessed further in section F.2.2. below.

As regards the EU manufacturers of thermal paper, they would have to make some tests on their products if they keep on using BPA in their product while being compliant to the concentration limit proposed. However and as already explained, at the very low level of the limit proposed, the thermal paper could no longer be efficient. The concentration limit proposed is thus considered to be equivalent to a total ban of BPA in thermal paper. As a consequence, if EU manufacturers no longer use BPA in their products after the entry into force of the restriction, they might not have in principle to test them. In that case, their compliance control costs are deemed around zero.

However, as explained in section B.2, some manufacturers of thermal paper do not carry out themselves the chemical formulations used for the thermal reactive layer of their paper and purchase 'ready-to-use' formulations from upstream suppliers. Those manufacturers might

thus face a lack of information as regards the formulations they buy. Nonetheless, this potential lack of information is not considered as strictly requiring a need for testing the thermal paper since it concerns the raw material used as an input in the manufacturing operation and should be addressed prior to its use in the process. The cost of obtaining information on BPA content in those formulations is not known and could therefore not be assessed herein.

F.2.2.2. Economic impacts on thermal paper convertors

As a reminder, converting consists of purchasing paper jumbo rolls from manufacturers and then slitting them to commonly used sizes for various industries and distributors. Thermal paper converting companies are not expected to bear substitution costs except maybe some increase in the purchasing price of the jumbo rolls if the manufacturers pass on their extra cost of substitution on the convertors. Whether the manufacturers will pass on this extra cost along the supply chain downstream or they entirely absorb it is however unknown.

Convertors can be expected to carry out some tests in order to check the compliance to the restriction of the jumbo rolls they buy from manufacturers.

As already mentioned, to date there is no EU standard analytical method to measure BPA in thermal paper. According to the consultation of the French SCL (see section G.4), there still have some possibilities to carry out these measures, based on existing methods already used to measure BPA in other materials and supports.

The dosage of BPA can be measured with LC-DAD or GC-MS with adaptations from the following existing standards.

Table 127. Existing standard methods to measure BPA

Analytical standard method	Description
XP CEN/TS 13130-13:2005-05-01: Materials and articles in contact with foodstuffs - Plastics substances subject to limitation - Part 13: determination of 2,2-bis(4-hydroxyphenyl) propane (Bisphenol A) in food	This standard provides the dosage of BPA with LC-DAD (Liquid Chromatography With Diode Array Detection) containing in food stimulants after contact with the material or article in contact with foodstuffs. The dosage of the extraction solution of the thermal paper is similar to the dosage carried out for

stimulants	food stimulants
NF EN ISO 18857-2:2012-01: Water quality - Determination of selected alkylphenols - Part 2: Gas chromatographic-mass spectrometric determination of alkylphenols, their ethoxylates and bisphenol A in non-filtered samples following solid-phase extraction and derivatisation	This standard provides the dosage of BPA after acidification of aqueous extract, extraction in solid phase, elution with a solvent, derivation and dosage through detection GC-MS (gas chromatographic-mass spectrometric).

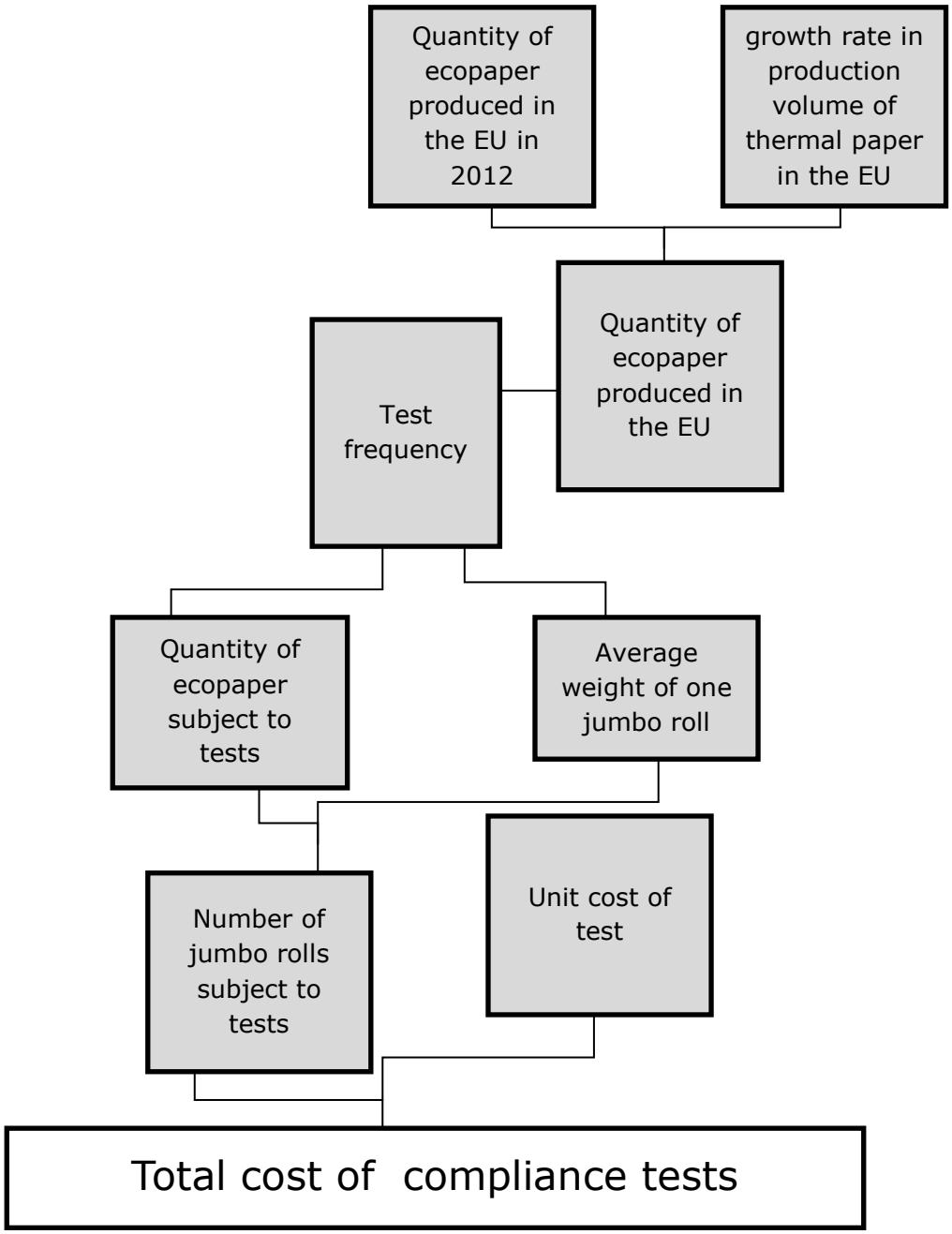
For both methods, the sample of thermal paper tested can be prepared in the following way: the extraction of BPA is carried out from a sample of thermal paper with an organic solvent (e.g. ethanol) into an ultrasound bath at room temperature. The extract got is then diluted with a solvant adapted to the dosage method.

For DGCCRF, 2011, the samples of thermal paper tested sized 10 cm² (around 3.1cm x 3.1cm) and were extracted from the middle of tickets weighted each around 50mg. The samples were kept in a dry and dark place at room temperature within two pieces of aluminium foil.

The costs associated to both these methods are related on one side, to the equipment used for the measurement (LC-DAD or GC-MS) and on the other side, to the cost of the tests themselves. As regards the former, **the equipments based on LC-DAD or GC-MS are common in chemical analysis laboratories and cost from €50,000 and €100,000.** Given that EU laboratories are already equiped with such technical devices, these costs are not considered as extra costs due to the restriction proposed. Regarding the unit cost of testing thermal paper samples, information has been collected from the SCL providing **a unit cost of €260 (excluding VAT) for one sample** (based on the pricing by private laboratories using the GC-MS technique).

In order to assess the total compliance cost for testing thermal paper, the following logigram has been followed.

Figure 46. Logigram used for the calculation of compliance control costs



The quantity of ecopaper produced is provided by ETPA and amounts to 65% of 540,000 tons in 2012, that is, 351,000 tons (INERIS, 2013).

As previously, the annual growth of thermal paper over 2019-2030 is thus estimated to be between 5% (low range) and 7% (high range) per year.

The information about the average weight of one jumbo roll could not be got during the consultation neither from the quotes made on specialised websites. As a result, this data is based on assumptions. Knowing that the jumbo format correspond to a very large paper roll, supplied and purchased on the market in important quantity (around 10-20 tons minimum on

the wholesale market), the weight is estimated to be between 50 kgs (low range) and 100 kgs (high range).

The test frequency is set at 1 per 1,000 jumbo rolls expected to occur the first year after the entry into force of the restriction (2019), 1 per 10,000 for the 5 subsequent years (2020-2024) and 1 per 100,000 for the 6 subsequent years (2025-2030). The test frequencies are based on assumptions. There is no data available on that kind of tests carried out on thermal paper in the EU.

The input data and assumptions made for this assessment are summarized in the table below.

Table 128. Summary of input data used for the assessment of testing costs

Assumptions/input data	**Value**
Test frequency first year, one per 1000 jumbo rolls (2019)	0.001
Test frequency for the 5 subsequent years, One per 10000 jumbo rolls (2020-2024)	0.0001
Test frequency for the 6 subsequent years, One per 100000 jumbo rolls (2025-2030)	0.00001
Assumed growth rate in production volume of thermal paper in the EU	5%-7%
Cost per test (SCL)	260€
average weight of a jumbo roll (assumption) kgs	50kgs-100kgs

Given those data, the results are presented in the table below. They vary according to the weight of jumbo rolls and the annual growth of the thermal paper production chosen.

Table 129. Compliance control costs expected from the restriction

Input data	Compliance control costs (Discount rate 4%)
Low range values: 5% annual growth 50 kgs weight of a jumbo roll	€1,755,056 over 2019-2030 (€146,255 per year)
High range values: 7% annual growth	€ 3,053,666 total over 2019-2030 (€254,472 per year)

100 kgs weight of a jumbo roll	

As a whole, the total costs of compliance control tests can be estimated between €1,755,056 and €3,053,666 over 2019-2030 or between €146,255 and €254,472 per year.

The year 2030 has been chosen as a bound for the calculation to be in line with the calculation of the health benefits for comparison purposes.

Another assessment can also be carried out to compute the impact on these costs on the average price. The assessment is based on the following additional input data and assumptions:

- the surface of thermal paper contained in one single jumbo roll: this data is not available. It has been inferred from the following available data: $2.4x10^9$ correspond to 168,000 tons thermal paper (EC, 2008) which is equivalent to 0.07kg for one m². Related to an average weight of one jumbo roll between 50kgs and 100kgs, the surface of one jumbo roll is estimated between 714m² and 1,429m².
- the average price of thermal paper: 0.069€/m² (INERIS, 2013)
- the unit cost of test: 260€ (VAT excluded).

Table 130. Relative price impact on thermal paper due to compliance control costs – illustrative examples

Value/impact on the price of thermal paper paper		
Test frequency	**Surface of thermal paper in one single jumbo roll**	**Relative impact on the price of one thermal paper jumbo roll**
1 per 1,000	714m²	0.53%
	1,429m²	0.26%
1 per 10,000	714m²	0.05%
	1,429m²	0.03%
1 per 100,000	714m²	0.01%
	1,429m²	0.003%

The relative impact on prices seems thus to be moderate, even very moderate for the highest surface assumed. Given that the restriction also covers other types of thermal paper than ecopaper, this other types would have in principle to be tested as well. Due to the lack of data

on the price of this other types of thermal paper, these potential additional tests have not been calculated.

For the convertors which are not in full control of their supply chain, testing may be the only option to ensure due diligence that they are in compliance with the proposed restriction. It may also be likely that these costs will be split to some extent between convertors and traders downstream the supply chain.

However, given the concentrated (oligopolistic) structure of the production market in the EU, it can be expected that convertors and manufacturers have trust and transparent relationships which may make the information disclosure on products (ecopaper and other types) easy along the supply chain. Taking this aspect into consideration, the compliance control costs assessed might be largely overestimated.

Moreover, if adopted, the proposal would in principle result with a total ban of the EU marketplacing but also with the stop of the manufacture of BPA-containing thermal paper. As already mentioned, it is not expected that the EU manufacturers will continue to produce BPA-containing thermal paper for exports only. Instead, they are expected to keep on producing thermal paper by switching to an alternative dye developer. Consequently, the convertors would be impacted by the stop of the manufacture of BPA-containing thermal paper but if the volume of thermal paper is considered as remaining constant, the convertors are expected to transfer their finishing activity to the finishing of alternative thermal paper newly produced in the EU. Except the potential testing to be carried out on the jumbo rolls purchased to the EU manufacturers, no additional cost is thus expected as a consequence of the restriction. Nevertheless, if non-EU countries keep on using BPA-containing thermal paper, the convertors who used to carry out the finishing steps for these non-EU markets could thus no longer meet their demand and these markets would be lost.

F.2.2.3. Economic impacts on thermal paper traders

Traders (distributors) can be also expected to carry out some tests in order to check the compliance to the restriction of the rolls they buy from convertors. Some large traders may be particularly proactive in ensuring conformity and may choose to test their products. In other cases, testing may be undertaken further upstream by wholesalers and distributors. The exact way the tests would be carried out along the supply chain is not known.

The compliance control costs likely to be borne by traders are the same as the costs calculated in the previous section. As said above, it is likely that these costs will be split between convertors and traders downstream the supply chain. However, likewise, given the concentrated (oligopolistic) structure of the thermal paper market in the EU, it can be expected that traders have also transparent relationships with their partners along the supply chain and may get the information about the composition of the products rather easily. To that respect, the compliance control costs assessed above might be largely overestimated.

As far as other potential impacts for traders are concerned, it can be expected some competition between EU suppliers and non-EU BPA-free thermal paper producers and, depending on the price of BPA-free thermal paper, the EU traders may get some advantage from that competition.

F.2.2.4. Economic impacts on thermal paper customers

The 'customers' of thermal paper are the downstream users (like large retailers, corner shops or banks) who use thermal paper for the tickets or receipts they provide their clients with. The customers are not expected to be significantly affected by the extra costs faced by the manufacturers. As explained above, these extra costs are likely to be either passed on along the supply chain either entirely absorbed buy the manufacturers themselves over their full range of products supplied. The exact way it would be done has not been communicated by industry. However, there is no indication that the downstream users would face major additional costs (due to higher prices of thermal paper) from the restriction since the cost of the thermal paper rolls they buy from distributors is likely to be a very tiny share of their total operating costs.

As regards the compliance controls, the downstream users of thermal paper are not expected to carry out themselves many tests. Some large retailers may be prone to practice some tests in order to ensure the compliance of the products they use and provide to their clients. However, the corner shops and small enterprises might not be proactive in doing so, mainly for economic reasons.

Moreover, some impacts could occur due to the replacement of BPA with alternatives on printer compatibility for downstream users. However, it is expected that manufacturers will produce BPA-free thermal paper adapted to existing printers, in order to maintain their customers. Furthermore, some alternative dye developers are already used in thermal paper today by several manufacturers in the EU, resulting in thermal paper compatible with existing printers. This information tends to be confirmed by Danish E.P.A., 2013 according to which for customers substituting to-BPA free thermal paper rolls in their thermal printers, there seem to be no technological challenges and the same thermal printers should be used regardless whether BPA, BPA-free or phenol free paper is used.

F.2.2.5. Economic impacts on thermal paper importers

Importers of thermal paper into the EU might be mainly concerned by compliance control costs. Indeed, they are expected to have to carry out some tests in order to check the compliance to the restriction of the jumbo rolls they buy from manufacturers outside the EU. These costs cannot be however assessed since the volume of thermal paper imported into the EU could not be obtained. Depending on the capacity of importers to get some precise information about the composition of the products they import, these costs can be high or low.

It is likely that the costs borne by importers will be of the same order of magnitude as the costs associated with the tests made by the customs services and the control authorities after the entry into force of the restriction.

F.2.2.6. summary of economic impacts on thermal paper market

Overall, depending on the annual growth rate of the production of thermal paper in the EU and the decreasing trend of the prices of alternatives, the annual substitution cost over 2019-2030 ranges from €1 million and €25 million in 2019 value (based on the average scenario, considered as the most realistic scenario). A sensitivity analysis has been performed in section F.2. on several sensitive parameters. As regards the compliance control costs, borne due to the conformity tests carried our

by the supply chain on the products, they are estimated between €146,255 and €254,472 per year over 2019-2030. As a whole, the costs of the restriction proposed for the thermal paper market (substitution and compliance control costs) are estimated to range from €1.2 million and €25.3 million in 2019 value (based on the average scenario, considered as the most realistic scenario). These average costs stand for between 0.18% and 4.60% of the total production value of thermal paper manufactured for POS applications (as shown in the table below).

In order to be able to judge about the relative magnitude of these costs, of the substitution costs in particular which weight the most, they can be compared to the total production value of the thermal paper manufactured in the EU. In section F.2.2.1.1 above, the total value of the thermal paper produced in the EU for POS applications (eco-paper) containing BPA has been computed at €308.2 million in 2013. Taking again into account an annual growth of the thermal paper market between 5% and 7% per year from 2013, the average value of the production of thermal paper over 2019-2030 equals to €558 million (with an annual growth of 5%) or €702 million (with an annual growth of 7%).

Table 131. Proportion of the restriction costs in the production value of POS thermal paper (for the medium -realistic- scenario)

		Annual growth of the thermal market = 5%	**Annual growth of the thermal market = 7%**
Average production value of thermal paper for POS applications over 2019-2030		€548 million	€689.6 million
Proportion of total cost in the production value	**BPS**	0.19%	0.18%
	D8	2.86%	2.78%
	Pergafast	3.26% (for a 15% refined cost increase) 4.60% (for a 20% refined cost increase)	3.17% (for a 15% refined cost increase) 4.48% (for a 20% refined cost increase)

In conclusion, the costs of the restriction stands for between 0.18% and 4.60% of the total production value of thermal paper manufactured for POS applications. In the situation where these extra costs would be entirely passed on along the supply chain (manufacturers, convertors, traders and finally endusers), it may be expected that the final extra cost for the downstream endusers (retailers, shops, etc.) would be unsignificant, taking into consideration that the thermal tickets rolls they purchase may cost very little comparatively to the whole range of supplies and consumables they use for their activities.

However, 42% of this production is exported outside the UE. Depending on whether the manufacturers will pass on the extra costs entirely on the non-EU customers or not, the substitution costs would be actually only 42% of the costs presented herein.

F.2.3. Economic impacts for the market of alternative dye developers

The market of alternative dye developers is expected to grow and capture the demand left by the BPA 'non use' after the entry into force of the restriction. Each alternative market may not be equally affected, depending on the alternative(s) chosen by the manufacturers of thermal paper.

The table below summarizes the expected impacts on the market of alternative dye developers.

Table 132. Likely economic impacts for the market of alternative dye developers

Market	Likely economic impacts
Market of alternative dye developers	-Higher demand for alternatives: higher profitability -prices are likely to decrease over time while demand grows -attractivity for new entrants into these markets -increase in employment (for R&D, for testing printer compatibility, for marketing the new products -positive impact in terms of "green" image -the development of new patents could lead to competitive advantages -not expected to be a need for technical adjustments for printers compatibility to the new products -might be some R&D expenses or investments to increase the production capacity -higher competition with non-EU alternatives suppliers: depending on the price of the non-EU alternatives, the EU suppliers will have a competitive advantage or not.

F.2.4. Economic impacts for the market of alternative printing/free-paper techniques

The market of alternative printing techniques is not expected to be significantly affected by the proposed restriction. As shown in section C, these techniques are quite different from direct thermal printing systems and might not meet the same technical requirements for endusers. These machines are generally bigger, slower and more expensive and used for very different ends (offices e.g.). To that respect, replacing all direct thermal printers in the whole EU is not considered to be economically feasible.

As regards the free-paper alternatives presented in section C, they are expected to grow in the future but the extent of this growth is uncertain. The market of e-tickets and mobile payments are new and increasing but they might not be considered as suitable alternatives in short or medium-term. Indeed, they might suffer from general acceptability (at least at short term) and might thus be hardly adopted at EU scale. Overall, the free-paper alternatives are expected to grow independently on the use or not of BPA in thermal paper.

The table below summarizes the expected impacts on the markets of alternative printing techniques and free-paper alternatives.

Table 133. Likely economic impacts for the market of alternative printing techniques/e-ticket

Markets	Likely economic impacts
Markets of alternative printing techniques	No significant impacts expected
Free-paper alternatives	-No major impact expected strictly due to the restriction -unlikely to be largely accepted in short- term -already a growing market

F.2.5. Uncertainties related to the economic impacts assessment

The uncertainties related to the economic impacts assessment can be summarised as follows:

- The following uncertainties might be overestimating:

  - The share of 70% taking into account in the substitution costs calculation (see sensitivity analysis carried out in section F.2.) might be to some extent overestimating. A sensitivity analysis has been carried out on that parameter.
  - The compliance control costs have been assessed for convertors and distributors and expected to be split between each other. However, given the concentrated (oligopolistic) structure of the production market in the EU, it can be expected that

convertors and distributors and manufacturers have trust and transparent relationships which may make easy the information disclosure about the products along the supply chain. Taking this aspect into consideration, the compliance control costs assessed may be largely overestimated.

- The calculation of substitution costs is mainly based on the price difference between BPA and 3 alternative chemicals (BPS, D8 and Pergafast). However, the prices of those chemicals are based on data collected from the stakeholders consultation and from quotes on specialised websites (quotes done in INERIS, 2013). They are few, difficult to double-check and the data (very high) on the price of pergafast is rather uncertain and probably overestimated. As a consequence, the substitution costs are likely to be overestimated, especially as regards the upper bounds related to Pergafast, even for the medium scenario.

- The following uncertainties might be underestimating:

- Some costs are not quantified such as the 'indirect costs' associated to substitution although. However, they are not considered to be major according to the stakeholders consultation.

- Due to the lack of data on the volume of imported thermal paper, the compliance control costs for EU importers and customs services have not been quantitatively assessed. Nonetheless, the calculation carried out for the compliance control costs potentially borne by the convertors and traders could provide an order of magnitude on these costs.

- Other uncertainties can also be reported which might be overestimating or underestimating:

- The cost impacts in the long term (after 2030) have not been assessed

- As already mentioned, the volume of 540,000 tons of thermal paper produced by ETPA in 2012 include 229,000 tons produced for the EU market and 311,000 tons exported. As a result, 42% of thermal paper produced only are dedicated for the EU marketplacing. From this, and from the expected restriction costs calculated above, only a fraction of these costs could be relevant if the boundary of the analysis is EU only. It is also dependent on whether the EU manufacturers will pass on the extra costs downstream the supply chain in the EU (and the way they will do it) and dependent on whether the extra costs for the 58% exported share are entirely passed on to non-EU consumers or not. The stakeholders consulted didn't provide any information on this point. It has to be noted that the calculation of the human health benefits does not raise the same questions since it is considered that, although the benefits are not

expressed as a function of the volume of thermal paper produced, they are still linked to the amount of thermal paper which are actually in use in the EU today (2013), which is 42% of the tonnage provided by ETPA.

F.3 Social impacts

No major change in employment is expected to occur on the BPA market since, as shown above, the BPA market might not be significantly affected buy the proposed restriction.

However, some increase in employment (R&D, workers for production, marketing, etc.) may be observed on the markets of alternative dye developers due to the increase in demand for those chemicals and the expected growth of these markets.

F.4 Wider economic impacts

No particular wider economic impact is expected. The increase in costs for EU supply chain of thermal paper is not of a magnitude that could generate macro-economic impact.

The non-EU manufacturers of thermal paper who export thermal paper into the EU would have to comply with the new regulation.

F.5 Distributional impacts

As explained above, the extra costs the EU manufacturers will have to cope with are likely to be either passed on along the supply chain either entirely absorbed by the manufacturers themselves over their full range of products supplied. The exact way it would be done has however not been communicated by industry.

F.6 Summary of the socio-economic impacts

The table below summarizes the socio-economic impacts expected from the restriction proposed.

Table 134. Summary of the socio-economic impacts expected from the restriction proposed

<table>
<tr><th>Type of impacts</th><th>Quantitative / Qualitative results</th><th colspan="4">Possible biases in net benefits (+ or -)</th></tr>
<tr><td>Health impacts</td><td>The human health impact assessment performed herein is semi-quantitative and address the 4 critical effects demonstrated in the risk assessment for workers and consumers:

Effects on the female reproductive system: the increase in endometriosis occurrence is quantitatively assessed but the disruption of ovarian cycles and the increase in ovarian cysts are analysed qualitatively

Effects on metabolism and obesity: the increase in body weight and the increase in cholesterol are quantitatively assessed

Effects on the mammary gland: the increase in breast cancer occurrence (due to the increase vulnerability of mammary gland) is quantitatively assessed

Effects on brain and behaviour: the alteration of memory and learning functions are analysed qualitatively</td><td colspan="4"><table>
<tr><td><u>Effects on the female reproductive system</u></td><td>◦ Ovarian cysts

◦ Disruption of ovarian cycles

◦ Endometriosis</td><td>Not quantified

Not quantified

Quantified</td><td>+

+

No bias expected</td></tr>
<tr><td><u>Effects on the brain and behavior</u></td><td>◦ learning function

◦ spatial</td><td>Not quantified

Not quantified</td><td>+

+</td></tr>
</table></td></tr>
</table>

	As a whole, the total quantified potential health benefits of the proposed restriction are estimated to range from (at least) €3.5 million to €5.2 million, keeping in mind that all the benefits have not been valued and that the total benefit would be actually higher. A sensitivity analysis has been carried out on this assessment.		memory			
		Effects on metabolism and obesity	◦ cholesterol ◦ Increase in BW	Quantified Quantified/ Overweight and obesity used as proxies	No bias expected -	
		Effects on mammary gland	◦ Structural changes and breast cancer ◦ no intangible costs taken into account (pain and suffering)	Quantified/ *Hyperplasia used as a proxy for the probability of getting breast cancer	+ or -? +	

			◦ no costs about the treatment of benign breast tumours included		+	
		Number of cashiers	2% of the EU population	Inferred from national statistical data but not directly available	+	
		Other workers who may handle thermal paper are not taken into account in the evaluation		Not quantified	+	
Environmental impacts	Not the concern of the dossier but some environmennal benefits are still expected from the restriction. They are related to: the reduction of BPA releases in water from the	Not Quantified			+	

<table>
<tr><td></td><td colspan="2">recycling of thermal paper

the reduction of BPA secondary poisoning from the re-use of thermal paper in other paper-based products</td><td></td><td></td></tr>
<tr><td rowspan="2">Economic impacts</td><td>Market of BPA</td><td>No major impact expected given the very little market share of BPA market for this specific use (0.16% in the EU)</td><td colspan="3"></td></tr>
<tr><td>EU Market of thermal paper</td><td>• manufacturers:

it is unlikely that they will keep on using BPA after the entry into force of the restriction given the very low concentration limit proposed (equivalent to a ban)

they will mainly face substitution costs due to the switch to alternative dye developers: depending on the annual growth rate of the</td><td>Substitution costs</td><td>◦the calculation is based on the total EU production of thermal paper. However, 42% of this production is exported outside the UE. Depending on whether the</td><td>+</td></tr>
</table>

		production of thermal paper in the EU and the decreasing trend of the prices of alternatives, the annual substitution cost over 2019-2030 ranges from €**1 million and €25 million in 2019 value (based on the average scenario, considered as the most realistic scenario), keeping however in mind that the upper bound might be overestimated.** A sensitivity analysis has been performed in section F.2. on several sensitive parameters **.** For those who do not formulate themselves the chemical thermal layers of their thermal paper, they might also bear costs of getting information from their suppliers about the compliance of these formulations.		manufacturers will pass on the extra costs entirely on the non-EU customers or not, the substitution costs would be actually only 42% of the costs presented herein ◦ Upper bound probably overestimated (the price of pergafast is based on only one claim and expected to be lower)	+ +		

		For those who formulate themselves the chemical thermal layers, they are not expected to bear compliance control costs if they no longer use BPA		
		• convertors: they are mainly expected to bear compliance control costs due to testing. **As a whole, the total costs of compliance control tests can be estimated between €1,755,056 and €3,053,666 over 2019-2030 or between €146,255 and €254,472 per year.** Assuming that the information circulates smouthly along the supply chain, especially between manufacturers and convertors, it can be expected that these controls might not be necessary and overestimated	Quantified/frequence based on assumptions (could not be checked)	+ or -?

		these costs might be split to some extent between convertors and traders downstream the supply chain		
		• traders/distributors: they are mainly expected to bear compliance control costs due to testing such as described for convertors. Some large traders may be particularly proactive in ensuring conformity		
		• customers/endusers: no indication that the endusers would face major additional costs (due to higher prices of thermal paper) from the restriction since the cost of the thermal paper rolls they buy from distributors is likely to be a very tiny share of their total operating costs		

		the endusers of thermal paper are not expected to carry out themselves many control tests, except some proactive large retailers.		
		• importers: they are expected to be mainly concerned by compliance control costs (not assessed) depending on the capacity of importers to get some precise information about the composition of the products they import, these costs can be high or low.	Not quantified	-
	Overall, the costs of the restriction proposed for the thermal paper market (substitution and compliance control costs) are estimated to range from €1.2 million and €25.3 million in 2019 value (based on the average scenario, considered as the most realistic scenario), keeping however in mind that the upper bound might be overestimated. These average costs stand for between 0.18% and 4.60% of the total			

	production value of thermal paper manufactured for POS applications.		
	Market of alternative dye developers	The market of alternative dye developers is expected to grow and capture the demand left by the BPA 'non use' after the entry into force of the restriction. Each alternative market may not be equally affected, depending on the alternative(s) chosen by the manufacturers of thermal paper.	
	Market of alternative printing techniques/free-paper techniques	The market of alternative printing techniques is not expected to be significantly affected by the proposed restriction. These techniques are quite different from direct thermal printing systems and might not meet the same technical requirements for endusers. These machines are generally bigger, slower and more expensive and used for	

		very different ends (offices e.g.). To that respect, replacing all direct thermal printers in the whole EU is not considered to be economically feasible. As regards the free-paper alternatives, they are expected to grow in the future but the extent of this growth is uncertain. The market of e-tickets and mobile payments are new and increasing but they might not be considered as suitable alternatives in short or medium-term. Indeed, they might suffer from general acceptability (at least at short term) and might thus be hardly adopted at EU scale. Overall, the free-paper alternatives are expected to grow independently on the use or not of BPA in thermal	

		paper.	
Social impacts	No major change in employment is expected to occur on the BPA market. Some increase in employment (R&D, workers for production, marketing, etc.) may be observed on the markets of alternative dye developers due to the increase in demand for those chemicals and the expected growth of these markets		
Wider economic impacts	No particular wider economic impact is expected. The increase in costs for EU supply chain of thermal paper is not of a magnitude that could generate macro-economic impact. The non-EU manufacturers of thermal paper who export thermal paper into the EU would have to comply with the new regulation.		
Distributional impacts	The extra costs the EU manufacturers will have to cope with are likely to be either passed on along the supply chain either entirely absorbed by the manufacturers themselves over their full range of products supplied. The exact way it would be done has however not been communicated by industry.		

G. Stakeholder consultation

This section presents the stakeholders consulted during the elaboration of this restriction proposal:

the industry actors of the thermal paper market in the EU and (to some lesser extent from outside the EU)

the REACH MSCAs

2 stakeholders involved in the enforcement and monitoring activities in France: the DGCCRF and the SCL

G.1. Consultation of Industry

INERIS 2013 survey (INERIS, 2013)

In the framework of this restriction proposal, ANSES and INERIS cooperate on a socio-economic analysis. For this purpose, INERIS conducted a European survey about the use of BPA in thermal paper and substitution in order to provide information for the assessment.

This study included 3 main areas:

1/ identification (through investigation of databases and business directories, Internet searches, documentation screening …) of the relevant stakeholders in the sector to be contacted in France and in Europe

2/ sending a questionnaire to the identified stakeholders, follow-up, targeted phone interviews and meetings, data collection and consolidation

3/ descriptive analysis of the results of the study

The identification of the relevant stakeholders in France and Europe was carried out based on a bibliographic study. It consisted primarily of querying and compiling the results of several databases listing the companies as well as their activities. Moreover a consolidated list of federations, unions and professional entities having a connection with thermal paper was established. The most important stakeholders of the thermal paper market are considered to have been reached during this survey (in particular four of the five main European thermal paper manufacturers, also members of ETPA).

Finally, this step brought a list of more than 5,000 contacts for whom electronic contact information had been set. A web-based questionnaire prepared by INERIS was then sent to them. In order to maximize the response rate to the questionnaire, the document was adapted to the audience surveyed and made user-friendly: depending on the activity of his company, the contacted person was directed through questions relevant in his situation.

The questionnaire was proposed to professionals for three months (from July to end of September 2013). The on-line survey was kept open until December 31st 2013 in order to leave extra time to stakeholders to answer.

The stakeholders identified were the following:

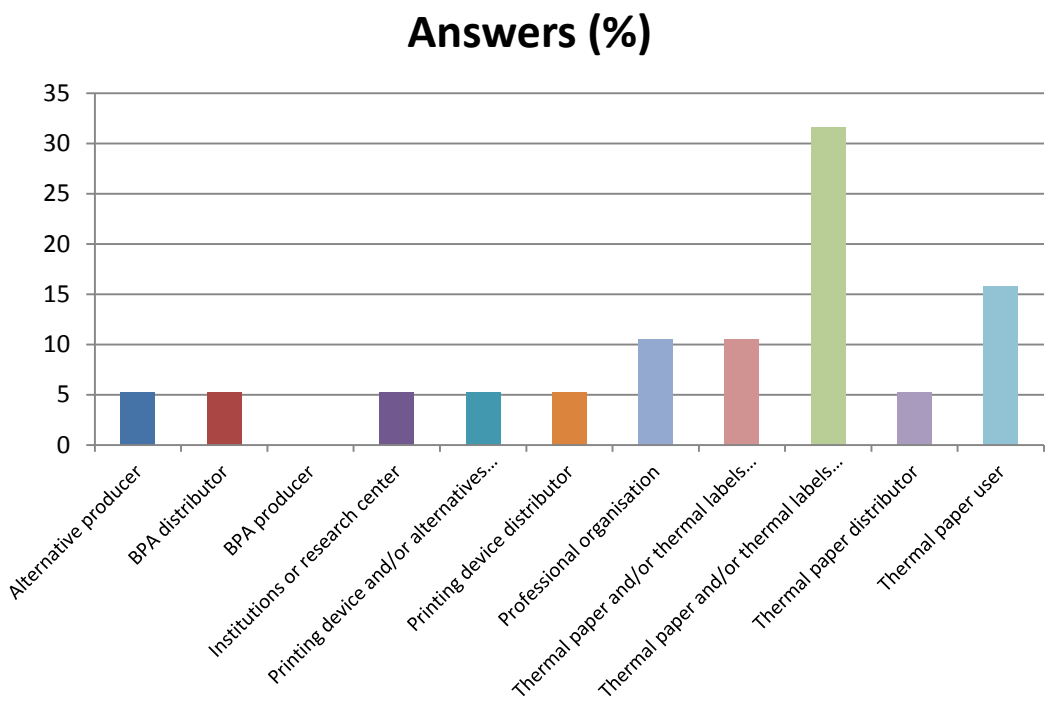
- BPA manufacturers ;
- BPA distributors ;
- Thermosensitive dyes manufacturers ;
- Thermosensitive dyes distributors ;
- Manufacturers of paper for thermal papers ;
- Thermal paper or thermal labels manufacturers ;
- Thermal paper or thermal labels distributors ;
- Printing device and technical alternative solutions manufacturers ;
- Printing device (including thermal paper) and technical alternative solutions distributors
- users of printing machines for tickets, receipts, industrial labels
- research or Regulation organizations (technical institutes, research centers, administration...)
- professional federations

The survey was also broadened to some companies located outside the European Union (Japan, USA, Turkey).

INERIS obtained the return of 21 completed questionnaires among 5,271 questionnaires sent, followed by 16 targeted telephone interviews and further email discussions. The survey covered the whole EU27 but a few countries, such as France, Germany, UK, Sweden, appear as the most participating countries in this survey.

The figure below represents the distribution of answers to the survey by sector of activity.

Figure 47. Distribution of answers to the survey by sector of activity (*n*= 21)



Source : INERIS, 2013

Although this result is not very significant concerning its numerical aspect, it still provided a certain amount of information, in particular regarding:

- the current use of BPA in this sector and the BPA content in thermal paper (such as described in section B.2)
- the consequences for stakeholders of a possible restriction of BPA use in thermal paper and the possible reduction of thermal paper use
- the evolution and trends of thermal paper market (such as described in section E.1)
- the possible use of alternatives to BPA in thermal paper (such as mentioned in section C)
- the cost of substitution which allowed an assessment of the difference of cost between BPA-containing paper and BPA-free paper (such as analysed in section F.2)

INERIS reports some difficulties encountered regarding the performance of this survey:

- The tight schedule for the study (operational survey for 3 months), which did not allow us to optimize the response rate;
- The survey was launched during the summer which is not a favorable period.
- The difficulty of obtaining electronic contact information for key players (for example, the list of players from the COPACEL did not contain this information and a manual search was necessary);
- The language barrier regarding English encountered for some countries.
- The received emails sometimes came from email addresses not present in the recipient lists. In fact, a general email may automatically redistribute messages to different partners having a personal address.
- Some answerers to the questionnaire were not able to be identified (they are then designated as "unknown" in the remainder of this report), and no useful information

were obtained through these "unknown" questionnaires. This is very probably intentional, the aim being to view the questionnaire without answering it.

ETPA meeting

In parallel to the survey carried out, INERIS met ETPA in October 2013 in order to discuss about the issue of the use of BPA in thermal paper in the EU and the upcoming restriction proposal. As already mentioned above, ETPA is the European thermal paper association, and gathers the 5 main European producers of thermal paper: three of them participated to the meeting.

This meeting was helpful in confirming some data previously got through the INERIS e-questionnaire and in providing some new qualitative and quantitative information about:

- the use of BPA in thermal paper in the EU
- the trends of the market of thermal paper
- the possibilities of substitution of BPA for that particular use

To summarize, ETPA concluded that a ban of BPA for the use in TP would have an extremely negative impact on the European TP producers, like serious competitive disadvantages towards the non-European competitors which would have unforeseeable consequences for the European producers, the market and its stakeholders. BPS would be the first substance used to replace BPA. Furthermore some other markets in the world could require BPA containing papers, so European producers would still like to be able to produce BPA containing papers for exportations. Another issue is the recycling of alternatives containing thermal paper, and the presence of other chemicals in recycled papers.

These different pieces of information have been integrated in the analysis performed in this restriction proposal above.

G.2. Consultation of the REACH Member States Competent Authorities

The REACH Competent Authorities of all EU28 Member States have been contacted by ANSES during the elaboration of this restriction proposal. A questionnaire has been sent to the contact persons for REACH Regulation and more generally for health and environment concerns related to chemicals. The consultation took place from July 2013 to October 2013. The questionnaire sent is presented in Annex 1.

This consultation aimed at collecting information about:

- the key actors of the thermal paper market in the EU
- the use and substitutes of BPA in thermal paper
- the risk and exposures related to BPA-containing thermal paper
- existing/planned national regulations in the EU MS

Feedbacks were very helpful and the answer rate was satisfactory:

- 17 MS participated to the survey and sent back the questionnaire filled in.

- 8 MS provided quantitative data on the number of manufacturers and/or importers of thermal paper in their country as well as some data on the content of BPA of this type of paper and some tonnage of thermal paper manufactured
- 4 MS participated very actively by enclosing to their reply several studies and reports to help the analysis (used in the sections B and C in particular)
- All provided information on possible chemical substitutes (such as presented in section C)
- All indicated information about national RMM/action on this specific use already implemented in their country (none) or in the pipeline (Sweden in particular), such as presented in section B.9.

- 1 MS was supportive but had no information to share

- 10 MS did not answer the survey

Overall, the information collected by ANSES through this consultation was very helpful and allowed to double-check the results of the data got from the INERIS, 2013 survey and vice-versa. However, very little information was collected about import of thermal paper in the EU.

G.3. Consultation of French directorate for Competition Policy, consumer Affairs and Fraud Control (DGCCRF)

As regards enforceability and monitorability issues, ANSES organized a meeting with the French directorate for Competition Policy, consumer Affairs and Fraud Control (DGCCRF) in early December 2013. The DGCCRF is in charge of controlling the compliance to regulations related to competition, consumption and customs services.

The DGCCRF expressed some concern about the capacity of the restriction proposed to be enforceable and monitorable while indicating that there is currently no TARIC or Prodcom code specifically targeting 'thermal paper'. They particularly drew ANSES' and the REACH French Competent Authority's attention on the importance to clearly define the scope of the restriction. This issue is addressed above in section E.2 and it has been proposed several existing TARIC codes under which 'thermal paper' might be covered.

G.4. Consultation of SCL

To supplement the issues discussed with the DGCCRF (French directorate for Competition Policy, consumer Affairs and Fraud Control), presented above in section G.3, the SCL has also been consulted by ANSES in early December 2013, regarding the analytical methods likely to be used to measure BPA content (in the framework of RMO 1 – the restriction proposed) and migration (in the framework of RMO 2). The French SCL (*Service Commun des Laboratoires*) is

in charge of providing expertise and analytical measures on products, scientifici and technical support to the French government and applied research.

The SCL provided very useful information about the analytical possibilities to measure the content and migration of BPA. They indicated that no standard method exists but some methods could be used for the purpose of the compliance to this restriction, which are currently used to measure BPA in food contact materials. Besides, one of these methods has been used to measure the content of BPA in thermal tickets in the framework of the study carried out by the SCL for ANSES in DGCCRF, 2011. These methods are presented above, in section F.2.

G.5. Public consultation on the Annex XV restriction report (18 June 2014 – 18 December 2014)

After submission of the Annex XV restriction report, ECHA organised a six-month public consultation on the restriction report from 18 June 2014 – 18 December 2014. During the consultation, almost 34 comments were received from stakeholders, representing industry, trade and NGOs, as well as Member State Competent Authorities. The comments (nonconfidential) received, as well as the responses from the dossier submitter (France) and from the rapporteurs of the Committees for Risk Assessment and Socio-economic Analysis are to be made available on the ECHA website.

http://echa.europa.eu/web/guest/previous-consultations-on-restriction-proposals

H. Other information

No other information.

References

1. Abbas S, Peter Ihle, Ingrid Köster, Ingrid Schubert, Prevalence and incidence of diagnosed endometriosis and risk of endometriosis in patients with endometriosis-related symptoms: findings from a statutory health insurance-based cohort in Germany, European Journal of Obstetrics & Gynecology and Reproductive Biology, Volume 160, Issue 1, January 2012, Pages 79–83.

2. Acevedo N., Davis B., Schaeberle C.M., Sonnenschein C. and Soto A.M. 2013. Perinatally Administered Bisphenol A as a Potential Mammary Gland Carcinogen in Rats, Environmental Health Perspectives, volume 121, number 9, September 2013.

3. Adewale HB, Jefferson WN, Newbold RR, and Patisaul HB. 2009. Neonatal Bisphenol-A Exposure Alters Rat Reproductive Development and Ovarian Morphology Without Impairing Activation of Gonadotropin-Releasing Hormone Neurons. *Biology of Reproduction* 81: 690-699.

4. Afssa (Agence française de sécurité sanitaire des aliments). 2010. Avis de l'Agence française de sécurité sanitaire des aliments relatif à l'analyse critique des résultats d'une étude de toxicité, sur le développement du système nerveux ainsi que d'autres données publiées récemment sur les effets toxiques du bisphénol A. Afssa-Saisine nø2009-SA-0270., -7. Maisons-Alfort.

5. Afssa (Agence française de sécurité sanitaire des aliments) (2010a) Annexe de l'avis de l'Afssa du 29 janvier 2010 relatif à l'analyse critique des résultats d'une étude de toxicité sur le développement du système nerveux ainsi que d'autres données publiées récemment sur les effets toxiques du bisphenol A. Afssa, Maisons-Alfort.

6. Alonso-Magdalena P, and Morimoto S RCFENA. 2006. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect.* 114(1): 106-112.

7. Alonso-Magdalena P, and Ropero AB CMCCBMGBNSSENA. 2008. Pancreatic insulin content regulation by the estrogen receptor ER alpha. *PLoS One.*

8. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, and Nadal A. 2010. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. *Environmental Health Perspectives* 118 (9): 1243-1250.

9. ANSES (Agence française de sécurité sanitaire de l'alimentation, de l'environnement et du travail). 2011. Connaissances relatives aux usages du bisphénol A., -68. Maisons-Alfort.

10. ANSES (Agence française de sécurité sanitaire de l'alimentation, de l'environnement et du travail). 2011a. Health effects of Bisphenol A. Maisons-Alfort.

11. ANSES (Agence française de sécurité sanitaire de l'alimentation, de l'environnement et du travail). 2012. Substances reprotoxiques et perturbateurs endocriniens;

Composés de la famille des bisphénols: Bisphénols M, S, B, AP, AF, F et BADGE. Maisons-Alfort.

12. ANSES (Agence française de sécurité sanitaire de l'alimentation, de l'environnement et du travail). 2013. Substitution of Bisphenol A. Maisons-Alfort.

13. ANSES (Agence française de sécurité sanitaire de l'alimentation, de l'environnement et du travail). 2013. Risk assessment of Bisphenol A (BPA) on human health. Maisons-Alfort.

14. Armor. 2012. "Pas de bisphénol A dans les rubans transfert thermique." 2012. Available from http://www.armor-tt.com/index.php/fr/actualites/article/227.html.

15. Asahi J, and Kamo H BRDYYAMDHAHT. 2010. Bisphenol A induces endoplasmic reticulum stress-associated apoptosis in mouse non-parenchymal hepatocytes. *LifeSciences* 87: 431-438.

16. Asano S, and Tune JD DG. 2010. Bisphenol A activates Maxi-K (KCa1.1) channels in coronary smooth muscle. *British Journal of Pharmacology* 160: 160-170.

17. Aschberger K, and Castello P HEKSMSPSSD. 2010. Bisphenol A and baby bottles: challenges & perspectives. *JRC scientific and technical reports.* (Publications Office of the European Union, Luxembourg).

18. Bai Y, Chang F, Zhou R, Jin PP, Matsumoto H, Sokabe M, and Chen L. 2011. Increase of anteroventral periventricular kisspeptin neurons and generation of E2-induced LH-surge system in male rats exposed perinatally to environmental dose of bisphenol-A. *Endocrinology* 152 (4): 1562-1571.

19. Balakrishnan, Kimiora Henare M, and Eric B.Thorstensen B. 2010. Transfer of bisphenol A across the human placenta. *Basic Science: Obstetrics* 1-7: 202-393.

20. Beery AK, and Zucker I. 2011. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav. Rev.* 35 (3): 565-572.

21. Benner JS, Smith TW, Klingman D, Tierce JC, Mullins CD, Pethick N, and O'Donnell JC. 2005. Cost-effectiveness of rosuvastatin compared with other statins from a managed care perspective. *Value in Health* 8 (6): 618-628.

22. Berger RG, Hancock T, and deCatanzaro D. 2007. Influence of oral and subcutaneous bisphenol-A on intrauterine implantation of fertilized ova in inseminated female mice. *Reproductive Toxicology* 23: 138-144.

23. Berger RG, Foster WG, and deCatanzaro D. 2010. Bisphenol-A exposure during the period of blastocyst implantation alters uterine morphology and perturbs measures of estrogen and progesterone receptor expression in mice. *Reproductive Toxicology* 30: 393-400.

24. Berkowitz G. 2006. Limitations of a case-control study on bisphenol A (BPA) serum levels and recurrent miscarriage. *Human Reproduction* 21: 565-566.

25. Betancourt AM, Eltoum IA, Desmond RA, Russo J, and Lamartiniere CA. 2010. In utero exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. *Environmental Health Perspectives* 118 (11): 1614-1619.

26. Betancourt AM, Mobley JA, Russo J, and Lamartiniere CA. 2010. Proteomic analysis in mammary glands of rat offspring exposed in utero to bisphenol A. *Journal of Proteomics* 73: 1241-1253.

27. Biedermann S, Tschudin P, and Grob K. 2010. Transfer of bisphenol A from thermal printer paper to the skin. *Analytical and Bioanalytical Chemistry* 398 (1): 571-576.

28. Bishop-Bailey D, Hla T, Warner TD (2000) Bisphenol A diglycidyl ether (BADGE) is a PPARgamma agonist in an ECV304 cell line. *British Journal of Pharmacology* **131,** 651-654.

29. Birnbaum LS, and Fenton SE. 2003. Cancer and developmental exposure to endocrine disruptors. Environmental *Health Perspectives* 111 (4): 389-394.

30. BFR-WUR. 2014. Analysis of alternatives for BPA in thermal paper, report 1515, Dec 2014

31. Blair Rm FAU, and Fang HF eal. 2014. The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. *(1096-6080 (Print))*.

32. Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, and Lanphear BP. 2009. Prenatal bisphenol A exposure and early childhood behavior. *Environmental Health Perspectives* 117 (12): 1945-1952.

33. Bonaccorsi L, Nosi D, Quercioli F, Formigli L, Zecchi S, Maggi M, Forti G, Baldi E. 2008. Prostate cancer: a model of integration of genomic and non-genomic effects of the androgen receptor in cell lines model. *Steroids* 73**,** 1030-1037.

34. Braun JM, Kalkbrenner AE, Calafat AM, Bernert JT, Ye X, Silva MJ, Barr DB, Sathyanarayana S, and Lanphear B. 2011. Variability and predictors of urinary bisphenol A concentrations during pregnancy. 119: 131-137.

35. Brown III HS, Pérez A, Li YP, Hoelscher DM, Kelder SH, and Rivera R. 2007. The cost-effectiveness of a school-based overweight program. *International Journal of Behavioral Nutrition and Physical Activity* 4.

36. Bruze M. 1986. Sensitizing capacity of dihydroxydiphenyl methane (bisphenol F) in the guinea pig, 228--232.

37. Cabaton N, and Chagnon MC LJCJZD. 2006. Disposition and metabolic profiling of bisphenol F in pregnant and nonpregnant rats. *J Agric Food Chem.* 56(26): 10307-10314.

38. Cabaton N, and Zalko D RECCDGCMCJPE. 2008. Biotransformation of bisphenol F by human and rat liver subcellular fractions. *Toxicol In Vitro.* 22(7): 1697-1704.

39. Cabaton N, and Dumont C SIPEZDC-MMCMC. 2009. Genotoxic and endocrine activities of bis(hydroxyphenyl)methane (bisphenol F) and its derivatives in the HepG2 cell line. *Toxicology.* 255(1-2): 15-24.

40. Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, and Soto AM. 2010. Perinatal Exposure to Environmentally Relevant Levels of Bisphenol-A Decreases Fertility and Fecundity in CD-1 Mice. *Environ Health Perspect*.

41. Campbell JD, and Ramsey SD. 2009. The costs of treating breast cancer in the US: A synthesis of published evidence. *Pharmacoeconomics* 27 (3): 199-209.

42. Cantonwine D. 2010. Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. *Environ Health.* Oct: 9.

43. Cariot A, Dupuis A, bouy-Llaty M, Legube B, Rabouan S, and Migeot V. 2012. Reliable quantification of bisphenol A and its chlorinated derivatives in human breast milk using UPLC-MS/MS method. 100: 175-182.

44. Carwile JL, Ye X, Zhou X, Calafat AM, Michels KB (2011). Canned soup consumption and urinary bisphenol A: a randomized crossover trial. *JAMA* 306 (20): 2218-2220.

45. CDC (Centers for Disease Control and Prevention). 2011. Fourth national report on human exposure to environmental chemicals, updated tables.

46. Chen MY, and Ike M FM. 2002. Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. *Environ Toxicol* 17(1): 80-86.

47. Chevallereau, V., Disposition du Bishpénol A chez le cheval : établissement d'une relation allométrique chez les mammifères. Thèse d'exercice, Médecine vétérinaire, Ecole Nationale Vétérinaire de Toulouse, http://oatao.univ-toulouse.fr/8651/1/chevallereau_8651.pdf

48. Chou Wei-Chun, Jyh-Larng Chen, Chung-Fen Lin, Yi-Chun Chen, Feng-Cheng Shih and Chun-Yu Chuang; Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environmental Health 2011, 10:94 http://www.ehjournal.net/content/10/1/94

49. Clifford S. 2011. Shopper Receipts Join Paperless Age. The New York Times.

50. Cobellis L, Colacurci N, Trabucco E, Carpentiero C, and Grumetto LD. 2009. Measurement of bisphenol A and bisphenol B levels in human blood sera from endometriotic women. *Biomedical Chromatography* 23: 1186-1190.

51. Colerangle JB, and Roy D. 1997. Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. *Journal of Steroid Biochemistry and Molecular Biology* 60: 153-160.

52. Collet SH, Picard-Hagen N, Viguie C, Lacroix MZ, Toutain PL, and Gayrard V. 2010. Estrogenicity of Bisphenol A: A Concentration-Effect Relationship on Luteinizing Hormone Secretion in a Sensitive Model of Prepubertal Lamb. *Toxicological Sciences* 117: 54-62.

53. Collet S. 2012. Développement d'une approche toxicocinétique/toxicodynamique basée sur des mécanismes physiologiques pour évaluer les effets oestrogéniques du Bisphénol A, Thèse, Institut National Polytechnique de Toulouse.

54. Cox KH, Gatewood JD, Howeth C, and Rissman EF. 2010. Gestational exposure to bisphenol A and cross-fostering affect behaviors in juvenile mice. *Hormones and Behavior* 58 (5): 754-761.

55. Danish E.P.A. 2014. Alternative technologies and substances to bisphenol A (BPA) in thermal paper receipts., Environmental Project No. 1553.

56. Danish E.P.A. 2013. Background for national legislation on bisphenol A (BPA) in EU and EFTA countries . In Press.

57. Danish E.P.A. 2013a. Survey of Bisphenol A and Bisphenol-A-diglycidylether polymer, Part of the LOUS-review, Environmental Project No. 1483. In Press.

58. Danish E.P.A. and COWI. 2011. Migration of bisphenol A from cash register receipts and baby dummies.

59. Demierre AL, Peter R, Oberli A, and Bourqui-Pittet M. 2012. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. *Toxicology Letters* (0).

60. DGCCRF. 2011. Recherche et quantification du bisphénol A et S dans les tickets de caisse. Laboratoire SCL de Lyon. Rapport non publié.

61. DHI, and Water and Environment. 2007. Study on enhancing the Endocrine Disrupter priority list with a focus on low production volume chemicals.

62. Dhimolea E., Wadia P.R., Murray T.J., Settles M.L., Treitman J.D., Sonnenschein C., Shioda T., Soto A.M. 2014. Prenatal Exposure to BPA Alters the Epigenome of the Rat Mammary Gland and Increases the Propensity to Neoplastic Development, PLOS One, volume 9, Issue 7, July 2014.

63. Doerge DR, Twaddle NC, Vanlandingham M, and Fisher JW. 2010a. Pharmacokinetics of bisphenol A in neonatal and adult Sprague-Dawley rats. *Toxicology and Applied Pharmacology* 247 (2): 158-165.

64. Doerge DR, Twaddle NC, Wooding KA, and Fisher JW. 2010b. Pharmacokinetics of bisphenol A in neonatal and adult rhesus monkeys. 248: 1-11.

65. Doerge DR, Vanlandingham M, Twaddle NC, and Delclos KB. 2010c. Lactational transfer of bisphenol A in Sprague-Dawley rats. 199: 372-376.

66. Doerge DR, Twaddle NC, Vanlandingham M, Brown RP and Fisher JW, 2011a. Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague-Dawley rats. *Toxicology and Applied Pharmacology* 255: 261-270.

67. Doerge DR, Twaddle NC, Vanlandingham M, and Fisher JW. 2011b. Pharmacokinetics of bisphenol A in neonatal and adult CD-1 mice: inter-species comparisons with Sprague-Dawley rats and rhesus monkeys. *Toxicology Letters* 207 (3): 298-305.

68. Doerge DR, and Twaddle NC VMFJW. 2012. Pharmacokinetics of bisphenol A in serum and adipose tissue following intravenous administration to adult female CD-1 mice. *ToxicolLett. 2012 Mar 20. [Epub ahead of print] Division of Biochemical Toxicology, National Center forToxicological Research, U. S. Food and Drug Administration, Jefferson, AR 72079, United States.*

69. Doherty L, and Bromer J ZYATTH. 2010. In Utero Exposure to Diethylstilbestrol (DES) or Bisphenol-A (BPA) Increases EZH2 Expression in the Mammary Gland: An Epigenetic Mechanism Linking Endocrine Disruptors to Breast Cancer. *Hormones and Cancer* 1: 146-155.

70. Dumont C., and Perdu E dSDLRRCJCM. 2011. Bis(hydroxyphenyl)methane-bisphenol F-metabolism by the HepG2 human hepatoma cell line and cryopreserved human hepatocytes., 34(4), 445-453.

71. Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, and Munoz-De-Toro M. 2007. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. *Environmental Health Perspectives* 115 (1): 80-86.

72. EC (European Commission). 2003. European Union Risk Assessment Report. 4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A) CAS No: 80-05-7 EINECS No: 201-245-8 RISK ASSESSMENT. Complete risk assessment in one document., -695. Luxembourg.

73. EC (European Commission). 2004. Guidance document on dermal absorption. Commission européenne, DG SANCO, Sanco/222/2000 rev. 7. Luxembourg.

74. EC (European Commission). 2008. European Union Risk Assessment Report. 4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A) CAS No: 80-05-7 EINECS No: 201-245-8 RISK ASSESSMENT. Complete risk assessment in one document.

75. EC (European Commission). 2010. European Union Risk Assessment Report. 4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A) CAS No: 80-05-7 EINECS No: 201-245-8 RISK ASSESSMENT. Complete risk assessment in one document., -695. Luxembourg.

76. EC (European Commission). 2012. Toxicity to reproduction.

77. ECHA (European Chemicals Agency). 1987. Genetic Toxicity in vitro. ECHA.

78. ECHA (European Chemicals Agency). 1989. Genetic Toxicity in vitro. ECHA.

79. ECHA (European Chemicals Agency). 1990. Genetic Toxicity in vitro. ECHA.

80. ECHA (European Chemicals Agency). 1991. Genetic Toxicity in vitro. ECHA.

81. ECHA (European Chemicals Agency). 1996. Genetic Toxicity in vitro. ECHA.

82. ECHA (European Chemicals Agency). 2007. Guidance on Socio-Economic analysis – Restrictions. ECHA

83. ECHA (European Chemicals Agency). 2010. Genetic Toxicity in vitro. ECHA.

84. ECHA (European Chemicals Agency). 2010a. Addendum to the guidance on socio-economic analysis (SEA) – Restrictions calculation of compliance costs.

85. Edlow AG, Chen M, Smith NA, Lu C, and McElrath TF. 2012. Fetal bisphenol A exposure: Concentration of conjugated and unconjugated bisphenol A in amniotic fluid in the second and third trimesters. *Reproductive Toxicology*.

86. EFSA (European Food Safety Agency). 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). Question number EFSA-Q-2005-100., -75.

87. EFSA (European Food Safety Agency). 2008. Toxicokinetics of Bisphenol A - Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC), -10.

88. EFSA (European Food Safety Agency). 2010. Scientific Opinion on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A, question number EFSA-Q 2009-00864, EFSA-Q-2010-01023, EFSA-Q-2010-00, adopted on 23 September 2010.

89. EFSA (European Food Safety Agency). 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, EFSA journal 2015;13(1):3978.

90. Elobeid MA, and Allison DB. 2008. Putative environmental-endocrine disruptors and obesity: a review. *Curr* Opin *Endocrinol Diabetes Obes.* 15(5): 403-408.

91. Evans NP, North T, Dye S, and Sweeney T. 2004. Differential effects of the endocrine-disrupting compounds bisphenol-A and octylphenol on gonadotropin secretion, in prepubertal ewe lambs. *Domest Anim Endocrinol* 26: 61-73.

92. EWG (Environmental Working Group). 2010. Synthetic estrogen BPA coats cash register receipts.

93. FAO (Food and Agriculture Organization)//WHO (World Health Organization). 2010. Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A Summary Report including report of Stakeholder Meeting on Bisphenol A.

94. Farbos M. 2012. Toxicocinétique comparée du bisphénol A chez cinq espèces et extrapolation de l'animal à l'homme, under review.

95. FBR-WUR. 2014. Analysis of alternatives for BPA in thermal paper, report 1515, Dec 2014.

96. FDA (Food and Drug Administration). 2008. Draft Assessment of Bisphenol A for Use in food contact Applications, -105.

97. Fenton SE. 2006. Endocrine-disrupting compounds and mammary gland development: Early exposure and later life consequences. *Endocrinology* 147 (6): S18-S24.

98. Fernandez M, Bianchi M, Lux-Lantos V, and Libertun C. 2009. Neonatal Exposure to Bisphenol A Alters Reproductive Parameters and Gonadotropin Releasing Hormone Signaling in Female Rats. *Environmental Health Perspectives* 117: 757-762.

99. Fernandez M, Bourguignon N, Lux-Lantos V, and Libertun C. 2010. Neonatal Exposure to Bisphenol A and Reproductive and Endocrine Alterations Resembling the Polycystic Ovarian Syndrome in Adult Rats. *Environmental Health Perspectives* 118: 1217-1222.

100. Fujimoto VY, Kim D, Vom Saal FS, Lamb JD, Taylor JA, and Bloom MS. 2011. Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization. *Fertility and Sterility* 95 (5): 1816-1819.

101. Funabashi T, Kawaguchi M, Furuta M, Fukushima A, and Kimura F. 2004. Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. *Psychoneuroendocrinology* 29 (4): 475-485.

102. Gayrard V, and Marlène Z.Lacroix 12SHC12CV12AB-M12P-LT12aNP-H2. 2013. High Bioavailability of Bisphenol A from Sublingual Exposure. ed Environ Health Perspect, 951-956.

103. Geens T, Neels H, and Covaci A. 2012. Distribution of bisphenol-A, triclosan and n-nonylphenol in human adipose tissue, liver and brain. *Chemosphere.*

104. Gehring M, Vogel D, Tennhardt L, Weltin D, and Bilitewski B. 2004. Bisphenol a contamination of wastepaper, cellulose and recycled paper products. 29 2004, at Rhodes.

105. Ghibellini G, and Leslie EMaBKL. 2006. Methods to evaluate biliary excretion of drugs in humans: an updated review. *Mol. Pharm.* 3: 198-211.

106. Ginsberg G RD. 2009. Does rapid metabolism ensure negligible risk from bisphenol A? *Environmental Health Perspectives* 117 (1639): 1643.

107. Grabowski DC, Lakdawalla DN, Goldman DP, Eber M, Liu LZ, Abdelgawad T, Kuznik A, Chernew ME, and Philipson T. 2012. The large social value resulting from use of statins warrants steps to improve adherence and broaden treatment. *Health Affairs* 31 (10): 2276-2285.

108. Gruber EV, Stock S, and Stollenwerk B. 2012. Breast Cancer Attributable Costs in Germany: A Top-Down Approach Based on Sickness Funds Data. *PLoS ONE* 7 (12).

109. Hajszan T, and Leranth C. 2010. Bisphenol A interferes with synaptic remodeling. *Frontiers in Neuroendocrinology* 31: 519-530.

110. Hanioka N, and Naito T NS. 2008. Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. *Chemosphere* 74 (33): 36.

111. Hashimoto Y, and Moriguchi Y OHNJNTNM. 2000. Estrogenic activity of chemicals for dental and similar use in vitro. *Journal of Materials Science: Materials in Medicine* 11(8): 465-468.

112. Hashimoto Y, and Moriguchi Y OHKMMKNM. 2001. Measurement of estrogenic activity of chemicals for the development of new dental polymers. *Toxicol. In Vitro* 15(4-5): 421-425.

113. Health Canada. 2008. Évaluation préalable finale pour le défi concernant le Phénol, 4,4-(1-méthyléthylidène)bis (Bisphenol-A). *(Santé Canada, Ottawa)*.

114. Higashihara N, and Shiraishi K MKOYMYYK. 2007. Subacute oral toxicity study of bisphenol F based on the draft protocol for the "Enhanced OECD Test Guideline no. 407", 825-832.

115. Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, Yokota H, and Taketani Y. 2004. Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia. *Endocr J* 51: 595-600.

116. Hirom PC, and Millburn PaSRL. 2014. Bile and urine as complementary pathways for the excretion of foreign organic compounds. *Xenobiotica* 6: 55-64.

117. Holoch K, and Lessey B. 2010. Treatment is Cost-effective and may Improve Fertility. *Fertility Weekly*.

118. Hong YC, Park EY, Park MS, Ko JA, Oh SY, Kim O, Lee KH, Leem JH, and Ha EH. 2009. Community level exposure to chemicals and oxidative stress in adult population. 184: 139-144.

119. Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, and Iguchi T. 2002. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reproductive Toxicology* 16 (2): 117-122.

120. Hormann AM, Vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, Ellersieck MR, Welshons WV, Toutain PL, Taylor JA. 2014. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioactive and urine total levels of bisphenol A (BPA), PLOS One. Oct 22; 9(10), eCollection 2014.

121. Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, and Vom Saal FS. 1999. Environmental toxins: Exposure to bisphenol A advances puberty. *Nature* 401 (6755): 763-764.

122. ICIS. 2009. Bisphenol A (BPA) Prices and Princing Information. ICIS Chemical Intelligence petrochemicals-aromatics: bisphenol a : pricing.

123. Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, and Taketani Y. 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Human Reproduction* 17 (11): 2839-2841.

124. INERIS (Institut national de l'environnement industriel et des risques). 2010. Données technico-économiques sur les substances chimiques en France: Le Bisphénol A. (INERIS-DRC-10-102861-01251A)., -200. Verneuil-en-Halatte: Ineris.

125. INERIS (Institut national de l'environnement industriel et des risques). 2010a. Le Bisphenol A - mise à jour juin 2010. Rapport d'étude. N° INERIS-DRC-10-109972-01125C, 1-23.

126. INERIS (Institut national de l'environnement industriel et des risques). 2013. Survey of the use of bisphenol A (BPA) in thermal papers and its alternatives in the European Union, REPORT N° DRC-13-136308-10844A. Ineris.

127. INERIS (Institut national de l'environnement industriel et des risques). 2013. Economic benefits of a BPA restriction in thermal paper: reference values for avoided cholesterol and overweight, REPORT N° DRC-13-45981-12954A . Ineris.

128. INRS. 2010. Bisph,nol A. Fiche toxicologique FT 279., -8. Paris.

129. INSERM (Institut national de la santé et de la recherche médicale). 2010. Bisphénol A. Effets sur la reproduction. Rapport 010. -90, Expertise collective. Inserm, Paris.

130. INSERM (Institut national de la santé et de la recherche médicale). 2011. Reproduction et environnement, Expertise collective. Inserm, Paris.

131. Iordanidou A, Hadzopoulou-Cladaras M, Lazou A (2010) Non-genomic effects of thyroid hormone in adult cardiac myocytes: relevance to gene expression and cell growth. *Molecular and Cellular Biochemistry* 340**,** 291-300.

132. Itoh H, and Iwasaki M HTSHTTTS. 2007. Urinary bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study. *EnvironmentalHealth and Preventive Medicine* 12: 258-264.

133. Jeffs J. 2011. Bisphenol-free tickets - Länstrafiken Jämtland - Market analysis. ed SSC Jegrelius.

134. Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, and Lamartiniere CA. 2009. Oral Exposure to Bisphenol A Increases Dimethylbenzanthracene-Induced Mammary Cancer in Rats. *Environmental Health Perspectives* 117: 910-915.

135. Ji K, Hong S, Kho Y, Choi K. Effects of bisphenol S exposure on endocrine functions and reproduction of zebrafish. Environ Sci Technol. 2013 Aug 6;47(15):8793-800

136. John J, Wolfenstetter SB, and Wenig CM. 2012. An economic perspective on childhood obesity: Recent findings on cost of illness and cost effectiveness of interventions. *Nutrition* 28 (9): 829-839.

137. Jones LP, and Sampson A KHKHYYKSBJWABI. 2010. Loss of BRCA1 leads to an increased sensitivity to Bisphenol A. *Toxicology Letters* 199: 261-268.

138. Kandaraki E, Chatzigeorgiou A, Livadas S, Palioura E, Economou F, Koutsilieris M, Palimeri S, Panidis D, and amanti-Kandarakis E. 2010. Endocrine Disruptors and Polycystic Ovary Syndrome (PCOS): Elevated Serum Levels of Bisphenol A in Women with PCOS. *J Clin Endocrinol Metab*.

139. Kass L, Altamirano GA, Bosquiazzo VL, Luque EH, and Munoz-De-Toro M. 2012. Perinatal exposure to xenoestrogens impairs mammary gland differentiation and modifies milk composition in Wistar rats. *Reproductive Toxicology*.

140. Kemi. 2013. Bisfenol A i kassakvitton. Redovisning av regeringsuppdrag.

141. Kidani T, Kamei S, Miyawaki J, Aizawa J, Sakayama K, and Masuno H. 2010. Bisphenol A downregulates Akt signaling and inhibits adiponectin production and secretion in 3T3-L1 adipocytes. *J Atheroscler. Thromb.* 17 (8): 834-843.

142. Kim K, and Son TG PHKSKHKHKTJKHSLJ. 2009. Potencies of bisphenol A on the neuronal differentiation and hippocampal neurogenesis. *Journal of Toxicology and Environmental Health, Part A* 72: 1343-1351.

143. Kim YH, Kim CS, Park S, Han SY, Pyo MY, and Yang M. 2003. Gender differences in the levels of bisphenol A metabolites in urine. 312: 441-448.

144. Kitamura S, and uzuki T eal. 2005. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. *Toxicological Sciences* 84: 249-259.

145. Klein S, D'Hooghe T, Meuleman C, Dirksen C, Dunselman G, and Simoens S. 2013. *What is the* societal *burden of endometriosis-associated symptoms? A prospective Belgian study*. In press.

146. Klopman G, and hakravarti SK. 2003. Screening of high production volume chemicals for estrogen receptor binding activity (II) by the MultiCASE expert system. *Chemosphere.* 51(6): 461-468.

147. Kobayashi K, and Ohtani K KHMM. 2010. Dietary exposure to low doses of bisphenol A: effects on reproduction and development in two generations of C57BL/6J mice. *Congenital Anomalies* 50: 159-170.

148. Krotz S.P., Carson SA, Tomey C, and Buster JE. 2012. Phthalates and bisphenol do not accumulate in human follicular fluid.

149. Kubo K, and Arai O OROMHTAS. 2001. Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. *Neuroscience Letters* 304: 73-76.

150. Kubo K, Arai O, Omura M, Watanabe R, Ogata R, and Aou S. 2003. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neuroscience Research* 45 (3): 345-356.

151. Kunju LP, and Cookingham C TKCWSMKC. 2011. EZH2 and ALDH-1 mark breast epithelium at risk for breast cancer development. *Modern Pathology* 24: 786-793.

152. Kurebayashi H, Harada R, Stewart RK, Numata H, and Ohno Y. 2002. Disposition of a low dose of bisphenol a in male and female cynomolgus monkeys. *Toxicological Sciences* 68 (1): 32-42.

153. Kurebayashi H, Betsui H, and Ohno Y. 2003. Disposition of a low dose of 14C-bisphenol A in male rats and its main biliary excretion as BPA glucuronide. *Toxicological Sciences* 73 (1): 17-25.

154. Kurebayashi H, Nagatsuka S, Nemoto H, Noguchi H, and Ohno Y. 2005. Disposition of low doses of 14C-bisphenol A in male, female, pregnant, fetal, and neonatal rats. *Archives of Toxicology* 79 (5): 243-252.

155. Kuruto-Niwa R, and Nozawa R MTSTTY. 2005. Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. *Environ. Toxicol. Pharmacol.* 19(1): 121-130.

156. Kuruto-Niwa R, Tateoka Y, Usuki Y, and Nozawa R. 2007. Measurement of bisphenol A concentrations in human colostrum. 66: 1160-1164.

157. Kwintkiewicz J, Nishi Y, Yanase T, Giudice LC (2010) Peroxisome proliferator-activated receptor-gamma mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environmental Health Perspectives* 118**,** 400-406.

158. Kwon S, and Stedman DB EBCRWF. 2000. Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. *Toxicological Sciences* 55: 399-406.

159. Lachaine J, Merikle E, Tarride JE, Montpetit M, and Rinfret S. 2007. A Model for Assessing the Cost-Effectiveness of Atorvastatin and Simvastatin in Achieving Canadian Low-Density Lipoprotein Cholesterol Targets. *Clinical Therapeutics* 29 (3): 519-528.

160. Lagarde F. 2013. Perturbateurs endocriniens et relations dose-effet non monotones : état des lieux, mécanismes impliqués et conséquences en toxicologie.

161. Lang I.A., Galloway T.S., Scarlett A., Henley W.E, Depledge, M., Wallace, R.B., Melzer, D., 2008. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. *JAMA* 300: 1303-1310.

162. Liao C, and Kannan K. 2012. Bisphenol S, a new bisphenol analogue, in paper products ans currency bills and its association with bisphenol A residues, 6515-6522.

163. Liao C, and Kannan K. 2011. Widespread Occurrence of Bisphenol A in Paper and Paper Products: Implications for Human Exposure. *Environ. Sci. Technol. p* 45: 9372-9379.

164. Longnecker MP. 2009. Human data on bisphenol a and neurodevelopment. *Environmental HealthPerspectives* 117: A531-A532.

165. Luisi S, Lazzeri L, Ciani V, and Petraglia F. 2009. Endometriosis in Italy: from cost estimates to new medical treatment. *Gynecol Endocrinol* 25: 734-740.

166. Machtinger, and Catherine M.H.Combelles2 SAM34KFCPRH7aCR. 2013. Bisphenol-A and human oocyte maturation in vitro. *Human Reproduction* 0 n0: 1-11.

167. Mahoney MM, and Padmanabhan V. 2010. Developmental programming: impact of fetal exposure to endocrine-disrupting chemicals on gonadotropin-releasing hormone and estrogen receptor mRNA in sheep hypothalamus. *Toxicol Appl Pharmacol* 247: 98-104.

168. Makris SL. 2011. Current assessment of the effects of environmental chemicals on the mammary gland in guideline rodent studies by the U.S. environmental protection agency (U.S. EPA), organisation for economic co-operation and development (OECD), and national toxicology program (NTP). *Environmental Health Perspectives* 119 (8): 1047-1052.

169. Marino P, Le Corroller AG, Palangié T, Janvier M, Fabbro M, Molinier L, Delozier T, Livartowski A, Moatti JP, and Viens P. 2003. Can sequential administration minimise the cost of

high dose chemotherapy? An economic assessment in inflammatory breast cancer. *Pharmacoeconomics* 21 (11): 807-818.

170. Markey CM, and Luque EH MDTMSCSA. 2001. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod.* 65(4): 1215-1223.

171. Markey CM, Wadia PR, Rubin BS, Sonnenschein C, and Soto AM. 2005. Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. *Biol Reprod* 72: 1344-1351.

172. Marmugi A, Ducheix S, Lasserre F, Polizzi A, Paris A, Priymenko N, Bertrand-Michel J, Pineau T, Guillou H, Martin PG, and Mselli-Lakhal L. 2012. Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. *Hepatology* 55 (2): 395-407.

173. Marmugi et al 2014, Adverse effects of long-term exposure to bisphenolA durng adulthood leading to hyperglycemia and hypercholesterolemia in mice. TOXICOLOGY 325 (2014) 133-143

174. Marquet F. 2011. In vivo and ex vivo percutaneous absorption of [^{14}C]-bisphenol A in rats: a possible extrapolation to human absorption? *Archives of Toxicology* A paraŒtre.

175. Martini M, Miceli D, Gotti S, Viglietti-Panzica C, Fissore E, Palanza P, and Panzica G. 2010. Effects of perinatal administration of Bisphenol A on the neuronal nitric oxide synthase expressing system in the hypothalamus and limbic system of CD1 mice. *Journal of Neuroendocrinology* 22 (9): 1004-1012.

176. Matsuda S, and Saika S AKSESJ. 2010. Changes in brain monoamine levels in neonatal rats exposed to bisphenol A at low doses. *Chemosphere* 78: 894-906.

177. Mazur CS, and Kenneke JF H-WJLJ. 2010. Differences between human and rat intestinal and hepatic bisphenol A glucuronidation and the influence of alamethicin on in vitro kinetic measurements. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 38 (2232): 2238.

178. Mazur CS, Marchitti SA, Dimova M, Kenneke JF, Lumen A, and Fisher J. 2012. Human and rat ABC transporter efflux of bisphenol a and bisphenol a glucuronide: interspecies comparison and implications for pharmacokinetic assessment. Toxicological Sciences 128(2): 317-325.

179. Medina D. 2007. Chemical carcinogenesis of rat and mouse mammary glands. *Breast. Dis.* 28: 63-68.

180. Melzer D, Rice NE, Lewis C, Henley WE, and Galloway TS. 2010. Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. *PLoS ONE* 5 (1): e8673.

181. Ménard S, Guzylack-Piriou L, Lencina C, Leveque M, Naturel M, Sekkal S, Harkat C, Gaultier E, Olier M, Garcia-Villar R, Theodorou V, Houdeau E, Perinatal exposure to a low dose of bisphenol A impaired systemic cellular immune response and predisposes young rats to intestinal parasitic infection, PLoS One. 2014 Nov 21;9(11):e112752. doi: 10.1371/journal.pone.0112752. eCollection 2014.

182. Menard S, Guzylack-Piriou L, Leveque M, Braniste V, Lencina C, Naturel M, Moussa L, Sekkal S, Harkat C, Gaultier E, Theodorou V, Houdeau E, Food intolerance at adulthood after perinatal exposure to the endocrine disruptor bisphenol A, FASEB J. 2014 Nov;28(11):4893-900. doi: 10.1096/fj.14-255380. Epub 2014 Aug 1,

183. Mendoza R, Garc+-a-Guzm+in M, Baranda-Avila N, Morimoto S, Perrot-Applanat M, and Ýn M. 2010. Administration of bisphenol A to dams during perinatal period modifies molecular and morphological reproductive parameters of the offspring. *Reproductive Toxicology* In Press, Uncorrected Proof.

184. Mendum T, Stole E, VanBenschoten H, and Warner J. 2010. Concentration of bisphenol A in thermal paper., 81-86. Green Chem Lett Rev.

185. Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, and D'Hooghe T. 2009. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. *Fertil Steril* 92: 68-74.

186. Mielke H, and Gundert-Remy U. 2009. Bisphenol A levels in blood depend on age and exposure. *Toxicology Letters* 190 (1): 32-40.

187. Miodovnik A, and Engel SM ZCYXSLSMCAWM. 2011. Endocrine disruptors and childhood social impairment. *Neurotoxicology* 32: 261-267.

188. Miyawaki J, Sakayama K, Kato H, Yamamoto H, and Masuno H. 2007. Perinatal and postnatal exposure to bisphenol a increases adipose tissue mass and serum cholesterol level in mice. *Journal of Atherosclerosis and Thrombosis* 14 (5): 245-252.

189. Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X, and Hauser R. 2010. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. *Internation Journal of Andrology* 33 (2): 385-393.

190. Monje L, Varayoud J, Munoz-De-Toro M, Luque EH, and Ramos JG. 2009. Neonatal exposure to bisphenol A alters estrogen-dependent mechanisms governing sexual behavior in the adult female rat. *Reproductive Toxicology* 28 (4): 435-442.

191. Moodie ML, Carter RC, Swinburn BA, and Haby MM. 2010. The cost-effectiveness of Australia's active after-school communities program. *Obesity* 18 (8): 1585-1592.

192. Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, and Russo J. 2008. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. *Journal of Endocrinology* 196: 101-112.

193. Morck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L, and Knudsen LE. 2010. Placental transport and in vitro effects of Bisphenol A. *Reproductive Toxicology* 30 (1): 131-137.

194. Mose T., and L.Mathiesen a VKbJKSNaEScMKTAMaKMbHPbKVbLEKaPM. 2012. Meta-analysis of data from human ex vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated European project NewGeneris. *Placenta* 33: 433-439.

195. Munoz del Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, and Soto AM. 2005. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. *Endocrinology* 146 (9): 4138-4147.

196. Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, and Soto AM. 2007. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reproductive Toxicology* 23 (3): 383-390.

197. Nadal A, and Alonso-Magdalena P SSQIRAB. 2009. The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. *Mol Cell Endocrinol.* 304(1-2): 63-8.

198. Naderi M, Wong MY, Gholami F. Developmental exposure of zebrafish (Danio rerio) to bisphenol-S impairs subsequent reproduction potential and hormonal balance in adults. 2014. Aquat Toxicol. Mar;148:195-203.

199. Nakagami A, Negishi T, Kawasaki K, Imai N, Nishida Y, Ihara T, Kuroda Y, Yoshikawa Y, and Koyama T. 2009. Alterations in male infant behaviors towards its mother by prenatal exposure to bisphenol A in cynomolgus monkeys (*Macaca fascicularis*) during early suckling period. *Psychoneuroendocrinology* 34 (8): 1189-1197.

200. Nakamura K, Itoh K, Yoshimoto K, Sugimoto T, and Fushiki S. 2010. Prenatal and lactational exposure to low-doses of bisphenol A alters brain monoamine concentration in adult mice. *Neuroscience Letters* 484 (1): 66-70.

201. Navarro VM, and anchez-Garrido MA CJRJG-GDPRAEPLT-SM. 2009. Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation. *Endocrinology* 150: 2359-2367.

202. Ndaw S., Robert A., Jargot D., Remy A., Bertrand N., Malard S., Lafon D. 2015. Occupational exposure to Bisphenol A via thermal paper: Urinary biomonitoring study, under press.

203. Newbold RR, Jefferson WN, and Padilla-Banks E. 2007. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reproductive Toxicology* 24: 253-258.

204. Newbold RR, Jefferson WN, and Padilla-Banks E. 2009. Prenatal Exposure to Bisphenol A at Environmentally Relevant Doses Adversely Affects the Murine Female Reproductive Tract Later in Life. *Environmental Health Perspectives* 117: 879-885.

205. Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, and Tsubura A. 2004. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reproductive Toxicology* 18 (6): 803-811.

206. Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, and Tsubura A. 2005. Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. *In Vivo* 19: 487-494.

207. Nishimura M, and Naito S. 2006. Tissue-specific mRNA expression profiles of human phase I metabolizing enzymes except for cytochrome P450 and phase II metabolizing enzymes. *Drug Metab. Pharmacokinet.* 21 (5): 357-374.

208. Nnoaham K, Hummelshoj L, Webster P, D'Hooghe T, de Cicco Nardone F, and de Cicco Nardone C. 2011. Impact of endometriosis on quality of life and work productivity: a multicenter study across ten countries . *Fertility and Sterility* 96 (2).

209. NTP CERH (National Toxicology Program). 2007. NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A., -396.

210. NTP (National Toxicology Program). 2008. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. NIH, No. 08-5994 (NIH, Research Triangle Park, NC)

211. National Toxicology Program (NTP). 2008a. Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. NIH Publication No. 08 - 5994.

212. National Toxicology Program (NTP). 2014. NTP Research Concept: Bisphenol S, Draft: NTP Board of Scientific Counselors Meeting, June 17- 18, 2014.

213. OECD (Organisation for Economic Co-operation and Development). 2009. Emission Scenario Document on Pulp, paper and board industry.

214. OEHHA (Office of Environmental Health Hazard Assessment). 2009. Evidence on the developmental and reproductive toxicity of bisphenol A., -302.

215. Office of Environmental Chemicals Safety Environmental Health. 1999. Toxicity testing reports of environmental chemicals. *Ministry of Health&Welfare, Japan.*

216. Okuda K, Takiguchi M, and Yoshihara S. 2010. In vivo estrogenic potential of 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, an active metabolite of bisphenol A, in uterus of ovariectomized rat. *Toxicology Letters* 197: 7-11.

217. Otaka H, Yasuhara A, and Morita M. 2003. Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and clean-up by solid-phase extraction. 19: 1663-1666.

218. Östberg T, and Noakson E. 2010. Bisfenol A i svenske kvitton. Analysresultat. Jegrelius for Jämtlands Läns Landsting.

219. Paap E., Broeders M.J.M., van Schoor G, Otten J.D.M., Verbeek A.L.M., Large increase in a Dutch woman's lifetime risk of developing breast cancer, European Journal of Cancer, Volume 44, Issue 11, July 2008, Pages 1485–1487.

220. Padmanabhan V, and Siefert K RSJTPJALTLKK. 2008. Maternal bisphenol-A levels at delivery: a looming problem? *Journal of Perinatology* 28: 258-263.

221. Palanza P, Gioiosa L, Vom Saal FS, and Parmigiani S. 2008. Effects of developmental exposure to bisphenol A on brain and behavior in mice. *Environmental Research* 108 (2): 150-157.

222. Patisaul HB, Fortino AE, and Polston EK. 2006. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. *Neurotoxicology and Teratology* 28: 111-118.

223. Patisaul HB, and Fortino AE PE. 2007. Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A. *Neurotoxicology (Amsterdam)* 28: 1-12.

224. Patisaul HB, Todd KL, Mickens JA, and Adewale HBD. 2009. Impact of neonatal exposure to the ER alpha agonist PPT, bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in male and female rats. *Neurotoxicology (Amsterdam)* 30: 350-357.

225. Patterson TA, Twaddle NC, Roegge CS, Callicott RJ, Fisher JW, and Doerge DR. 2013. Concurrent determination of bisphenol A pharmacokinetics in maternal and fetal rhesus monkeys. *Toxicology and Applied Pharmacology* 267 (1): 41-48.

226. Perez P, Pulgar R, Olea-Serrano F, Villalobos M, Rivas A, Metzler M, Pedraza V, Olea N. 1998. The estrogenicity of bisphenol A-related diphenylalkanes with various substituents at the central carbon and the hydroxy groups, 167-174.

227. Pfeiffer E, and osenberg B DSMM. 1997. Interference with microtubules and induction of micronuclei in vitro by various bisphenols. *Mutation Research* 390(1-2): 21-31.

228. Poimenova A, Markaki E, Rahiotis C, and Kitraki E. 2010. Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A. *Neuroscience* 167 (3): 741-749.

229. Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, and Waechter JM, Jr. 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicological Sciences* 54 (1): 3-18.

230. Prins GS, Ye SH, Birch L, Ho SM, and Kannan K. 2011. Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. *Reproductive Toxicology* 31 (1): 1-9.

231. Radice D, and Redaelli A. 2003. Breast cancer management: Quality-of-life and cost considerations. *Pharmacoeconomics* 21 (6): 383-396.

232. Richter CA, Taylor JA, Ruhlen RL, Welshons WV, and Vom Saal FS. 2007. Estradiol and bisphenol a stimulate androgen receptor and estrogen receptor gene expression in fetal mouse prostate mesenchyme cells. *Environ Health Perspect* 115: 902-908.

233. Ropero AB, and Alonso-Magdalena P G-GERCFENA. 2008. Bisphenol-A disruption of the endocrine pancreas and blood glucose homeostasis. *Int J Androl.* 31(2): 194-200.

234. Rosenmai AK, Dybdahl M, Pedersen M, Alice van Vugt-Lussenburg BM, Wedebye EB, Taxvig C, Vinggaard AM, Are structural analogues to bisphenol A safe alternatives? 2014. Toxicol Sci. May;139(1):35-47. Epub 2014 Feb 22.

235. RPA. 2003. Interim Risk Reduction Strategy and Analysis of Advantages and Drawbacks for Bisphenol-A.

236. Rubin BS, Murray MK, Damassa DA, King JC, and Soto AM. 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ Health Perspect* 109: 675-680.

237. Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, and Soto AM. 2006. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology* 147 (8): 3681-3691.

238. Rudel RA, Fenton SE, Ackerman JM, Euling SY, and Makris SL. 2011. Environmental exposures and mammary gland development: State of the science, public health implications, and research recommendations. *Environmental Health Perspectives* 119 (8): 1053-1061.

239. Russo IH, and Russo J. 1996. Mammary gland neoplasia in long-term rodent studies. Environmental Health Perspectives 104 (9): 938-967.

240. Ryan BC, Hotchkiss AK, Crofton KM, and Gray LE. 2010. In Utero and Lactational Exposure to Bisphenol A, In Contrast to Ethinyl Estradiol, Does Not Alter Sexually Dimorphic Behavior, Puberty, Fertility, and Anatomy of Female LE Rats. Toxicological Sciences 114: 133-148.

241. Ryan KK, Haller AM, Sorrell JE, Woods SC, Jandacek RJ, and Seeley RJ. 2010. Perinatal exposure to bisphenol-a and the development of metabolic syndrome in CD-1 mice. Endocrinology 151 (6): 2603-2612.

242. Salian S, and Doshi T VG. 2009. Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring. Life Sciences 85: 742-752.

243. Sandor Fz. 1996. Plastiques et composites - Polyéthylènes basse densité, - A3310. Paris: Techniques de l'ingénieur.

244. Sargis RM, Johnson DN, Choudhury RA, and Brady MJ. 2010. Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. *Obesity. (Silver. Spring)* 18 (7): 1283-1288.

245. Savabieasfahani M, Kannan K, Astapova O, Evans NP, and Padmanabhan V. 2006. Developmental programming: Differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function. *Endocrinology* 147: 5956-5966.

246. Schreder E. 2010. On the Money: BPA on Dollar Bills and Receipts. Washington Toxics.

247. Seifried HE, Seifried RM, Clarke JJ, Junghans TB, San RH.. 2006. A compilation of two decades of mutagenicity test results with the Ames Salmonella typhimurium and L5178Y mouse lymphoma cell mutation assays. *Chemical Research in Toxicology* 19(5): 627-644.

248. Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y, Ozawa S. 2002. Sulphation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicol In Vitro 16: 549-556.

249. Signorile PG, Spugnini EP, Mita L, Mellone P, D'Avino A, Bianco M, Diano N, Caputo L, Rea F, Viceconte R, Portaccio M, Viggiano E, Citro G, Pierantoni R, Sica V, Vincenzi B, Mita DG, Baldi F, and Baldi A. 2010. Pre-natal exposure of mice to bisphenol A elicits an endometriosis-like phenotype in female offspring. *General and Comparative Endocrinology* 168: 318-325.

250. Simoens S, Hummelshoj L, and D'Hooghe T. 2007. Endometriosis: cost estimates and methodological perspective. *Hum Reprod* 13: 395-404.

251. Simoens S, Hummelshoj L, Dunselman G, Brandes I, and Dirksen C. 2011. Endometriosis cost assessment (the EndoCost study): a cost-of-illness study protocol. *Gynecol Obstet Invest* 71: 170-176.

252. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, Brodszky V, Canis M, Colombo GL, Deleire T, Falcone T, Graham B, Halis G, Horne A, Kanj O, Kjer JJ, Kristensen J, Lebovic D, Mueller M, Vigano P, Wullschleger M, and Dhooghe T. 2012. The burden of endometriosis: Costs and quality of life of women with endometriosis and treated in referral centres. *Human Reproduction* 27 (5): 1292-1299.

253. Singh M, McGinley JN, and Thompson HJ. 2000. A comparison of the histopathology of premalignant and malignant mammary gland lesions induced in sexually immature rats with those occurring in the human. *Laboratory Investigation* 80 (2): 221-231.

254. Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SC, Fennell TR.. 2000. Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol 168: 225-234.

255. Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, and Huppi PS. 2009. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. *Environmental Health Perspectives* 117 (10): 1549-1555.

256. Spearow JL, Doemeny P, Sera R, Leffler R, Barkley M (1999) Genetic variation in susceptibility to endocrine disruption by estrogen in mice. *Science* 285**,** 1259-1261.

257. Stroheker T, Chagnon MC, Pinnert MF, Berges R, Canivenc-Lavier MC. 2014. Estrogenic effects of food wrap packaging xenoestrogens and flavonoids in female Wistar rats: a comparative study, 421--432.

258. Stump DG, Beck MJ, Radovsky A, Garman RH, Freshwater LL, Sheets LP, Marty MS, Waechter JM, Jr., Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Chappelle AH, and Hentges SG. 2010. Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. *Toxicol Sci.* 115 (1): 167-182.

259. Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, and Suzumori K. 2005. Exposure to bisphenol A is associated with recurrent miscarriage. *Hum Reprod* 20: 2325-2329.

260. Sun Y, Irie M, Kishikawa N, Wada M, Kuroda N, and Nakashima K. 2004. Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. 18: 501-507.

261. Tachibana T, Wakimoto Y, Nakamuta N, Phichitraslip T, Wakitani S, Kusakabe K, Hondo E, and Kiso Y. 2007. Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. *Journal of Reproduction and Development* 53: 509-514.

262. Takagi H, Shibutani M, Masutomi N, Uneyama C, Takahashi N, Mitsumori K, Hirose M.. 2004. Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life. *Archives of Toxicology* 78: 97-105.

263. Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. 2004. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. *EndocrineJournal* 51: 165-169.

264. Taylor JA, Welshons WV, and Vom Saal FS. 2008. No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. *Reproductive Toxicology* 25 (2): 169-176.

265. Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM, and Vandevoort CA. 2011. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environmental Health Perspectives* 119 (4): 422-430.

266. Teeguarden JG, Calafat AM, Ye X, Doerge D, Churchwell MI, Gunawan R, and Graham MK. 2011. Twenty-four hour human urine and serum profiles of bisphenol A during high-dietary exposure. 123: 48-57.

267. Tharp AP, Maffini MV, Hunt PA, Vandevoort CA, Sonnenschein C, and Soto AM. 2012. Bisphenol A alters the development of the rhesus monkey mammary gland. *Proceedings of the National Academy of Sciences of the United States of America*.

268. Thayer KA, and Foster PM. 2007. Workgroup report: National Toxicology Program workshop on Hormonally Induced Reproductive Tumors - Relevance of Rodent Bioassays. *Environ Health Perspect* 115 (9): 1351-1356.

269. Tian YH, Baek JH, Lee SY, and Jang CG. 2010. Prenatal and postnatal exposure to bisphenol a induces anxiolytic behaviors and cognitive deficits in mice. *Synapse* 64 (6): 432-439.

270. Tominaga T, Negishi T, Hirooka H, Miyachi A, Inoue A, Hayasaka I, and Yoshikawa Y. 2006. Toxicokinetics of bisphenol A in rats, monkeys and chimpanzees by the LC-MS/MS method. *Toxicology* 226 (2-3): 208-217.

271. Truffi B. 2000. Le papier thermique, EFPG, available on : http://cerig.pagora.grenoble-inp.fr/icg/Dossiers/Papier_thermique/chapitre_6.htm

272. Tsukioka T, Terasawa J, Sato S, Hatayama Y, Makino T, Nakazawa H. 2004. Development of analytical method for determining trace amounts of BPA in urine samples and estimation of exposure to BPA. J Environ Chem. 14:57–63.

273. Tsutsui T, and Tamura Y YEBJ. 2014. Involvement of genotoxic effects in the initiation of estrogen-induced cellular transformation: Studies using syrian hamster embryo cells treated with 17β-estradiol and eight of its metabolites., 8-14.

274. UBA. 2010. Bisphenol A. An industrial chemical with adverse effects.

275. UK. 2008. Transitional dossier Annexe XV on BPA.

276. US EPA. 2012. "BPA Alternatives in Thermal Paper Partnership - Design for the Environment." Available from http://www.epa.gov/dfe/pubs/projects/bpa/index.htm.

277. Vandenberg LN, Hauser R, Marcus M, Olea N, and Welshons WV. 2007. Human exposure to bisphenol A (BPA). *Reprod Toxicol.* 24 (2): 139-177.

278. Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, and Soto AM. 2007a. Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. *Endocrinology* 148: 116-127.

279. Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS, and Soto AM. 2008. Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. *Reproductive Toxicology* 26: 210-219.

280. Vehmas K, Kariniemi M, Linna H, Jokiaho K, and Torniainen E. 2011. Future of european printing industry.

281. Völkel W, Colnot T, Csanády GA, Filser JG, Dekant W. 2002. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. ed Chemical Research in Toxicology, 1281-1287.

282. Volkel W, Bittner N, and Dekant W. 2005. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. 33: 1748-1757.

283. Volkel W, Kiranoglu M, and Fromme H. 2008. Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. 179: 155-162.

284. Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, and Soto AM. 2007. Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. *Environmental Health Perspectives* 115: 592-598.

285. Walton K, Dorne JL, Renwick AG. 2001. Uncertainty factors for chemical risk assessment: interspecies differences in glucuronidation. Food and Chemical Toxicology 39: 1175-1190.

286. Wang LY, Denniston M, Lee S, Galuska D, and Lowry R. 2010. Long-term Health and Economic Impact of Preventing and Reducing Overweight and Obesity in Adolescence. *Journal of Adolescent Health* 46 (5): 467-473.

287. Weber LK, and Keri RA. 2011. Bisphenol A increases mammary cancer risk in two distinct mouse models of breast cancer. *Biology of Reproduction* 85 (3): 490-497.

288. WHO (World Health Organization). 2010. Background Paper on Sources and Occurrence of Bisphenol A Relevant for Exposure of Consumers.

289. Wei J, Sun X, Chen Y, Li Y, Song L, Zhou Z, Xu B, Lin Y, Xu S. 2014, Perinatal exposure to Bisphenol A exacerbates non-alcoholic steatohepatitis-like phenotype in male rat offspring fed a high-fat diet. Journal of Endocrinology 222, 313-325

290. Willhite CC, and Ball GLaMCJ. 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. J. Toxicol. Environ. Health B Crit Rev. 11: 69-146.

291. Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, Kushi LH, Erdmann C, Hiatt RA, Rybak ME, Calafat AM. 2010. Investigation of Relationships between Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols and Pubertal Stages in Girls. *Environmental Health Perspectives* 118.

292. Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. 2008. Environmental exposures and puberty in inner-city girls. *Environmental Research* 107: 393-400.

293. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, and Calafat AM. 2008. Prenatal phenol and phtalate exposures and birth outcomes. 116: 1092-1097.

294. Xu XH, Zhang J, Wang YM, Ye YP, and Luo QQ. 2010. Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus in male offspring mice. *Hormones and Behavior* 58 (2): 326-333.

295. Yamasaki K, Takeyoshi M, Yakabe Y, Sawaki M, Takatsuki M. 2003. Comparison of the reporter gene assay for ER-alpha antagonists with the immature rat uterotrophic assay of 10 chemicals, Toxicology Letters, 142(1-2):119-31.

296. Yamasaki K, Noda S, Imatanaka N, Yakabe Y. 2004. Comparative study of the uterotrophic potency of 14 chemicals in a uterotrophic assay and their receptor-binding affinity. *Toxicology Letters* 146(2): 111-120.

297. Yang YJ, Hong YC, Oh SY, Park MS, Kim H, Leem JH, and Ha EH. 2009. Bisphenol A exposure is associated with oxidative stress inflammation in postmenopausal women. 109: 797-801.

298. Ye X, Kuklenyik Z, Needham LL, and Calafat AM. 2005. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance mass spectrometry. 383: 638-644.

299. Ye X, Kuklenyik Z, Needham LL, and Calafat AM. 2006. Measuring environmental phenols and chlorinated organic chemicals in breats milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry. 831: 110-115.

300. Ye X, Pierik FH, Hauser R, Duty S, Angerer J, Park MM, Burdorf A, Hofman A, Jaddoe VWV, Mackenbach JP, Steegers EAP, Tiemeier H, and Longnecker MP. 2008. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phtalates among pregnant women in Rotterdam, the Netherlands: the generation R study. 108: 260-267.

301. Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, Maekawa A. 2004. Maternal exposure to low doses of bisphenol a has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. *Journal of Reproduction and Development* 50: 349-360.

302. Zalko D, Jacques C, Duplan H, Bruel S, and Perdu E. 2011. Viable skin efficiently absorbs and metabolizes bisphenol A. *Chemosphere* 82 (3): 424-430.

303. Zoeller RT, and Bansal R PC. 2014. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology* 146: 607-612.

Annexes

Annex 1. Questionnaire sent to MSCAs during the 2013 ANSES consultation

QUESTIONNAIRE

BISPHENOL-A (BPA) – CAS N° 80-05-7 – USE IN THERMAL PAPER

Objective:

Survey/Collect of information for a **Restriction proposal** according to **Art. 68** of REACH Regulation (Registration, Evaluation, Authorisation and Restriction of Chemical substances).

Context:

The French Ministry of environment has decided to submit a European restriction proposal for the use of Bisphenol-A (BPA) (CAS n°80-05-7) in thermal papers (used for point-of-sale, cash registers and bank receipts, logistic labels, etc.) in the framework of REACH regulation.

In this context, ANSES (French Agency for Food, Environmental and Occupational Health & Safety) is in charge of elaborating the restriction dossier and is carrying out a survey (questionnaire enclosed) about this specific use of BPA.

This survey aims at:

1/ identifying the actors of the thermal papers markets

2/ collecting information on the use and substitutes of BPA in thermal papers

3/ collecting information on risk and exposures to BPA from thermal papers

4/ identifying existing national regulations

As indicated in the ECHA Register of Intentions for restrictions, this dossier is planned to be submitted to ECHA mid-January 2014. Given that deadline, we kindly ask you to sending back the questionnaire enclosed **by September the 20th 2013**.

The questionnaire is structured as follows:

Section A	Contact details
Section B	National market(s) of BPA-containing thermal papers
Section C	Use and exposure to BPA from thermal papers
Section D	Substitutes of BPA in thermal papers
Section E	National regulations of BPA in thermal papers

SECTION A: CONTACT DETAILS

Name:

Organisation Name:

Address:

Country:

Telephone number:

Fax number:

E-mail:

SECTION B:

NATIONAL MARKET(S) OF BPA / BPA-CONTAINING THERMAL PAPERS

Question 1.	
Where known to you, could you kindly provide the following information:	
The number (or approximate number) of manufacturers of BPA-containing thermal papers in your country	
The number (or approximate number) of importers of BPA-containing thermal papers in your country	
The volume (or approximate volume) of BPA-containing thermal papers placed on the market in your country	(please specify the unit)
The volume (or approximate volume) of BPA used as developer for dyes in thermal papers in your country	(please specify the unit)
The proportion (%) of thermal papers that may contain BPA in your country? Please also indicate the basis for this percentage (guess, estimate or market data).	

Question 2
Where known to you, could you kindly provide the market price of BPA: (please specify the unit)

Question 3
Where known to you, could you kindly also provide the same information as questions 1 & 2 for European level:

SECTION C: USE AND EXPOSURE TO BPA FROM THERMAL PAPERS

Question 4.
Do you have any information about the concentration of BPA in thermal papers? **☐Yes. Please provide details (and units) below ☐No**

Question 5.
Are/Have been <u>some measurements campaigns of BPA in thermal papers</u> being carried/carried out in your country? **☐ YES. Please specify the results and details (including analytical methods and units) ☐No**

Question 6.
Are/Have been <u>some measurements campaigns of exposure to BPA in thermal papers</u> being carried/carried out in your country such as workers/consumers impregnation measurements ? ☐ **YES. Please specify the results and details (including analytical methods and units)** ☐**No**

Question 7.
Do you have any information about the <u>frequency and number of tickets/receipts workers/consumers may handle (daily, weekly, monthly)</u>? ☐ **YES** ☐**No**

SECTION D: SUBSTITUTES OF BPA IN THERMAL PAPERS

Question 8.
Do you have any information about substitutes to BPA <u>already used as developer for dyes</u> in thermal papers (other bisphenols, e.g. BPS, or other than bisphenols)? Could you please provide your view about the advantages and disadvantages of those compared to BPA? ☐**Yes. Please provide details below** ☐**No**
Efficiency *Price* *Technical feasibility* *...*

Question 9.
Do you have any information about <u>potential substitutes to BPA</u> (under development e.g.) <u>likely to be used as developer for dyes</u> in thermal papers (other bisphenols, e.g. BPS, or other than bisphenols)? Could you please provide your view about the advantages and disadvantages of those compared to BPA? ☐**Yes. Please provide details below** ☐**No**
Efficiency *Price* *Technical feasibility* *...*

Question 10.
Do you have any information about <u>substitutes to thermal papers</u>? Could you please provide your view about the advantages and disadvantages of those compared to thermal papers? ☐**Yes. Please provide details below** ☐**No**
Efficiency *Price* *Technical feasibility* *...*

Question 11.
As regards all the substitutes mentioned in questions 5-7 (already used or likely to be used as developer for dyes or substitutes to thermal papers themselves), and when known to you, could you please provide <u>some contact details for these BPA-free markets (companies, industry unions, etc.)</u>?

SECTION E: NATIONAL REGULATIONS OF BPA IN THERMAL PAPERS

Question 12.		
Is there currently a <u>national regulation</u> which bans, restricts or controls the manufacturing, import, use and/or market placing of BPA-containing thermal papers? ☐**Yes.** Please provide the relevant information below ☐**No**		
Materials/Substances regulated	**Concentration/migration limit of the substance** **(if relevant)**	**Legal reference**

Question 13.	
Are there currently <u>non-regulatory actions</u> aiming at banning, restricting or controlling the manufacturing, import, use and/or market placing of BPA-containing thermal papers? ☐**Yes.** Please provide the relevant information below ☐**No**	
Materials/Substances targeted	**Type of non-regulatory actions and actors involved** (please specify year and details)

***

Please also indicate below any other relevant national bodies (and their contact information) which could assist us in this study:

Feel free to enclose any study, document, report which can be be helpful

Feel free to add any comments on issues raised by this questionnaire in the space below:

Annex 2 – Method of computation of excess risks for the human health impact assessment

In the present analysis it is proposes a modification of the current approach used for non cancer effect; similar to the approach for cancer risk assessment (adjusting animal doses to equivalent human doses, deriving the point of departure by fitting a mathematical model to the data, and linearly extrapolating from the point of departure to lower doses).

This extrapolation does not use UF (for example, a UF of 10 for human to animal extrapolation); but a straight line (from the point of departure for the observed data) to the origin. The slope of this straight line (the slope factor), is use to estimate risk at exposure levels.

Risk = Exposure x Slope Factor.

Using this approach, the probability (risk) of an individual with an adverse level can be estimated directly as a function of dose. It is assumed that the relationship between exposure and response observed in animal is similar to human.

Dose-response assessment

The following studies were chosen by the experts Committee of ANSES (ANSES, 2013) for BPA risk assessment (Table below).

From data selected, a mathematical concentration-response modeling was used to predict a response level that will serve as the basis of a health assessment.

Study	**Type of data**	**endpoint**
Miyawaki, 2007	continuous	Total cholesterol
Miyawaki, 2007	continuous	Body weight
Signorile *et al.* 2010	quantal	endometriosis
Murray, 2007	quantal	Preneoplastic lesion (mammary gland)
Moral, 2008	quantal	Undifferentiated Terminal end bud(TEB) (mammary gland)
Moral, 2008	quantal	Terminal duct(TD) (mammary gland)

Fitting the Models

The first step is to select model that describe the data using appropriate model structures for the type of data (continuous or quantal, Table above).

A mathematical model is applied to the experimental data to produce a dose-response curve of best fit. Detailed of the full process on this approach are presented in BMD Software technical guidance from US EPA (http://www.epa.gov/ncea/bmds/).

For the dichotomous (or quantal) data, the response or effect may be reported as either the presence or absence of an effect. The dose-response models describe how the probability or frequency of a specified response changes with the dose level.

As shown in the Figure A below, each data point represents the percent response at each dose. In the first low dose, a 0% response can be seen (0 out of n animals are affected due to the exposure). At higher doses, there is an increase in the % response.

For continuous data, a hybrid approach has been used (Equation 1), described by Gaylor and Slikker (1990), which fits dichotomous models to continuous data.

By dichotomizing the continuous data (dividing the population into two categories affected or none affected), the probability of an individual with an adverse level can be estimated directly as a function of dose.

For this purpose it is necessary to define what level of cholesterol or body weight increased is considered adverse. After defining the adverse cutoff, it can be estimated how many additional individuals in a population with an increased exposure to BPA will have adverse outcomes over baseline (Equation 1).

To estimate risks for continuous end points, it is necessary to define a response level considered adverse or abnormal. In this report, it has been defined adverse (cut off) as values ≥ 90th percentile of the control response distribution (Figure B). The relative excess response was found by subtracting the baseline response from the predicted response, and an estimation of the probability that an individual is in the adverse range is performed.

For example, the human dose can be calculated at which the increase in cholesterol is 6% in X % of the population.

$P(di) = \Phi\, x[c-\mu(di)/\sigma]$

Φ = cumulative standard normal distribution fonction

C = cut-off value

μ(di) = mean response at dose di

σ= standard deviation

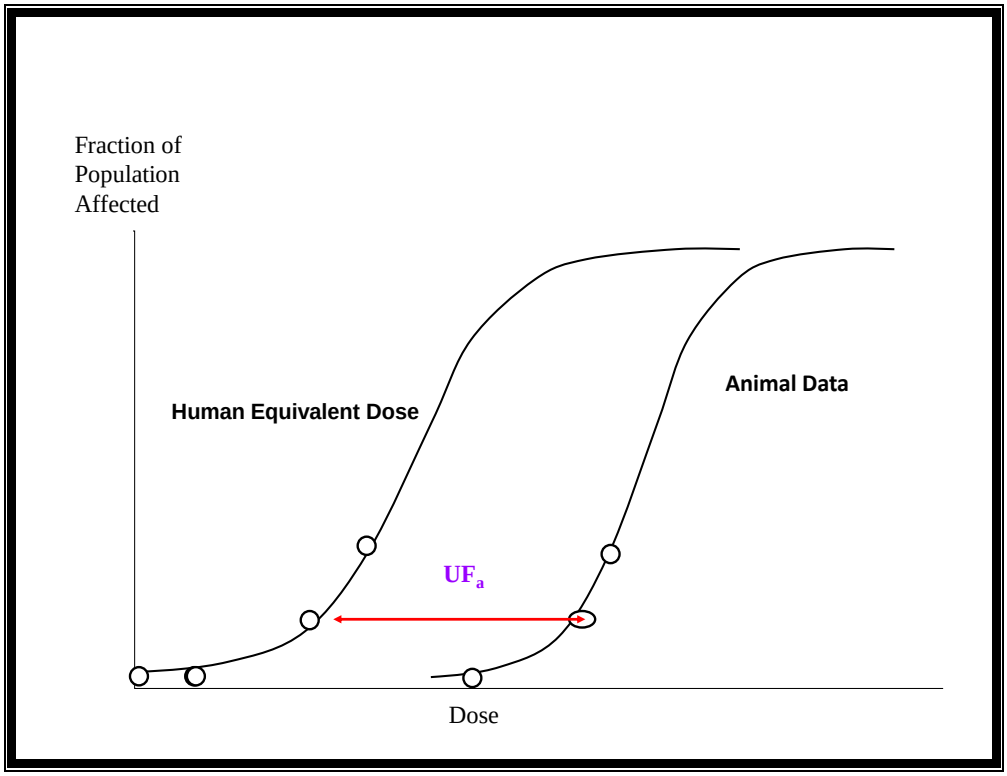


Figure A: the curve is a best fit line; it does not necessarily go through each and every data point

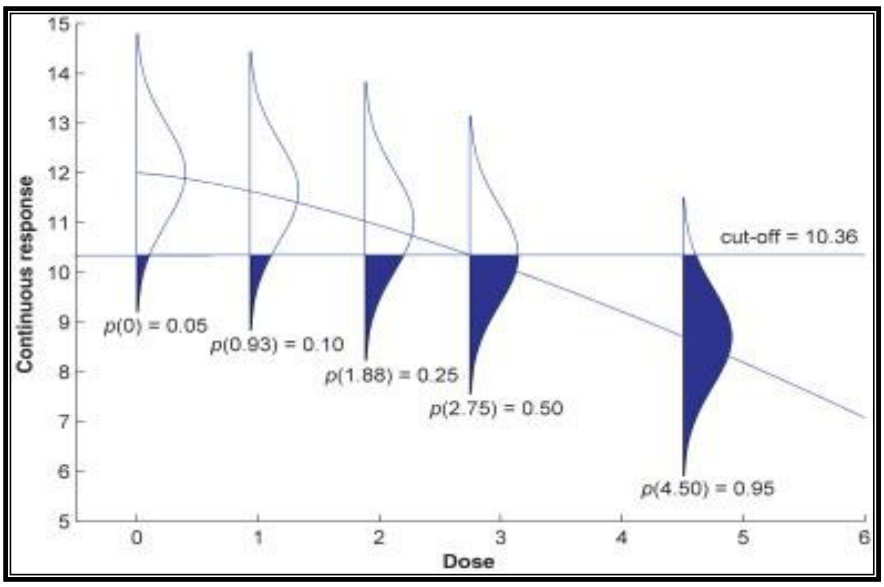


Figure B: example of the hybrid approach

Population variability:

Incorporating population variability into dose–response assessment and low-dose extrapolation was not applied in this work. Because adverse (cut off) have been defined as values ≥ 90th percentile of the control response distribution, the choice of a 10% rate of adverse effects in an unexposed population somewhat accounts for a susceptible portion of the population.

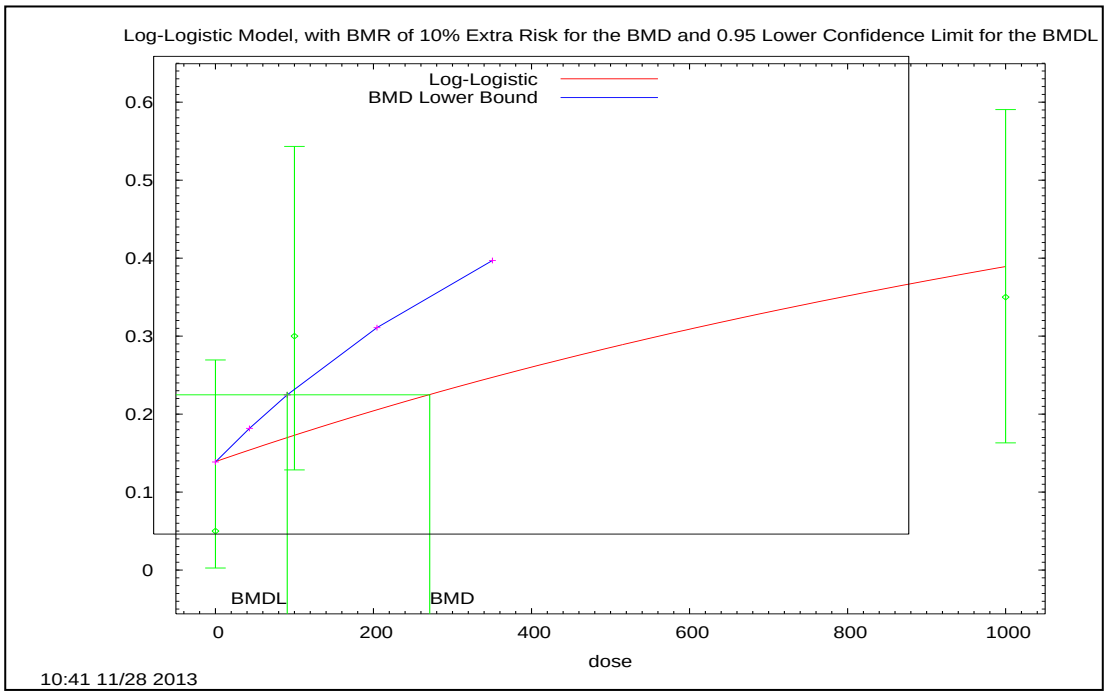
For the following end point (quantal): pre neoplastic lesion, undifferentiated terminal end bud, and terminal duct, it has been performed an additional transformation in order to modeling the dose response.

The number of animal with at least one TEB was calculated from the original data from the authors.

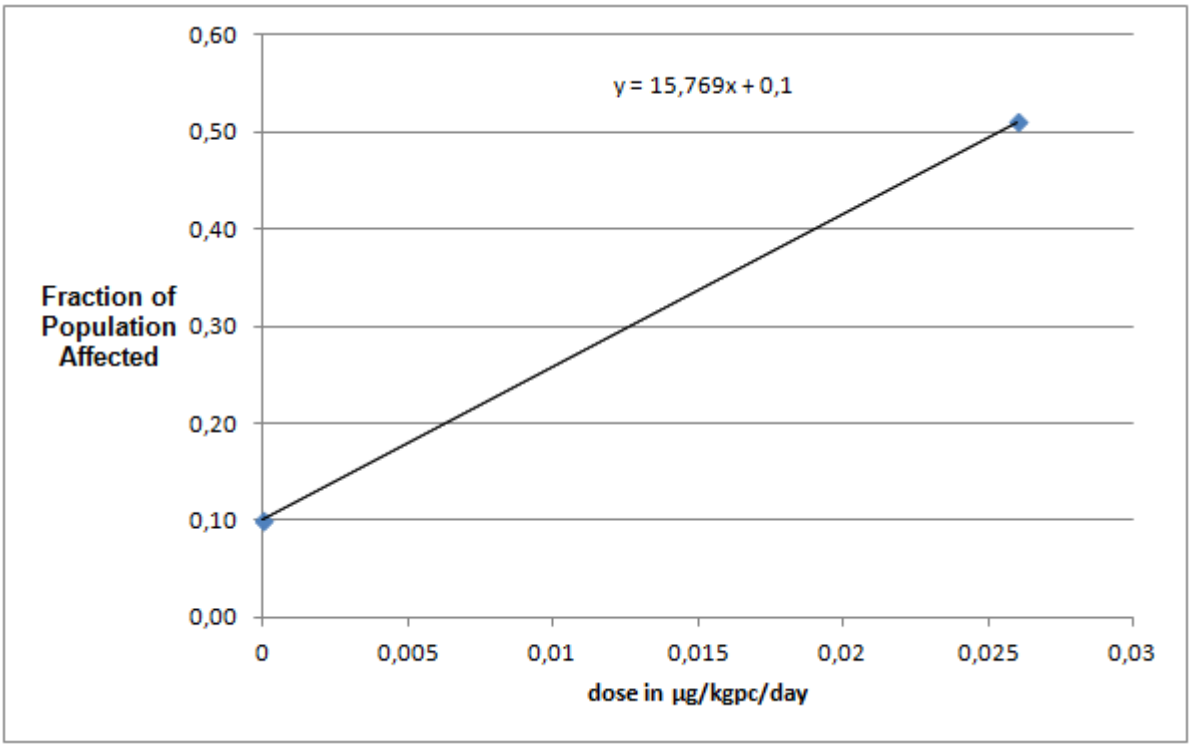
The number of animal with a number of TD > 66; 5 was calculated from the original data from the authors. This value corresponds to the upper limit value in the IC 95 % of control group.

The number of animal with a % of pre neoplastic lesion > 9.3 % was calculated from the original data from the authors. This value corresponds to the upper limit value in the IC 95 % of control group.

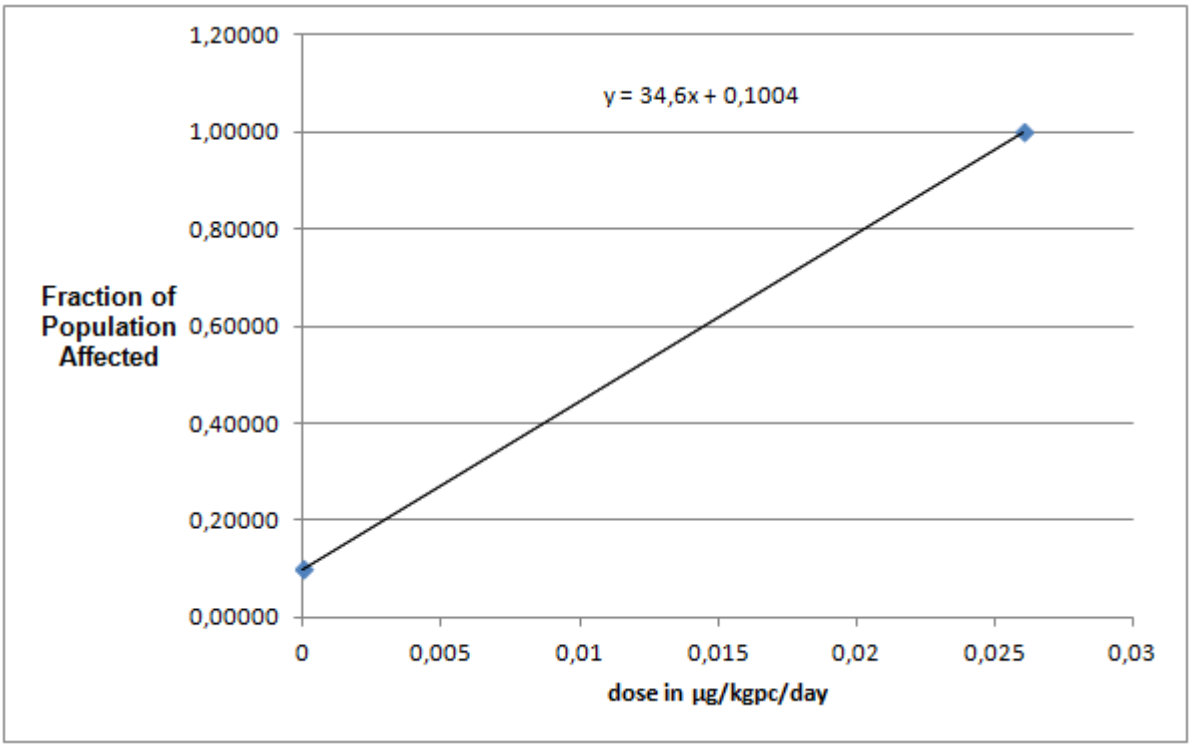
From Signorile et al. (2010) for endometriosis, the following dose-response relationship is established.



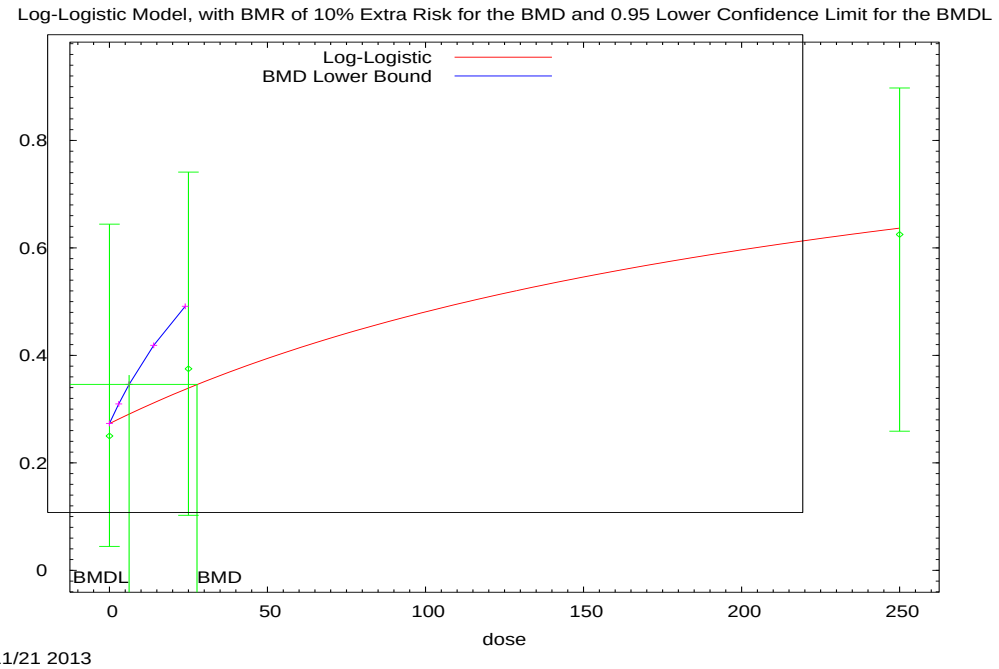
From Miyawaki, 2007 for the increase in body weight, the following dose-response relationship is established.



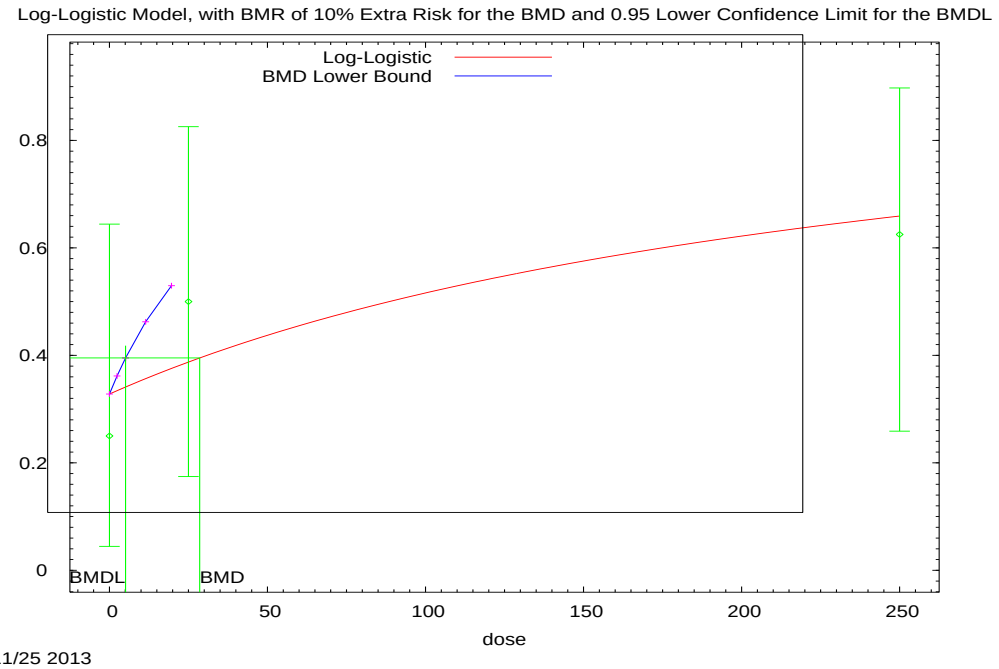
From Miyawaki et al. (2007) for the increase in cholesterol, the following dose-response relationship is established.



From Moral, 2008 (TEB), the following dose-response relationship is established.

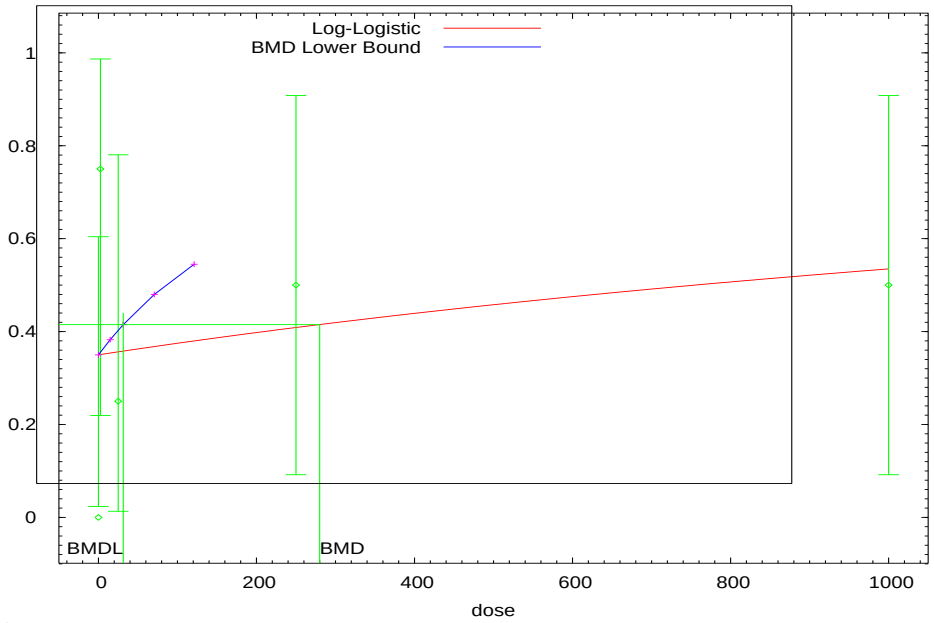


From Moral, 2008 (TD), the following dose-response relationship is established.



From Murray, 2007 (95 days), the following dose-response relationship is established.

Log-Logistic Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



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Annex 3: Comparison of BPA risk assessments between ANSES, BfR-DE (in the framework of the evaluation under REACH) and Denmark

	ANSES	**BfR-DE**	**DK**
Dates	MARS 2013	EVAL 2012	2011
Source study/ Starting point	**Mammary gland**: (Moral et al, 2008); **Cerebral development**: (Xu et al., 2011c); **Female reproductive system**: (Rubin et al, 2001); **Metabolism and obesity**: (Miyawaki et al, 2007);	dietary three-generation study performed in the rat (Tyl et al., 2002)	studies of 2 generations of rodents where the critical effects were changes in the body weight and organ weight (in breed and adult rats) and liver effects (mice) (Tyl 2001, Tyl 2006)

NOAEL	**Mammary gland:** NOAEL = **25** 5AEL = gland: nerations of rodents where the critical effects were changes in the body weight and organ weight (**Cerebral development:** NOAEL = **50** 0OAEL = development:ns of rodents where the critical effects were changes i**Female reproductive system:** LOAEL = **100** 100L =reproductive system: rodents where the critical effects were changes in the body w **100** 100L =reproductive system: rodents where the critical effects were changes in the**100** µ00 L =reproductive system: rodents where the critical effects wer**Metabolism and obesity:** LOAEL = **260** 60EL = sm and obesity: em: rodents where the critical effects were changes in tCalculated NOAEL= **86,7** (LOAEL/3) NOAEL=	5 mg/kg bw/d based on effects on body weight and body weight gain (value was also taken by SCF (2002) for the derivation of the TDI)	5 mg/kg bw/d based on liver effects (Tyl 2001 and 2006)
First pass metabolism	Absolute factor of biodisponibility by oral route of FREE BPA of 3% based on studies of Doerge and Collet : eg for mammary gland: 25 ats) and	factor of 10 to give an internal NAEL of 0.5 mg/kg bw/d	none

	liver 0.75 µg/kg/j (= internal NOAEL)		
Dermal absorption	"For pregnant professional woman:**Parameters**: Flow of absorption: uniform distribution [0,026 g bw/d BPA of 3% based on studies of Doerge antriangular distribution [3h/j - 6,5h/j - 10h/j]; Body weight: discrete distribution.1) Systemic bioavailability after dermal absorption = the most influential parameter.2) There are situations of risk for every BD factors tested (5%, 10%, 30%, 50%, 75%) and for the four critical types of effects.3) There is no longer observed risks for the following BD: 0.58% for the mammary gland, the lowest value.For the pregnant woman consumers:**Parameters**: Absorption rate: triangular distribution [10% - 27% - 60%]; Quantity of substance: uniform distribution [0,035 - 3,75]; fingers number: uniform distribution [1 cm² - 12 cm²]; Absorption duration: uniform distribution [2 h]; body weight: discrete distribution.1)	Dermal absorption in both animals and humans is assumed to be 50 %, whereas the absorption following oral administration is estimated at 100 %.	50% (based on Zalko et al., 2011 study)

		Systemic bioavailability after dermal absorption = the most influential parameter.2) For these three values y after dermal absorption = the most influential parameter.niform distribution [0,035 - 3,75]; fingers number: uniform distribution [1 cm² - 12 cm²]; Absorption duratio		
Assesment factors	species factorsko et al., 2011 st	An additional factor inter species is applied (by default when there is no data specific to the substance) of 2.5.	much lower levels of free BPA reach the systemic circulation in humans compared to the levels observed in rats and mice indicating that humans are likely to be less sensitive to the effects of BPA it is not considered necessary to apply an additional factor	2,5 (for general interspecies differences)

			of 2.5 to take account for other species differences (UK transitional dossier, 2008)	
	Interspecies AF	4 (extrapolation from the rat to humans)	4	7 (mice)
	Intraspecies ion from the rat to humans)s)stemic circulation in h	10	10	10
	0traspecies ion from the	1	1	1
	dose-response and endpoint specific/ severity issues	3 because of the severity of the effects	1	1
	quality of the database	1	1	1

DNEL calculated	Internal DNELs: Mammary gland: 0,0025 gland: 0,0025 ctsrity issuesculation in humans compared to the levels observed in rats and mice indicating that humans are likely to	dermal internal DNEL is 0.025 mg/kg bw/d	0,029 mg/kg bw/d, not internal DNEL
Worker exposure of Thermal Paper page	P95 = 0.43 sure of Thermal Paper page /dsrity issuesculation in humans compared to the levels observed in rats and mice indicating that humans are likely to be less sensitive to the effeduration of expo (triangular distribution) (Biedermann), constant amount of BPA regardless of the time (between 5 and 60 sec) and repeat (between 3 and 10) of the contact.	based on the occupational exposure level, no initial concern has been identified for worker => no detailed risk assessment for worker	conservative scenario: humid fingers all day, 100 individual contacts, 5 seconds, 5 cmorker => no detailed risk assessment for w**0,0215 mg/kg bw/d** (50% absorption)
Consumer exposure of Thermal Paper page	P95 = 0.08 mg / kg BW / day (consuming pregnant woman and her baby) (calculated with the cutaneous absorption rate estimated by Biedermann for a period of 2h)	By ECETOC TRAAC 8: $1,84*10^{-3}$ mg/kg bw day	Exposure of consumers: Realistic worst case scenario 0,00103 mg/cm2/sec (expo of 10 cm^2/8 fingers for 10 seconds)Max internal dose in the worst case scenario 0,004 mg/kg/d

Consumer RCR Thermal Paper page	There is a risk for consumers and professionals for the 4 critical effects, ie P95>DNEL: cf below for example	Risk characterisation Ratio for Dermal Exposure: $7,36*10^{-2}$	RCR dermal (tier 1 scenario estimate based on migration to sweat simulant) = 0,07/0,37 RCR dermal (realistic worst case scenario based on measure migration to wet fingers) = 0,03/0,14
Professional RCR thermal paper		not evaluated	worst case scenario: RCR dermal = 0,15/0,74

Annex 4: Tables summarising the reasons for having selected the studies for the health risk assessment

Effects on the female reproductive system

Summary of animal studies on the effects of bisphenol A on the female reproductive system

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
Studies by the oral route								
Mendoza-Rodríguez *et al.*, 2011	Wistar rats	Oral	10 mg/L in drinking water, estimated intake of 1.2 mg/kg bw/d GD6 - PND21	**F1** Increase in thickness of the epithelium and uterine stroma. Decrease in apoptosis in the uterine epithelium. Disorders of the oestrous cycle (increased frequency of irregular cycles). Decrease in ER-α receptor expression in the epithelial cells of the uterus during the oestrus phase.	$LOAEL_u$ = 1.2 mg/kg bw/d in F1	Disorders of the oestrous cycle and endometrial hyperplasia	**Recognised**	Single dose * **Limit of the study: no controlled exposure except for drinking water** **Study selected because administered dose inferior to the EFSA's NOAEL (5 mg/kg**

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
								bw/d)
Ryan *et al.*, 2010a	Long-Evans rats	Oral	2 - 20 or 200 µg/kg bw/d GD7 - PND18	No effect on the number of live pups, birth weight of pups, anogenital distance at PND2, age at vaginal opening, fertility, fecundity of F1 generation. Confirms the results of multigenerational studies (Tyl *et al.*, 2002).	NOAEL: 200 µg/kg/d (highest dose tested)	No effect on the age at vaginal opening after prenatal and postnatal exposure.	-	No GLP nor OECD guideline study Study chosen because of the low doses employing several test doses including one less than 5 mg/kg bw/d
Rubin *et al.*, 2001	Sprague-Dawley rats	Oral	1 and 10 mg/L drinking water, estimated intake of 0.1 mg/kg bw/d to 1.2 mg/kg bw/d GD6 - pups					*Strength:* Water consumption was measured *Weaknesses:* - The number of mated dams (n=6) was low.

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			were weaned					- Not reported whether the litter was used as statistical unit
				F1 (OVX-Ovariectomised):				
				Decrease in LH secretion suggesting a neuroendocrine effect.	NOAEL could not be determined (animal not intact)	Disruption of ovarian cyclicity		Animal not intact
				F1 (Intact animals):				
				- No effect on the average number of pups/litter on the age at vaginal opening and the anogenital distance.	NOAEL 1.2 mg/kg bw/d			
				- Increased frequency of irregular oestrous cycles, which results in a decrease in the average number of cycles per animal over a period of	NOAEL: 0.1 mg/kg bw/d** (result taken into account for the health risk	Disruption of ovarian cyclicity	**Recognised**	**** Critical effect and NOAEL taken into account for**

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				18 days.	assessment) LOAEL 1.2 mg/kg bw/d**			**the HRA**
				- Increase in body weight of male and female pups from PND0 to PND11 at both doses tested. - From PND11 to PND22, increased body weight persisted in animals treated with the low dose (male and female) and from PND28, only females treated at the low dose had a higher body weight. This effect persisted until PND114.	LOAEL: 0.1 mg/kg bw/d	Increased body weight in early postnatal period	-	
				- Uterotrophic test in pubescent females treated for three days at doses of 1, 10 and 100 mg/L: No increase in weight of the uterus with	NOAEL could not be determined (animal not intact)			Animal not intact

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				secretion.				
Berger *et al.*, 2007	CF-1 mice	Oral	Administration of BPA by addition to peanut butter in an amount of 0.11-9% or by addition to the feed in an amount of 3 and 6%. GD1 - GD5	No change in litter size or parturition rate The dose of 68.84 mg of BPA/d/animal (corresponding to a BPA supplementation of 6%) caused the abortion of all gestations.	Study not used for the determination of NOAELs.			Study not used for the HRA, as the doses showing an effect were much higher than the applicable NOAEL. Methodological limitations.
Kobayashi *et al.*, 2010	C57BL/6J mice	Oral	Transgenerational study F0-F1 F2 F0 treated at 0.05 - 0.5 or 5 mg/kg bw/d (ingested doses	<u>Study of several generations:</u> The animals were exposed over several generations from F0 to F2. Results: No change in body weight, body weight gain, feed consumption, duration of gestation,	NOAEL: 5 mg/kg bw/d (estimated highest dose tested)	No significant effect on the measured parameters.	-	

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			approximately corresponding to food supplementation of respectively 0.33 - 3.3 to 33 ppm) GD6 - PND22	litter size, or survival of pups in the F0 animals. No difference between the sex ratio and the viability in the F1 animals. No change in body weight, feed consumption, developmental parameters, anogenital distance, or organ weight (liver, kidney, heart, spleen, thymus, testis, ovaries and uterus) in F1 and F2 adults. No change in sperm number or motility in F1 and F2 animals.				
Tyl *et al.*, 2008	Mice	Oral	0.003 - 0.03 – 0.3 - 5 - 50 and 600 mg/kg bw	In the wide range of doses studied, particularly at doses consistent with human exposure, no effect on	NOAEL: 50 mg/kg/d LOAEL: 600 mg/kg bw/d	No effects on the female reproductive system	Suspected	A large amount of research studies report effects of BPA

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			/d Exposure 10 weeks before mating until adulthood	reproduction. Presence of effects at higher doses (not relevant to human exposure).		(no effects on ovarian primordial follicle counts, estrous cyclicity, or reproductive function).		at very low doses in animal studies, below those that are used.for the calculation of the EFSA's TDI.
Tyl *et al.*, 2002	Rats	Oral	0.001 - 0.02 - 0.3 - 5 - 50 and 500 mg/kg bw/d Exposure 10 weeks before mating until adulthood	In the wide range of doses studied, particularly at doses consistent with human exposure, no effect on reproduction. Presence of effects at higher doses (not relevant to human exposure).	NOAEL: 5 mg/kg/d LOAEL: 500 mg/kg bw/d	Delayed vaginal patency was observed only at very high doses No other effects on female reproduction parameters (no	Suspected	Enumeration of ovarian primordial follicles of both ovaries of ten females at high dose and control only (30% of animals). No examination of the lower doses does not allow to

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
						effects on ovarian primordial follicle counts, estrous cyclicity or reproductive function).		conclude on the non-monotonic dose-response relationship.
Studies by the subcutaneous route								
Adewale *et al.*, 2009	Long-Evans rats	Subcutaneous	50 and 50,000 µg/kg bw/d PND0-PND3	F1 as adults:				Non-monotonic dose-response relationship (2 doses) No GLP nor OECD guideline study?
				↘ in age at puberty (age of vaginal opening), stronger effect at lower doses.	LO(A)EL: 50 µg/kg bw/d	Age at puberty	**Recognised**	*

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				Modification in ovarian morphology (appearance of cysts, ↘ in number of corpora lutea, degenerated follicles). ↗ in proportion of acyclic animals after six months of age.	NOAEL: 50 µg/kg/d** LOAEL: 50 mg/kg/d	Disruption of ovarian cyclicity and ovarian cysts	**Recognised**	* Great uncertainty about the value of the NOAEL/ LOAEL because of the large gap between the doses tested.
				No change in sexual behaviour. No change in the response of GnRH neurons to oestradiol positive feedback (C-FOS expression at the pre-ovulatory peak).	NO(A)EL: 50 mg/kg bw/d		-	
Berger *et al.*, 2007	CF-1 mice	Subcutaneous	0.0005 - 0.0015 - 0.0046 - 0.0143 - 0.0416 - 0.125 - 0.375	↘ in litter size at 3.375 mg/d ↘ in the proportion of females to be parturient at the 10.125 mg/d dose	NOAEL: 1.125 mg/animal/d (corresponding approximately to 34 mg/kg/d) Study not used	-	-	Study not used for the HRA, as the doses showing an effect were

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			- 1.125 - 3.375, and 10.125 mg/animal/d GD1 - GD4	↘ in the number of implantation sites at the 10.125 mg/d dose	for the determination of NOAELs.			higher than the applicable NOAEL and also due to methodological limitations.
Cabaton *et al.*, 2010	CD-1 mice	Subcutaneous	25 ng, 250 ng or 25 µg/kg bw/d GD8 - PND16 Pups exposed postnatally *via* lactation	↘ in fertility and fecundity (↘ in the number of gestations over a period of 32 weeks, in the number of pups per birth and in the total number of pups born over the 32 weeks of study)	LOAEL = 25 ng/kg bw/d	Decrease in fertility after 6 months		No GLP nor OECD guideline study Study not replicated by another team. Non-monotonic dose-response relationship (3 doses).
Fernandez *et al.*, 2010	Sprague-Dawley rats	Subcutaneous	5 (0.25 - 0.62 mg/kg), 50 (2.5 - 6.2 mg/kg), 500 µg/50µL					** No GLP nor OECD guideline

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			(25 - 62.5 mg/kg) PND1 - 10 Treatment of pups					study Uncertainty about the value of the NOAEL determined due to the variation in the dose administered over time by injection of a constant volume.
				↗ in serum testosterone and oestradiol and ↘ in progesterone in adulthood and ↗ in pulsatility of GnRH from hypothalamic explants *ex vivo*	LOAEL: ~0.25 mg/kg/d** (for the serum progesterone marker) NOAEL ~0.25	Effect on the hypothalamic-pituitary-gonadal axis	**Recognised**	**

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
					mg/kg/d LOAEL ~2.5 mg/kg/d (other serum markers)			
				↓ Number of pups/litter (0 gestation in the group treated at the highest dose)	NOAEL ~0.25-0.62 mg/kg/d** LOAEL~ 2.5-6.2mg/kg/d	-	-	
				↓ Number of oocytes/oviduct (= 0 in the group treated at the highest dose) ↓ Number of corpora lutea and ↗ in the number of atretic ±	NOAEL ~2.5 at 6.2 mg/kg/d** LOAEL 25-62.5mg/kg/d	Ovarian effects	**Recognised**	**

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				cystic follicles				
				↓ Antral follicles on the ovaries at the dose of 2.5 to 6.2 mg/kg/d (lowest dose tested for this parameter).	LOAEL~ 2.5 -6.2 mg/kg/d** (lowest dose tested for this parameter)	Ovarian effects	**Recognised**	**
Markey *et al.*, 2005	CD-1 mice	Subcutaneous	0.025 and 0.25 µg/kg bw/d GD9 – PND3	**F1** No results observed on the ovaries at 3 and 6 months				No GLP nor OECD guideline study
				Vagina: decrease in absolute and relative weight of the vagina.	NOEL: 25 ng/ kg bw/d LOEL: 250 ng/kg bw/d		Suspected	This study is compared with the study of Mendoza-Rodriguez for the endometrial hyperplasia

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
								effects
				Uterus: decrease in absolute weight of the lamina propria - increase in BrdU incorporation rate and in expression of ERα and PR in the uterine epithelium.	LOEL: 25ng/kg bw/d			
Newbold *et al.*, 2007	CD-1 mice	Subcutaneous	10 - 100 - 1000 µg/kg bw/d PND1- PND5 Treatment of pups					No GLP nor OECD guideline study
				- No difference between **body weight** of the treated and control animals, irrespective of the dose.				
				Ovaries - significant increase in frequency of polycystic ovaries but only at the	NOAEL 10 µg/kg/d LOAEL 100	Ovarian cyst and endometrial	**Recognised**	Non-monotonic dose-response

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				dose of 100 µg/kg bw/d. - significant increase in cystic endometrial hyperplasia but only at the dose of 100 µg/kg bw/d.	µg/kg/d	hyperplasia		relationship (3 doses) *
				Appearance and/or increased incidence of a series of genital tract abnormalities, some of which are pre-neoplastic or neoplastic in nature. Due to the low incidence reported and sample size (16 to 23 mice/group), the impact of treatment on the occurrence of these anomalies is not statistically significant. These effects are listed below:	NOAEL/LOAEL cannot be determined			

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				- Decrease in ovulation rate - Paraovarian mesonephric cysts - Progressive proliferative lesions of the oviduct - Development of endometrial glands in the myometrium - Atypical uterine hyperplasia - Leiomyomas - Neoplastic polyps (stroma) - Persistence of Wolffian ducts				
Newbold *et al.*, 2009	CD-1 mice	Subcutaneous	0.1 - 1 - 10 - 100 and	For all diseases combined, increase in	$LOAEL_{nm}$: 0.1 µg/kg/d but			Non-monotonic

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			1000 µg BPA/kg bw/d GD9 - GD16	the frequency of genital tract abnormalities.	not statistically significant at higher doses.			dose-response relationship (4 doses)
				If dissociated by type of anomaly, the only significant difference relates to the increased **incidence of ovarian cysts**:	$LOAEL_{nm}$: 1 µg/kg/d but not statistically significant at higher doses.	Polycystic ovary	**Recognised**	Non-monotonic dose-response relationship (5 doses) *
				Appearance and/or increased incidence of a series of genital tract abnormalities, some of which are pre-neoplastic or neoplastic in nature. Due to the low incidence reported and sample size, the impact of treatment on the occurrence of these anomalies is not statistically significant. These effects are listed	NOAEL/LOAEL cannot be determined	Pre-neoplastic lesions of the genital tract	-	

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				below: - Paraovarian mesonephric cysts (10 µg/kg bw/d), - Neoplastic lesion in ovary including cystadenocarcinoma found at BPA - 10, 100 and 1000 µg/kg bw/d (not significant, NS) - Progressive proliferative lesions of the oviduct observed in all treated groups but not in controls (NS) - Increased incidence of cystic endometrial hyperplasia (CEH) for all groups except BPA-0.1 (even the control) – (NS) - Adenomatous hyperplasia with CEH in BPA-1, BPA-100 but not				

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				in controls (NS) - Atypical uterine hyperplasia, considered a precursor of uterine adenocarcinomas, found in BPA-0.1 BPA-1 and BPA-1000, but not in controls (NS) - Persistence of Wolffian ducts. - Uterine polyps observed in BPA-0.1, BPA-1 and 10 (NS). This kind of lesion has been reported as being associated with the development of stromal cell sarcomas in rodents.				
Nikaido *et al.*, 2005	CD-1 mice	Subcutaneous	10 mg/kg bw/d PND15-PND19	No change in the age of puberty. No macroscopic change in the uterus, vagina, and breast development. Anovulatory state for 80% of the animals	-	-		Study not used for the HRA, as the dose showing an effect was higher than the applicable NOAEL.

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				treated with BPA *versus* control group. No modification of ovarian cyclicity.				Moreover, study not selected because the exposure is post natal.
Signorile *et al.*, 2010 (see description above)	BALB-C mice (dams: 6 females/treatment group; female offspring: 20/treatment group)	Subcutaneous	100 and 1000 µg/kg bw/d GD1 - PND7 Postnatal exposure of pups *via* lactation	Increased frequency of cystic and adenomatous endometrial hyperplasia. Trend towards increased incidence of atypical endometrial hyperplasia. Appearance of endometriosis type lesions (glands and stroma expressing ER and Hoxa10). Increased frequency of ovarian cysts.	LOAEL: 100 µg/kg bw/d	Endometrial hyperplasia. Ovarian cysts.	**Recognised**	****** **Although not GLP, study of good quality based on the Klimisch score, estrogenic contamination controlled, administration by subcutaneous route.**
Mahoney and	Sheep	Subcut	5 mg/kg bw/d	↗ in the expression of	$LOEL_u$:	Effect on	**Recog**	*****

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
Padmanabhan, 2010		aneous	GD30 - G90	ESR1 and ↘ in the expression of ESR2 in medial preoptic area during oestrus. ↗ in expression of GnRH (gonadotropin-releasing hormone) in the medial preoptic area during oestrus.	5 mg/kg/d	the hypothalamic-pituitary-gonadal axis.	**nised**	
Berger *et al.*, 2010	CF-1 mice	Subcutaneous	100 - 200 - 300 mg/kg bw/d GD1 - GD4	↘ in implantation sites. Histological modifications to the wall of the uterine cavity. Decrease in ERα and PR receptor expression.	-	-		Study not used for the HRA, as the doses showing an effect were higher than the applicable NOAEL
Tachibana *et al.*, 2007	ICR mice	Subcutaneous	10 mg/kg bw/d GD0 - GD7	↘ in the embryo number. ↘ in the weight of the uterus and marked modifications to placental structure.	-			Study not used for the HRA, as the dose showing an effect was higher than

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
								the applicable NOAEL.
Nikaido *et al.*, 2004	CD-1 mice	Subcutaneous	0.5 and 10 mg/kg bw/d GD15 - GD18	Acceleration of weight gain in F1 females.	LOEL 10 mg/kg bw/d LOEL 0.5 mg/kg bw/d NOAEL 10 mg/kg bw/d (no observed adverse effect)			
				Precocious vaginal opening	NOEL: 0.5 mg/kg bw/d LOEL: 10 mg/kg bw/d	Age at puberty	**Recognised**	**
				Disruption of the oestrous cycle (the percentage of time spent in diestrus phase is significantly higher than	LOEL: 0.5 mg/kg bw/d	Cycle disruption	**Recognised**	**

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
				that ofthe negative controsl)- increased frequency of anovulatory state)				
				Acceleration of mammary gland differentiation	*Section on the mammary gland*			
Patisaul *et al.*, 2009	Long Evans rats	Subcutaneous	50 µg/kg bw/d and 50 mg/kg bw/d PND1 - PND5 Treatment of pups	Decreased expression of KiSS (immunostaining) in ovariectomised females under steroid treatment.	NOEL: 50 µg/kg bw/d LOEL: 50 mg/kg bw/d	Effect on the hypothalamic-pituitary-gonadal axis		The animals were ovariectomised after treatment with BPA, consequently this study was not rejected. Moreover, the steroid treatment applied can mimic a physiological condition. Uncertainty due to the

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
								gap between the two doses administered.
				Disruption of the oestrous cycle with prolonged oestrus.	LOEL: 50 µg/kg bw/d	Cycle disruption	**Recognised**	*
Navarro *et al.*, 2009	Wistar rats	Subcutaneous	100 - 500 µg/animal PND1 – 5 Treatment of pups	Suppression of KiSS-1 messenger RNA levels in the hypothalamus at PND30.	LOEL: 100 µg/animal	Effect on the hypothalamic-pituitary-gonadal axis	**Recognised**	Study not used for the HRA, as the dose showing an effect was probably higher than the applicable NOAEL
Studies by the intramuscular route								
Evans *et al.*, 2004	Ewes	Intramuscular	3.5 mg/kg twice a week 4-week-old ewes (prepubertal) treated for 5	↘ in the frequency and amplitude of LH pulsatility after ovariectomy. No modification of ovary weight.	$LOEL_u$: 3.5 mg/kg	Effect on the hypothalamic-pituitary-gonadal axis	**Recognised**	***** Single dose

References	Species	Route	Doses Exposure periods	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study/ effects limitations
			weeks					
Savabieasfahani *et al.*, 2006	Ewes	Intra-muscular	5 mg/kg bw/d GD30 - GD90	Hypersecretion of LH in the prepubertal period. Modification of the preovulatory peak of LH.	$LOEL_u$: 5 mg/kg/d	Effect on the hypothalamic-pituitary-gonadal axis	**Recognised**	* Single dose
Studies by the intravenous route								
Collet *et al.*, 2010	Ewes	Intra-venous	5 - 10 - 20 - 40 and 80 mg/kg bw/d Prepubertal ovariectomised sheep treated for 4 days	Inhibition of LH pulse-generating system qualitatively similar to the effects of 17β-oestradiol (positive control).	LOEL: 20 mg/kg/d corresponding to a plasma concentration ~30 ng/ml = 10X levels in humans)	Effect on the hypothalamic-pituitary-gonadal axis		Animal not intact

Summary of animal studies on the effects of bisphenol A on vaginal opening and on age at first oestrus

Exposure period	**References**	**Species**	**Routes**	**Exposure period**	**Exposure dose**	**Effect assessed on vaginal opening and age at first oestrus**	**NOAEL/LOAEL**	**Remarks / Study limitations**
Studies by the oral route								
Gestation	Howdeshell *et al.*, 1999	CF-1 mice	Oral gavage	GD11 - GD17	BPA: 2.4 µg/kg bw /d	<u>Vaginal opening:</u> no effect	$NOEL_u$: 2.4 µg/kg bw/d	
						Interval between vaginal opening and age at first oestrus: decreased by 2~4d	$LOEL_u$: 2.4 µg/kg bw/d	* Single dose
Gestation and postnatal	Yoshida *et al.*, 2004	Donryu rats	Oral gavage	GD2 - PND21 Treatment of mothers i.e. PN: orally, low dose	BPA: 6 µg/kg bw/d 6 mg/kg bw/d	<u>Vaginal opening</u> No effect of BPA	NOEL: 6 mg/kg/d	Not retained for the health risk assessment because of major methodological limits : validity of the method of dosage not

								known, which form of BPA tested not known, how much time after administration of the substance the dosage is performed not known, food contamination, no control group and no historical control group.
	Takagi *et al.*, 2004	Sprague-Dawley rats	Oral Feed	GD15 - PND10 Treatment of mothers i.e. PN: orally, low dose	BPA feed: 60 - 600 - 3000 ppm or ~6 - 300 mg/kg b w/d Ethinyl E2 0.5 ppm	<u>Vaginal opening</u> No effect of BPA	NOEL: 300 mg/kg/d (3000 ppm)	-
Gestation and postnatal	Kwon *et al.*, 2000	Sprague-Dawley rats	Oral gavage	GD11 - PND20 Treatment of mothers i.e. PN: orally, low	BPA: 3.2 - 32 - 320 mg/kg b w/d DES 15	<u>Vaginal opening and age at first oestrus:</u> **No effect of BPA nor of**	NOEL: 320 mg/kg bw/d	Late post-natal exposurecompared to Rubin study by oral route (2001)

				dose	µg/kg bw/d	**DES.**		
	Ryan *et al.*, 2010a	Rats	Oral gavage	GD7 - PND18	EE2: 0.05 - 0.5 - 1.5 - 5 - 15 - 50 µg/kg bw/d BPA: 2 - 20 - 200 µg/kg bw/d	<u>Vaginal opening</u> EE2 at the dose of 5 µg/kg led to a 4d advance in vaginal opening. BPA did not cause any effect.	NOEL 200 µg/kg bw/d	-
	Rubin *et al.*, 2001 (see description above)	Sprague-Dawley rats (18 dams _ 30 OVX Male offsrping : newborn,n = 12 – 3 months old, n= 12 – 5 month old, n=18 Female offspring: newborn, n=12 – 8 months,	Oral	GD6 – pups were weaned	1 and 10 mg/L in drinking water, intake estimated at 0.1 mg/kg b w/d to 1.2 mg/kg b w/d	- No effect on the average number of pups/litter, on the age at vaginal opening and the anogenital distance.	F1 (intact animals): NOAEL: 1.2 mg/kg bw/d	

		n= 24 – 12 to 16 months, n= 34						
Studies by the subcutaneous route								
Gestation	Nikaido *et al.*, 2004	CD-1 mice	Subcutaneous	GD15 - GD19	BPA: 0.5 or 10 mg/kg bw/d DES: 0.5 or 10 µg/kg bw/d	<u>Vaginal opening:</u> BPA 0.5 mg/kg bw/d: no effect BPA 10 mg/kg bw/d : advance of 1.2 d DES: advance of 1.5 and 1.9 d at the doses of 0.5 and 10 µg/kg bw/d respectively	NOEL: 0.5 mg/kg bw/d LOEL 10mg/kg bw/d	** But study not retained for the health risk assessment because of the subcutaneous route of administration
Gestation	Honma *et al.*, 2002	ICR Jcl mice	Subcutaneous	GD11 - GD17	BPA: 2 or 20 µg/kg DES: 0.02 - 0.2 or 2 µg/kg	<u>Vaginal opening and age at first oestrus:</u> BPA 20 µg/kg: advance (~1d) DES: advance	NOEL 2 µg/kg/d LOEL 20 µg/kg/d	**

						1.5 d minimum		
Gestation	Savabieasfahani *et al.*, 2006	Sheep	Subcutaneous	GD30 - GD90 (2/5th of gestation)	BPA: 5 mg/kg	No effect: on age at first oestrous cycle determined by the level of progesterone	$NOEL_u$: 5 mg/kg/d	- The lack of control of photoperiodic conditions may have hidden effects, which invalidates measurement of the parameter investigated. High dose.
Early postnatal	Adewale *et al.*, 2009	Rats	Subcutaneous	PND0 - PND3 Treatment of pups	EB*: 25 µg BPA: 50 µg/kg BPA: 50 mg/kg PPT: 1 mg/kg	<u>Vaginal opening:</u> EB: Advance 4d BPA: 50 µg/kg: Advance 2d BPA: 50 mg/kg: NS PPT 1 mg/kg: Advance 1d	$LOEL_{nm}$: 50 µg/kg/d but no effect at higher dose	* Non-monotonic dose-response relationship (2 doses) Significant gap in doses.
	Fernandez *et al.*, 2009	Sprague-Dawley rats	Subcutaneous (castor oil)	PND1 - PND10 Treatment of pups	1st dose tested of BPA: 2.5 – 6.2 mg/kg bw	<u>Vaginal opening:</u> 2.5 d advance 4.8 d advance	LOEL 2.5 - 6.2 mg/kg bw/d	** Uncertainty about the value of the NOAEL determined due

					2nd dose tested of BPA: 25 to 62.5 mg/kg bw			to the variation in the dose administered over time by injection of a constant volume.
Peripubertal	Nikaido *et al.*, 2005	CD-1 mice	Subcutaneous	PND15-19 prepubertal	BPA: 10 mg/kg bw/d DES: 10 µg/kg bw/d	<u>Vaginal opening</u> No effect with BPA 10 mg/kg bw/d DES 10 µg/kg bw/d: advance	$NOEL_u$: 10 mg/kg/d	-

Effects on the brain and behaviour

Studies showing effects on the brain and behaviour are specific for these type of effect and there is no negative studies for these effects.

Summary of studies on the effects of bisphenol A on brain and behaviour

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
Studies by the oral route								
Poimenova *et al.*, 2010	Wistar rats	Oral	40 µg/kg bw/d GD1 - weaning (42 days)	↗ in levels of corticosterone and ↘ in GR levels in males in basal state and in both sexes after stress. No effects on the MR receptor in normal conditions, but ↘ in MR level in females, in both groups of females. ↘ in spatial memory in both sexes. ↘ in exploratory behaviour in females	$LOAEL_{u:}$ 40 µg/kg bw/d	Decreased exploratory behaviour and anxiety in F1 females (reduced stress adaptation). ↓ spatial memory in both sexes.	*Controversial*	

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				and appearance of anxious behaviour.				
Stump *et al.*, 2010	CD-SD rats	Oral	0.15 - 1.5 - 75 - 750 and 2250 ppm feed Gestation: 0.01 - 0.12 - 5.85 - 56.4 - 164 mg/kg bw/d Lactation: 0.03 - 0.25 - 13.1 - 129 - 410 mg/kg bw/d GD0 - PND21	No effect on exploratory behaviour				
				<u>For systemic effects:</u> Decrease in body weight gain in mothers from 56.4 mg/kg bw/d during gestation and from 129 mg/kg bw/d during lactation.	NOAEL: 5.85 mg/kg/d for gestation and 13.1 mg/kg bw/d for	Body weight		

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
					lactation			
				P (Parents):		No neurotoxicity (OECD 426)		
				For neurodevelopmental and neurotoxic effects in F1: Some seizures were observed in this study at PND11 at 750 ppm (2 pups) and at 2250 ppm (4 pups), but these effects were not replicated in a further study.	NOAEL (F1): 13.1 mg/kg bw/d NOAEL at 410 mg/kg/d for lactation.	Transient reduction in body weight in pre-weaning No neurotoxicity (OECD 426)	No neurotoxicity at doses without systemic effects	
Kubo *et al.*, 2001	Wistar rats	Oral	1.5 mg/kg bw/d GD0 - PND21	Abolition of sexual dimorphism compared to the control. No change in the	$LOAEL_u$: 1.5 mg/kg bw/d		**Recognised**	* A single dose administered

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				reproductive organs or sex hormones.				
Kubo *et al.*, 2003	Wistar rats	Oral (water *ad libitum*)	Perinatal GD0 - PND21 BPA at 0.1 mg/L and 1 mg/L drinking water *ad libitum*, equivalent to around 0.03 - 0.3 mg/kg bw/d	<u>Effect on sexual dimorphism</u>: - Elimination and reversal of differences in open-field behaviour (locomotive activity, hyperactivity, exploratory behaviour and anxiety). - Increase in body weight in females on the day of vaginal opening with BPA at 1 mg/L (300 µg/kg/d). - Change in exploratory behaviour of males towards that of	LOAEL: 30 µg/kg bw/d**	Impaired sexual behaviour in the male from 30 µg/kg bw/d. Decrease in exploratory behaviour in males at 30 µg/kg bw/d. Inversion of differences in volume of the locus coeruleus (LC) nucleus from 30 µg/kg bw/d.	**Recognised**	**

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				females (increased distances covered, increased number of rearings). - Demasculinisation of males and defeminisation of females regarding anxiety (↑ in time spent in the central area in males and ↓ in females when compared to males and females of the control group) at the doses of 30 and 300 µg/kg/d - Decreased sexual behaviour in males exposed to BPA at 30 µg/kg/d and to RVT (Resveratrol).				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				- Increase in testicular weight (9%) with BPA at the dose of 300 µg/kg bw/d and decrease with DES. - Inversion of differences in volume of the locus coeruleus (LC) nucleus, involved in sexual dimorphism, increased in males and decreased in females exposed to BPA at the doses of 30 and 300 µg/kg bw/d, to RVT and DES. Identical changes in the number of LC neurons.				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
Funabashi *et al.*, 2004	Wistar rats	Oral (drinking water)	10 mg/L drinking water, equivalent to 2.5 mg/kg bw/d GD0 - PND21 (GD0 not clearly indicated) Observations in F1 animals from 4-7 months	- Difference in the number of corticotropin-releasing hormone-immunoreactive (CRH-ir) neurons between females and males in the preoptic area (PAO) but no difference in the bed nucleus of the stria terminalis (BST). - No significant difference in the number of CRH-ir neurons between exposed and non-exposed animals, all sexes combined. - The number of CRH-ir (corticotropin-releasing hormone-immunoreactive) neurons in the PAO	$LOAEL_u$: 2.5 mg/kg/bw	Abolition of CRH (corticotropin-releasing hormone) neuron sexual dimorphism in the BST (bed nucleus of the stria terminalis)	**Recognised**	* A single dose administered. No details on the materials used for breeding, the presence of phyto-oestrogens in the diet and the presence of EDs in drinking water

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				and BST was higher in female rats than in males. - BPA abolished CRH-ir neuron sexual dimorphism in the BST (anterior and posterior) by increasing the number of CRH neurons in males and decreasing it in females. - No effect of BPA was observed in the PAO.				
Ryan *et al.*, 2010a	Long-Evans rats	Oral	2 - 20 or 200 µg/kg bw/d GD7 - PND18 EPA protocol	No effect on behavioural sexual dimorphism.	NOAEL: 0.2 mg/kg bw/d	No effect in the EPA protocol.		Effects on brain and behavior were not investigated in this study.

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
Cox *et al.*, 2010	Mice	Oral	8 mg/kg bw/d (BPA administered in feed) GD9 - PND0	- Suppression of behavioural sexual dimorphism in young exposed during embryogenesis. - No effects on dietary intake, caring behaviour or urinary marking in pups irrespective of the mother's origin (treated or not). - Increased anxiety (plus shaped maze test). - No effect of BPA exposure during gestation on the weight of the gonads of male or female offspring. - No effect on the level of corticosterone in male or female	$LOAEL_{u:}$ 8 mg/kg bw/d (corresponding to 50 mg/kg feed)	↑ anxiety in young Suppression of behavioural sexual dimorphism	*Controversial*	

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				offspring.				
Tian *et al.*, 2010	ICR mice	Oral	100 and 500 µg/kg bw/d GD7 - PND36	<u>Behavioural and histochemical tests:</u> - ↗ in dopamine D2 receptors and decreased dopamine transporters (DAT) in the putamen. - ↘ in NMDA receptors in the frontal cortex, dentate gyrus (DG) and cornu ammonis 1 and 3 (CA1 and CA3) regions of the hippocampus.	LOAEL: 0.1 mg/kg bw/d	Cognitive deficits (Y maze) observed without monotonic dose-response relationship; ↘ in expression of hippocampal NMDA receptors (dose-response relationship)	**<u>Recognised</u>** (biochemical effect)	Small sample size: pups from two mothers treated per dose level.
				- Cognitive deficits (Y maze) observed without monotonic dose-response relationship.			Controversial (cognitive deficit)	

References	**Species/ strain**	**Routes**	**Dose** **Exposure period**	**Effects**	**NOAEL/ LOAEL**	**Critical effect**	**Rating of effects**	**Remarks / Study limitations**
				-Anxiolytic effect at 100 µg/kg bw/d			Controversial (anxiolytic)	
Xu *et al.*, 2010b	Rats n = 10 animals/group Gender and number of animals per category: -10 mothers randomly exposed par modality of treatment (n =10). Females were	Oral	0 – 0.05 – 0.5 – 5 – 50 and 200 mg/kg bw/d in rats GD7 - PND21	- Decreased expression of NMDA receptors (NR1, NR2A and NR2B subunits), ER□ oestrogen receptors and increase in aromatase in the hippocampus in F1 male rats <u>at the doses of 0.05 to 50 mg/kg bw/d</u>. - The decrease in expression of NMDA receptors was also observed at 200 mg/kg bw/d but with a lesser effect compared to lower doses.	LOAEL: 50 □g/ kg bw/d**	Dose-dependent inhibition of expression of NMDA receptors, ER□ oestrogen receptors and increased aromatase in the hippocampus.	**Recognised**	**

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
	placed and exposed individually. -10 pups selected per litter by trying to respect the male/female ratio. Pups individually identified at PND7 -At weaning (PND21), the pups were separated into same-sex littermates and			+ see detailed description and further justification in section B.5.10.1.3				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
	housed							
Mahoney and Padmanabhan, 2010	Sheep	Subcutaneous	5 mg/kg bw/d GD30 - GD90	- Increase in expression of ESR1 and ☐ in expression of ESR2. - Increased expression of gonadotropin-releasing hormone.	$LOAEL_u$: 5 mg/kg bw/d		**Recognised**	A single dose tested.
Palanza *et al.*, 2008	CD-1 mice	Oral	10 µg/kg bw/d 3 scenarios 1) GD14 - GD18 2) during gestation (from GD11) and continued until PND73) only after birth until adulthood	- Changes in maternal behaviour in F1 offspring only after *in utero* or adult exposure (scenarios 1 and 3), but not in scenario 2. -↘ in time spent by mothers caring for their pups and ↗ in time where they remained alone in the cage (isolated resting time). no effect on the	$LOEL_u$: 10 µg/kg/d fractionated administration: either gestation or lactation	Changes in maternal behaviour during fractionated exposure (i.e. gestation or lactation).	Suspected	Questions on the impact of changes in maternal behaviour in terms of human health. No effect during continuous

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				weight of offspring at birth.				exposure; however, an effect on maternal behaviour was observed during fractionated exposure during gestation or lactation.
Xu et al., 2010c	ICR mice	Oral (gavage)	Doses 0 – 0.05 – 0.5 – 5 and 50 mg/kg bw/d Perinatal GD7-PND21					

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				<u>Significant effects on body weight:</u> - At PND21 decrease in body weight at the dose of 0.05 mg/kg bw/d and increase at 50 mg/kg/d. - At PND56 decrease in body weight at the doses of 0.05 and 0.5 mg/kg bw/d.	LOAEL: 50 µg/kg bw/d			
				<u>Impaired memory functions and learning:</u> - Impaired spatial memory at the doses of 5 and 50 mg/kg/d at PND21, and at the doses of 0.5, 5 and 50 mg/kg bw/d at	NOAEL: 50 µg /kg bw/d LOAEL: 500 µg/kg bw/d	Impaired memory functions and learning NOAEL: 50 µg/kg bw/d		** Non-monotonic dose-response relationship

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				PND56. - Impaired learning abilities at the doses of 5 and 50 mg/kg bw/d at PND21, and 50 mg/kg bw/d at PND56.		LOAEL: 500 µg /kg bw/d		
				<u>Decreased expression of hippocampal NMDA receptors:</u> - Significant dose-dependent fall in expression of the NR1 subunit of hippocampal NMDA receptors at PND21 and PND56. Effect observed from 0.05 mg/kg bw/d with a fall of about 40%.	LOAEL: 50 µg /kg bw/d	Decreased expression of hippocampal NMDA receptors LOAEL: 50 µg/kg bw/d	**Recognised**	**

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				- Significant decrease in expression of the NR2A subunit at 5 and 50 mg/kg bw/d at PND21 and at all dose levels at PND56 (approx. 41% at the dose of 0.05 mg/kg/d and 61% at the dose of 5 mg/kg/d). - Significant decrease in expression of the NR2B subunit at 0.5, 5 and 50 mg/kg bw/d at PND21 and at all dose levels at PND56 (around 42% at the dose of 0.05 mg/kg/d.				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				Significant decrease in expression of ERβ receptors at the doses of 0.5, 5 and 50 mg/kg bw/d at PND21 and PND56	NOAEL 50 µg /kg/d**[80] LOAEL: 500 µg /kg/d			
Martini *et al.* 2010	CD-1 mice	Oral	Perinatal GD11 - PND8 0 - 10 - 20 - 40 µg/kg bw/d	- Decrease in body weight at birth at the dose of 20 µg/kg bw/d. No effect at weaning. - Increased NOS (Nitric Oxide	NOAEL $_{nm}$[81]: 10 µg/kg bw/d LOAE L_{nm}: 20 µg/kg bw/d	Modulation of NOS in the MPON (↑ females) and in the BST (↓ males)		Possible non-monotonic dose-effect relationship (3 doses).

[80] **: good quality study

[81] nm: non-monotonic

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				Synthase) immunoreactivity at 20 µg/kg/d in the median preoptic nucleus (MPON) in females. - Decreased NOS immunoreactivity in the ventromedial subdivision of the BST (bed nucleus of the stria terminalis) at 20 µg/kg/d in males.				Use of polypropylene cage.
Studies by the subcutaneous route								
Nakagami *et al.*, 2009	Cynomolgus monkeys	Subcutaneous	10 µg/kg bw/d (blood level equivalent to that from ingestion of 5 mg/kg bw/d in rats) from GD20 and until	Univariate analysis: significant effects on 3 infant behaviours and 1 maternal behaviour: - in F1 ♂: 'embracing' and 'social exploration'	$LOAEL_u$ in mothers: 10 µg/kg bw/d	Behaviour of mothers with regard to F1♂ becoming more like behaviour of mothers of	Suspected	Questions on the impact of changes in maternal behaviour (outward

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
			the end of gestation (around GD160) Observations at the following periods: PND31-60 (2 MAB - Months After Birth) and PND61 - 90 (3 MAB)	behaviours ↘ at 2 months and 'outward looking' behaviour ↗ at 2 and 3 months. - In mothers of ♂, 'outward looking' behaviour ↗ at 2 and 3 months. Multivariate analysis: discriminant scores of F1 ♂ were closer to the F1 ♀ controls than the F1 ♂ controls. No effects in ♀. Regarding maternal behaviour, the mothers of F1 ♂ had discriminant scores closer to those of the control mothers of F1 ♀ than those of the control mothers of F1 ♂.	$LOAEL_u$ in F1: 10 µg/kg bw/d	F1♀. Behaviour of F1♂ becoming more like behaviour of F1♀.		looking) in terms of severity of effects.
Patisaul *et*	CD-SD rats	Subcut	500 µg/animal/	Demasculinisation of neuron sexual		Neuron sexual	**Recognis**	*

References	**Species/ strain**	**Routes**	**Dose** **Exposure period**	**Effects**	**NOAEL/ LOAEL**	**Critical effect**	**Rating of effects**	**Remarks / Study limitations**
al., 2006		aneous	d PND1 - PND2	dimorphism by increased immunoreactivity of tyrosine hydroxylase (TH) in males in the anteroventral periventricular nucleus of the hypothalamus Decrease in the percentage of TH immunoreactive cells which co-express ER□ receptors (in both sexes).		dimorphism and modulation of ESRs	**ed**	A single dose administered
Patisaul *et al.*, 2007	CD-SD rats	Subcutaneous	500 µg/animal/d PND1 - PND2	- No change in volume of the sexually dimorphic nucleus (SDN) in the preoptic area. - Increased number of calbindin neurons in the SDN. - No demasculinisation of the volume of the				* A single dose administered

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				anteroventral periventricular nucleus of the hypothalamus.				
Rubin *et al.*, 2006	CD-1 mice	Subcutaneous	0 - 25 - 250 ng/kg bw/d GD8 - PND16	- ↘ in the intersex difference in the number of neurons expressing tyrosine hydroxylase (TH) due to a ↘ in the number of TH neurons in females. - Impaired neuron **sexual dimorphism** in the exposed animals	LOAEL = 25 ng/kg bw/d	Neuron sexual dimorphism	**Recognised**	**
Adewale *et al.*, 2011	Long-Evans rats	Subcutaneous	50 µg/kg bw/d and 50 mg/kg bw/d PND0 - PND3 (4 injections)	No change in sexual behaviour ↗ in body weight at the age of 99 days, only at the dose of 50 mg/kg bw/d	NOAEL: 50 µg/kg bw/d / LOAEL: 50 mg/kg bw/d	Increase in body weight		
				No change in	LO(A)EL:	Neurogenesi	**Recognis**	**

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				serotonin fibre density or in the density of ERα receptors in the ventrolateral subdivision of the ventromedial nucleus ↗ in the number of oxytocin neurons in the paraventricular nucleus at BPA 50 µg/kg bw/d and 50 mg/kg bw/d.	50 µg/kg bw/d	s (↗ in the number of oxytocin neurons in the paraventricular nucleus)	**ed**	
Kim *et al.*, 2009	ICR mice	Subcutaneous	5 - 10 - 20 mg/kg bw/d GD14.5 - GD18.5 then injection of 20 mg/kg twice a day for 3 days from PNW8	**F1** At PNW3, ↗ in body weight at 5 mg/kg and ↘ at 20 mg/kg but not at PNW8 Accelerated formation of the dentate gyrus at PND1 at the dose of 20 mg/kg. → BPA may block the				Study not used for the ERS: - the doses showing an effect (on the dentate gyrus) were much higher

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				proliferation of neural stem cells and promote cell differentiation at a relatively early stage. BPA had no observed effects on the cortical structure of the neural cells, hippocampus or cell density. In adult mice, BPA had no observed effects on hippocampal neurogenesis.				than the applicable NOAEL
Bai *et al.* 2011	Sprague-Dawley rats	Subcutaneous	Single dose 2 µg/kg bw/d GD10 - PND7	Increase in the number of neurons expressing kisspeptin in the anteroventral periventricular (AVPV) nucleus in males. Increase in the number of neurons	$LOAEL_U$: 2 µg/kg bw/d	Neuron sexual dimorphism (Increased number of neurons expressing kisspeptin and GnRH in males)	**Recognised**	* A single dose tested

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				expressing GnRH in the preoptic area (POA) in males. Increase in plasma LH and oestradiol in males. Decrease in plasma testosterone in males (around 30%).				
Nakamura *et al.* 2010	ICR/Jlc mice	Subcutaneous	20 µg/kg/d GD0 - PND21	At PNW14-15, increased DA in the putamen and the dorsal raphe nucleus, and increased DOPAC = 3,4-dihydroxyphenylacetic acid) in the putamen (CP). Increased serotonin in the thalamus and the substantia nigra (SN) at PNW3 and in the dorsal raphe nucleus (DRN) and	$LOAEL_U$: 20 µg/kg/d	Impaired aminergic system (increase in the concentration of DA and 5-HT brain monoamines and their metabolites)	**Recognised**	* A single dose tested

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				the SN at PNW14-15. Increase in 5-HIAA (5-hydroxyindole acetic acid) in the dorsal raphe nucleus at PNW14-15, in the putamen at PNW3 and PNW14-15, and in the preoptic area (LH/POA) at PNW3.				
Zhou *et al.* 2011	Sprague-Dawley rats	Subcutaneous	2 µg/kg/d GD10 - PND7	No effect of BPA on the size of the basolateral amygdala (BLA) (n=25). No histological or cytological differences in the BLA between control and BPA rats (n=47). Changes in the plasticity of cortical	$LOAEL_U$ 2 µg/kg/d	Hyperactivity and attention deficit associated with significant changes in neuronal plasticity in the basolateral amygdala**.**	**Recognised**	* Very good study. However it was only carried out with a single dose. Lack of dietary phyto-oestrogen controls,

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				BLA in exposed rats: - Multiple potentials after single stimulation (mean 4.15 ± 0.53 for the BPA rats vs. 1 potential in the controls) with gradual decrease in amplitude (n=18 slices, 8 rats). - Higher amplitude in first potentials in BPA rats (curves: Amplitude of the 1st potential is function of the stimulation intensity) - Induction of long-term potentiation				and presence of BPA in restraining cages and drinking bottles.

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				(LTP) after stimulation at high frequencies in BPA rats and not in controls. (n=14 slices, 8 rats for controls, 10 rats for BPA). - No effects on the number of potentials, amplitude of potentials and on the induction of LTP in BLA neurons after stimulation of thalamic afferents. **Conclusion**: - BPA induced an increase in neuronal excitability and facilitated the induction of LTP in				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				the BLA. - Involvement of dopamine receptor D1 (DRD1) in long-term potentiation induced by BPA (DRD1 antagonist SCH23390 reduced LTP in BPA rats and DRD1 agonist SFK-81297 increased LTP in controls). - Induction of a deficit in GABAergic pathways in the cortical BLA. - Synaptic inhibition after paired-pulse and synaptic facilitation in BPA				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				rats (n=12 slices, 6 rats) related to a modification in the inhibitory effect of GABA receptors in cortical BLA. - The LTP induced by BPA is due to a malfunction of the GABAergic pathway in the cortical BLA. - Rats exposed to BPA exhibited hyperactivity compared to control rats, and attention deficit. - Hyperactivity and attention deficit were associated with the				

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				potentiation induced by BPA in the cortical BLA (decreases in these behaviours with SCH23390 (DRD1 antagonist), muscimol ($GABA_AR$ antagonist) and MK801 (NMDAR antagonist)).				
Studies by the intracranial route								
Matsuda *et al.*, 2010	Rats	Intracranial	0.1 - 1 - 10 µg/kg Single injection at PND2 (1st experiment) 1000 µg/kg single injection at PND2 (2nd experiment)	- Significant ↗ in serotonin in the hippocampus, 5-HIAA and 5-HT in the brain stem, dopamine and DOPAC in the striatum 28 days after the injection. Seven days after the injection, ↗ in 5-HT and norepinephrine (NE) and ↘ in DOPAC	Study showing persistence of biochemical effects in situ (but doses injected in situ: problem of metabolism and			Study not used for the HRA due to the fact that the doses administered in the brain were doses related to the body

References	Species/ strain	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				and 5-HIAA were observed in the hippocampus. - BPA disappeared from brain tissues within 5 hours of the injection, even at the highest dose of 1000 µg/kg. BPA may have effects on cerebral monoamine levels in the 28 days after its disappearance.	bioavailability irrelevant for the HRA…)			weight of individuals that do not enable the extent of exposure to be assessed.

Effects on metabolism and the cardiovascular system

Summary of studies on the effects of bisphenol A on metabolism and the cardiovascular system

Reference	**Species / strains**	**Routes**	**Dose** **Exposure period**	**Effects**	**NOAEL/ LOAEL**	**Critical effect**	**Rating of effects**	**Remarks / Study limitations**
Studies by the oral route								
Rubin *et al.*, 2001	Sprague-Dawley rats	Oral	1 and 10 mg/L in drinking water, estimated intake of 0.1 mg/kg bw/d to 1.2 mg/kg bw/d GD6 - pups were weaned	**F1 (Intact animals):** - Increase in body weight of male and female pups from PND0 to 11 at both doses tested. - From PND11 to 22, increase in body weight persisted in animals treated at the low dose (male and female) and from PND28, only females treated at the low dose had a higher body weight. This effect persisted until PND114.	LOAEL: 0.1 mg/kg bw/d	Increased body weight in early postnatal period	Effects on increased body weight confirmed by the studies of Ryan *et al.* 2010b, Somm *et al*. 2009, Miyawaki *et al.,* 2007 and Alonso-Magdale	** *Strengths:* -water consumption was measured *Weaknesses:* -low number of mated dams (n=6) -not reported whether

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
							na *et al.,* 2010	the litter was used as statistical unit
Ryan *et al.*, 2010b	CD-1 mice	Oral	0.25 µg/kg bw/d GD0 to PND21	In F1 animals, ↗ in body weight in males and females at 3 weeks (increase of around 7%). ↗ in body length in males at 4 weeks; these biometric differences disappeared in adulthood. No significant effect on glucose tolerance was observed.	$LOAEL_u$: 0.25 µg/kg bw/d	↗ in body weight in males and females at 3 weeks of age.	**Recognised**	* Weakness: -Single dose

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
Somm *et al.*, 2009	Sprague-Dawley rats	Oral	70 µg/kg bw/d (administered in drinking water) GD6 - PND21					
				At birth: BPA treatment during gestation did not affect sex-ratio or litter size. Newborns (♀ and ♂): ↗ in body weight				
				PND21 ↗ in body weight in females Parametrial fat more abundant (3-fold increase *vs.* control group) and with hypertrophied adipocytes in which	$LOEL_u$: 70 µg/kg bw/d	Parametrial fat more abundant (3-fold increase *vs.* control group) and with hypertrophied	**Recognised**	* Strengths: phytoestrogen content in food measured, and

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				lipogenic genes and enzymes are overexpressed		adipocytes in which lipogenic genes and those of lipogenic enzymes are overexpressed		water content of BPA measured, Weaknesses: Single dose Statistical procedures not clearly described.
				In the liver, increased RNA levels of C/EBP-α, SREBP-1C, ACC and FAS k. Circulating lipids and glucose levels were normal.	$NOAEL_u$: 70 µg/kg bw/d	In the liver, increased RNA levels of C/EBP-α, SREBP-1C, ACC and FAS k,		
				4 to 14 weeks: the	$LOEL_u$: 70	Increase in		

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				change in body weight of males treated with BPA receiving a standard diet was similar to that of controls. ↗ in weight (about 7% calculated on the last point at 14 weeks) in males exposed to BPA + high fat diet *versus* control group, ↗ in weight in females (about 7% calculated on the last point at 14 weeks) for both types of diet tested. In males receiving the high-fat diet, the response to the glucose tolerance test was normal.	µg/kg bw/d	body weight in males and females.		

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				Conclusion: Perinatal exposure to BPA. ↗ in adipogenesis at weaning in ♀. In adult ♂, ↗ in body weight observed if high-fat diet.			**Recognised**	
Miyawaki et al. 2007	ICR mice (The number of dams is 3 per group, but statistical data are made with the number of pups ranging from 16 to 19) (1 control group with 3	Oral	0, 1 and 10 µg/kg bw/d, administered in drinking water, GD10 to PND30 (n=3 per dose group) **(+see detailed description above as well as weaknesses and strengths)**	**0.26 mg/kg/d** In F1 females: - Increase in body weight (↑ 13%) - Increase in adipose fraction (↑ 32%) - Increase in cholesterol (↑ 33%) In F1 males: - Increase in body weight (↑ 59%)	LOAEL= 0.26 mg/kg/d LOAEL= 0.26 mg/kg/d	Increase in female body weight and cholesterol Increase in male body weight and cholesterol.	**Recognised (comforted by Somm and Weiet al., 2009 – see description above) which shows comparable**	** Strengths: -Glass bottle, cage in polypropylene, use of a food with 30% of fat Weaknesses: -small

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
	dams and a total of 16 pups)			- Increase in cholesterol (↑ 23%) - Increase in triglycerides (↑ 34%) **2.72 mg/kg bw/d:** In F1 females: - Increase in body weight (↑ 11%) - Increase in cholesterol (↑ 17%) In F1 males: - Increase in body weight (↑ 59%) - Increase in cholesterol (↑ 18%)			**effects at lower doses and supported by Marmugi et al;, 2012, which show that low doses of BPA induce lipid synthesis through increased expression of lipogenic genes.)**	sample size -litter effect not considered -diet not tested for phyto-estrogens

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
Studies by the subcutaneous route								
Alonso-Magdalena *et al.*, 2010	OF-1 mice	Subcutaneous	0 - 10 and 100 µg/kg bw/d GD9 to GD16					
				In mothers,				
				GD 18: ↗ in insulin resistance induced by gestation and ↘ in glucose tolerance. dose-dependent ↗ in plasma levels of insulin, from 10µg/kg bw/d and in leptin, triglycerides and glycerol at 100 µg/kg bw/d. ↘ in insulin-stimulated Akt	LOAEL= 10 µg/kg bw/d	Increased insulin	Controversial (with the study of Ryan *et al.*, 2010: - no indications of increased susceptibility to high-fat diet-induced obesity	

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				phosphorylation in gastrocnemius skeletal muscle and liver at 10µg/kg bw/d (only dose tested).			and glucose intolerance in adult mice exposed prenatally at 0.25 µg/kg bw/d).	
				4 months post-partum: - higher body weight (significant at 100 µg/kg bw/d), - higher concentrations of triglycerides (from 10µg/kg bw/d) and insulin, leptin and glycerol at 100µg/kg bw/d.	LOAEL= 10 µg/kg bw/d	Increased triglycerides	**Recognised**	
				<u>In F1 offspring</u>,				

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				3 months: No significant changes in males and females.	NOAEL = 100 µg/kg bw/d			
				In males:				
				6 months: - ↘ in glucose tolerance, ↗ in insulin resistance, and ↗ in plasma levels of insulin, leptin, triglycerides and glycerol, - altered calcium signalling in islets of Langerhans - ↘ in BrdU incorporation into insulin-producing β cells, whereas their surface was unchanged.	LOAEL= 100 µg/kg bw/d	Increased insulin resistance induced by gestation and ☐ in glucose tolerance	Controversial (with the study of Ryan *et al.,* 2010: - no indications of increased susceptibility to high-fat diet-induced obesity and glucose	

Reference	Species / strains	Routes	Dose Exposure period	Effects	NOAEL/ LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
							intolerance in adult mice exposed prenatally at 0.25 µg/kg bw/d).	
				<u>6 months:</u> - ↗ in plasma levels of triglycerides and glycerol		Increased triglycerides	**Recognised**	**

Effects on the mammary gland

Summary of studies on the effects of bisphenol A on the mammary gland

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
Studies by the oral route								
Betancourt *et al.*, 2010a	Sprague-Dawley rats	Oral	0 - 25 - 250 µg BPA/kg F0: Exposure of mothers to BPA from GD10 to GD21 followed by single dose of DMBA on PND50 or PND100 F1: exposure not checked	**<u>Effects observed:</u>** - *In utero* exposure to 250 µg/kg of BPA **associated with a single exposure to DMBA** at 100 days postnatally (but not at PND50), produced an increase in the incidence of mammary tumours and a shorter latency time compared to the control group. - **Without DMBA and at the dose of 250 µg BPA/kg**, an increase in cell	LOAEL 250 µg/kg NOAEL 25 µg/kg bw/d	Increased carcinogenic effect of an initiator (DMBA) and delay in window of susceptibility to DMBA (Cell proliferation)	Suspected (animal)	** Shift in the period of susceptibility to DMBA (carcinogenic initiator) positive effects when DMBA injected at PND 100 but not at PND 50 Strengths: -large sample size ; - phytoestrogen-free diet (all)

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				proliferation and overexpression of some proteins involved in cell proliferation was observed. **<u>Critical effect:</u>** - Amplification of breast tumour development (number/rat and time to occurrence) in a DMBA model - Expression of proteins involved in cell proliferation - Changes in proteins influencing cell proliferation at PND100 (250 µg/kg)				-use of non-PC cages and of non plastic bottles diet (all) Weaknesses : insufficient study reporting (e.g. tumour incidence, timing of necropsy)

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				- ERα, PR-A, Bcl-2, steroid receptor coactivators, (SRCs), EGFR, IGF-1R and phospho-c-Raf.				
Betancourt *et al.*, 2010b	Rats	Oral	0 – 25 - 250µg BPA/kg GD10 - GD21 Female descendants were sacrificed at PND21 and PND50.	↗ phospho-AKT, ↗ c-Raf, phospho-ERKs-1 and 2, ↘ TGF-β in breast tissues at 50 days postnatally Important signalling pathways were disrupted by BPA. Prenatal exposure to BPA resulted in deterioration of protein expression in the mammary gland	NOAEL/LOAEL could not be determined (mechanistic)	No NOAEL for the endpoints observed		Proteomic analysis at PND21 and PND50. Study cannot be used for selecting the key study in the HRA.

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				postnatally.				
Jenkins *et al.*, 2009	Female Sprague-Dawley rat pups	Oral	0 - 25 and 250 µg/kg bw/d, 5 d/w Administered to lactating mothers (n=5-8) from PND2 to PND202 (corresponding to 15 administrations/mother). The female rat pups (n=24-34) were treated with a single dose of DMBA at PND50.	↗ in tumour incidence with co-exposure at high dose **NOAEL 25 µg/kg bw/d** **LOAEL 250 µg/kg bw/d**	NOAEL: 25 µg/kg bw/d LOAEL: 250 µg/kg bw/d	Tumour-promoting effect in postnatal period. (because only one study not confirmed)	Suspected	** Strengths: -large sample size, -oral administration by gavage - phytoestrogen-free diet (all) -use of non-PC cages and of non plastic bottles diet (all) Weaknesses: -Study design *(*cell proliferation and

Reference	**Species**	**Routes**	**Dose** **Exposure period**	**Effects**	**NOAEL/LOAEL**	**Critical effect**	**Rating of effects**	**Remarks / Study limitations**
								apoptosis was measured at 12 months of age in TEB only
Moral *et al.*, 2008	Sprague-Dawley rats (10 animals per group)	Gavage	0, 25 and 250 µg/kg bw/d (n=10) GD10 to GD21	Effect: Increase in the number of undifferentiated epithelial structures (TEB and TD). No effects on proliferation; BPA exposure changed the gene expression signature: - altered gene expression, maximal at 100 d with the high dose (genes up-	NOAEL: 25 µg/kg bw/d	Increase in TD (at D21 and D100) and TEBs at D21 only, and modulation of gene expression maximum at D50.	**Recognised**	** *Strengths:* *-large sample size* *-oral administration by gavage* *- phytoestrogen-free diet* *-study design (comprehensive histology of TEB, AB and lobules*

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				modulated at both doses, including a cluster related to immune response; underexpressed genes including differentiation-linked genes at high dose). - At low dose, the expression profile was changed most at 50 d. (+see detailed description and strengths and weaknesses of the study in section B.5.9.2.3.)				*types 1)* *Weaknesses:* *The type of epithelial cells undergoing proliferation was not specified*
Tharp et al, 2012	Female monkeys (female			see detailed description and strengths and weaknesses of	LOAEL = 400 µg BPA/kg bw			

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
	offspring n=4, controls = 5)			the study in section B.5.9.2.4.	per day			
Studies by the subcutaneous route								
Durando *et al.*, 2007	Female Wistar rats	Subcutaneous pump	0 - 25 µg/kg GD8 to GD23 (GD1 corresponds to the day the sperm plug was in the females) Female pups were treated with a single dose of NMU at PND50 and sacrificed at PND110 and 180.	↗ in cell proliferation/apoptosis ratio.	$LOAEL_u$: 25 µg/kg		**Recognised**	*Strengths* -use of non-PC cages and of non plastic bottles -multiple tests performed to address the same endpoint -correlation between morphological and functional changes assessed -mechanistic plausibility

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				↗ in signs of desmoplasia	$LOAEL_u$: 25 µg/kg		-	*Weaknesses* -single dose level study -animal diet and phytoestrogen content not measured - Low number of animals tested for histological examination -The type of epithelial cells undergoing proliferation was not specified

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				↗ in ductal hyperplasia	$LOAEL_u$: 25 µg/kg		**Recognised**	
				↗ in neoplastic lesion	$LOAEL_u$: 25 µg/kg		Suspected	
Jones *et al.*, 2010	BRCA1-deleted mice	Subcutaneous pump	250 ng BPA/kg bw/d	BRCA1 deletion followed by BPA exposure stimulated the mammary gland leading to hyperplasia compared to the control	$LOAEL_u$: 250 ng BPA/kg bw/d		-	Animal not intact Transgenic mice. Results difficult to interpret.
Munoz del Toro *et al.*, 2005	CD1 mice	Subcutaneous pump	0 - 25 - 250 ng/kg bw dissolved in DMSO GD9 to PND4	↗ in response to oestrogens from the dose of 25 ng/kg bw on a batch of ovariectomised animals (n=10). ↗ in expression of progesterone	LO(A)EL: 25 ng/kg bw NB: LOEL 250 for number of TEBs	↑ in branching of ducts, epithelial PR and ↓ in markers of apoptosis	**Recognised**	** Strengths: -intact animal -phytoestrogen

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				receptors from the dose of 25 ng/kg bw. ↗ in branching of terminal ducts and ↓ in markers of apoptosis in TEBs at the dose of 25 ng/kg bw.		in TEBs		contamination of food evaluated by E-screen - Weaknesses: -only 2 doses -6 to 10 animals treated
Murray *et al.*, 2007	Wistar-Furth rats (number of dams exposed not reported; apparently, ≤6 offspring/group were examined histopathologically)	Subcutaneous pump	0 - 2.5 - 25 - 250 - 1000 µg/kg bw GD9 to PND1	↗ number of intraductal hyperplasia in mammary gland at all doses (more pronounced at PND50 compared to PND95). +see detailed description and strengths and weaknesses of	LOAEL: 2.5 µg/kg bw.	Intraductal hyperplasia	**Recognised**	** *Strengths:* *-number of doses* *- phytoestrogen-free diet* *-use of non polycarbonate (PC) cages and of non plastic*

Reference	Species	Routes	Dose Exposure period	Effects	NOAEL/LOAEL	Critical effect	Rating of effects	Remarks / Study limitations
				the study in section B.5.9.2.3.				*bottles*
				CIS present in mammary glands of animals exposed to the highest doses at puberty and at 3 months.	LOAEL: 250 for CIS (NOAEL of 25 for CIS)	Intraductal carcinoma *in-situ*	Suspected	Due to the observed pathology (CIS), even though the number of animals used is low
Vandenberg *et al.*, 2007b	Female CD1 mice	Subcutaneous pump	0 - 250 ng BPA/kg bw/d (n=40 foetuses vs n= 36) GD8 to GD18	↗ in ductal area ↘ in cell size Delay in intra-duct lumen formation Adverse changes in mammary gland phenotype	LO(A)EL 250 ng BPA/kg bw/d	Effects on the phenotype of the mammary gland	**Recognised**	The number of mothers treated was not clearly specified.
Vandenberg *et*	Female CD1	Subcutane	0 - 0.25 - 2.5 -	Impaired development of	LOAEL 0.25	Ductal hyperplasi	**Recognis**	Non-monotonic

Reference	**Species**	**Routes**	**Dose** **Exposure period**	**Effects**	**NOAEL/LOAEL**	**Critical effect**	**Rating of effects**	**Remarks / Study limitations**
al., 2008	mice	ous pump	25 µg/kg bw/d GD8 to PND16	mammary glands: ductal hyperplasia ↗ in proliferation indices compared to control group	µg/kg/d	a non-monotonic dose/effect relationship	**ed**	hyperplasia/dose relationship at 12-15 months.
Wadia *et al.*, 2007	Outbred CD-1 mice Inbred C57B16 mice	Subcutaneous pump	0 - 250 ng/kg bw/d Mixed exposure to BPA and E2 GD8 to PND2	Perinatal exposure to BPA does not adversely affect the uterine response to E2 administered from PND25 to PND35 but does adversely affect the uterine response of the mammary gland.	No $NOAEL_u$ / $LOAEL_u$ could be identified			Co-exposure with E2
Studies by other routes								
Doherty *et al.*, 2010	CD1 mice	Intra-peritoneal	0 - 5 mg/kg DES: 10 □g/kg GD9 to GD26	↗ in histone H3 trimethylation ↗ in EZH2 (2X) expression in	$LOAEL_u$: 5mg/kg of BPA via IP has similar effects to			The dose administered is not suitable for consideration

Reference	**Species**	**Routes**	**Dose** **Exposure period**	**Effects**	**NOAEL/LOAEL**	**Critical effect**	**Rating of effects**	**Remarks / Study limitations**
				mammary tissues compared to the control	those induced by 10 µg/kg of DES			in the HRA Mechanistic study

Key:

nm: non-monotonic

$NOAEL_u$ ($_u$ for a single dose)

NOAEL: No Observed Adverse Effect Level

$LOAEL_u$ ($_u$ for a single dose)LOAEL: Low Observed Adverse Effect Level

**** good quality study

* quality study of lesser quality

Annex 5: ANSES comments on EFSA draft opinion (2014)

OPINION

of the French Agency for Food, Environmental

and Occupational Health & Safety

in response to the consultation of the European Food Safety Authority

on its draft Opinion regarding the assessment of risks to human health related to dietary exposure to Bisphenol A

BACKGROUND OF THE REQUEST

On 9 June 2009, the Agency received a formal request from the Directorate General for Health (DGS) for a health risk assessment (HRA) of exposure to category 3[82] (R3) reprotoxic (according to Directive 67/548/EC) and/or endocrine disrupting (ED) substances found in consumer products marketed in France. This expertise covered the general population, including vulnerable populations and people in the workplace handling so-called 'mass-market' consumer products in the context of their professional activity (excluding production, processing, distribution and disposal).

In this context, in 2013, ANSES published an Opinion on the risks to human health associated with bisphenol A (BPA) taking into account not only exposure related to consumer products but also exposure from other media (drinking water, foodstuffs, domestic dust, air). This Opinion presented the expertise work undertaken by a Working Group on endocrine disruptors and category 3 reprotoxic substances (ED WG) created by ANSES in 2010. The expert appraisal report on the health effects of BPA produced by the ED WG was submitted to several expert groups at ANSES and validated by the Expert Committee on the Assessment of the risks related to chemical substances in February 2013 (ANSES, 2013).

From 25 July to 15 September 2013, EFSA published an interim report on the assessment of BPA exposure on its website for public consultation. All interested stakeholders were invited to submit their written comments before 15 September 2013.

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[82] Substances classified as category 3 reprotoxic according to Directive 67/548/EEC are now classified as toxic to reproduction, category 2 according to (EC) Regulation no. 1272/2008, known as the CLP (Classification, Labelling, Packaging) Regulation. In this document, substances are classified based on the CLP Regulation.

ANSES contributed to this public consultation by analysing the online report and attaching the observations of the French National Agency for Medicines and Health Products Safety (ANSM), more specifically on the theme of cosmetics.

A table summarising the 42 comments made by ANSES and ANSM that were submitted online on the EFSA website can be found in the annexes of this Opinion (Annex 1).

On 17 January 2014, an EFSA draft Opinion on the risks to health related to BPA in foodstuffs was published on the EFSA website for consultation. This draft Opinion drew on an analysis of published data on BPA up to the end of 2013. The experts conclude that BPA does not pose risks to consumers at the current levels of exposure through food and the handling of thermal receipts containing BPA. In this draft Opinion, EFSA proposes a temporary TDI that relies on the results of the study by Tyl *et al.* (2002, 2008).

On 7 February 2014, ANSES issued an internal request to analyse certain points of the EFSA draft Opinion.

EXPERT APPRAISAL METHOD

Given the limited time-frame to respond to the consultation, the expert appraisal was undertaken by several expert rapporteurs from the ED WG with expertise in toxicology, modelling (*PB-PK* and *BMD* modelling in particular), uncertainty analysis and kinetics, as well as experts specialising in effects on the mammary gland, the central nervous system, the female reproductive system and metabolic diseases. Each expert was mandated to assess a specific part of the EFSA draft Opinion.

The expert appraisal primarily focused on the main differences in the interpretation of data versus the ANSES reports published in 2011 and 2013. It also specifically addressed new aspects of the risk assessment process proposed in the EFSA draft Opinion.

The results of the expert appraisal presented below take into account the experts' comments. They cover specific points of the EFSA draft Opinion that can influence risk assessment results: the choice of publications taken into account, the selection of the critical effect(s), BMD calculation, the estimation of internal exposure in humans and the treatment of uncertainty. Comments specific to certain studies and additional information provided by ANSES have been attached to this Opinion. Quotes from the EFSA draft Opinion appear italicised and in quotation marks.

Given the short time-frame provided for the consultation organised by EFSA and the considerable background work undertaken by this agency, the experts were mobilised in a context of urgency. This Opinion does not intend to present a full expert assessment of the safety of BPA but highlights some major questions and identifies potential improvements to be made following a reading of the EFSA draft Opinion.

RESULTS OF THE EXPERT APPRAISAL

General comments

Publications taken into account

The analysis of epidemiological studies is not particularly covered in this Opinion given that EFSA and ANSES interpret the results of these studies in a similar manner. The observations in this section only apply to experimental data for which there are differences in interpretation between the two agencies.

ANSES observes that this new health risk assessment for BPA not only takes into account studies on oral exposure but also studies on subcutaneous exposure, which was not the case in previous EFSA reports. Most of the studies undertaken to examine the toxicity of BPA were not carried out according to the OECD guidelines and/or did not adhere to 'Good Laboratory Practice' (GLP). These studies were nonetheless taken into account in the EFSA assessment, even though EFSA gave greater weight to OECD studies undertaken in accordance with GLP (e.g. Tyl, 2002, 2008). Several recent studies published after the ANSES expert appraisal were also included in the assessment, potentially providing additional information, particularly on certain critical effects such as metabolism for which little information was available until recently. That said, ANSES considers that so far, none of these studies fundamentally call into question the conclusions of its expert appraisal on the nature of the health effects of BPA. Specific comments by type of effect are given in the rest of this Opinion, although the articles not taken into account in the ANSES 2013 report have not been specifically analysed for this call for comments.

ANSES notes that most of the non-OECD/GLP publications assessed in the EFSA draft Opinion have been criticised for various criteria such as the number of animals and control animals, consideration or non-consideration of the 'litter effect', animal housing conditions such as types of cages and diets (e.g. phyto-oestrogen-free or not), BPA exposure conditions including route of exposure, number of doses, blind evaluation, correlation between biochemical effects and anatomical or functional lesions, etc. However, it is unfortunate that these criteria have not been classified. Furthermore, other criteria that are nonetheless essential for the interpretation of results, such as the exposure period, hormonal sensitivity during development and puberty, etc. appear not to have been given the same importance.

Weight-of-evidence assessment

The hazard assessment of BPA proposed by ANSES in 2011 relies on a classification of effects as effects that are 'recognised', 'suspected', 'controversial' or 'effects for which no conclusion can be drawn on the basis of the available data' depending on the number and quality of available studies.

The approach used by EFSA is based on the weight of evidence estimated by the experts considering the quality of the data *corpus* by type of effect. However, while this approach has the advantage of systematically analysing lines of evidence in response to a specific issue, it can cause the *corpus* of data and publications to become over-fragmented, ultimately meaning that there is not sufficient perspective to judge a set of arguments that may be part of a *continuum* of similar effects. For example, regarding the effects of BPA on metabolism,

subdivisions are made by period of exposure for animal testing (prenatal exposure and exposure in adulthood), and for each exposure period, new subdivisions are made for each study parameter (weight, glucose tolerance, insulin sensitivity). All of these subdivisions lead to the fragmentation of information included in the same scientific article and can cause confusion for the reader. The same is true for other effects such as effects on the mammary gland and brain. Conversely, grouping together several different effects in the final weight-of-evidence analysis can result in a lack of consistency in the data analysis (e.g. for the mammary gland, grouping of morphological changes, cell proliferation and atypical ductal lesions under the same item).

The classification of effects based on plausibility criteria ("*likely*", "*as likely as not*", etc.) is not clearly justified in the draft Opinion, even though the expert assessment is intended to draw conclusions based on the available data. Therefore, it would be desirable, for the transparency of the expert assessment, to further stress these assessment criteria in the final report. For example, no criteria are offered to consider that the available studies for a given line of evidence have low, medium or high reliability. This is even more surprising considering that, for certain lines of evidence, there are studies that only have weaknesses (see Table 29, "*Starting point*", page 421, and *Line 5*, page 423), while for others, there are studies that have both strengths and weaknesses (see *Lines* 1 to 4, pages 421-423). That said, for the vast majority of the lines of evidence, EFSA grants a low level of reliability to the data, whether the lines of evidence are strong or limited. This approach therefore focuses on the limitations of studies in terms of their level of evidence.

The way in which the studies as a whole have been included to address the issue raised and assess reliability so as to conclude as to the likelihood of an effect ("*overall conclusion on likelihood*") is not clearly described (see Table 30, p. 427).

It is stated (page 208) that the assessment of terms for expressing likelihood ("*very likely, likely, etc.*") fully relies on expert judgement. Two issues remain unclear:

- For each line of evidence, a scientific judgement must be made by experts specialising in the issue (ECHA, 2010). However, in the EFSA draft Opinion, this process is not described.
- The method for addressing potentially diverging opinions among the working group's members is not clearly explained. Did all of the group's experts assess these criteria ("*likely*", etc.) in the same way for the same line of evidence? If that was not the case, how were divergences taken into account, or not?

This degree of subjectivity is supported by the abundant use of terms such as "*acceptable*", "*convincing*", "*evidence...too weak*" used without being defined.

Non-monotonic relationships

Several experimental studies on BPA exposure have reported non-monotonic dose-response relationships (Jenkins *et al*., 2011; Jones *et al*., 2011; Marmugi *et al.,* 2012, etc.). These studies were taken into account in the ANSES expert appraisal and the statistical and biological likelihood of there being non-monotonic relationships was assessed and confirmed in a number of cases. However, no scientific consensus has been achieved as to the quality of the studies or the extent of evidence supporting the assumption of non-monotonic relationships for BPA. Therefore, EFSA has taken into account, with a lower level of evidence, studies that did not show an increasing dose-response relationship.

Hazard characterisation: choice of critical effects

ANSES observes that some of the critical effects deemed "recognised" in its 2013 expert appraisal are considered "as likely as not" or even "unlikely" by EFSA. Specific comments on this type of effect can be found in the sections that follow. The sections of the EFSA draft Opinion dealing with effects not addressed in the ANSES expert appraisal on health risks related to BPA (ANSES, 2013) are not specifically analysed in this Opinion.

Effects on the female reproductive system

In the ANSES expert appraisal (ANSES, 2013), the following effects observed in animals with pre- and/or post-natal exposure were considered sufficiently worrying and relevant to be taken into account:

- Increase in the occurrence of ovarian cysts;
- Increase in the frequency of endometrial hyperplasias;
- Disruption of ovarian cycles.

The study ultimately chosen by ANSES for the HRA was the study by Rubin *et al.* (2001) which showed a disruption of the ovarian cycle with lengthening of the oestrous cycle. This study on oral exposure gave a NOAEL of 100 µg/kg bw/day and a LOAEL of 1200 µg/kg/day after treatment from GD6 until weaning in Sprague-Dawley rats.

The divergences in the scope of conclusions between the ANSES report and the EFSA draft Opinion are due to different methodologies. It appears that the classification established by the EFSA working group requires there to be a negative biological modification in conjunction with the effects observed. And yet studies rarely explore in detail gonadotrope activity function in terms of fertility. It also appears that some divergences in the classification of studies are linked to the way in which the two groups approach methodological biases. Indeed, the EFSA working group considers that not considering properly the litter effect or the statistical analysis is a major methodological limitation that impacts in particular the strength of the study by Rubin *et al.* (2001) chosen by ANSES's experts as a key study for the identification of hazards to the female reproductive system.

The two agencies use different methodological bases to classify effects. The ANSES ED WG established a classification based on a structured decision tree whereas EFSA issues an overall score by system (see page 436 overall conclusion on the effects of BPA on the male and female reproductive system) for exposure to BPA in the development phase while ANSES's assessment is based on an analysis by type of effect (effects on the genital tract and ovaries, effects on the hypothalamic-pituitary-gonadal axis, effects on the onset of puberty, etc.). EFSA mentions that the lack of convergence between studies is a source of too much uncertainty. This assessment may appear justified when considering the system as a whole. However, this uncertainty is significantly reduced if the data in the literature are analysed effect by effect. As for the EFSA analysis of the functional significance of the observed effects, it is undeniable that this type of information may be the cornerstone to hazard assessment. However, rejecting effects because this information is not available can mean disregarding recognised scientific facts where knowledge of functional physiology suggests they may have negative consequences on the effectiveness of this function.

The analysis of the scientific literature from 2011 to 2012 undertaken by the ED WG highlighted an effect on folliculogenesis with developmental exposure. According to the decision tree adopted by the ED WG that was used for the classification of effects, these effects could be considered "recognised". The EFSA experts rightly point out that the functional significance of this type of effect, particularly in terms of fertility impairment, remains to be determined. It still remains true that the mechanisms highlighted in the various studies undertaken in different species are often associated with changes in follicular dynamics and sometimes depletion of follicular reserves. A good-quality publication identified by the ED WG indicates that bisphenol A at low doses (25 ng/kg subcutaneously) with exposure during the development phase (GD8-PND16) could accentuate the decline in ageing-related fertility in CD-1 mice (Cabaton *et al*., 2011). Although it is impossible, in the current state of knowledge, to establish a direct cause-and-effect relationship, the assumption that such an effect could be related to changes in follicular dynamics underlines the importance of not neglecting the possible impact of BPA on folliculogenesis. Furthermore, it appears that the effects of BPA on ovarian follicles can also appear with exposure in adulthood. For example, the EFSA assessment mentions a good-quality study that shows that subchronic (90 days) oral exposure to low doses (1 and 100 µg/kg bw/day) in young adult female rats (Lee *et al*., 2013) caused augmentation of follicular atresia and luteal regression while reducing ovarian steroidogenesis and stimulating apoptosis. These ovarian changes were associated with an increase in the synthesis and release of pituitary LH and lengthening of the oestrous phase. According to the rules for the classification of effects adopted by the ED WG, these effects cannot be classified as recognised due to a lack of other converging data on effects on fertility decline and effects on ovarian follicles in adults. However, the string of assumptions and the likelihood of an impact on fertility are sufficiently significant to draw the attention of experts to the effects of BPA on ovarian follicles and their possible consequences in terms of fertility.

Effects on the central nervous system

Of all of the observed effects regarding the toxicity of BPA to the central nervous system, the critical effect selected by the ANSES experts involves the impairment of memory and learning, concurrent with a decrease in the expression of various subunits of glutamate NMDA (N-methyl-D-aspartate) receptors, which are particularly involved in synaptic and neuronal plasticity and in memory and learning processes. These effects are also reinforced by the action of BPA in neural systems expressing nitric oxide synthase (NO synthase) with sex- and region-dependent effects in the hypothalamus and limbic system (Martini *et al.*, 2010).

The study by Xu *et al.* (2010a) was chosen by ANSES as the key study. This study was undertaken by oral administration (gavage) in ICR mice (n=10 animals/group) and included four exposure doses in addition to the control group: 0.05; 0.5; 5 and 50 mg/kg bw/day. Ten gestating mice per dose level were exposed from GD7 to PND21. This study did not adhere to the OECD guidelines or GLP. Nonetheless, the study protocol is clearly described and many molecular (NMDA receptors, oestrogen receptor β) and physiological effects were investigated. The reduced expression of NMDA receptors observed in the hippocampus in this study was reproduced by the same team in Sprague Dawley rats (Xu *et al.*, 2010b), in similar conditions, and by other teams (Tian *et al.*, 2010).

The choice of the Xu *et al.*, 2010a study is supported by studies whose results provide a string of assumptions on the brain damage induced by BPA in relation to cognitive effects. The study

by Martini *et al.* (2010) shows changes in the expression of cerebral NO synthase (NOAEL 10 µg/kg/day) in mice exposed orally. The study by Tian *et al.* (2010) highlights changes in the dopaminergic and glutamatergic systems (NMDA) together with cognitive deficits and decreased anxiety in mice exposed orally (LOAEL 100 µg/kg/day). The study by Xu *et al.* (2010b) shows a decrease in the expression of certain glutamate NMDA receptor subunits and oestrogen receptor β (ER β) in rats exposed orally (LOAEL 50 µg/kg/day). Studies on subcutaneous exposure, such as that by Zhou *et al.* (2011), make a connection between changes in synaptic and neuronal plasticity and behaviour in rats with an LOAEL of 2 µg/kg/day.

In its draft Opinion(see page 303, EFSA identifies several weaknesses in the study by Xu *et al.* (2010a):

- "*However, in the absence of a correlation with a functional adverse effect, the Panel did not consider the available data as convincing evidence of neurobehavioural toxicity of BPA*."

ANSES comments: one of the strengths of the study by Xu *et al.* (2010a) is precisely that it showed a link between changes in synaptic and neuronal plasticity mechanisms in specific cerebral regions (hippocampus) and functional behavioural impairment (spatial learning and conditioning). This is a surprising comment from the EFSA experts, since this study does indeed link various aspects of cerebral function in molecular and behavioural terms.

- "*Study design (no wash-out period between different test procedures)"*

ANSES comments: the wash-out period between different procedures has never been given special attention by EFSA in the studies taken into account in previous reports. It could be considered that a wash-out period would be necessary if successive tests were undertaken with the same study parameter, which is not the case of the key study chosen by ANSES. Indeed, even though the tests carried out by Xu *et al*. (2010a, 2010b) studied the learning and memorisation capacities of animals, two types of memory were successively explored on PND21 and PND56 in the same animals that had been exposed early on to BPA: spatial memory with the Morris water maze and emotional and contextual memory with a conditioning test associating negative reinforcement with the reinforcement context. The Morris water maze, which is above all dependent on the plasticity of the hippocampus, a key region for spatial learning, was used 1st while the 2nd test examined emotional memory and the activity of the limbic system involving the amygdalae, even though this system interacts with the hippocampus. These considerations suggest that successively undertaking the two tests in the same animals does not bias the results and that the lack of a wash-out period between the two tests is not a study weakness. As a precaution, Xu *et al.* could have alternated the order in which the groups took the two tests so as to offset the effects of potential interactions between them. However, even if different tests investigate the same type of memory, it is not at all mandatory to have a wash-out period between them. Indeed, an experimental protocol can be designed so as to successively carry out various tests using the same parameter to see if different types of events can modify the same parameter (e.g. working memory, anxiety, depression, etc.). Thus, the lack of a wash-out period is not a study weakness.

- *"Test performed in one sex only (only male offspring)"*

ANSES comments: a study performed in males only is not a weakness but is intended to focus on effects that can be induced in a specific population. Furthermore, the results obtained by Xu *et al.* are reliable enough to be used for the expert appraisal even if they only apply to males.

- *"Insufficient study reporting (reproductive outcome not shown, e.g. maternal bw, no pre-weaning body weight data shown)"*

ANSES comments: the data on the body weight of pups, produced on PND21, show a significant decrease at the lowest dose of BPA (0.05 mg/kg bw/day) and a significant increase at the highest dose (50 mg/kg bw/day) versus the controls. The same variations were observed on PND56 but the difference at the highest dose was no longer significant. It is indeed unfortunate that data on litter growth in the first three weeks of life were not provided by the authors so as to be able to attribute these variations to BPA exposure only and not other biases, such as differences in litter weight at birth depending on the number of pups and differences in maternal behaviour. Even so, the cerebral and behavioural differences observed in the groups exposed to BPA were such that they could not be attributed to differences in body weight related to a larger litter or under-developed maternal behaviour. Indeed, the brain is an organ whose growth is preserved in the early phase of development in the event of under-nutrition for example.

- *"Statistical analysis (litter effect not considered, i.e. no information about one male pup/litter)"*

ANSES comments: although they did not adhere to the OECD 426 guideline, the authors included ten gestating female mice per exposure group in the study and used one male per litter to make up the experimental groups whose behaviour was tested. By doing so, Xu *et al.* (2010a) considered the mother as the statistical unit. The inclusion of ten mothers per group, each represented by one pup from each litter, thus eliminated the litter effect, which would not have been the case and would have made testing necessary if all of the pups in each litter had been evaluated for their behaviour. The study's only weakness is the lack of information about the selection of the pup in each litter.

- *"Information about type of water bottles is missing"*

ANSES comments: in the study by Xu *et al*. (2010a), no information is given regarding the materials used for the water bottles. However, the study was chosen based on the following arguments:

- The study links the effects of BPA on memory to significant changes in NMDA receptor expression in the hippocampus, a cerebral structure involved in memory and learning (40% decrease in the expression of some of this receptor's subunits). A shortage of NMDA receptors induces considerable and sometimes permanent cognitive impairment.

- Although the effects on NMDA receptor expression are the most significant, the effects on memory were chosen by the ED WG as critical effects since it is always difficult to know whether a physiological, cellular or biochemical change can have harmful consequences for an individual.

- These effects are part of a *continuum* of effects, also observed in other studies, on cognitive function and causing histochemical changes in various cerebral structures (Adewale *et al.* 2011; Martini *et al.,* 2010; Bai *et al.*, 2011; Zhou *et al.*, 2011; Rubin *et al.*, 2006).

Lastly, the study was also taken into account by ANSES's experts, despite the poorly controlled BPA environment, considering the following two cases: (i) Environmental BPA induces the same effects as those described in the study. In this case, the BPA received experimentally aggravates the effects induced by environmental BPA, which leads to differences in effects between the controls and exposed individuals. (ii) Environmental BPA induces effects opposite to those observed. In this case, the BPA received experimentally first cancels out these effects and then induces opposite effects, which also leads to a difference between the controls and treated individuals.

It is surprising that this study was not taken into consideration in the weight-of-evidence analysis, given that other studies with this type of weakness were used in the EFSA draft Opinion (see *11.2 Table* 34).

A more recent study by the same team (Xu *et al.*, 2013) was evaluated by the EFSA experts, who mention that one of this study's weaknesses is that the doses were not adjusted to the weight of the animals, whereas the doses do seem to have been adjusted to the weight of the individuals: *"The body weight of each mouse was weighed every week to adjust the drug volume"*. According to ANSES's experts, this was a well-conducted study in which the authors took many precautions to avoid environmental contamination with phyto-oestrogens and BPA. The results are in line with the study by Xu *et al.*, 2010 in mice. Effects on the markers of synaptic function were observed from 0.4 mg/kg/day. Effects on glutamate receptors were observed at 0.4 and 40 mg/kg/day. This study has the advantage of combining cognitive effects with histological changes. ANSES's experts are surprised by this comment regarding the study by Xu *et al.* published in 2013 in *Hormones and Behavior,* as the same statistical procedure was used in other studies published by the same authors and mentioned by EFSA with no such comments. Substantively, Xu *et al.* 2013, like in the key study chosen by ANSES, used Tukey's test to make *a posteriori* comparisons in the various variance analysis models used. Tukey's test is a conservative test that was developed to guarantee the probability of risk □ for all possible comparisons unlike the Newman-Keuls test for example.

Other studies reported effects of BPA on learning and memory after a single exposure (Eilam-Stock, 2012; Inagaki, 2012), which the EFSA experts consider to be a weakness. According to ANSES's experts, this type of exposure is not necessarily a weakness insofar as the aim is to take into account the toxicity induced by acute exposure, which can be quite relevant when considering that single exposure can induce harmful effects that are sometimes irreversible.

Furthermore, the use of positive controls is considered a strength in the studies evaluated by the EFSA expert committee. And yet the inclusion of a positive control in a study implies that the positive control and the substance of interest induce the same type of effect. Thus, the types of effects induced by the substance of interest are prejudged and any deviation from the effects induced by the positive control reduces the level of confidence attributed to the effects induced by the substance.

Thus, the lack of a positive control is considered to be a weakness for a study while its presence is a strength. However, several objections limit the usefulness of a positive control:

- The use of a positive control prejudges the substance's mode of action which is far from being characterised and therefore far from being known.

- For bisphenol A, it is clear that there are effects not related to oestrogenic action.

- In the event that the positive control and substance have the same mode of action, the doses (and therefore the internal concentrations) at which effects are induced may be different depending on the affinity of the positive control or substance for the same targets.

- The same substance can induce different effects at different doses since the biological targets are not the same depending on their affinity for the substance. This is particularly true for hormones and endocrine disruptors. Thus, it is difficult to compare the effect of a substance, which could be the same as that of the positive control at one dose and different at another dose. This is true for both the substance and the positive control. For example, LHRH agonists first induce an increase in testosterone and then at high doses or with extended exposure almost completely reduce plasma testosterone.

More broadly, ANSES's experts consider it unfortunate that the EFSA expert assessment does not consider the effects of BPA in terms of impaired cerebral development further to pre- or peri-natal exposure to be relevant effects for the risk assessment. Whereas significant consideration is given to studies reporting this type of effect in Section 3.4.2.2 of the EFSA draft Opinion (sub-section "*Effects on brain biochemistry, neurogenesis, neuroanatomy and gene expression*", pages 96-97), these effects are not included in the WoE approach in Section 11 of the same draft Opinion.

Other comments on studies assessing the effects of BPA on cerebral function not considered in the ANSES report published in 2013 can be found in the annexes of this Opinion.

Effects on metabolism

As stated above in the general comments, the approach used by EFSA on the weight of evidence for a given effect separates various effects that can be related and be part of a *continuum* that should also be analysed as a whole. For example, a sub-section of the draft Opinion is devoted to the 'weight gain' parameter and the EFSA experts cite various articles reporting or not reporting weight changes. The following point, 'insulin', reports whether changes in insulin secretion and glucose tolerance have been described. The various studies that monitored this parameter are reported. And yet it is obvious that if an animal gains weight after a treatment, this could have repercussions on insulin resistance and glucose tolerance. It is therefore important to analyse all study parameters to have an overall idea of the metabolic impact of BPA. With the subdivision presented in the EFSA draft Opinion, it is difficult for the reader to form an opinion of the effects of BPA on metabolism, even more so given that lipid metabolism is closely related to carbohydrate metabolism.

In addition, the EFSA expert committee draws the following conclusions for each sub-section:

- the lack of a dose-response relationship (see line 4763),
- obtaining contradictory results that are difficult to reconcile (see line 4768, line 4797),
- a non-conclusive statistical analysis (see line 4805),
- a small magnitude of effects (see lines 4817-4818),
- a 'litter' effect not taken into account,
- that it is difficult to understand the underlying mechanisms (see line 4919).

Some other terms used should be clarified, such as: "*not clear cut*" (see line 4847), "*unclear*" (see line 4851) and "*methodological deficiencies*" (see line 4869). In the end, the expert committee indicates "*the assumption of non-monotonicity is not supported by the data*" (see line 4961) and "*the high fat feed intake cannot be considered as a good model for human health assessment*" (see line 4963).

Moreover and regarding *in vitro* studies, EFSA recognises that it is highly likely that nanomolar concentrations of BPA can affect insulin secretion *in vitro* (see line 5008) but that considering the limitations of *in vitro* models, the relevance of results obtained on the impact of BPA on the physiology of pancreatic β cells remains to be specified ("*is currently unclear*", see line 5010).

Regarding non-monotonic relationships, the EFSA expert committee rejects studies for two reasons:

- U-shaped or bell curves cannot be superimposed with the various biological parameters studied. And yet hormonal sensitivity depends on the tissue that is studied and the hormonal context (development, puberty, adulthood) and the use of feedback in tissues.

- effects observed in response to a fatty diet cannot be taken into account. The diets given to rodents are very different even when considered as standard as opposed to fatty diets, particularly due to their level of soya and dietary fibres. This is a significant point since the metabolism of animals closely depends on diet (Zimmermann C *et al.*, 2012). There is therefore no reason to discard fatty diets and only consider standard diets, especially when studying the obesogenic action of BPA. Moreover, a number of metabolic changes are only highlighted in response to a fatty diet, i.e. when an animal is subject to an imbalanced diet to see its ability to adapt to a new nutritional environment.

In the end, the EFSA expert committee concludes that metabolic effects are "*as likely as not*" while ANSES considers that the available experimental data are sufficient to consider that BPA can have effects on metabolism.

The EFSA expert committee concludes that there is "*reasonable evidence*" that BPA has effects on glucose and insulin regulation and/or pancreatic morphology and function, based on the results of short-term studies, while long-term studies do not show any effects (see line 5020). Even so, in the end, the expert committee concludes that the effects of BPA on metabolism are

"*as likely as not*". It would be worthwhile to explain why the effects observed with short-term studies are not relevant.

Effects on the mammary gland

In its expert appraisal published in 2011, ANSES considered that the effects of BPA on mammary gland maturation were recognised effects in animals and should be taken into account to assess risks to human health. ANSES observes that in its draft Opinion, EFSA also considers that the effects of BPA on mammary gland development are "*likely*" and that these effects can be transposed to humans. However, ANSES considers that it is important to acknowledge the possibility of increased cancer risk in the descendants of women who have a high level of endogenous oestrogens or xeno-oestrogens during pregnancy and are then exposed to tumour-initiating agents. And yet the EFSA experts only include the analysis of the direct carcinogenic effects of BPA on the development of neoplastic lesions in their criteria. They do not take into consideration enhanced susceptibility after early pre- and/or postnatal exposure to BPA, even at low doses, followed by exposure to a carcinogenic agent (e.g. DMBA or NMU) during puberty. This notion, which was already explained in previous reports (EFSA 2006, 2010), is a point of disagreement with ANSES. Effects on the mammary gland are the most significant effects identified by ANSES to assess the risks of BPA. Situations of at-risk exposure have been identified based on these effects.

Moreover, the arguments set out in the EFSA report according to which the rodent model is not a good model for mammary carcinogenesis because it only develops a limited number of cancer sub-types compared to the thirty or so sub-types of human tumours are not justified (page 139, lines 5822-5824). Firstly, in nature, no studies have estimated the diversity of tumours in rodents exposed to a complex environment. Secondly, no animal models used in specific conditions with little variety can mimic the diversity of mammary cancers in women exposed to a complex environment. Most international experts consider that mammary development and carcinogenesis are similar in rodents and humans (Russo and Russo, 1996; Singh *et al.,* 2000; Rudel *et al*., 2011). Furthermore, different rodent strains (rats and mice) can have different sensitivity and susceptibility to carcinogenesis, which should be taken into account in the interpretation of experimental studies.

More specifically regarding effects on maturation and architectural modifications in the mammary gland, after foetal or neo-natal exposure to BPA, changes reported in the terminal ducts (TEBs, where carcinogenesis is likely initiated) and mammary branches at puberty are clearly described in the report (pages 139-140). However, other changes in the organisation of the mammary gland, such as changes in the epithelial-stromal organisation or the maturation of adipose tissue, hormonal changes and metabolic changes that can result in abnormalities in adulthood, are not described in the EFSA report.

ANSES notes that the EFSA report includes the preliminary results of a recent study on chronic carcinogenesis undertaken in 2013 in the USA (US FDA/NCTR, 2013) in Sprague-Dawley rats. Since ANSES's experts have not assessed this study, it is difficult to comment on EFSA's analysis of it but ANSES considers that it should be analysed against other recent publications that appear to show neoplastic lesions (Acevado and Soto, 2013). Furthermore, other publications have not been taken into account, such as the study by Lamartinière *et al*. (2011) which shows an increase in proliferation after exposure during lactation in Wistar rats, while

this study does not have the weaknesses noted by EFSA for studies from the same group (Betancourt *et al.,* 2010 and Jenkins *et al*., 2009).

The spread of data on the mammary gland in the EFSA report is unfortunate as it makes them difficult to interpret and integrate into effects on the mammary gland, an organ that is highly complicated to study and whose particularities should be taken into account. Conversely, the grouping of morphological changes (TEBs, Abs), cellular proliferation (including simple ductal hyperplasia) and atypical ductal lesions in the same line of evidence can interfere with the interpretation of data.

Estimation of exposure

Toxicokinetics and metabolism

An analysis of recent data does not show major differences in interpretation between ANSES and EFSA regarding the absorption, distribution, metabolism and elimination of BPA. However, the following explanations and comments should be made:

- **"***Because of the high activity of the conjugation enzymes the percentage of unconjugated BPA in the blood is only a few percent of total BPA (sum of conjugated and unconjugated BPA)".*

ANSES comments: to comment on the free *versus* total ratio in the blood, it is not enough to describe the activity of conjugation enzymes; it would be better to speak of clearance and write "due to the relatively high BPA clearance compared to the relatively low BPA-glucuronide clearance, the percentage of unconjugated BPA in the blood is only a few percent of total BPA (sum of conjugated and unconjugated BPA)".

- *"Based on the analysis of oral versus intravenous toxicokinetic data, the oral systemic bioavailability of unconjugated BPA in rats is 2.8%, in mice 0.2% and in monkeys 0.9%."*

ANSES comments: this point also appears questionable and is not supported by the recent study by Gayrard *et al*. (2013). The bioavailability values that appear here are those measured after gavage and not by contamination of food. It would therefore be best to write: "Based on the analysis of oral (gavage) *versus* intravenous toxicokinetic data, the oral systemic bioavailability of unconjugated BPA in rats is 2.8%, in mice 0.2% and in monkeys 0.9%".

Moreover, an article in press by Vom Saal (2014) in monkeys indicates oral (bolus) bioavailability of 5%.

More specifically regarding the study by Gayrard *et al.* (2013), ANSES's experts are surprised that EFSA rejected the only study that has explored sublingual absorption, on the pretext that this exposure scenario is unlikely with oral treatment. Gavage is not a likely route of exposure either and the significance of this study is precisely that it shows the possibility of a high-peak concentration of free BPA near the mouth, for example when holding a receipt, plastic pen or polycarbonate spoon in the mouth. In this case, the brain or thyroid can be exposed to high concentrations for a short time and a direct or indirect effect on these organs cannot be excluded.

Exposure scenarios

The exposure scenarios taken into account in the ANSES and EFSA expert assessments are different in that EFSA only took into account a 'consumer/general population' scenario while ANSES also assessed risks to people in the workplace who handle thermal receipts as part of their job. ANSES particularly assessed risks related to BPA exposure for women holding cashier positions subject to much higher exposure levels than the general population.

There are differences between the exposure scenarios assessed by ANSES in its expert appraisal and those taken into account by EFSA.

In its expertise work, ANSES calculated exposure for children over the age of three years (3 to 18 years old), adults (men and women combined) and pregnant women. For these three population categories, the exposure sources taken into account when calculating exposure doses were as follows: food, the ingestion of settled dust and the inhalation of air (exterior and interior). For these three exposure media, an aggregated internal exposure dose was calculated. Regarding the handling of thermal receipts, an internal exposure dose was calculated for pregnant women and adults as consumers, excluding situations of exposure in the workplace.

Exposure scenarios corresponding to people in the workplace handling thermal receipts (pregnant women and adults), such as cashiers, were also developed. The internal doses calculated through skin contact with thermal paper were not aggregated with the other exposure doses calculated by ANSES due in particular to a lower level of confidence associated with these results.

All exposure calculations were made applying a probabilistic approach.

In the end, ANSES undertook a risk assessment for pregnant women only, with three exposure scenarios: pregnant women exposed through food, the ingestion of dust and the inhalation of air; pregnant women as consumers exposed dermally by handling thermal paper; and pregnant women in the workplace (cashiers) exposed dermally by handling thermal paper.

In its "*DRAFT scientific opinion on the risks to public health related to the presence of BPA in foodstuffs – Part: exposure assessment*", EFSA calculated the following BPA exposure sources:

Table 1: exposure sources and population sub-groups considered by EFSA for the assessment of BPA exposure

	Infants (0-6 months) Maternal milk			Infants Infant formula	Childr en	Child ren	Child ren	Adolesc ents	Wom en	Me n	Oth er adul ts	Elde rly peo ple
	1-5 da ys	6 days -3 mont	4-6 mont hs	0-6 mont hs	(6-12 mont hs)	(1-3 years)	(3-10 years)	(10-18 years)	18-45 year s	18-45 yea rs	45-65 yea rs	+ 65 year s

		hs										
Ingestion												
dust		x	x	x	x	x	x	x	x	x	x	x
toys		x	x	x	x	x						
food	x	x	x	x	x	x	x	x	x	x	x	x
Inhalation												
air	x	x	x	x	x	x	x	x	x	x	x	x
Skin contact												
thermal paper							x	x	x	x	x	x
cosmetics		x	x	x	x	x	x	x	x	x	x	x
Total exposure		x	x	x	x	x	x	x	x	x	x	x

All exposure calculations were made using external doses and a deterministic approach.

For each exposure estimate, a mean level and high level were calculated.

ANSES comments:

As stated in Appendix VI of the EFSA report, even though all of the comments received on its "*Exposure assessment*" report had been examined, EFSA was not able to revise this specific part on exposure assessment so it could be included in its risk assessment report covered in this Opinion. This amendment work is currently underway at EFSA.

Therefore, it is not possible to evaluate whether the comments submitted to EFSA by ANSES in September 2013 regarding requests for clarifications, justifications, reformulations, details and additional references to be inserted in the text have been taken into consideration. However, Appendix VI of the document states that the EFSA experts considered that some of the comments received were relevant, and could lead to a change in the calculations. This Appendix presents the changes taken into account that resulted in new exposure figures. It also presents the EFSA experts' rationale for not taking into account certain comments such as those indicating that the assessment should not include some instances of occupational exposure, exposure from medical devices and exposure from dental sealants.

The comments made by ANSES can be found below, although it is not possible to assess whether or not they have been taken into account. Comments on dermal exposure are not addressed here, since this item is covered in a separate part of this Opinion.

Regarding the overall approach to the estimation of exposure, ANSES recommended implementing a probabilistic approach to calculate exposure rather than the deterministic approach used by EFSA. The risk assessment undertaken by EFSA is based on a deterministic approach to calculate exposure, with a mean level and a high level.

EFSA does not take into account any scenarios in the workplace (cashiers handling thermal receipts), considering that this is not part of its scope of expertise.

Regarding BPA exposure through cosmetic products, given that BPA may be found in containers, ANSES's comments generally insisted on uncertainties regarding the presence of BPA in cosmetic products, such that it did not seem possible to calculate a reliable and representative level of exposure to BPA through the use of these products (only six products, choice of body lotions as a benchmark for exposure, etc.). EFSA considers that the assumptions used are the most reliable that can be made based on the current data. An assessment of exposure through the use of cosmetic products is maintained.

Regarding the respiratory volume used in EFSA's calculations, taken from the publication by Trudel *et al*, 2008 and considered to be over-estimated and not representative of a daily respiratory volume as required in the calculation, ANSES recommended referring to the *Exposure Factor Handbook – 2011 edition*. This comment was taken into consideration and the calculations for respiratory exposure were amended by EFSA (see Tables 23A and 23B in Appendix VI).

Regarding the level of ingestion of settled dust used in EFSA's calculations, taken from the publication by Trudel *et al*, 2008, as for respiratory volume, ANSES considered that the values used were unsuitable and taken from an inappropriate publication. This comment was taken into consideration. The calculations for the ingestion of settled dust were amended by EFSA in its report (see Tables 23A and 23B of Appendix VI).

Biosurveillance data

Although exposure is generally determined by assaying BPA in urine, where it is mainly found in conjugated form, a number of studies also report blood concentrations of BPA in adults and in the umbilical cord blood of newborns. In its expert appraisal report on BPA (ANSES, 2013), ANSES thus devoted a paragraph to blood assays and particularly the share of the various forms of BPA (conjugated and unconjugated) in this matrix. Since the toxicity of BPA has been attributed to its unconjugated form, the share of this form in the blood, related among other things to the individual's metabolising capacity, is an essential parameter to be taken into account when assessing the potential effects of exposure.

In its expert appraisal report, ANSES presented mean values of blood concentrations of unconjugated BPA reported by various studies undertaken between 2002 and 2012 in Asia, Europe and the USA ranging from 0.32 to 2.5 ng/mL in adults. A study carried out in Taiwan in a sample of 97 pregnant women (Chou *et al.*, 2011) reported a maximum value of 29.4 ng/mL.

In umbilical cord blood, the study by Fénichel *et al.* (2012) cited in the ANSES report (ANSES, 2013) presented, for a population of 152 newborns, blood concentrations of unconjugated BPA ranging from 0.14 to 4.76 ng/mL, with a mean greater than 1.1 ng/mL.

In its report (Section 3.1.2.4, pages 42 to 44), EFSA concludes that the data published since 2010 confirm the fact that, after oral exposure to BPA, the unconjugated form of BPA in the plasma is so low that it cannot be detected/quantified with analytical methods having a limit of detection below 0.3 ng/mL. These conclusions, at odds with the ANSES report (ANSES, 2013), are based on a single study (Teegarden *et al.*, 2011) undertaken in the USA in 20 subjects in whom successive blood assays over a 24-hr. period had shown concentrations of unconjugated BPA below the 0.3 ng/mL limit of detection for all of the 320 serum samples analysed.

The study by Teegarden *et al.* (2011), also taken into account in ANSES's expert appraisal, was the only one of the studies that reported such low values. The other studies cited in the ANSES report are not taken into account in the EFSA report.

In the paragraph devoted to BPA in the blood of pregnant women and umbilical cord blood, the EFSA report cites the study by Kosarac *et al.* (2012), reporting serum concentrations of total BPA in 12 pregnant women ranging from <0.026 ng/mL to 10.4 ng/mL (median = 0.548 ng/mL, detection frequency: 67%) at mid-pregnancy and from <0.026 ng/mL to 3.05 ng/mL (median = 1.46 ng/mL, detection frequency: 58%) at delivery. Umbilical cord blood concentrations ranged from <0.026 ng/mL to 2.57 ng/mL (median = 1.82 ng/mL, detection frequency: 42%). Most of the detected total BPA was considered unconjugated BPA since conjugated BPA was only detected in two out of 12 serum samples at concentrations of 0.12 ng/mL and 0.22 ng/mL respectively (this last point is not specified in the EFSA report).

However, the EFSA experts consider that, despite the good quality of the analytical methodology, the data in the study by Kosarac *et al.* have low credibility due to a lack of information with respect to sample collection and handling, and discrepancies with the study by Teegarden *et al.* (2011), in which free BPA was never detected and total BPA was only detected in six out of 20 subjects who had peak concentrations of 0.6 to 1.3 ng/mL. In Appendix II of the EFSA report, the low number of subjects in the Kosarac study is also considered a weakness.

In general, the conclusions of the EFSA report on blood concentrations of total BPA and free BPA and the ratio of these two forms are based only on the results of the study by Teegarden *et al.* (2011). The few studies cited in the report that present high concentrations of unconjugated BPA in biological fluids are all considered as having many methodological shortcomings. This position is particularly questionable insofar as the study by Teegarden *et al.* ultimately appears to be an exception in the literature compared to the vast majority of other studies, most of which are not covered in the EFSA report.

Skin penetration of BPA

In its report, EFSA considers that the diet (oral route) is the main source of exposure in the general population, while dermal exposure from thermal paper is considered the second source of exposure in the population above three years of age (see line 373). Of the five *in vitro* publications on the percutaneous penetration of BPA, EFSA relied on the article by Demierre *et al.* (2012) to estimate the contribution of the dermal route to total daily exposure. For EFSA, the total absorbed quantity over a 24-hr. period is 10% of the dose applied on the skin based on the 8.6% absorbed within 24 hrs. (quantity in the receptor fluid) and the 0.6% in the skin

(excluding the *stratum corneum*). According to EFSA, the quantity of BPA in the *stratum corneum* (39.4% of the applied dose) should not be taken into account for systemic absorption (see line 2370).

The study by Demierre *et al.* (2012) is considered the key study for EFSA for whom it is a good-quality publication. Likewise, the use by Demierre *et al.* (2012) of water as a vehicle of BPA is more comparable to a scenario of consumer exposure to thermal paper than acetone (Marquet *et al.,* 2011) or diluted hydro-ethanol solutions (Mork *et al.,* 2010, Zalko *et al.,* 2011), and the applied surface density of 1.83 µg/cm^2 is comparable to exposure estimates as derived for thermal paper (1.37-5.5 µg/cm^2 finger tip).

For ANSES, the choice of the study by Demierre *et al*. (2012) as the key study and the rejection of the study by Zalko *et al*. (2011) (see line 18936) are questionable. First of all, the study by Demierre *et al.* (2012), which was supposedly undertaken in accordance with the OECD TG 428 guideline, has several weaknesses (see Annex 5). Secondly, a comparison of the results obtained by Mork *et al.* (2010), Zalko *et al*. (2011) and Demierre *et al*. (2012) does not favour a study undertaken with a diluted aqueous solution of BPA (Demierre *et al.*, 2012) over studies undertaken with varying concentrations of hydro-ethanol solutions of BPA (Mork *et al*., 2010, Zalko *et al*., 2011). Indeed, the permeability coefficient of BPA is independent of the type of vehicle used (aqueous or hydro-alcohol) or the concentration of BPA in the applied BPA solution. Thus, the Kp calculated from the experimental data reported by Zalko *et al.* (2011) is 0.9 10^{-4} cm/h. This Kp value is the same as the value obtained with Demierre *et al.* (2012) (kp=1.1 10^{-4} cm/h) who used a 194 µg/mL aqueous solution of BPA, and Mork *et al.* (2010) (kp=1.75 10^{-4} cm/h) who used a 3995 µg/mL hydro-ethanol solution. Likewise, the fraction of BPA absorbed within 24 hrs. is comparable for Mork *et al*. (2010) (approximately 6.5%= 13 X 24 h/48 h), Demierre *et al.* (2012) (8.6%) and Zalko *et al.* (2011) (15.2%= 45.6% X 24 h/72 h).

EFSA's affirmation that the use of water as a vehicle for BPA is more comparable to a scenario of exposure to thermal paper than acetone needs to be justified. Marquet *et al.* (2011) applied BPA as a solution in acetone. The acetone immediately evaporated. In these conditions, BPA in solid form was directly put into contact with the *stratum corneum,* as in the case of BPA transferred from thermal paper to the *stratum corneum* of the finger. The absorption flux of BPA (0.12 µg/cm^2/h) applied at a rate of 200 µg/cm^2 of skin (after evaporation of acetone) was approximately 6-7 times smaller than the BPA flux of 0.70 µg/cm^2/h (13%/48h X 259 µg/cm^2) obtained after applying BPA in a hydro-alcoholic solution at a rate of 259 µg BPA/cm^2. This difference in flux can be attributed to the need to first dissolve solid BPA before it penetrates the skin.

EFSA estimates that only 10% of the BPA dose applied on the skin is bioavailable within 24 hrs. This value is based on the quantity found in the receptor fluid (8.6% of the dose) and the skin (0.6% of the dose) reported by Demierre *et al.* (2012). This quantity in the skin is small compared to the values reported by Kaddar *et al.* (2008) and Mork *et al.* (2010) which are, excluding the *stratum corneum* and epidermis, 8.8% after 10 hrs. of exposure and 17.2% after 48 hrs. of exposure respectively. A significant reservoir effect was also reported *in vivo* in rats in which over 80% of the quantity of BPA in the skin after 8 hrs. of exposure was absorbed within 68 hrs. (Marquet *et al*., 2011). In light of the data in the literature, failure to take into account a skin reservoir effect could cause the daily dose of absorbed BPA to be under-estimated.

In its 2013 expert appraisal report, ANSES used a triangular distribution for skin penetration rates with 27% as the most likely value and 10% and 60% as the lower and upper limits, weighted by the daily duration of skin penetration. ANSES's experts considered 27% to be the most likely value as it was taken from a study in volunteers handling receipts in exposure conditions similar to those of real life (Biedermann *et al.*, 2010). The study by Demierre *et al.* used by EFSA was undertaken using human skin explants on which BPA was applied in the form of an aqueous solution. This formulation was different from that of receipts, and therefore the choice of this study for this assessment did not adhere to the guidelines (OECD 428, EHC235), which underline the need for studies to reflect real-life exposure conditions in terms of doses, durations and formulations.

The choice of the study by Demierre *et al.* as the key study and the estimate of 10% as a conservative value are defended but remain questionable considering the methods and results reported in the BPA skin absorption studies (see Annex 5).

High uncertainty remains as to the fate of BPA after skin penetration and the degree of metabolisation by the skin. Few studies have properly investigated the metabolism of BPA and ANSES approves EFSA's recommendations as to the need to further explore this issue (see line 6876). No toxicokinetic studies have measured the dermal bioavailability of BPA. Therefore, the evidence once again seems limited to affirm, as stated in the EFSA report, that the value of 10% skin penetration is conservative (see line 6489 lines 6860-6862).

For information, ANSES's approach resulted in a percutaneous absorption rate of 0.02% to 27% (probabilistic approach) over a 24-hr. period, which can be compared to EFSA's rate of 10% (deterministic approach)[83]. In the end, this difference between the EFSA and ANSES approaches hardly influences the difference in results between the respective risk assessments, which is mainly related to the choice of toxicological benchmark dose. Furthermore, ANSES observes that in the recent SCENIHR Opinion on the safety of bisphenol A in medical devices, the experts chose a skin penetration value of 25-30% taken from the study by Demierre *et al.* based on the same *corpus* of data.

Risk assessment

Use of a BMD

To model the dose-response relationship from the study by Tyl *et al.* (2008), EFSA chose to use RIVM's PROAST software (www.proast.nl) in which the choice of response level (or BMR) is defined as a percent change in the response compared to the response observed in the controls. The idea is to choose a value above which the observed response is considered abnormal. This choice of BMR must be clearly explained.

To calculate the BMD (and BMDL) based on the study by Tyl *et al.* (2008), EFSA chose a BMR of 10% related to an increase in absolute kidney weight. EFSA defends this choice of 10%

[83] In the ANSES exposure model, taking into account the penetration period used and the absorption rate of 27%, the 24-hr. absorption rate is approximately 0.02% to 27%, which is a range of equally probable outcomes (this is an estimate and the model would need to be run again with triangular distribution for an exact result (mode: 27%, min 10% and max 60%)).

(page 67), considering that below 10%, the effects observed are not harmful to health ("*less than 10% should not be regarded as adverse*") which may indeed be justified given the lack of histopathologically visible kidney lesions.

However, according to the EFSA recommendations[84], a default 5% BMR is recommended for continuous data (see Section 5.2 "*For continuous data the BMR could be defined in various ways. The way recommended here is to define it as a percent change in the average magnitude of the response variable as compared to the predicted background response. The recommended default value is a BMR of 5%*"). Depending on the choice made in terms of BMR (5% or 10%), the BMD and BMDL values differ greatly. An alternative choice of BMR could have been made based on the upper limit of (95% or 99%) confidence intervals around mean values for increases in kidney weight in male and female control animals (F0 and F1). BMR calculations for these various choices are given in the annexes (see Annex 6).

EFSA chose to calculate BMD and BMDL values using sex and generation (F0 and F1) as covariates. This makes it possible to see whether either the two sexes or two generations is more sensitive to BPA. Table 54 of the EFSA report shows that generation F0 males are most sensitive to BPA, with a BMDL rounded to 4 mg/kg bw/day.

For clarity purposes, it would have been helpful to present the same calculations in the appendices, modelling F0 males (the most sensitive) and then F0 males with generation F1 males as the covariate and lastly F0 males with F0 females as the covariate. This approach would make it possible to compare the various BMD and BMDL pairs and choose the most reliable one.

Regarding the choice of data on the critical effect (absolute weights *versus* relative weights), the study by Tyl *et al*. (2008) provides figures on the relative weights of each organ (Tables 19-20).

It would have been beneficial to perform the same calculations comparing absolute weights and relative weights.

In conclusion, the data modelling on kidney weight of Tyl *et al* (2008) was performed with the software PROAST, distinguishing between four subgroups (F0 and F1 males, F0 and F1 females). The appeal of this approach (taking into account covariates) is that it measures the influence of sex (male or female) and generation (F0 or F1) on equation parameters (exponential and Hill). The EFSA analysis shows that generation F0 males are more sensitive to BPA than F0 females and F1 males.

The table below shows BMD and BMDL values recalculated by ANSES based on the use of covariates, a BMR of 10% or 5% and the effect (absolute weight and relative weight).

The values vary by several orders of magnitude depending on the choices made. It should be noted that the BMD/BMDL ratios are all less than ten when relative weight is considered as the

[84] Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1-72

critical effect (and so these values have a lower level of uncertainty than if absolute weight were the critical effect).

It can be noted that a 5% BMR (as recommended by EFSA in its methodological guide[85]) with an increase in relative weight as the critical effect results in a $BMD_{5\%}L_{90\%}$ of 286 µg/kg/day, i.e. a value that is one tenth of that used by EFSA.

Table 2: Summary of BMD and BMDL values based on the use of covariates (F1 and sex), the effect (absolute versus relative weight) and the response level (BMR).

Effect	Covariate	BMR (CES)	BMD (µg/kg.bw/day)	BMDL (µg/kg.bw/day)	BMD/BMDL ratio
Increase in absolute (left) kidney weight in F0 males	**Females, and F0/F1**	**10%**	**23600**	**3633**	**6.5**
		5%	1040	43	24
	F1 males	10%	19000	2732	6.9
		5%	1050	33	31
	F0 females	10%	48900	9272	5.2
		5%	4520	262	17
	none	10%	48400	9694	5
		5%	5740	348	16
Increase in relative (% of total weight) (left) kidney weight in F0 males*	Females, and F0/F1	10%	35500	10000	3.5
		5%	2170	286	7.6
	F1 males	10 %	36400	10520	3.4
		5%	2300	260	8.8
	F0 females	10 %	54900	14250	3.8
		5%	5370	539	9.9
	none	10 %	51900	16720	3
		5%	10600	1316	8

*Note: for the calculation of BMD values with relative weight, the F1 generation (males) is the most sensitive, but the values described in the table are those for F0 (for comparison purposes).

[85] Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1-72

Only values taken from the exponential equation are shown in this table. Irrespective of the model used (exponential or Hill), the results have the same order of magnitude.

The report does not consider effects on the mammary gland (*mammary gland ductal proliferation*) for the risk assessment on the grounds that the $BMDL_{10}$ obtained with the various models varies significantly (more than ten orders of magnitude) (see p. 161, p. 515). The fact that the choice of models has an impact on BMD results is known (Foronda *et al.*, 2007, Sand *et al.*, 2008). This is not reason enough to not use this critical effect for the risk assessment. To address the impact of the model on BMD values, a sensitivity analysis could have been undertaken and a range of values could have been included in the risk assessment for this critical effect.

Animal - human extrapolation: PBPK modelling

The approach used by EFSA consists in calculating a human equivalent dose from the critical dose (BMDL) established in mice according to the study by Tyl *et al.* (2008). To do so, an equivalence factor was calculated from area-under-the-curve (AUC) ratios for free BPA in serum for the same single dose of 100 µg/kg body weight/day.

Like EFSA, ANSES recommends using allometric adjustment by default based on the ratio of body weights between mice and humans to the ¼ power. However, if one or more PBPK (physiologically-based pharmacokinetic modelling) models are available, they are preferably used to establish the human equivalent dose. EFSA therefore used the PBPK models of Yang *et al.* (2013) and Fisher *et al.* (2011) (same team) to calculate a human equivalent dose factor (HEDF) (ratio of animal AUCs/human AUCs) from a single dose of 100 µg/kg bw/day for the two species, which assumes a linear toxicokinetic dose response which is far from being certain, particularly due to the possible saturation of metabolism. A table listing uncertainties and their potential impact on HEDF determination is presented in the report (see Table 50, page 499).

From ANSES's perspective, the approach would have involved converting the external exposure dose in mice (the BMDL already established) (Tyl *et al.* 2008) into an internal dose using the mouse PBPK model (Yang *et al.*, 2013). This internal dose corresponds to an AUC. In humans, it can be expected that this same AUC would have similar effects (or no effects), provided that a 2.5 uncertainty factor is applied for the toxicodynamic component. A human PBPK model (Yang *et al.*, 2013) could then be used to establish the corresponding exposure dose for BPA.

In general, the requirements for using a PBPK model can be summarised through these 'guidelines' taken from the WHO document[86] (see Figure 1).

The level of confidence associated with a model relies on an analysis of the model's overall structure, a simulation and validation, and lastly an evaluation of reliability including a sensitivity and uncertainty analysis (see Figure 1).

[86] Characterization and application of physiologically based pharmacokinetic models in risk assessment, WHO 2010.

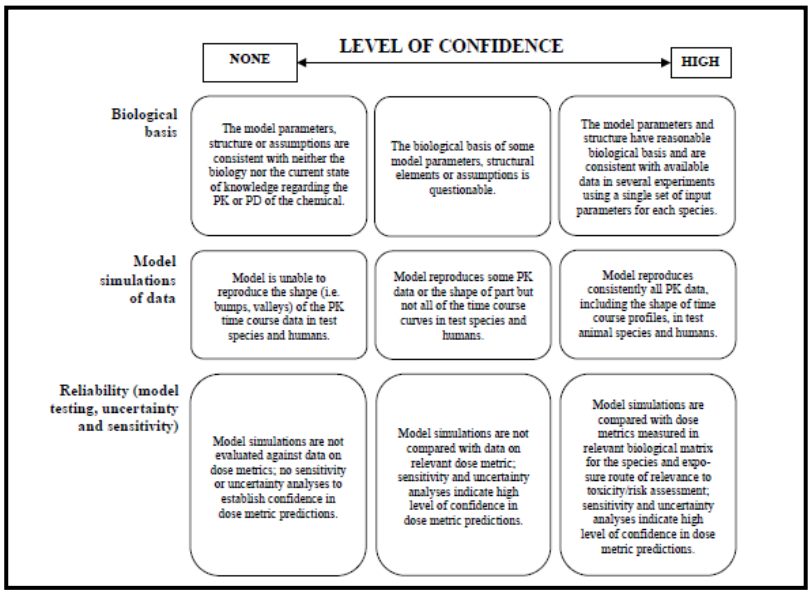


Figure 1: level of confidence in a PBPK model – source WHO[5], 2013

Description of the PBPK models used

The models (rats and humans) used in the EFSA report are those described in the articles of Fisher *et al.* (2011) for humans and Yang *et al.* (2013) for rats, in addition to one human model from Mielke *et al.* (2011).

Fisher group models

- **Description of the Fisher team's PBPK models**

The first two PBPK models used were intended to identify the starting dose resulting from the work of the Fisher group. This group first produced a PBPK model for monkeys and extrapolated it to humans, and then for rats exposed to BPA.

The model developed for monkeys and humans has a structure with seven compartments: the blood compartment (serum), reproductive tract (gonad), brain, fatty tissues, richly perfused tissues, slowly perfused tissues and liver. This model also has three pseudo-compartments: the small intestine, stomach and a compartment that the authors call volume of distribution (Vd). This last pseudo-compartment represents the metabolised fraction of BPA as BPA-c (Fisher *et al.,* 2011). However, it does not take into account the enterohepatic cycle (Fisher *et al.* 2011).

The rodent PBPK model, published by Yang *et al*. (2013), is the same as that of Fisher *et al*. (2011). For the metabolite (BPA-c), the authors described three compartments: the plasma, 'body' and liver, and a pseudo-compartment called the digestive tract. Note that the plasma compartment and liver are the same compartments as those given for the parent product (free BPA) but that the 'body' compartment is an agglomeration of the other compartments. In this version, the model contains a description of an enterohepatic cycle (Yang *et al.*, 2013). Moreover, each of the compartments is described as having limited perfusion ("*well-stirred model*"), meaning that the quantity of BPA distributed in the tissues is related to the perfusion capacity of the organ, which implies and assumes that the BPA that enters the compartments is evenly and instantly distributed.

The physiological parameters are those traditionally found in the literature. The metabolic parameters used for rodents (Vmax and Km) have been taken from a review of the literature or optimised from the published kinetic data. The physicochemical parameters (partition coefficient) used for the two models have been taken from two prior publications by these same authors (Doerge *et al.* 2011; Fisher *et al.* 2011). The equations are described in an attachment (for Yang *et al.* 2013) and do not appear to include syntactic errors: they are basic equations for PBPK models.

In conclusion, the physiological basis for the two models appears acceptable. However, we did not analyse or audit the equations and parameters of the said models. Based on Figure 1 in reference to the WHO document, the level of confidence for the physiological basis of the models would be 'medium' (WHO-IPCS, 2010).

- **Calibration, evaluation and predictability of the models**

The PBPK model for rodents and humans is used to estimate the internal plasma concentration (Cb) of BPA and its area under the curve (AUC) according to various exposure scenarios. The models were calibrated from a single dose of 100 µg/kg bw/day in rats and monkeys (Doerge *et al.,* 2010a; Doerge *et al.,* 2010b). However, a calibration has no predictive value for the model and it is necessary to compare the measured data with those calculated by the model. Fisher's model was calibrated by visual inspection for several parameters and therefore its calibration remains questionable. It could have been optimised with the software used (ACSLX), which would have increased the confidence level.

Visual examination of Figures 7, 8 and 9 in the model by Fisher *et al.* (2011) is satisfactory[87] for exposure to varying concentrations of 10 mg/kg bw/day, 400 mg/kg bw/day or a total of 5 mg. There is good fit between what is measured and what is modelled. Visual examination of Figures 7, 8 and 10 in Yang's model is satisfactory[4]: for exposure to varying concentrations (1 mg/kg bw/day, 10 mg/kg bw/day), there is good fit between what is measured and what has been calculated.

In conclusion, based on Figure 1 (WHO-IPCS document, 2010), the level of confidence for the 'Simulation and validation' of the models would be 'medium-low'.

- **Reliability analysis including a sensitivity and uncertainty study**

[87] It is recommended that the WHO-IPCS ratio between predicted value and measured value be less than two. ANSES does not have access to gross data to establish this ratio.

The following uncertainty factors are discussed in the EFSA report:

- *Uncertainty as to the measurement of concentrations in animals.*

Analytical accuracy is 20% for the method used for all the studies. Moreover, the method used protects from risks of exterior contamination of samples.

- *Uncertainty as to the calculation of AUCs*

This uncertainty stems from the variability between animals and the calculation method that introduces uncertainties, particularly for the calculation to infinity. The authors consider that taking into account the standard deviation covers these two aspects, which is acceptable. Another source of uncertainty relates to the handling of missing values (below the limit of detection), underestimating the value of the AUC.

The oral absorption procedure appears consistent between the experimental studies in animals and the human PBPK model, which does not generate additional uncertainty. For the human model, only the impact of inter-individual variability is evaluated. Therefore, several evaluations of uncertainty are missing, particularly regarding the PBPK model in humans.

To first legitimise the calculation of the equivalence factor at the concentration of 100 µg/kg/day, this assumes a linear toxicokinetic dose response which is far from being certain, particularly due to the possible saturation of metabolism. The starting concentration for the hazard characterisation is greater than 3500 µg/kg/day (Tyl *et al.*, 2008). The use of PBPK models for each species (mice and humans), valid over a range including this starting concentration for the extrapolation, would have eliminated this uncertainty factor which is ignored here.

- *Uncertainty as to PBPK modelling*

Monte Carlo analysis is the most commonly used probabilistic approach with PBPK models since it incorporates variability into these models. The aim of this Monte Carlo analysis is to qualitatively and quantitatively characterise variability and uncertainty in estimations. It is possible to measure uncertainty, by changing a physiological parameter, a (physicochemical) partition coefficient or a biochemical parameter with realistic values. It is then possible to theoretically consider how these changes influence the outputs. In this case, the result is not a single concentration but rather a distribution of probability, with a median and 95th percentile.

According to a WHO report on PBPK modelling, the ratio of the 95th percentile and the median easily provides a measure of this uncertainty, which is high, medium or low[88] (WHO/IPCS 2010). However, this ratio does not appear in the EFSA report.

Sensitivity analysis makes it possible to determine the parameters that most influence the measured indicator (e.g. Cb, AUC). The approach consists in changing one parameter at a time (perhaps a physiological, physicochemical or biochemical parameter) and seeing how this change influences the measured indicator. The closer the value is to 1 in absolute value, the more the parameter influences the measured indicator. According to the WHO criteria, this

[88] Uncertainty analysis results are summarised as high uncertainty (value could be a factor of 2 or higher), medium uncertainty (value could be a factor between 0.3 and 2) or low uncertainty (value could be a factor of 0.3 or lower)

sensitivity can be classified as high, medium or low[89] (WHO-IPCS 2010). The authors of the original articles carried out a sensitivity analysis for each of the rat (Yang *et al.* 2013) and human (Fisher *et al.* 2011) models.

For the Fisher group's rat model and human model

CRITERIA	CONFIDENCE LEVEL
PHYSIOLOGICAL BASE	Medium to high
SIMULATION AND VALIDATION	Medium to low
RELIABILITY (UNCERTAINTY AND SENSITIVITY ANALYSIS)	Medium

Model of Mielke *et al.* 2011

- **Description of the PBPK model of Mielke *et al.* 2011**

The human model (which was used for dermal exposure (Mielke *et al.* 2011)) has eight compartments: muscle, skin, adipose tissue, skeleton, brain, kidneys, liver and an 'other organs' compartment. Two routes of exposure are described including oral and dermal exposure. All of the compartments are perfusion-limited. Metabolism occurs only in the liver.

- **Reliability analysis including a sensitivity and uncertainty study**

The authors of the 2011 publication indicate that a sensitivity analysis was performed in the 2009 publication (Mielke and Gundert-Remy, 2009). However, a review of the article does not show any sensitivity analysis. This is a limitation for using this model in a risk assessment and does not reflect a standardised WHO strategy. The model of Mielke *et al.* (2011) is worthwhile to generate assumptions but is a significant source of uncertainty that EFSA does not explain.

All things considered, the level of confidence that can be associated with a model is a combination of sensitivity and uncertainty analyses on a scale from low to high according to the criteria set by WHO. In conclusion, based on the WHO recommendations, the following confidence levels can be assigned:

[89] High (absolute value greater than or equal to 0.5), medium (absolute value greater than or equal to 0.2 but less than 0.5) or low (absolute value greater than or equal to 0.1 but less than 0.2)

For Mielke's model

CRITERIA	CONFIDENCE LEVEL
PHYSIOLOGICAL BASE	Medium
SIMULATION AND VALIDATION	Low
RELIABILITY (UNCERTAINTY AND SENSITIVITY ANALYSIS)	Very low, no evidence that this was performed

Conclusion

The two models of the team of Fisher *et al.* (2011 and 2013) give a good physiological description and have predictability for blood only. This model is not predictive for the other compartments. This poses a problem of confidence in the model. This limitation is partly due to the lack of data in the literature and possibly a methodological limitation. The use of pseudo-compartments also reduces confidence in the model. However, the authors nonetheless have good predictability for the blood compartment (serum or plasma).

Regarding the model of Mielke (2010), i.e. the PBPK model in humans that establishes overall exposure (oral and dermal), it would have been simpler to use the same model (with the same physiological basis) to determine this aggregate exposure, by including, for example, dermal exposure from Fisher's model for which a predictability assessment and sensitivity study were undertaken.

Mielke's model appears less reliable than Fisher's model for the following reasons:

- Fisher's model was compared to experimental data, which means the model can be tested

- The predictability of Mielke's model is demonstrated by comparing a measured point taken from the findings of Volkel. Furthermore, no sensitivity studies appear to have been performed with Mielke's model, which does not increase the level of confidence in the model.

Application of an additional uncertainty factor

ANSES, in its expert appraisal report published in March 2013, chose to apply an additional uncertainty factor of 3 to take into account all the uncertainties in connection with the effects of BPA observed at lower doses than those selected for the HRA and the existence of non-monotonic dose-response relationships, the existence of *in vitro* and *ex vivo* data in favour of a much greater sensitivity (beyond a factor of 10 already considered in the inter-species variability factor) of tissues of human origin with respect to BPA, compared to animal tissues. In the end, an overall uncertainty factor of 300 was applied in ANSES's expert appraisal.

In the EFSA report, uncertainties as to effects are described in several places in narrative mode. This is the case for effects on reproduction and development (p. 5), neurotoxic effects (p. 6), effects on immunity (p. 6), cardiovascular effects (p. 6), effects on metabolism (p. 7) and carcinogenic effects (p. 7). One might have expected for these uncertainties to be taken into consideration in the risk assessment, for example with a specific uncertainty factor to take

into account the state of knowledge. Such is not the case, based on the argument that the calculation of the human equivalent dose covers this due to its conservative nature. The report specifies that the HEDF of 0.03 that is used is conservative. And yet this argument is questionable; just because the HEDF developed for one effect (increase in kidney weight) is conservative, does not mean that it is conservative for all other effects.

Overall consideration of uncertainty

Despite what is said (see p. 9), uncertainty is only partially evaluated in the EFSA report. It would have been helpful to define the term 'uncertainty' and better describe the method used to choose uncertainties. The reasons why some uncertainties are described and others are not are not clear upon reading the report.

The aim of any risk assessment is to draw conclusions when 'perfect' and therefore 'certain' information is not available. In other words, a risk assessment is intended to produce a conclusion in a situation of uncertainty. It is therefore questionable to refuse to consider available knowledge on the pretext that it is uncertain. And yet, in the EFSA report, uncertainty is often used as an argument to consider that an effect is not likely (effects on reproduction and development, p. 5) or even exclude an effect that is considered likely from the risk assessment (effect on mammary hyperplasia, p. 8). In addition, when the uncertainty as to the effect is high (see p. 5), what arguments did the experts use to consider effects unlikely?

CONCLUSIONS OF THE EXPERT APPRAISAL

ANSES agrees with the observations made by the rapporteurs of the Working Group on endocrine disruptors and category 3 reprotoxic substances further to the analysis, on a complex topic in a short time-frame, of the draft opinion on the health risks related to BPA submitted to public consultation by EFSA on 17 January 2014.

This analysis dealt with the assessment approach developed by EFSA, hazard and exposure characterisation, biokinetic data and risk assessment. It covered specific points of the EFSA report that can influence the results of the risk assessment: the choice of publications taken into account, the selection of the critical effect(s), BMD calculation, the estimation of internal exposure in humans and the treatment of uncertainty. Comments specific to certain studies and additional information provided by ANSES have been attached to this Opinion.

Regarding the characterisation of effects, ANSES acknowledges the systematic nature of the approach used by EFSA to characterise, study by study, lines of evidence associated with the effects of BPA. Nonetheless, the approach implemented has a number of limitations, such as the sometimes over-fragmentation of the data analysis, making it difficult to characterise effects by organ or system (reproductive system, mammary gland, etc.) in a consistent manner. Furthermore, biochemical and/or histological signs that can lead to biological changes preceding effects harmful to health are not considered by EFSA as significant enough to be taken into account for the risk assessment. ANSES considers that some of these effects (e.g. effects on the central nervous system, effects on the mammary gland) should be taken into consideration for the assessment of risks related to BPA. Effects on the mammary gland are the most significant effects identified by ANSES to assess the risks of BPA. Situations of at-risk exposure have been identified based on these effects. Likewise, uncertainties as to the effects of BPA related to the quality of the studies analysed are mentioned several times in the EFSA

report. In this context of uncertainty, it would be helpful if the choices made by the EFSA experts throughout the expert assessment process were better described, documented and justified. In the EFSA report, uncertainty is often used as an argument to consider that an effect is not likely or even exclude an effect that is considered likely from the risk assessment.

ANSES observes that this new health risk assessment for BPA not only takes into account studies on oral exposure but also studies on subcutaneous exposure, which was not the case in previous EFSA opinions. Most of the studies undertaken to examine the toxicity of BPA were not conducted in accordance with the OECD guidelines and did not systematically adhere to 'Good Laboratory Practice' (GLP); these studies were nonetheless taken into account in the EFSA expert assessment, even though EFSA gave greater weight to studies following the OECD recommendations and/or carried out according to GLP (e.g. Tyl, 2002, 2008). Many studies have been published since June 2012, the deadline for publications taken into account by ANSES in its expert appraisal report on the assessment of health risks related to BPA published in March 2013. These recent studies included in the EFSA expert assessment provide additional information, particularly on certain critical effects such as metabolism for which fairly little information was available until recently.

Subject to an assessment of these new publications, which have not been analysed in this Opinion by the Working Group's experts, ANSES considers that the conclusions of its assessment published in March 2013 remain valid. ANSES nonetheless takes note of the number of publications since its report on the health effects of BPA (ANSES, 2011), which is justification for maintaining an active watch to update the data on this substance.

Lastly, ANSES considers it is necessary to define objective criteria to qualify studies investigating the effects of potential endocrine disrupting substances, given the differences in interpretation noted by the experts particularly with regard to the methodological limitations of BPA toxicity studies, the number of necessary doses and animals, the lack of positive controls and the lack of increasing dose-response relationships. These criteria should be standardised between EFSA and national health and safety agencies.

Annex 6: Thermal Paper containing BPA: EXPOSURE ASSESSMENT - EXPLANATIONS & ARGUMENTS I

A- Introduction

On the occasion of the risk assessment conducted by ANSES, thermal receipts have been identified as a potential source of exposure to BPA. Indeed, thermal paper type "eco-paper" used mainly for receipts and credit cards receipts, BPA is present as free monomer, and does not offer significant resistance to abrasion (Mendum *et al*., 2011). It can be transferable by contact with the skin (Biedermann *et al*., 2010 Zalko *et al*., 2011). Workers like cashiers may thus constitute a population of workers particularly exposed.

Considering this, it has been conducted a risk assessment for two kinds of situation concerning pregnant women:

1) **Worker Scenario**: Pregnant women who work as cashiers and handle receipts ;
2) **Consumers Scenario**: Pregnant women who are consumers and can handle receipts.

For these two situations, ANSES has modeled the exposures. The goal was to determine an internal exposure dose (IED) in order to compare it with different internal toxicological mark doses (ITD or "internal DNELs") derived from critical doses determined in key studies.

Four toxicological key studies were selected to conduct the risk assessment. The target population is pregnant women. Indeed, selected key studies are studies that exposed pregnant female animals and their offspring over a given period.

These four studies represent four types of health effects:

- Critical effect on the brain and behavior;
- Critical effect on the female reproductive system;
- Critical effect on metabolism and obesity;
- Critical effect on the mammary gland.

In the following, the aspects of selected toxicological key studies as well as internal reference toxicological liabilities are not covered. Only those aspects concerning the calculation of internal exposure doses (IED) over the handling of thermal receipts are addressed and argued.

B- Material and methods

In order to assess exposure over the handling of thermal receipts containing BPA, ANSES has implemented a probabilistic approach.

1) Probabilistic characterization of the exposure: general framework

Within the scope of works relating to BPA, it has been chosen to model the doses of exposure in accordance with a probabilistic approach for optimum management of the variability. By contrast with a conventional deterministic approach, for which only occasional estimations of exposure are calculated, a probabilistic approach takes into account all of the possible modalities of an entry variable through the intermediary of its distribution of probabilities. So any possible modality of an entry variable of a model can be combined with the modalities of the other entry variables depending on their probability of occurrence.

Random samples using the Monte Carlo approach (10000 iterations) are then done on each of the entry distributions of the model to define distribution of the exposure doses, represented in the form of histograms or accumulated distributions.

The probabilistic approach presents numerous advantages:

- It enables the percentage of overrun of the reference toxicological doses to be determined.
- The sensitivity analyses enabling the identification and ranking of the most influential exposure parameters on exposure models are facilitated (Pouillot *et al.*, (2002) or Cullen & Frey (1999)).

2) Specification of the probability distributions

As previously mentioned the probabilistic approach is based on allocation of a distribution of probabilities to each of the variables to then carry out a random draw in these distributions using the Monte Carlo method.

The major difficulty of this appraoch is defining the distributions of probabilites of the entry variables of the models. This information is generally not given in the literature and not all the variables collected in the population are available. The theoretical distribution of probabilities attributed to an exposure parameter and the underlying hypotheses to this choice therefore rely on the level of information available on the data (literature, subsidiary survey, etc.). It is therefore advisable to establish hypotheses to be able to define a theoretical distribution which is as close as possible to distribution of the data observed and to check these hypotheses using statistical tools such as the tests of Kolmogorov-Smirnov or Anderson-Darling and graphs (quantile-quantile graphs or comparison of histograms or of distribution functions), which give information on the validity and plausibility of this adjustment. However, a limit of the theoretical adjustment of a distribution of probabilities is that, although a distribution "sticks" correctly to the data, the latter will never be perfectly adjusted, notably at the level of the distribution queues. Therefore, it is advisable to integrate the discrete distributions within the models of exposure, constructed from all of the possible modalities and their probability of respective occurrence.

The different levels of information available for an entry variable as well as the hypotheses made in order to be able to allocate it a distribution are presented below. Four different situations are encountered, in addition to the case where only a single value is available and is integrated as in the model. They are ranked below from the one with the least information to the one with the most information, the ideal situation for specification of a distribution of probabilities, thus resulting in a different strategy to be adopted.

- A variation interval

Certain studies only give the variation interval of the parameter studied. In this case, a uniform distribution is allocated with the interval given as all of the possibilities of the parameter studied, characterized by the fact that all the intervals of the same length included in this variation interval have the same probability of occurrence.

- A variation interval and a central value

Other studies may give more and more info on the variation interval of the parameter, a central value, central, average or median, of the sample. In this situation, the distribution of probabilities specified is a triangular distribution which is characterized by a central value

which has the highest probability and minimum and maximum values which have a zero probability.

- A set of percentiles

In the majority of the studies selected, several percentiles are given, notably the percentiles 0 (minimum) and 100 (maximum), thus giving the variation interval. It is then a case of creating a distribution function from the couples *(xi; pi)* of cumulative data available, with *pi* the probability of obtaining a value lower than or equal to *xi*. The following step consists of simulation of a sample of values taken randomly on this cumulative distribution and integrating it as "input" in the model for the exposure parameter.

- A set of raw data

It may be that the raw data from a survey are available. In this situation, it is necessary to have an occurrence percentage of each individual. This weighting is at times specified by the study. If this is not the case, it is allocated an appearance percentage of 100/*n*, *n* being the individual number of the survey. Then the different values taken by the parameter are organized and the probabilities are added in the box where the same value is measured on several occasions. A set of couples *(Xi; pi)* with *{X1, X2, …, Xm}* is then obtained with all of the possible modalities of the parameter, and *{p1, p2, …, pm}* their respective probability of occurrence. However, it is at times preferable to group possible issues by class, so as to avoid having too large a number of different modalities with a percentage of low combined occurrences. Lastly, a sample of values taken randomly on the discreet distribution is simulated, defined from the occurrence probabilities of each of the realizations or classes of possible realizations for the parameter studied.

Comment 1: all the distributions of probabilities used in the exposure calculations are constructed from a Monte Carlo simulation of 10,000 iterations carried out using the software @Risk 5.0.

Comment 2: To assess the risk, P95 of the distribution describing the internal exposure dose (IED) via the manipulation of receipts by pregnant women cashiers is compared to the different ITD ("internal DNELs"). This choice is justified by the fact that the values beyond this percentile are the result of the combination of random draws in the distribution queues of each of the parameter of exposure and may in this way be judged as extreme cases, not representative of realistic exposure situations.

Results interpretation:

- If P95 < ITD ➔ risk is considered negligible;
- If P95 > ITD ➔ situations of exposure to risk exist.

C- Exposure assessment : results for workers

1) Exposure estimation for worker (pregnant women) : cashiers handling receipts

The equation used to model the dose of exposition via the handling of thermal receipts for a professional is based on the hypothesis of skin exposure to BPA over the work period. This hypothesis is based on the works of Biedermann *et al*., 2010 which show a constant quantity

of BPA transferred to the surface of the skin of the finger, whatever the duration (between 5 and 60 seconds) or the repetition (between 3 and 10) of contact with the receipts. The exposure depends on the percutaneous absorption flow; the duration of exposure assimilated to the work duration, the surface in contact with the paper and the body weight.

$$IED = \frac{F \times D \times S}{BW} \times f\ systemic\ bioavailability$$

With:

IED: Internal (exposure) daily dose [$\mu g/kg_{BW}/d$]

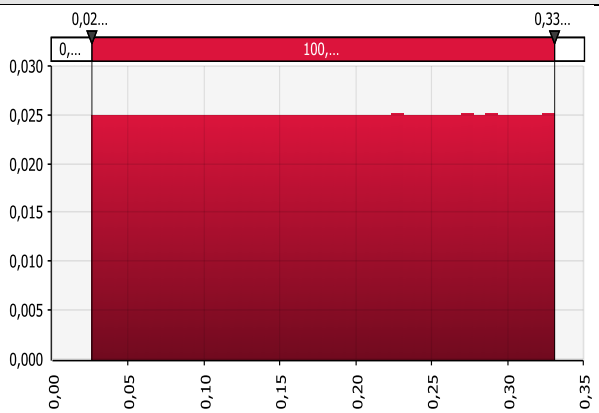
F: Absorption flow [$\mu g/cm^2/h$]

D: Duration of exposure to the till receipt [h/d]

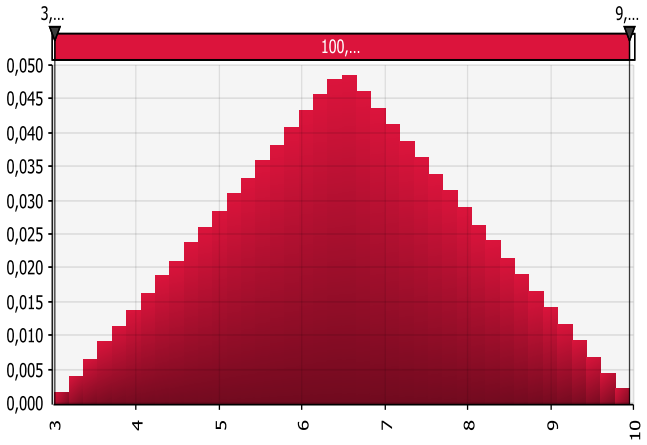
S: Surface in contact with the till receipt [cm^2]

BW: Body weight [kg_{BW}]

$f_{systemic\ bioavailability}$ [%]

Input variables	Distribution or deterministic value applied		
F: Absorption flow [µg/cm²/h] The study retained for the **percutaneous absorption flow** parameter was the one by Marquet *et al.* (Marquet, 2011) which aimed to determine a percutaneous absorption flow for BPA. It gave 15 data readings of BPA crossing the cutaneous barrier in µg.cm-2.h-1. On the basis of this information, it was then decided to allocate a **uniform distribution** with, as the distribution variation interval, the **minimum (0.026 µg.cm-2.h-1) and maximum (0.331 µg.cm-2.h-1)** values measured.		Min	0,03
		P25	0,10
		P50	0,18
		P75	0,26
		P90	0,30
		P95	0,32
		P99	0,33
		Max	0,33
		Mean	0,18

<table>
<tr>
<td>D: Duration of exposure to the till receipt [h/d]

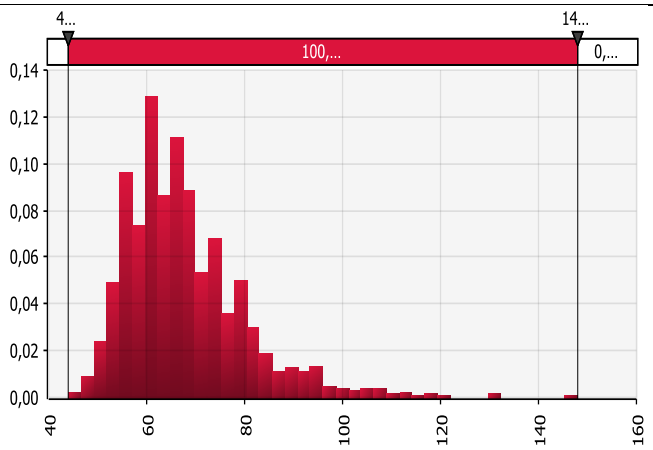
For the exposure duration, the specified distribution was based on the assessment of experts from the data from the collective agreement of the retail trade and the wholesale trade with dietary predominance. It was decided to allocate a triangular distribution of probabilities with as a minimum value 3 h.d-1 and as a maximum value 10 h.d-1, corresponding respectively to the minimum and maximum values of the daily work duration on the days worked, and lastly as an average value, taken as a mode of distribution, 6.5 h.d-1.</td>
<td> 
</td>
<td>Min
P25
P50
P75
P90
P95
P99
Max

Mean</td>
<td>3,02
5,48
6,50
7,53
8,44
8,89
9,50
9,95

6,50</td>
</tr>
<tr>
<td>S : Surface in contact with the till receipt [cm^2]

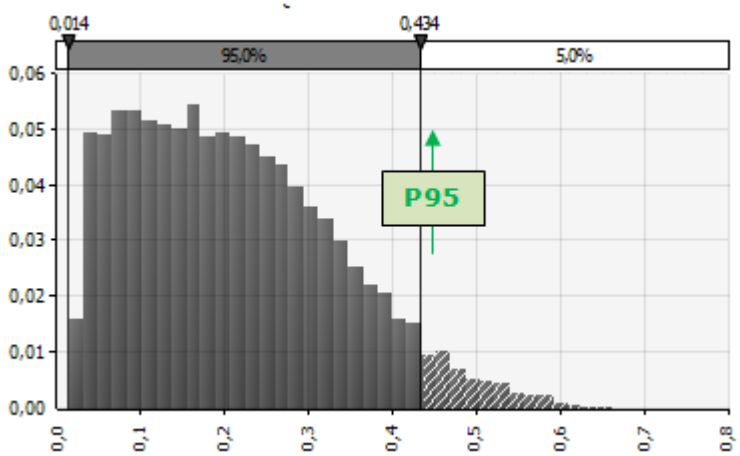
The distribution allocated to the surface in contact with a thermal receipt was based on the assessment of experts who proposed taking a total surface area corresponding to the cumulated surface area of the pads of the ten fingers (last phalanxes). It was decided to allocate a value of 12 cm², relying on US EPA (1986) which gives by default a surface area of 2 cm² for the thumb and 1 cm² for each of the other fingers.</td>
<td>Deterministic value of 12 cm^2</td>
<td colspan="2">/</td>
</tr>
<tr>
<td>BW : Body weight [kg$_{BW}$]

To determine the distribution of probabilities illustrating the body weight for the pregnant woman, the entire period of pregnancy must not be taken into consideration in calculation of the average weight of each of</td>
<td></td>
<td>Min
P25
P50
P75
P90</td>
<td>44
59
65
74
83</td>
</tr>
</table>

the individuals. The study of pre- and post-natal determinants of development and health of the Child (EDEN[90]), gives the body weights of the pregnant women at different stages of the pregnancy and was used to document this parameter, with the similar exception of the weights measured taken into account in order to calculate the average weight of the women are those given from the start of the pregnancy until the **7th month and a ½,** i.e. up to the **35th** week of amenorrhea.		P95 P99 Max Mean	92 109 148 67,74
$F_{systemic\ bioavailability}$ (%) There is no experimental analysis available to determine a factor of absolute bioavailability for dermal. Furthermore, no estimate of the relative bioavailability for subcutaneously could be performed, which does not allow to estimate the semi quantitatively factor bioavailability.	Default value : 100%	/	

Result: Probability distribution describing the internal exposure dose (IED) via the manipulation of receipts by pregnant women cashiers:

[90] The EDEN study was initiated by several teams of epidemiologists from the Institut Fédératif de Recherche 69, as well as participating clinicians from the CHU *(University Hospitals)* of Poitiers and Nancy. Their aim was to better define the characteristics of foetal development and the first few months of life which influence the development and the subsequent health of the child. 2002 women agreed to participate. Amon the very large amount of data available from this study, a distribution of discrete probabilities was simulated from the pairs "average weight/probability of occurrence".

IED distribution [µg.kg$_{BW}^{-1}$.d^{-1}]	**descriptive statistics**	
	Min	0,01
	P5	0,05
	P25	0,11
	P50	0,20
	P75	0,29
	P90	0,38
	P95	0,43
	Max	0,71
	Mean	0,21

2) Sensitivity analysis

In view of these results, a sensitivity analysis was carried out:

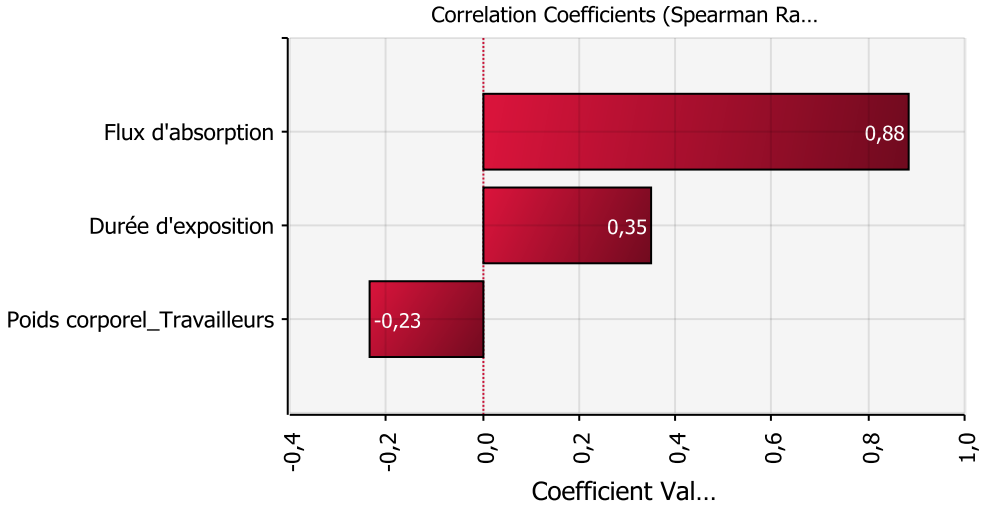
- To identify the influence of the input variables variability on the variability of the internal exposure dose (IED) calculated at the output;
- To test the influence of different systemic bioavailability values after the cutaneous absorption.

2.1) Parametric uncertainty

In view of the results of the risk characterization for pregnant women handling thermal receipt containing BPA at their workplace, an analysis of sensitivity by tornado graph was made to prioritize the "influence of different input variables of the exposure model". The tornado graphs are in fact a representation of the "influence of the variability of different input probability distributions in the model on the variability of the output". The software "@Risk 5.0" offers two types of statistical analysis to calculate the indices measuring the impact of each parameter on the model output: regression analysis and calculation of rank correlation. In the case of this work, the sensitivity analysis is based on the calculation of rank correlation coefficients of Spearman. The main factors influencing the result are presented first. Indeed, the link between each value of distributions and the result of the model is analyzed by the correlation coefficient. The rank correlation coefficients of Spearman were then preferably used as oblivious to the fact that the distributions of parameters follow or not a normal distribution, whereas the "hypothesis" of a normal distribution is underlying the use of correlation coefficients conventionally used.

The model used to calculate the exposure and the different input variables used (distribution or deterministic values) are mentioned above.

The graph below shows the sensitivity analysis by tornado graph of modelled internal dose for pregnant women handling thermal receipt containing BPA in the workplace (cashiers) by application of the model mentioned above:



Sensitivity analysis by tornado graph of the modelled internal exposure dose for pregnant women cashiers handling thermal paper containing BPA

What does it show?

↳ This graph reflects that the flow of percutaneous absorption is the most influential parameter on the internal dose calculated, given the variability of different probability distributions included in the model.

2.2) Uncertainty on the value of systemic bioavailability factor after dermal absorption

As described above, the model includes the percutaneous absorption but does not involve the systemic bioavailability factor after the absorption. Indeed, there is no data to determine this bioavailability factor in the scientific literature, thus it was considered by default that BPA absorbed through the skin was then bioavailable in the body, with a systemic bioavailability factor of 100%.

In the following, the influence of the bioavailability factor *f* on the daily intake ("1st exercise") and on the risk assessment ("2nd exercise") was tested.

The first exercise conducted no longer considers a factor of systemic bioavailability after dermal absorption of 100% by default but introduces into the model for this factor, a uniform distribution of probabilities ranging from 0.01% to 100%.

$$IED = \frac{F \times D \times S}{BW} \times \underbrace{f}_{systemic\ bioavailability}$$

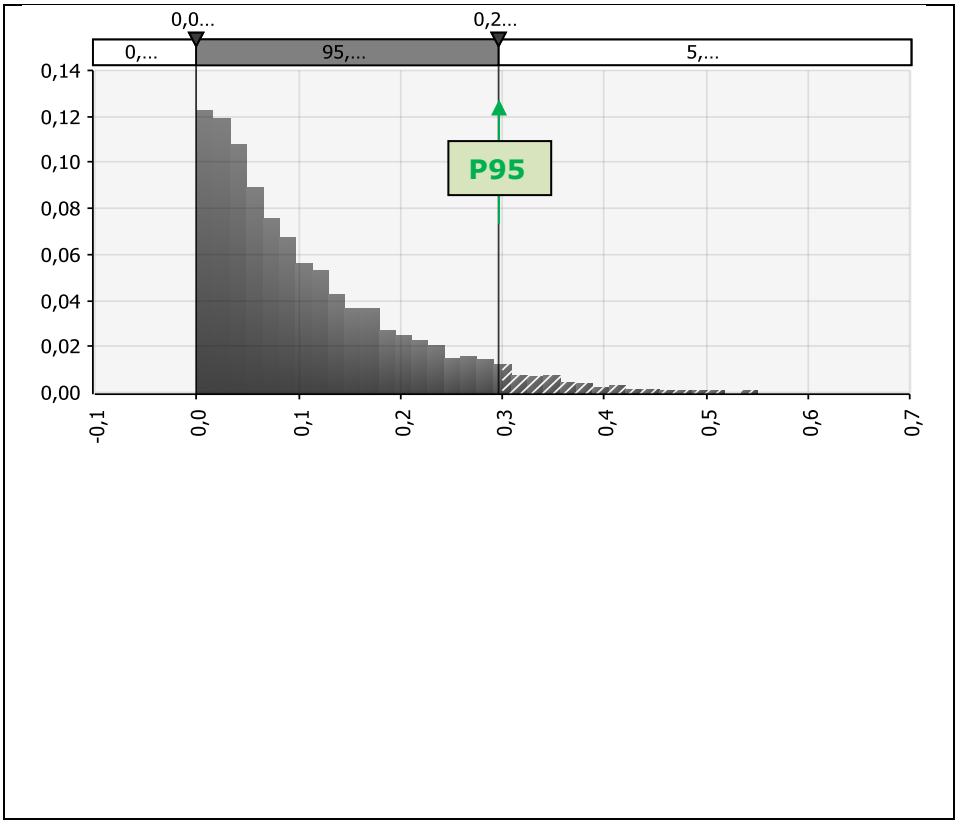
Same distributions or deterministic values than before

Uniform distribution [0.01% - 100%] :

Minimum	1,02E-03
Maximum	1,00E+00
Mean	5,00E-01
Std Dev.	2,88E-01
Variance	8,32E-02
5% Perc	5,09E-02
25% Perc	2,51E-01
50% Perc	5,00E-01

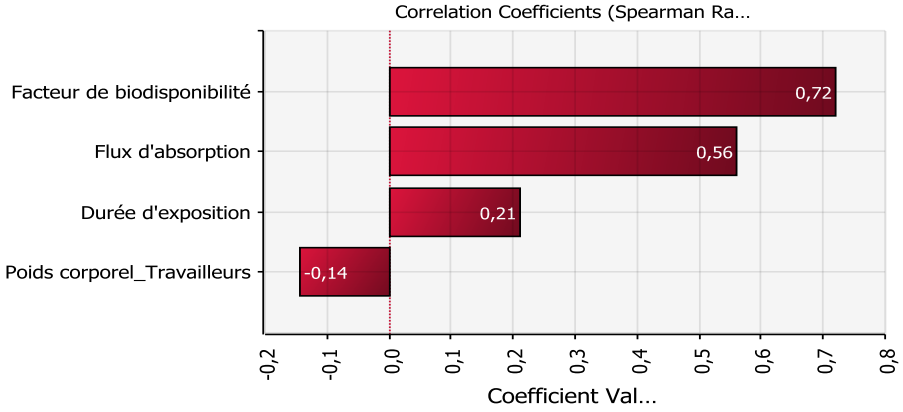
Probability distribution describing the internal exposure dose (IED) via the manipulation of receipts by pregnant women cashiers using a uniform distribution for systemic bioavailability [0.01% - 100%]

IED distribution [$\mu g.kg_{BW}^{-1}.d^{-1}$]	**descriptive statistics**



Min	9.38E-05
Max	6.48E-01
Mean	1.06E-01
5% Perc	6.73E-03
25% Perc	3.36E-02
50% Perc	7.80E-02
75% Perc	1.52E-01
90% Perc	2.42E-01
95% Perc	2.97E-01

This exercise is only a theoretical exercise conducted to assess, via a sensitivity analysis by tornado graph on the obtained results, the extent to which factor of systemic bioavailability after dermal absorption is an influential parameter.



Sensitivity analysis by tornado graph of the influence of the systemic bioavailability factor after dermal absorption

What does it show?

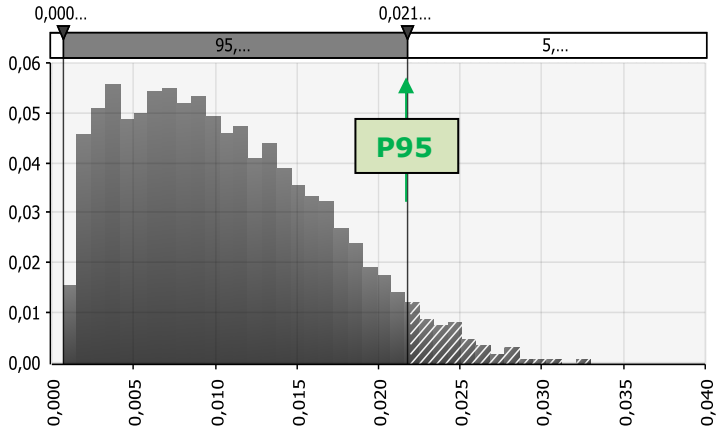
⇘ The sensitivity analysis shows that the factor of systemic bioavailability after dermal absorption is the most influential parameter on the result of the calculation.

This analysis confirms that the lack of data to determine a factor of dermal bioavailability is a major uncertainty.

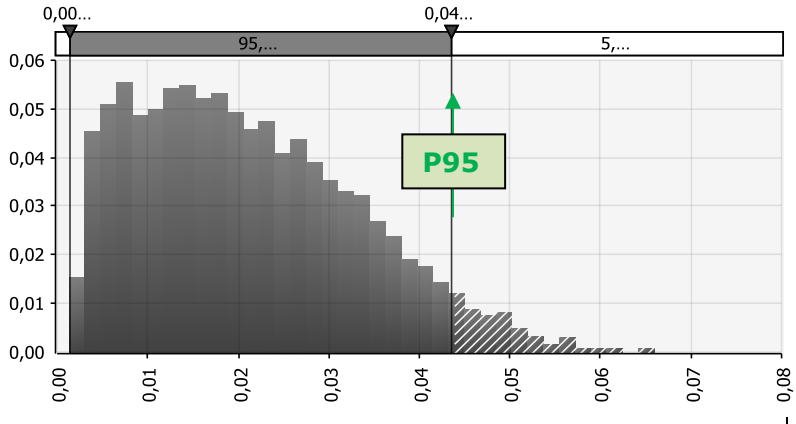
The second exercise conducted consists in the test in the model of different values of systemic bioavailability factor after dermal absorption. The arbitrarily chosen tested values are the following: 5%, 10 %, 30 %, 50 % and 75%. For these five values, the respective internal doses distributions were calculated, the other parameters of the model remaining unchanged.

$$IED = \underbrace{\frac{F \times D \times S}{BW}}_{\text{Same distributions or deterministic values than before}} \times \underbrace{f\ systemic\ bioavailability}_{\text{5 values are tested: 5%, 10%, 30%, 50% and 75%.}}$$

The graphics below show the 5 different probability distributions describing the internal exposure dose introducing respectively 5%, 10%, 30%, 50% and 75% for the systemic bioavailability factor in the model:

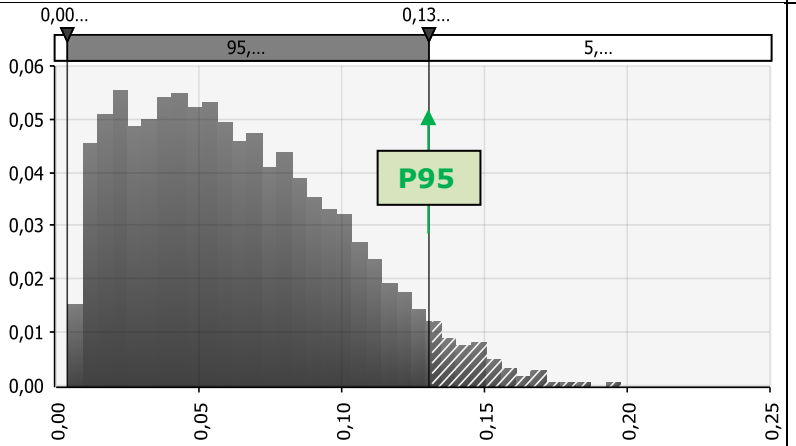
Factor of systemic bioavailability	IED distribution [µg.kg$_{BW}^{-1}$.d^{-1}]	Descriptive statistics
5%		Min 6.74E-04 Max 3.56E-02 Mean 1.06E-02 5% Perc 2.22E-03 25% Perc 5.62E-03 50% Perc 9.71E-03 75% Perc 1.47E-02 90% Perc 1.91E-02 95% Perc 2.17E-02

10%



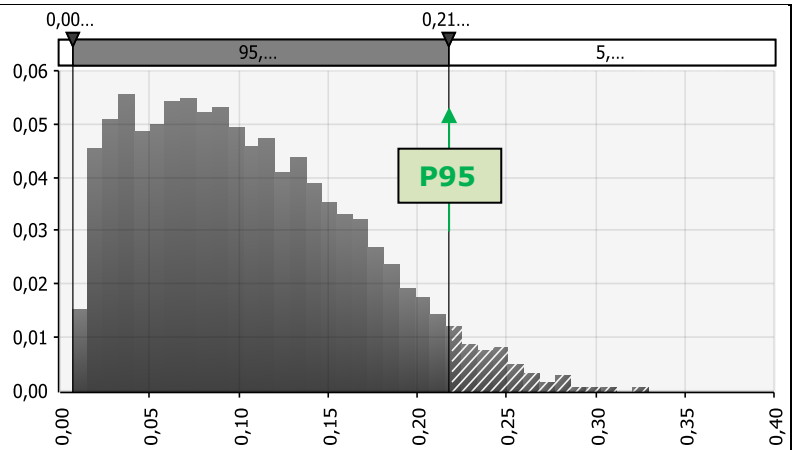
Min	1.35E-03
Max	7.11E-02
Mean	2.11E-02
5% Perc	4.45E-03
25% Perc	1.12E-02
50% Perc	1.94E-02
75% Perc	2.95E-02
90% Perc	3.81E-02
95% Perc	4.35E-02

30%



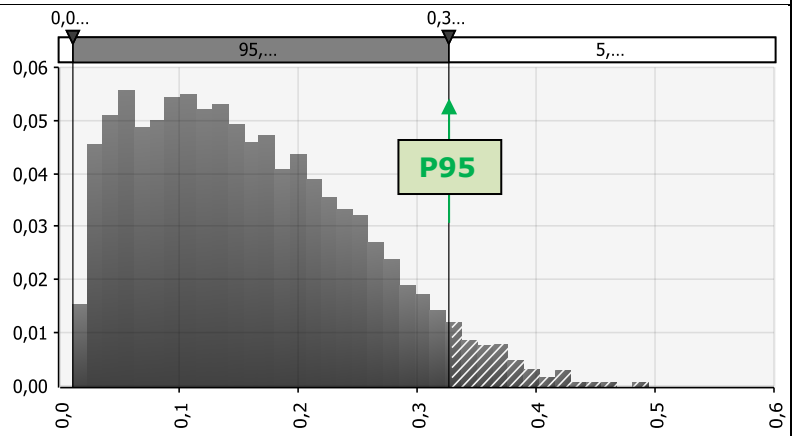
Min	4.05E-03
Max	2.13E-01
Mean	6.34E-02
5% Perc	1.33E-02
25% Perc	3.37E-02
50% Perc	5.83E-02
75% Perc	8.84E-02
90% Perc	1.14E-01
95% Perc	1.30E-01

50%



Min	6.74E-03
Max	3.56E-01
Mean	1.06E-01
5% Perc	2.22E-02
25% Perc	5.62E-02
50% Perc	9.71E-02
75% Perc	1.47E-01
90% Perc	1.91E-01
95% Perc	2.17E-01

75%



Min	1.01E-02
Max	5.34E-01
Mean	1.59E-01
5% Perc	3.33E-02
25% Perc	8.43E-02
50% Perc	1.46E-01
75% Perc	2.21E-01
90% Perc	2.86E-01
95% Perc	3.26E-01

D- Exposure assessment: results for consumers

1) Exposure estimation for consumers (pregnant women)

In the event of exposure of a consumer to thermal papers, it was decided to model the exposure according to two different approaches, using on the one hand an **absorption flow** expressed **in µg.cm^{-2}.h^{-1}**, and on the other an **absorption rate** expressed **in percentage absorbed** of the quantity of BPA transferred onto the skin. Unlike the professionals, the consumer will touch relatively few receipts over the course of a day and it is likely that the quantity of bisphenol A on the fingers is not constant through time. It appeared therefore justified to use an approach based on the level of absorption (absorption rate) combined with contact with a thermal receipt with BPA.

Given the uncertainties associated with each of these two approaches, and with a view to being more conservative for the health of consumers, the internal daily dose were calculated *via* the two models, but only distribution of the highest doses was retained to undertake the HRA (corresponding to the approach by level of absorption).

The model using an absorption flow (model a) depends on this flow, on the absorption duration, on the surface in contact with the thermal receipt and on body weight. The one using an absorption level (model b) depends on this level, on the quantity of BPA deposited onto the fingers by contact, on the number of fingers in contact with the receipt, on the absorption duration and lastly on body weight.

In the following, only the model used in the HRA is detailed. This is the model b.

$$IED = \frac{Rabs \times Qsubs \times N \times D}{2 \times BW} \times f\ systemic\ bioavailability \qquad \text{(Model b)}$$

With:

IED: Internal (exposure) daily dose [µg/kg$_{BW}$/d]

R_{abs}: Level of absorption (absorption rate) established for absorption duration of 2 hours [%]

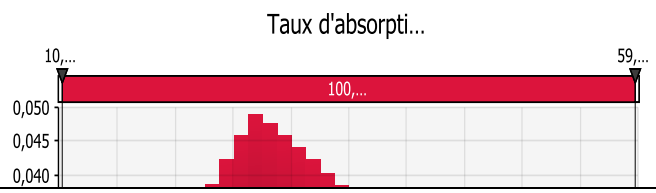
Q_{subs}: Quantity of the substance deposited by contact [µg/finger]

N: Number of fingers in contact with the till receipt [finger]

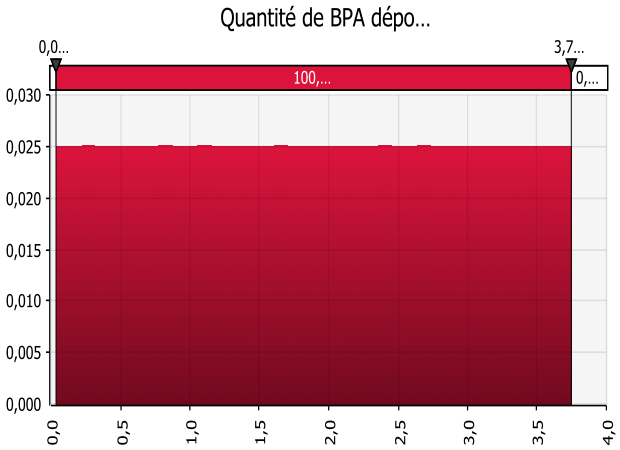
D: Absorption duration [h/d]

BW: Body weight [kg$_{BW}$]

$f_{systemic\ bioavailability}$ [%]

Input variables	Distribution or deterministic value applied		
R_{abs}: Absorption rate [%] The probability distribution assigned to this parameter is based on expert judgment from two		Min P25 P50	10,22 24,58 31,28

bibliographic sources, the RAR of the European Commission (EC, 2010) and the study of Biedermann *et al*., (2010). It is then proposed to specify a **triangular distribution** with as a **minimum of 10%,** value used by default in the RAR, a mode which corresponds to estimated rate of **27%** in the study by Biedermann et al., (2010) from the amount of BPA transferred onto the skin of the finger after 5 seconds of contact with a ticket, and the amount of BPA which was no longer removable from the skin by water and soap 2 hours after this contact, and as a **maximum of 60%** which corresponds to the estimated rate by Biedermann et al. (2010) 2 hours after the immersion of the finger in an ethanol solution containing BPA.		P75	39,69
		P90	47,15
		P95	50,91
		P99	55,94
		Max	59,83
		Mean	32,33

Q_{subs}: Quantity of the substance deposited by contact [µg/finger] For this parameter, it was decided by expert judgment to specify a **uniform distribution** whose limits are defined from two sources studies. These studies are Biedermann et al. (2010) and the Danish EPA (2011). The measurements were made using a similar protocol. The first study was performed on 14 measures five types of thermal papers and obtained amounts of BPA deposited on a finger ranging from 0.035 to 3 µg. The second measured on four thermal receipt amounts of BPA ranging from 0.58 µg to 3.75 µg. Therefore, it was decided to give a uniform distribution with a variation range of **from 0.035 to**		Min	0,04
		P25	0,96
		P50	1,89
		P75	2,82
		P90	3,38
		P95	3,56
		P99	3,71
		Max	3,75
		Mean	1,89

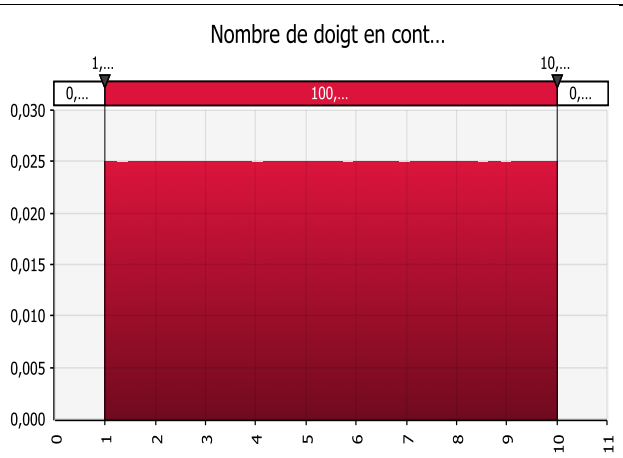
3.75 µg per finger.			

N: Number of fingers in contact with the till receipt

The **uniform distribution** specified for this parameter is also based on expert judgment.

Min: 1 finger, corresponding to the fact that the ticket can only be held with the thumb in contact with one face containing BPA.

Max: 10 fingers.

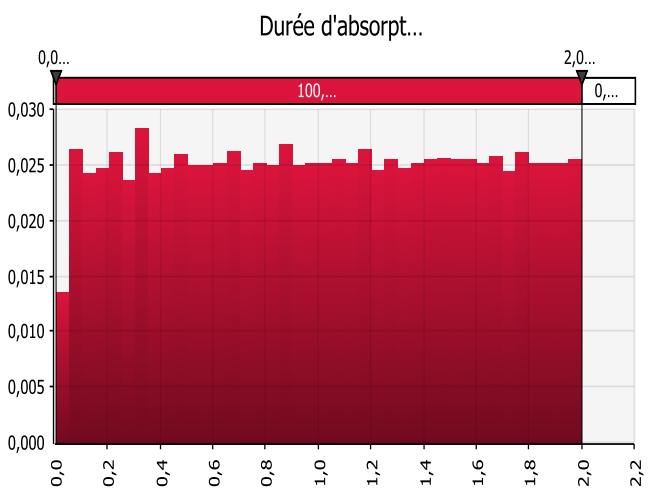


Min	1
P25	3,25
P50	5,50
P75	7,75
P90	9,10
P95	9,55
P99	9,91
Max	10
Mean	5,50

D: Absorption duration [h/d]

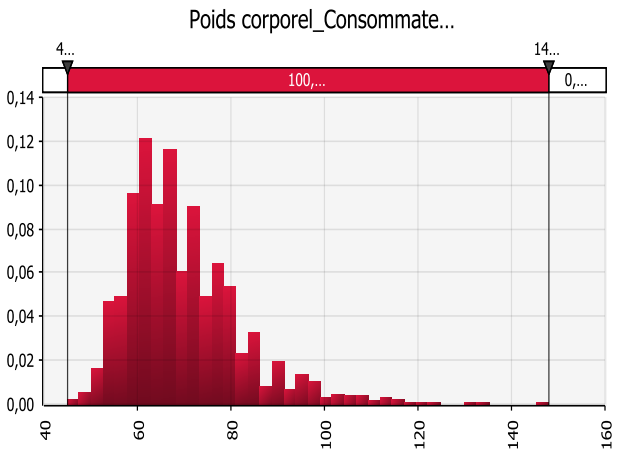
It was decided to assign a **uniform distribution** with a range varying minimum daily hours of contact with the ticket (the result from the multiplication of the duration of contact with the daily frequency of contact) to 2 hours maximum. It is thus considered that the absorption time of the BPA is a minimum to the duration of contact with the tickets and at most 2 hours. This maximum value was selected by expert judgment.

Probability distributions specified for the contact time with a ticket and the daily frequency of contact with a ticket for a consumer are from expert judgment based on the study of the Danish EPA (2011) where it is considered a contact time ranging from 5 to 66 seconds per contact, and a daily frequency of 1 to 5 contacts. This frequency of contact was estimated by the Danish EPA from data on the number of credit card



Min	7.10^{-3}
P25	0,52
P50	1,01
P75	1,51
P90	1,80
P95	1,90
P99	1,98
Max	2
Mean	1,02

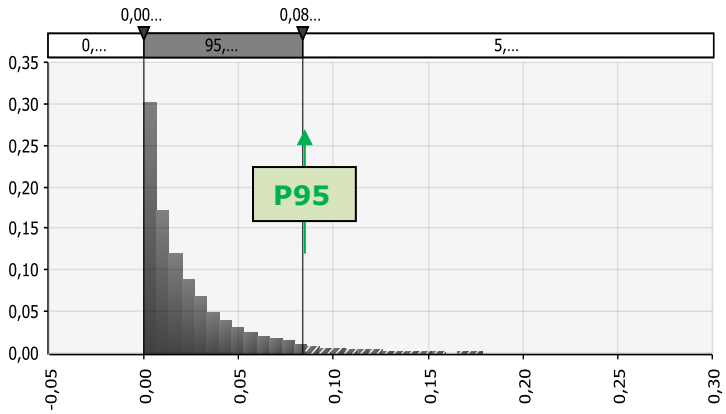
transactions in Denmark, on the distribution of payment methods, and the percentage of thermal paper receipts containing BPA (EU data).			

Input variables	Distribution or deterministic value applied		
BW: Body weight [kgBW] To determine the distribution of probabilities illustrating the **body weight for the pregnant woman as consumers**, the entire period of pregnancy has been taken into consideration in calculation of the average weight of each of the individuals. The study of pre- and post-natal determinants of development and health of the Child (EDEN[91]), gives the body weights of the pregnant women at different stages of the pregnancy and was used to document this parameter.		Min	45
		P25	62
		P50	67
		P75	76
		P90	85
		P95	93
		P99	111
		Max	148
		Mean	69,71

$F_{systemic\ bioavailability}$ (%) There is no experimental analysis available to determine a factor of absolute bioavailability for dermal. Furthermore, no estimate of the relative bioavailability for subcutaneously could be performed, which does not allow to estimate the semi quantitatively factor bioavailability.	Default value : 100%

Result: Probability distribution describing the internal exposure dose (IED) via the manipulation of receipts by pregnant women consumers:

[91] The EDEN study was initiated by several teams of epidemiologists from the Institut Fédératif de Recherche 69, as well as participating clinicians from the CHU *(University Hospitals)* of Poitiers and Nancy. Their aim was to better define the characteristics of foetal development and the first few months of life which influence the development and the subsequent health of the child. 2002 women agreed to participate. Amon the very large amount of data available from this study, a distribution of discrete probabilities was simulated from the pairs "average weight/probability of occurrence".

IED distribution [µg.kg$_{BW}^{-1}$.d^{-1}]	**Descriptives statistics**	
	Min	9,13.10^{-6}
	P5	8,82.10^{-4}
	P25	5,12.10^{-3}
	P50	0,01
	P75	0,03
	P90	0,06
	P95	0,08
	Max	0,26
	Mean	0,02

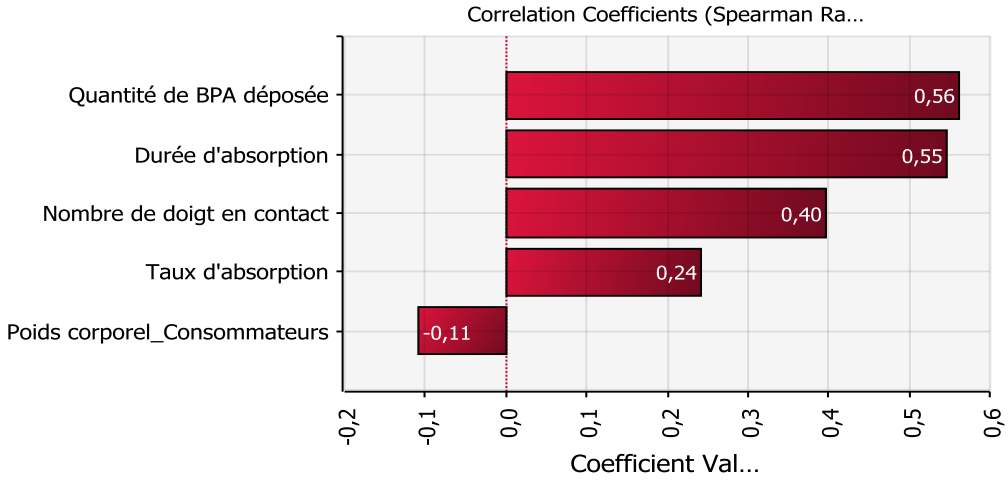
2) Sensitivity analysis

The same exercises as those made for the "workers"scenario were performed.

Only the results are presented below.

2.1) Parametric uncertainty

The graph below shows the sensitivity analysis by tornado graph of modelled internal dose for pregnant women handling thermal receipt containing BPA as consumers:



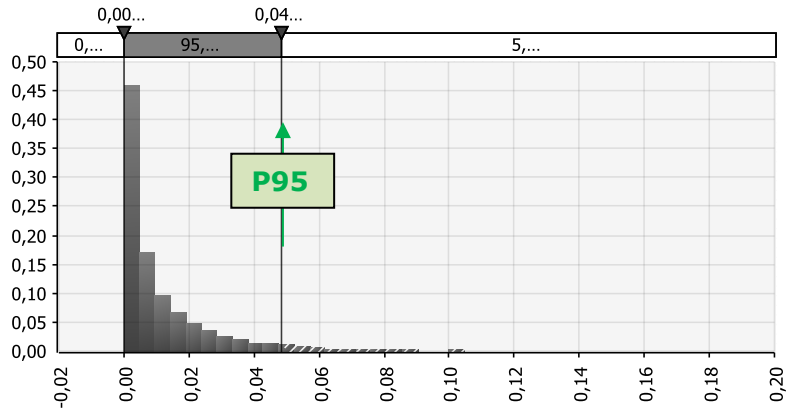
Sensitivity analysis by tornado graph of the modelled internal exposure dose for pregnant women consumers handling thermal paper containing BPA

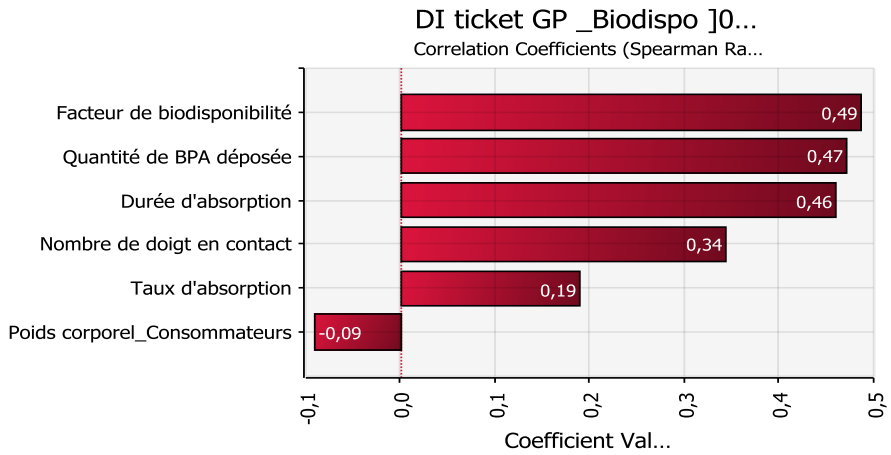
This graph shows that the amount of BPA deposited on the fingers and the duration of absorption are the most influential parameters on the internal dose calculated, taking into account the variability of different probability of distributions in the model.

2.2) Uncertainty on the value of systemic bioavailability factor after dermal absorption

First exercise:

Probability distribution describing the internal exposure dose (IED) via the manipulation of receipts by pregnant women consumers using a uniform distribution for systemic bioavailability [0.01% - 100%]

IED distribution [µg.kg$_{BW}^{-1}$.d^{-1}]	**Descriptives statistics**
	*To be completed*



Sensitivity analysis by tornado graph of the systemic bioavailability factor after dermal absorption compared to the other parameters

This exercise is a theoretical exercise conducted only to see through a sensitivity analysis by tornado graph on the obtained results, the extent to which factor systemic bioavailability after dermal absorption is an influential parameter.

The sensitivity analysis shows that the factor of systemic bioavailability after dermal absorption is the most influential parameter on the result of the calculation, given the high variability attributed to this factor.

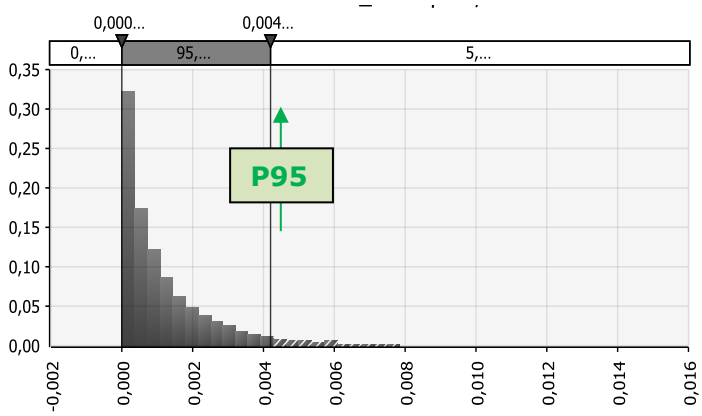
However, the influence of the parameters "amount of BPA deposited" and "duration of the absorption" are of almost equal influence. The sensitivity of these parameters, the documentation of which is based on limited data in the model, tends to give more uncertainties to the scenario of "consumer exposure handling thermal receipt" than to the scenario of "cashiers/workers exposure".

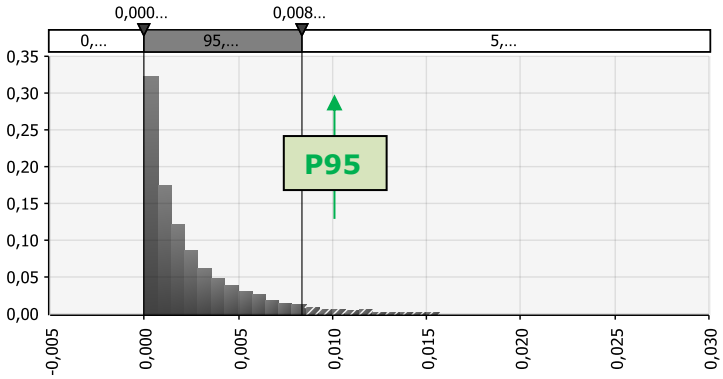
Second exercise:

$$IED = \underbrace{\frac{Rabs \times Qsubs \times N \times D}{2 \times BW}}_{\text{Same distributions than before}} \times \underbrace{f\ systemic\ bioavailability}_{\text{5 values are tested: 5\%, 10\%, 30\%, 50\% and 75\%.}}$$

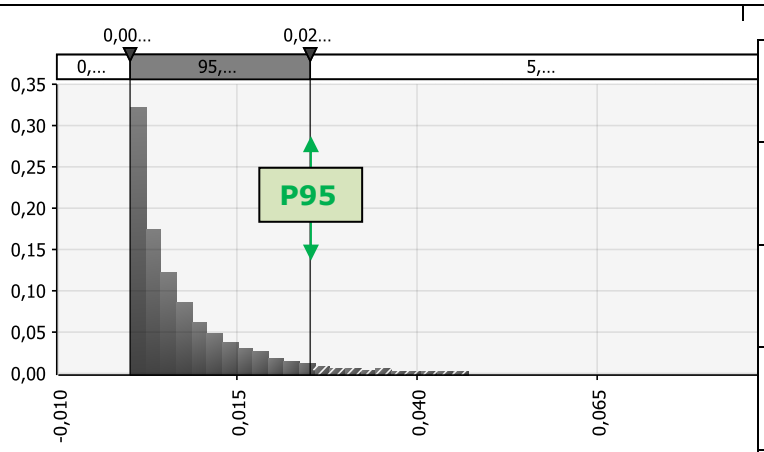
The graphics below show the 5 different probability distributions describing the internal exposure dose introducing respectively 5%, 10%, 30%, 50% and 75% for the systemic bioavailability factor in the model:

Factor of systemic bioavailabilit	**IED distribution [µg.kg$_{BW}^{-1}$.d^{-1}]**	**Descriptive statistics**

y		
5%		Min 4,56E-07 Max 1,32E-02 Mean 1,24E-03 5% Perc 4,43E-05 25% Perc 2,56E-04 50% Perc 7,24E-04 75% Perc 1,66E-03 90% Perc 3,13E-03 95% Perc 4,18E-03

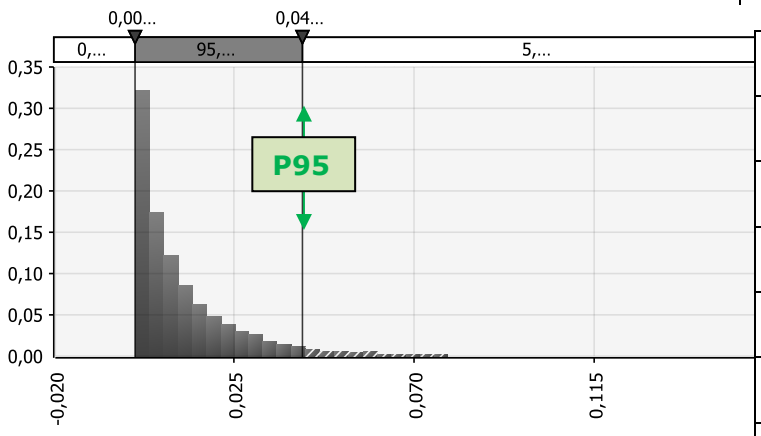
Factor of systemic bioavailability	**IED distribution [µg.kg$_{BW}^{-1}$.d^{-1}]**	**Descriptive statistics**
10%		Min 9,13E-07 Max 2,65E-02 Mean 2,48E-03 5% Perc 8,87E-05 25% Perc 5,12E-04 50% Perc 1,45E-03 75% Perc 3,31E-03 90% Perc 6,26E-03 95% Perc 8,37E-03

30%



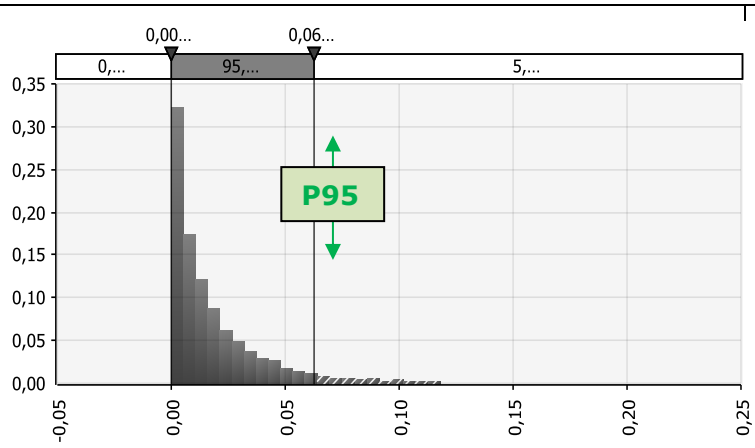
Min	2,74E-06
Max	7,95E-02
Mean	7,45E-03
5% Perc	2,66E-04
25% Perc	1,54E-03
50% Perc	4,34E-03
75% Perc	9,94E-03
90% Perc	1,88E-02
95% Perc	2,51E-02

50%



Min	4,56E-06
Max	1,32E-01
Mean	1,24E-02
5% Perc	4,43E-04
25% Perc	2,56E-03
50% Perc	7,24E-03
75% Perc	1,66E-02
90% Perc	3,13E-02
95% Perc	4,18E-02

75%



Min	6,84E-06
Max	1,99E-01
Mean	1,86E-02
5% Perc	6,65E-04
25% Perc	3,84E-03
50% Perc	1,09E-02
75% Perc	2,48E-02
90% Perc	4,69E-02
95% Perc	6,28E-02

E- Discussion and arguments

1) Cutaneous absorption

Differences between professionals and consumers for the exposure modelling by cutaneous contact in the HRA:

Professionals and consumers are differently exposed to BPA in term of duration and frequency of exposure. Indeed, the professionals is assumed to be continuously exposed over the duration of exposure to the till receipt, to a constant quantity of BPA on the skin surface of the finger whatever the duration (between 5 and 60 seconds) or the number (between 3 and 10) of contacts with the receipts, based on the study of Biedermann, 2010. Thus, the percutaneous absorption flow based on the study of Marquet, 2010 has been used to model the professionals' exposure. At the contrary, the consumer will touch relatively few receipts over the course of a day and it is likely that the quantity of bisphenol A on the fingers is not constant through time. It appeared therefore justified to use an approach based on the rate of absorption combined with contact with a thermal receipt with BPA for the consumers based on the study of Biedermann, 2010. The absorption rate is expressed in percentage absorbed of the quantity of BPA transferred onto the skin.

Regarding absorption via the cutaneous route, the European Commission considers that only 10% of the dose in contact with the skin is absorbed (European Commission, 2004, 2010). This estimation is confirmed by a study using a pig skin model (Kaddar et al., 2008). However, new *in vitro* studies may suggest that the cutaneous absorption of BPA could be greater (Morck *et al*., 2010; Zalko *et al*., 2011, Marquet *et al*., 2011; Demierre *et al*., 2012).

These studies are detailed below.

In the study **of Mørck *et al*., 2010** percutaneous absorption was measured on a static diffusion model (Franz cell) with human skin of 0.8 to 1 microns thick in contrast to the recommendations of the OECD guideline 428 which recommend a thickness of 200-400 microns (Mørck et al., 2010). The anatomical region of the skin is not specified, nor the number of donors. The cells are maintained at 35° C to maintain the skin at a temperature of 32° C. The integrity of the skin is checked by measuring the electric capacity. Skin is exposed to 17.5 mmol of BPA (422 µg with a concentration of 4mg.L^{-1}) for 48 hours and the receptor fluid is withdrawn at intervals. After 48 hours, 13% of BPA were found in the receiving room, 7.4% and 17.2% respectively were found in the epidermis and dermis. The percentage found in the receiving room is of the same order of magnitude as the UE default value of 10 % for dermal absorption (European, 2004, 2010). However, the estimation of skin penetration was not the original purpose of the study. Therefore, very few details are provided about the methodology. Nevertheless, the study generally follows the OECD recommendations 428 for estimating a rate of percutaneous absorption.

Research **Zalko *et al*, 2011** aims to assess skin penetration and metabolism of BPA in two *ex vivo* models: pig ear skin and human abdominal skin (Zalko *et al*, 2010).

The static diffusion model used a cell surface of 23 mm diameter. BPA diffusion and metabolism in fresh skin explants from pig ear were studied at different doses of radiolabeled BPA. The administered doses are 50, 100, 200, 400 and 800 nmol, corresponding to 2.75; 5.5; 11; 22 and 44µg.cm^{-2} in a buffer solution ethanol/phosphate. Skin samples used were previously dermatomed 500 microns thick. The same study was performed with 3 doses of BPA (50, 200 and 800 nmol) on skin explants of pig ear previously frozen at -20 ° C, which induced inactivation of biotransformation enzymes of phase I and phase II (method of *Kao et al*., 1985).

Finally, the operation is repeated with a single dose of 50 nmol, on human skin explants derived from 3 Caucasian women.

The radioactivity was determined in the different compartments: skin surface, skin explant, culture media, wells and inserts. The measurements were made at 24, 48 and 72 hours after application, but only the data to 72 hours are described. First, the skin is washed with a cotton swab impregnated with an ethanol / water solution, and then the swab is treated to extract the amount of residual BPA on the skin surface. The culture medium was collected and analyzed. The wells are washed and the amount of BPA in the washing liquid is determined.

The BPA within the skin is extracted and its amount is determined, as well as BPA trapped in the skin pellet, which is considered as "non-extractable radioactivity."

For each experiment and for each compartment, research and possible metabolite levels of BPA are determined.

After 72 hours of incubation, most of the radioactivity was recovered in the culture media for the frozen and fresh skin models from pig ear. For the pig ear fresh skin the values ranged from 53 ± 3.7% (800 nmol BPA) to 65.3 ± 8.2% (50 nmol BPA) of BPA applied on skin. Almost all BPA deposited penetrates into the skin whatever the dose, less than 1% of the dose being recovered at the skin surface. 20.8 ± 7.1% (50 nmol BPA) to 31.9 ± 5.7% (800 nmol BPA) were found within the skin. The results of the distribution of radioactivity in the different compartments depending on the model for the applied dose of 50 nmol after 72 hours are summarized in Table below.

	pig ear frozen skin	pig ear fresh skin	human fresh skin
Skin surface	< 3%	< 1%	2,5% ± 0,8%
Skin explant	28,8 ± 8,3%	20,8 ± 7,1%	41,5 ± 10,8%
Culture media	58,1 ± 3,6%	65,3 ± 8,2 %	45,6 ± 6,2%
Wells & inserts	< 3,2%	< 1,5%	NC

NC : not communicated

The study of Zalko *et al*., 2011 does not correspond to a penetration study as recommended by the OECD Guideline 428 and the doses used are not saturating doses. The incubation time is 72 hours beyond the 24 hours recommended to preserve the integrity of the explants and no test was performed to ensure this integrity. The exposure protocol using an ethanol solution is different compared with a human exposure to BPA in tickets as assumed. The SCCS and the OECD point out that the result of a dermal absorption study can be considered relevant for a risk assessment only if the dose application was performed according to a protocol mimicking the actual exposure conditions (SCCS 2010 OECD, 2004b).

The study conducted **by Marquet *et al*., 2011** includes an *in vivo* study of percutaneous penetration in Sprague-Dawley (SD) rats, and *ex vivo* studies of skin penetration in SD rats and human skin explants. These studies were used to determine the percutaneous absorption in vivo and ex vivo flow in rats and humans. The results obtained for the two species were compared.

The percutaneous absorption in vivo in the rat was performed as follows. One day prior to administration, rat hair has been clipped on the back and shoulders and the skin surface was cleaned with acetone to remove sebum. The exposed surface (10 cm^2) is bounded by affixing an aluminium ring. BPA is applied with a syringe containing a solution of BPA 4 $mg.mL^{-1}$ (diluted with acetone) and 50µL.cm^{-2} (0.2mg.cm^{-2}). Actual deposited doses are determined by weighing the syringe before and after administration. After administration of the solution of BPA, experimenters have left the animal treated skin in the open air so that the acetone to evaporate. Then, the animals were placed in a metabolism cage to collect the urine and feces. After a variable period of exposure (1 to 30 hours), batches from 3 to 5 rats were euthanized and the skin around the metal ring washed with a swab wetted with ethanol to detect the diffusion of BPA outside the treated area. Five hundred microliters of ethanol are introduced through the membrane to dissolve the occlusion on the skin remaining BPA. The membrane was cut, and the non-absorbed fraction of BPA is harvested. The skin is then dried with swabs and the radioactivity is counted separately by scintillation. The amount of radioactivity is

measured in the following samples: plasma, exposed skin, skin around the exposed area, ring + membrane, swab, urine, feces (for an exposure of 24 and 30 hours) and the carcass.

The absorbed dose (dose applied measured in urine, feces and carcass) and the penetrated dose (absorbed dose + dose in exposed skin) increased linearly with time of exposure. The absorption flow and the penetration flow, expressed in $\mu g.cm^{-2}.h^{-1}$ flow are determined from the slope of the curve of cumulative doses absorbed or penetrated as a function of exposure time. The penetration and absorption flows are identical ($2.5 \pm 0.2 \mu g.cm^{-2}.h^{-1}$). Furthermore, the content of the skin does not change significantly between 1 and 30 hours exposure, with a mean value of $31 \pm 10 \mu g.cm^{-2}$. These results indicate that there is no accumulation of BPA in the skin.

The maximum flow penetration is obtained after one hour of exposure. Furthermore, 64 hours after an exposure of 8 hours, the amount of BPA in the skin goes from 33 to $6 \mu g.cm^{-2}.h^{-1}$. During the same period, the absorbed dose increases from 25 to $46 \mu g.kg^{-1}.h^{-1}$. These results show that at the end of 8 hours of exposure, the BPA in the skin can be further absorbed. The skin acts as a reservoir, which would explain the difference in half-life of urinary excretion by transcutaneous compared to intravenous administration (28 hours vs. 10 hours). Elimination is 3-6 times higher in the feces than in the urine. The performance is satisfactory as it is between 90 and 100% depending on the doses administered.

Percutaneous absorption ex vivo rat and human skin was evaluated with the use of a static Franz cell. In rats euthanized, the entire back region is cut and the subcutaneous tissue is removed. The fresh human skin samples are obtained from explants of patients undergoing abdominal plastic surgery.

The skin was dermatomed to approximately 400 microns thick and is cut into 1.76 cm^2 circular sections which are placed on the diffusion cell such that the epidermis is facing up. The diffusion cell was maintained at a temperature of 36 ° C, which corresponds to a temperature of 32 ± 1 ° C at the surface of the skin. The administrated dose is $200 \mu g.cm^{-2}$ from a solution of BPA in acetone containing $50 \mu L.cm^{-2}$. At the end of the experiment, the fraction of the non absorbed dose is removed from the skin with ethanol, and a dry swab as previously described in the experiments in vivo. Radioactivity is determined in the different fractions by liquid scintillation.

The integrity of each skin sample is measured before the experiment by the measure of the transepidermal water loss (TEWL) as recommended by the guideline No. 428 OECD). The rate of metabolism of the tetrazolium salt into formazan is used to assess cell viability.

At the dose tested, for 24 hours, the viability and integrity of the samples treated skin showed no differences with the control group (skin sample without BPA).

The value of the rat absorption flow measured ex vivo is in the same order of magnitude as that found in vivo ($1.48 \mu g.cm^{-2}.h^{-1}$ versus $2.5 \mu g.cm^{-2}.h^{-1}$) (Table below). The maximum percutaneous absorption flow measured in humans is about 10 times lower than the flow measured in rats. A similar difference in permeability between humans and rats has been found for the skin penetration of BPA di glycidyl ether (BADGE) on the fresh skin in vitro (Boogaard *et al*., 2000, cited in Marquet *et al*., 2011).

Cutaneous absorption flow

Percutaneous absorption study	*In vivo* study in SD rat	E*x vivo* study on fresh SD rat skin	E*x vivo* study on fresh human skin
Absorption flow ($\mu g.cm^{-2}.h^{-1}$)	2,5	1,48	0,12

In the study of **Demierre *et al.*, 2012** the skin used is from the upper thighs of two human cadavers. After thawing, 3 sections from donor n°1 and 4 sections from donor n°2 of 200 microns thick were made. This study was conducted according to Good Laboratory Practice and according to OECD Guideline 428. Demierre et al., 2012 used a dynamic model with an exposure area of 0.64 cm^2. The device was maintained at 30-32°C. The receptor fluid is composed of saline 0.9% NaCl with an adjusted flow to 3 $mL.h^{-1}$. The integrity of the skin was evaluated before the start of the experiment for determining the coefficient of permeability (Kp). Membranes whose Kp was over 2.5 x 10^{-3} $cm.h^{-1}$ were excluded from the study. An aqueous solution of BPA radiolabeled 193.6 $mg.L^{-1}$ activity with 3.9 $GBq.mmol^{-1}$ was applied at 1.82$\mu g.cm^{-2}$/membrane. This BPA concentration does not correspond to a saturating concentration. The receptor fluid was collected hourly for the first 6 hours, then every 2 hours to 24 hours. After 24 hours, the membrane was rinsed three times prior to a cleaning solution and then with water. The stratum corneum was split into 15 layers. The first five were analyzed individually and the layers 6 to 10 and 11-15 were pooled. The remainder of the unfractionated skin is called residual membrane. The radioactivity emitted by the BPA in the surface of the skin, in the different layers of the skin (stratum corneum fractions and residual membrane) and in the receptor fluid was determined by scintigraphic counting.

After 8 hours of application, 0.093$\mu g.cm^{-2}$ (5.1% of the applied dose) was found in the receptor fluid. After 24 hours, the rate is 0.157$\mu g.cm^{-2}$, corresponding to 8.6% of the applied dose (Table below).

The maximum flow of skin penetration, based on the linear part of the curve (withdrawals 1:00 to 4:00), is estimated at 0,022 $\mu g.cm^{-2}.h^{-1}$. In the stratum corneum, 34.9% of the dose was recovered, mainly in the outer layers.

Fraction	Amount of BPA (% of BPA applied)
Surface de la peau (liquide de rinçage)	56,9 ± 4,9
Liquide dans le compartiment donneur	0,5 ± 0,4
Stratum corneum	34,9 ± 6,6
Skin residual membrane	0,6 ± 0,3
Fluide receveur	8,6 ± 2,1
Total	101,5 ± 1,6

Studies conducted by teams Marquet *et al*., 2011 and Demierre *et al*., 2012 aimed to determine a value of transcutaneous absorption flow for BPA while the main purpose of the study Zalko *et al*. 2011 was the analysis of the metabolites of BPA and the comparison of pig ear skin model with human skin model. As the aims of the studies do not fit exactly, it is difficult to compare these results (Table below).

Comparative table of the studies which evaluated the cutaneous penetration of in vitro BPA on human explants

	Zalko *et al.*, 2011	Marquet *et al.*, 2011	Demierre *et al.*, 2010	Mørck *et al.*, 2010
Number of specimens	NC	15	7	11
Number of donors	NC	6	2	NC
Nature of the skin	cold	cold	Defrosted	NC
Thickness of the skin	500 µm	400 µm	200 µm	800 - 1000 µm
Anatomical region of the skin	Abdomen	Abdomen	Thigh	NC
Dose	2,75 µg.cm^{-2}	200 µg.cm^{-2}	1,82 µg.cm^{-2}	422 µg
Solvent	Ethanol/phosphate buffer	Acetone	Physiological serum	NC
Number of points to evaluate the flow	-	NC	4	-
Flow ± standard deviation	-	0,12 ± 0,09 µg.cm^{-2}.h^{-1}	0,022 ± 0,011 µg.cm^{-2}.h^{-1}	-
% of absorption	45,6 ± 6,2 % in 72 h	-	-	17,2 ± 6,45 % in 48 h

NC = not communicated

With regard to the HRA on workers exposed by skin contact with thermal papers containing BPA, percutaneous absorption of BPA is considered to be continuous during the period of work.

This hypothesis is based on the observations of Biedermann *et al*., 2010 which shows a constant quantity of BPA transferred to the surface of the skin on the finger, regardless of the duration (between 5 and 60 seconds) and number (between 3 and 10 contacts) of contact with the receipts. The absorption values used the HRA on workers correspond to the interval of the percutaneous absorption flow values measured in the Marquet *et al*., 2011 in vitro study on human explants. Although in vitro models cannot replace an in vivo model, it enables the mechanisms of interaction during absorption to be investigated, the tests to be multiplied, and work to be carried out on human skin. Moreover, the validity of the experimental protocol used by Marquet et al on human skin explants is supported by data on rats with an absorption flow value measured in vitro(1.5 $\mu g.cm^{-2}.h^{-1}$) of the same order of magnitude as that measured in vivo(2.5 $\mu g.cm^{-2}.h^{-1}$).

With regard to the HRA for the general population relating to cutaneous contact with thermal papers containing BPA, the estimate of the percutaneous absorption rate (expressed in % absorbed by the dose transferred onto the skin, and not in the quantity absorbed by surface unit of skin and time) corresponds to values of the least probable rate of a minimum of 10 % and a maximum of 60 %, encompassing a most probable value of 27 %. The rate of 27 % was used in an experimental study (Biedermann, 2010). The data from this study cannot be considered as representative on a population scale. However, the experimental protocol is considered to be similar to the conditions of exposure for a person handling cashier's tickets on an occasional basis during the day, different to cashiers. This rate was estimated from the quantity of BPA transferred to the skin of the finger after a single contact of 5 seconds with a ticket, and the quantity of BPA which was no longer removable from the skin by soap and water 2 hours after this contact. The maximum rate of 60 % corresponds to the rate estimated by Biedermann *et al*., (2010) 2 hours after immersion of the finger in an ethanol solution containing BPA ; while the minimum level of 10 % corresponds to a (default) recommended value by the European Commission when a substance has a molecular weight over to 500 $g.mol^{-1}$ and an octanol-water distribution coefficient lower than -1 or higher than 4 (European, 2004). As the absorption rates being estimated by Biedermann, 2010 correspond to an exposure duration of the skin surface to BPA of 2 hours after one ticket contact, they must be weighted by an appropriate duration of exposure for the general population handling BPA-based thermal papers.

Bibliography :

Boogaard PJ, Denneman MA, Van Sittert NJ. Dermal penetration and metabolism of five glycidyl ethers in human, rats and mouse skin. Xenobiotica.2000 May; 30 (5):469-83

Demierre AL, Peter R, Oberli A, Bourqui-Pittet M. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. Toxicol Lett. 2012 Sep 18; 213(3):305-8.

CE (2004) Guidance document on dermal absorption. Commission européenne, DG SANCO, Sanco/222/2000 rev. 7

CE (2010) European Union Risk Assessment Report - 4,4'-isopropylidenediphenol (Bisphenol A). Complete risk assessment in one document. . Commission européenne, Joint Research Centre. Final approved version awaiting publications.

Kao J, Patterson FK, Hall J. Skin penetration and metabolism of topically applied chemicals in six mammalian species, including man: an in vitro study with benzo[a]pyrene and testosterone. Toxicol. Appl. Pharmacol. 1985 Dec; 81: 502-516.

Marquet Kao J, Patterson FK, Hall J. Skin penetration and metabolism of topically applied chemicals in six mammalian species, F, Payan JP, Beydon D, Wathier L, Grandclaude MC, Ferrari E. In vivo and ex vivo percutaneous absorption of [14C]-bisphenol A in rats: a possible extrapolation to human absorption? Arch Toxicol. 2011 Sep ; 85(9):1035-43.

Mørck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L, Knudsen LE. Placental transport and in vitro effects of Bisphenol A. Reprod Toxicol. 2010 Aug;30(1):131-7.

OCDE (Organisation de coopération et du développement économiques), 2004. Ligne directrice de l'OCDE pour les essais de produits chimiques n°428: absorption cutanée, méthode in vitro.

SCCS (Scientific Committee of Consumer safety), 2010. The SCCS's notes of guidance for the testing of cosmetic ingredients and their safety evaluation. 7th revision Scientific Committee on Consumer Safety.

Zalko D, Jacques C, Duplan H, Bruel S, Perdu E. Viable skin efficiently absorbs and metabolizes bisphenol A. Chemosphere. 2011 Jan;82(3):424-30.

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2) (Extract of Annex 5 of the BD) ANSES opinion regarding skin penetration of BPA considerations in the EFSA HRA (Source : extract from the opinion of the ANSES in response to the consultation of the European Food Safety Authority, published on the 3rd April 2014)

In its report, EFSA considers that the diet (oral route) is the main source of exposure in the general population, while dermal exposure from thermal paper is considered the second source of exposure in the population above three years of age (see line 373). Of the five *in vitro* publications on the percutaneous penetration of BPA, EFSA relied on the article by Demierre *et al.* (2012) to estimate the contribution of the dermal route to total daily exposure. For EFSA, the total absorbed quantity over a 24-hr. period is 10% of the dose applied on the skin based on the 8.6% absorbed within 24 hrs. (quantity in the receptor fluid) and the 0.6% in the skin (excluding the *stratum corneum*). According to EFSA, the quantity of BPA in the *stratum corneum* (39.4% of the applied dose) should not be taken into account for systemic absorption (see line 2370).

The study by Demierre *et al.* (2012) is considered the key study for EFSA for whom it is a good-quality publication. Likewise, the use by Demierre *et al.* (2012) of water as a vehicle of BPA is more comparable to a scenario of consumer exposure to thermal paper than acetone (Marquet *et al.,* 2011) or diluted hydro-ethanol solutions (Mork *et al.,* 2010, Zalko *et al.,* 2011), and the applied surface density of 1.83 µg/cm^2 is comparable to exposure estimates as derived for thermal paper (1.37-5.5 µg/cm^2 finger tip).

For ANSES, the choice of the study by Demierre *et al*. (2012) as the key study and the rejection of the study by Zalko *et al*. (2011) (see line 18936) are questionable. First of all, the study by Demierre *et al.* (2012), which was supposedly undertaken in accordance with the OECD TG 428 guideline, has several weaknesses (see Annex 5). Secondly, a comparison of the results obtained by Mork *et al.* (2010), Zalko *et al*. (2011) and Demierre *et al*. (2012) does not favour a study undertaken with a diluted aqueous solution of BPA (Demierre *et al.*, 2012) over studies undertaken with varying concentrations of hydro-ethanol solutions of BPA (Mork *et al*., 2010, Zalko *et al*., 2011). Indeed, the permeability coefficient of BPA is independent of the type of vehicle used (aqueous or hydro-alcohol) or the concentration of BPA in the applied BPA solution. Thus, the Kp calculated from the experimental data reported by Zalko *et al.* (2011) is 0.9 10^{-4} cm/h. This Kp value is the same as the value obtained with Demierre *et al.* (2012) (kp=1.1 10^{-4} cm/h) who used a 194 µg/mL aqueous solution of BPA, and Mork *et al.* (2010) (kp=1.75 10^{-4} cm/h) who used a 3995 µg/mL hydro-ethanol solution. Likewise, the fraction of BPA absorbed within 24 hrs. is comparable for Mork *et al*. (2010) (approximately 6.5%= 13 X 24 h/48 h), Demierre *et al.* (2012) (8.6%) and Zalko *et al.* (2011) (15.2%= 45.6% X 24 h/72 h).

EFSA's affirmation that the use of water as a vehicle for BPA is more comparable to a scenario of exposure to thermal paper than acetone needs to be justified. Marquet *et al.* (2011) applied BPA as a solution in acetone. The acetone immediately evaporated. In these conditions, BPA in solid form was directly put into contact with the *stratum corneum,* as in the case of BPA transferred from thermal paper to the *stratum corneum* of the finger. The absorption flux of BPA (0.12 µg/cm^2/h) applied at a rate of 200 µg/cm^2 of skin (after evaporation of acetone) was approximately 6-7 times smaller than the BPA flux of 0.70 µg/cm^2/h (13%/48h X 259 µg/cm^2) obtained after applying BPA in a hydro-alcoholic solution at a rate of 259 µg BPA/cm^2. This difference in flux can be attributed to the need to first dissolve solid BPA before it penetrates the skin.

EFSA estimates that only 10% of the BPA dose applied on the skin is bioavailable within 24 hrs. This value is based on the quantity found in the receptor fluid (8.6% of the dose) and the skin (0.6% of the dose) reported by Demierre *et al.* (2012). This quantity in the skin is small compared to the values reported by Kaddar *et al.* (2008) and Mork *et al.* (2010) which are, excluding the *stratum corneum* and epidermis, 8.8% after 10 hrs. of exposure and 17.2% after 48 hrs. of exposure respectively. A significant reservoir effect was also reported *in vivo* in rats in which over 80% of the quantity of BPA in the skin after 8 hrs. of exposure was absorbed within 68 hrs. (Marquet *et al*., 2011). In light of the data in the literature, failure to take into account a skin reservoir effect could cause the daily dose of absorbed BPA to be under-estimated.

In its HRA, ANSES used a triangular distribution for skin penetration rates with 27% as the most likely value and 10% and 60% as the lower and upper limits, weighted by the daily duration of skin penetration. ANSES's experts considered 27% to be the most likely value as it was taken from a study in volunteers handling receipts in exposure conditions similar to those

of real life (Biedermann *et al.*, 2010). The study by Demierre *et al.* used by EFSA was undertaken using human skin explants on which BPA was applied in the form of an aqueous solution. This formulation was different from that of receipts, and therefore the choice of this study for this assessment did not adhere to the guidelines (OECD 428, EHC235), which underline the need for studies to reflect real-life exposure conditions in terms of doses, durations and formulations.

The choice of the study by Demierre *et al.* as the key study and the estimate of 10% as a conservative value are defended but remain questionable considering the methods and results reported in the BPA skin absorption studies (see Annex 5 of the opinion).

High uncertainty remains as to the fate of BPA after skin penetration and the degree of metabolisation by the skin. Few studies have properly investigated the metabolism of BPA and ANSES approves EFSA's recommendations as to the need to further explore this issue (see line 6876). No toxicokinetic studies have measured the dermal bioavailability of BPA. Therefore, the evidence once again seems limited to affirm, as stated in the EFSA report, that the value of 10% skin penetration is conservative (see line 6489 lines 6860-6862).

For information, ANSES's approach resulted in a percutaneous absorption rate of 0.02% to 27% (probabilistic approach) over a 24-hr. period, which can be compared to EFSA's rate of 10% (deterministic approach)[92]. In the end, this difference between the EFSA and ANSES approaches hardly influences the difference in results between the respective risk assessments, which is mainly related to the choice of toxicological benchmark dose. Furthermore, ANSES observes that in the recent SCENIHR Opinion on the safety of bisphenol A in medical devices, the experts chose a skin penetration value of 25-30% taken from the study by Demierre *et al.* based on the same *corpus* of data.

3) Exposure assessment : limits of the exercise

The uncertainties linked to assessing exposure to BPA of consumers and professionals via handling of thermal-printed receipts are described here above.

Uncertainties linked to the scenarios:

The scenario of occupational exposure is centered on exposure via the cutaneous route of cashiers handling receipts with a particular focus on pregnant women. So, other professions exposed to thermal papers (lottery tickets, self-adhesive labels) were not taken into account.

Other routes of exposure to BPA such as hand-mouth contact are possible but were not able to be modelled taking into account the insufficiency of the available data.

[92] In the ANSES exposure model, taking into account the penetration period used and the absorption rate of 27%, the 24-hr. absorption rate is approximately 0.02% to 27%, which is a range of equally probable outcomes (this is an estimate and the model would need to be run again with triangular distribution for an exact result (mode: 27%, min 10% and max 60%)).

Only contact with the skin of the pads of the fingers was taken into account and not a surface in greater contact (inner side of the hands) which may not be excluded during changing of the roll or folding receipts for example.

The occupational exposure is assumed to be continuous and constant for the entire work duration on the basis of the observations of (Biedermann, 2010 which show a constant quantity of BPA transferred to the surface of the skin of the finger, whatever the duration (between 5 and 60 seconds) and repetition (between 3 and 10 contacts) of contact with the receipts.

Uncertainties linked to the structure of the models:

For the scenario "consumers" linked to the handling of thermal receipts, two models were developed:

The first which consisted of evaluating the internal dose from the flow of percutaneous penetration (as is the case for professionals) and with a non-continuous exposure over one day,

The second which consisted of evaluating the internal dose from a percutaneous absorption rate (in % of the amount on the skin surface) and the amount of BPA deposited on the skin surface linked to the contact with receipts over one day.

It has been decided to carry out calculations according to the two approaches considering the limits and the advantages of each one. Due to the uncertainties linked to use of either model, the most conservative results of exposure for the HRA orientated towards the choice of the second model (by use of a percutaneous absorption rate).

For the scenario "workers", the model retained cannot take into account the absorption of residual BPA in the skin tissue after the working day, which constitutes a factor of underestimation of exposure.

The models considered do not take into account the processes of metabolisation, distribution and elimination by the body. This constitutes a major uncertainty. One of the main limits is that they consider by default that 100 % of the dose absorbed by the skin is then bioavailable in the absence of robust toxicokinetic data for the cutaneous exposure route, and contrarily to the oral route which included the effect of initial hepatic passage. This hypothesis contributes to overestimate exposures calculated in connection with the handling of thermal receipts. The metabolisation of BPA linked strictly to passage through the skin barrier may be considered as an insignificant overestimation factor. The rate metabolized of the absorbed dose is estimated at 6 % after 10h of exposure according to the data from a study on explants of human skin (Zalko, 2011). These choices tend to increase the estimation of the internal dose.

More generally, knowledge of exposures via the cutaneous route is limited: contrarily to concentrations in the air, generally measurable by sampling and chemical analysis, there is not, to date, a standardised method, enabling sampling and therefore quantifying of the surface deposits of chemical products onto the skin. The only data of exposure that we have therefore comes from physical and toxicological models or from studies implementing experimental protocols.

Uncertainties linked to the input data of the models:

Concerning the value of the entry variables, the data of percutaneous penetration flow comes from 15 data *in vitro* on explants of human skin (Marquet, 2011). Extrapolation to a situation *in vivo* is reinforced by effective coherence of the estimations *in vitro* and *in vivo* in rats obtained according to the same experimental protocol (Marquet, 2011).

For the scenario of exposure of consumers using an absorption rate (in % absorbed of the dose transferred onto the skin), the percutaneous absorption of BPA corresponds to the least probable rate values of 10 % at the least and 60 % at the most, encompassing the most probable value of 27 % (Biedermann, 2010). This rate of 27 % was retained from an experimental study (Biedermann, 2010), the data of which cannot also be considered as representative to a population scale. However, the experimental protocol is considered as similar to conditions of exposure of a consumer who handles till receipts on an occasional basis during the day, different to cashiers. With the absorption rates therefore being estimated by Biedermann, 2010 for a duration of exposure of the skin surface to BPA of 2 hours, they were then weighted in the model of calculation by an exposure duration in the consumer varying at least from the daily duration of contact with the receipt (produced from the duration of contact with the daily frequency of contacts) to 2 hours at the most.

Taking into account the data and hypotheses used, the scenario of exposure of consumers handling till receipts appears subject to more uncertainties that the scenario of exposure of cashiers.

Annex 7: Thermal Paper containing BPA: EXPOSURE ASSESSMENT - EXPLANATIONS & ARGUMENTS II – Responses to RAC and SEAC rapporteurs requests

A – Introduction

From the second rapporteur's dialogue (26 September: 10:00-16:00), different questions and action points have been notified. They are summarized below:

Point n°1: The SEAC rapporteur mentioned the need for SEAC to know whether the modeled distribution was relevant at all for the real-life exposure in the population. This is important to know in the assessment of the disease burden.

Point n°2: It was asked what would change in testing 100000 iterations instead of 10000.

Point n°3: It was asked why we chose a uniform distribution for the percutaneous absorption flow.

Point n°4: It was asked why we use a value of 100% for the systemic bioavailability.

Point n°5: It was asked to provide geometric means.

Point n°6: The RAC co-rapporteur stressed the importance of a correct assumption for the absorption flow as it is the most sensitive input parameter in addition to the dermal bioavailability assumption. He stressed that the members had been very critical to the assumptions during the plenary meeting.

Point n°7: ECHA-S repeated the request to the DS to consider if the exposure modeling could be done with different input parameters. Because RAC will assume different parameters (possibly all the parameters used, alternative values and perhaps alternative distributions), the current probabilistic assessment could not be used.

B – Material and methods

In the following, we will distinguish two aspects:

- Aspects that need supplement explanations, test and arguments related to the models and the choices for input parameters that we've done in our HRA. They're provided in section C below that develops responses to point 1 to 6.
- Action points that were asked by the RAC rapporteur and related to new runs of the models with different input parameters. In section D below, we provide the different results obtained on the basis of inputs parameters provided by the RAC rapporteur. This corresponds to the point 7 mentioned above. These results are provides as illustrated points.

C – Explanations, arguments, new tests and calculations in response to the point 1 to 6

Response to point n°1: The SEAC rapporteur mentioned the need for SEAC to know whether the modeled distribution was relevant at all for the real-life exposure in the population. This is important to know in the assessment of the disease burden.

To assess exposure to BPA from handling thermal receipts, we've taken into account information and recommendations from an IPCS-OMS document title "Uncertainty and data quality in exposure assessment", and thus we've developed a probabilistic approach in order to improve our methodology for exposure assessment. The extracts from this document that are presented below, should help to understand how the probabilistic approach that we've developed allows for greater exposure likelihood estimates.

Context and background

Extract from "Uncertainty and data quality in exposure assessment", IPCS, WHO 2008 (http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf?ua=1):

The complexity of exposure assessments necessarily varies, depending on their purpose. Quantitative approaches to exposure assessment have evolved over time as new methodologies have developed, which has led to increasing detail and transparency and the potential for greater harmony in the results obtained from different exposure models.

In early exposure assessments used in risk characterization, practitioners often used single point estimates of the maximum exposure of individuals to compare with measures of dose–response. Such estimates often lacked transparency in the context of the assumptions on which they were based and led to confusion in terminology (employing concepts such as "upper-bound exposure" and "maximally exposed individual").

The limited transparency of traditional exposure assessments has been an obstacle to harmonization. The use of simple bounding estimates in exposure assessment, for example, precluded harmonization among agencies using different bounding assumptions. The limited transparency also failed to provide decision-makers with important information relevant to risk characterization and risk management, such as the (population) distribution of exposures and uncertainties. As a result, the impact of critical data gaps and research priorities were often not clearly articulated, and transparency around the incorporated degree of conservatism was often insufficient. This may have resulted in less than optimum efficiency in allocation of resources to risk management versus data generation to better inform the characterization of risks.

More recently, there has been increasing emphasis on the characterization of the exposure of different individuals in the population. For example, the United States Environmental Protection Agency's (USEPA) guidelines for exposure assessment, issued in 1992, called for both high-end and central tendency estimates for the population (USEPA, 1992). The high end was considered as that which could occur for the 90th percentile or higher of exposed individuals and the central tendency might represent an exposure somewhere near the median or mean of the distribution of exposed individuals.

Through the 1990s, there has been increasing emphasis also on characterization of the distinction between inter-individual variability and uncertainty in exposure assessments (e.g.NRC, 1994). During this time, there was also growing interest and use of probabilistic simulation methods, such as those based on Monte Carlo or closely related methods, as the

basis for estimating differences in exposures among individuals or, in some cases, in estimating the uncertainty associated with any particular exposure estimate (USEPA, 1997b).

These converging developments have brought the field of probabilistic exposure assessment from the background to a central part of exposure assessment today in many applications. The transparency afforded by probabilistic characterization and separation of uncertainty and variability in exposure assessment offers potential benefits in the context of increasing common understanding as a basis for greater convergence in methodology.

Variability versus uncertainty

Exposure assessment informs decision-making regarding protection of human health. As Cullen and Frey (1999) point out, decision-making regarding control of exposures is typically aimed at protecting a particular group, such as the entire population of a country, a highly exposed subpopulation, random individuals within a population or specific individuals with a particular characteristic in common, such as children. As pointed out by NRC (1994), there is certainty that different individuals will have different exposures. For example, each individual may have a different behavioral (activity) pattern, dietary pattern and physiological characteristics (e.g. breathing rates). For a given individual, these can change over time; at any given time, these vary between individuals. These differences lead to variability in the exposure levels of the individuals.

However, the true exposures are rarely known for a given individual and are estimated using modeling procedures based upon available data. Uncertainty regarding exposure estimates arises as a result of the limited availability of empirical information, as well as limitations in the measurements, models or techniques used to develop representations of complex physical, chemical and biological processes. As described by NRC (1994), "uncertainty forces decision makers to judge how probable it is that risks will be overestimated or underestimated for every member of the exposed population". Furthermore, because every individual can have a different exposure level, it is also possible that the estimate of uncertainty can differ among individuals.

Thus, the notions of intra-individual variability and uncertainty are distinct concepts, because they arise for different reasons. Variability is an inherent property of the system being modeled. In contrast, uncertainty can be conceptualized as dependent on the current state of knowledge regarding the system being modeled. From this perspective, uncertainty is more a property of the data than it is of the system being modeled. For example, the ability to make predictions regarding exposure will depend on the data and models available at the time that the prediction is made. Over time, perhaps "better" data might become available. Data could be considered better if they are more representative, more precise or both. A model would be better than another if it had less systematic error and greater precision. As the quality of data and models improves, the amount of uncertainty inherent in a prediction decreases. Thus, uncertainty is reduced as the result of developing an improved knowledge base.

Two important considerations in probabilistic exposure assessment are whether to quantify uncertainty and whether to separate it from variability within the analysis and output:

- *Option 1: When only variability is quantified, the output is a single distribution representing a "best estimate" of variation in exposure. This can be used to estimate*

exposure for different percentiles of the population but provides no confidence intervals and may give a false impression of certainty.

- *Option 2: When input distributions representing variability and uncertainty are combined (e.g. by "one-dimensional" or 1D Monte Carlo), the output is again a single distribution, but now represents a mixture of variability and uncertainty. It can be interpreted as an uncertainty distribution for the exposure of a single member of the population selected at random. This can be used to read off the probability of a randomly chosen individual being exposed to any given level.*
- *Option 3: When variability and uncertainty are propagated separately (e.g. by "two-dimensional" or 2D Monte Carlo), they can be shown separately in the output. For example, the output can be presented as three cumulative curves: a central one representing the median estimate of the distribution for variation in exposure, and two outer ones representing lower and upper confidence bounds for the distribution). This can be used to read off exposure estimates for different percentiles of the population, together with confidence bounds showing the combined effect of those uncertainties that have been quantified.*

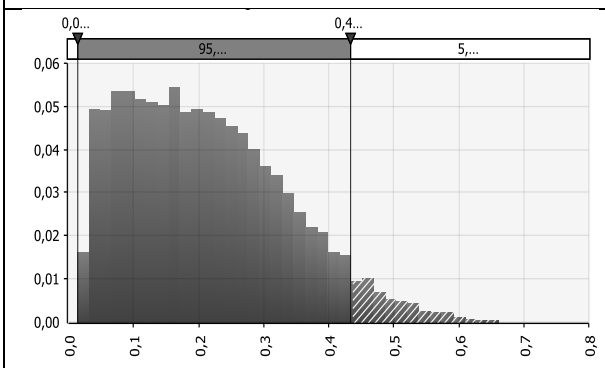
In our exposure assessment, we've developed the option 2: by adapting a distribution for the input variables of the model, the distribution of exposure levels based on their probability of occurrence takes into account the variability and some uncertainty, and thus the exposure estimations are more likely.

Response to point n°2: It was asked what would change in testing 100000 iterations instead of 10000.

In order to response to this question, we've re-run the models with 100000 iterations rather than 10000 for the two scenarios (workers and consumers). The graphics below show the results obtained. Considering those results, we can say that running the model with 100000 iterations rather than 10000 **do not influence the results and the conclusions of the risk assessment conducted.**

Worker scenario : initial simulation with 10000 iterations

IED distribution (µg/kgBW/d)



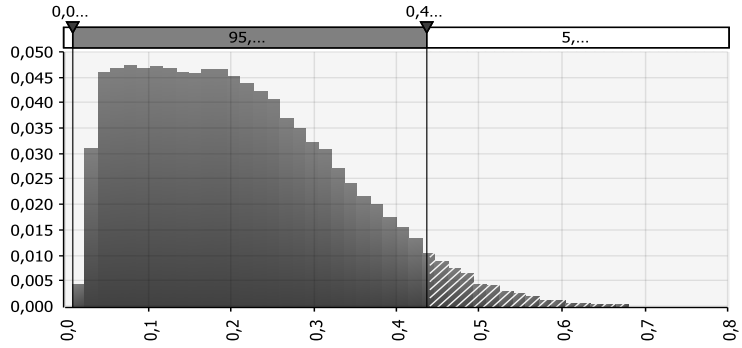
Descriptive statistics

Min	0.01
P5	0.05
P25	0.11
P50	0.20
P75	0.29
P90	0.38
P95	0.43
Max	0.71
Mean	0.21

		Geometric mean	0.172	

Worker scenario : new simulation with 100000 iterations

IED distribution (µg/kgBW/d)

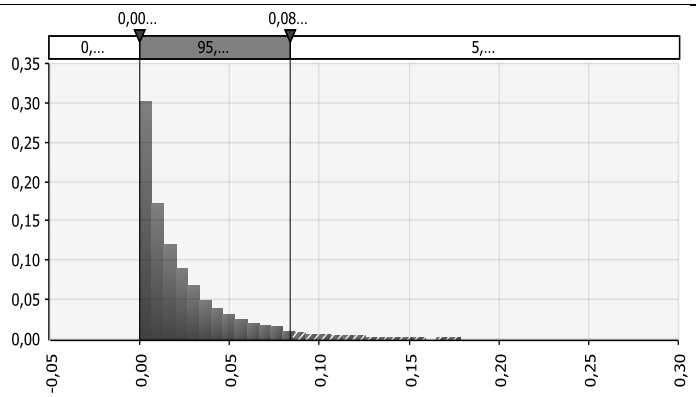


Descriptive statistics

Min	0.010
P5	0.045
P25	0.112
P50	0.196
P75	0.293
P90	0.382
P95	0.435
Max	0.817
Mean	0.211
Geometric mean	0.172

Consumer scenario : initial simulation with 10000 iterations

IED distribution (µg/kgBW/d)

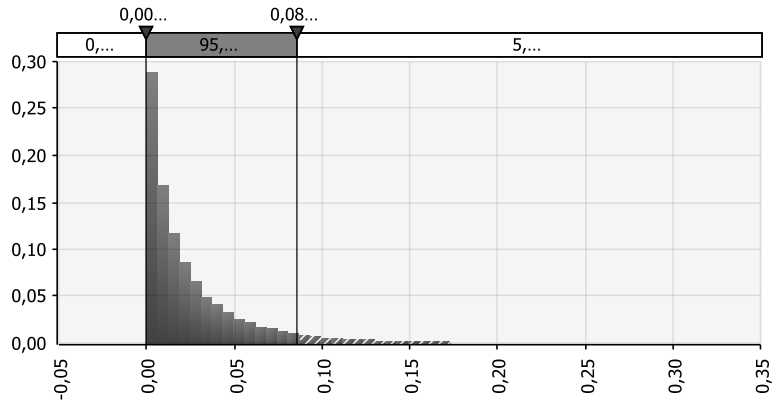


Descriptive statistics

Min	$9.13.10^{-6}$
P5	$8.82.10^{-4}$
P25	$5.12.10^{-3}$
P50	0.01
P75	0.03
P90	0.06
P95	0.08
Max	0.26
Mean	0.02
Geometric mean	0.012

Consumer scenario : new simulation with 100000 iterations

IED distribution (µg/kgBW/d)



Descriptive statistics

Min	1.82E-06
P5	8.40E-04
P25	0.005
P50	0.014
P75	0.034
P90	0.064
P95	0.086
Max	0.303
Mean	0.025

		Geometric mean	0.012	

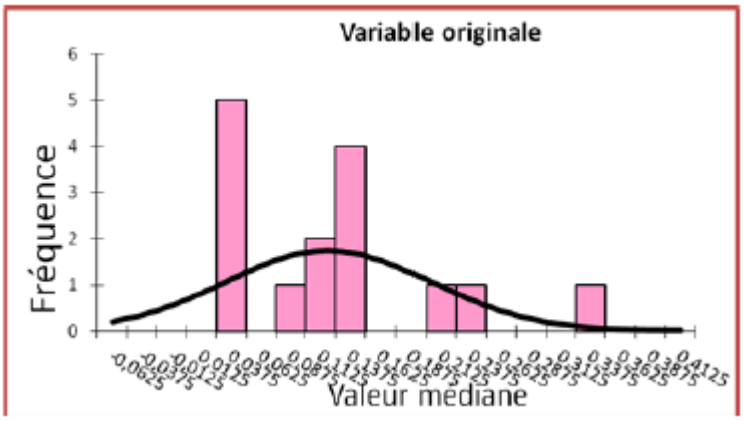
<u>Response to point n°3</u>: It was asked why we chose a uniform distribution for the percutaneous absorption flow.

The basic data used for this parameter (percutaneous absorption flow) are from the publication of Marquet et al. where 15 experimental results are available. From these 15 results, our experts have not considered they had sufficient data to extrapolate another distribution than a uniform one. To take into account the observation of the RAC rapporteur, we've re-analyzed the 15 results in order to see whether or not a different type of distribution was emerging.

Marquet et al, 2010: results from ex vivo study of skin penetration on fresh human skin explants (6 donors, duplicate or triplicate measurements):

	Percutaneous absorption flow of BPA ($\mu g/cm^2/h$)		
	Value 1	Value 2	Value 3
Donor 1	0.331	0.212	0.136
Donor 2	0.101	0.131	0.026
Donor 3	0.13	0.116	0.029
Donor 4	0.026	0.043	-
Donor 5	0.136	0.226	-
Donor 6	0.081	0.049	-

From the 15 results, development of the discrete distribution:



Comment: Shapiro & Wilk test → for α risk of 0.05, the distribution can be considered normal. In view of the data, a triangular distribution can be tested (very rough form of a log-normal distribution).

We have re-run the model with a triangular distribution rather than a uniform one for this parameter. The internal exposure doses distribution obtained is presented below. Considering the result, **it doesn't change the conclusions of the RA conducted.**

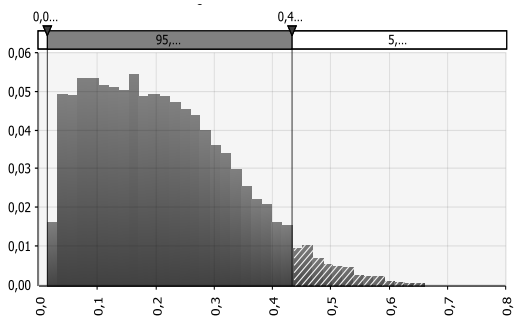
Initial simulation (workers scenario):

<table>
<tr><th>Input parameters</th><th>IED distribution (µg/kgBW/d)</th><th colspan="2">Descriptive statistics</th></tr>
<tr>
<td>Absorption flow : uniform (0.026 - 0.331 µg/cm^2/h)

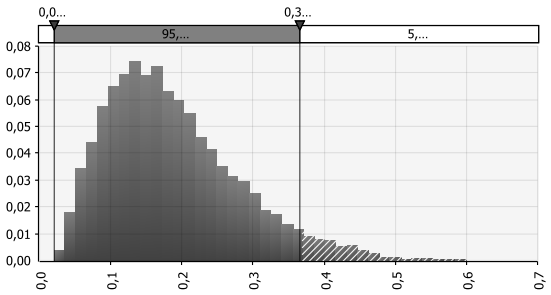
Exposure duration : triangular (3h/d - 6.5h/d - 10h/d)

Surface in contact : 12 cm^2

BW (pregnant women) : specific distribution from EDEN study

Systemic bioavailability : 100%</td>
<td> 
</td>
<td colspan="2">
<table>
<tr><td>Min</td><td>0.01</td></tr>
<tr><td>P5</td><td>0.05</td></tr>
<tr><td>P25</td><td>0.11</td></tr>
<tr><td>P50</td><td>0.20</td></tr>
<tr><td>P75</td><td>0.29</td></tr>
<tr><td>P90</td><td>0.38</td></tr>
<tr><td>P95</td><td>0.43</td></tr>
<tr><td>Max</td><td>0.71</td></tr>
<tr><td>Mean</td><td>0.21</td></tr>
<tr><td>Geometric mean</td><td>0.172</td></tr>
</table>
</td>
</tr>
</table>

New simulation (workers scenario):

Input parameters	IED distribution (µg/kgBW/d)	Descriptive statistics
Absorption flow : triangular (0.026 - 0.118 - 0.331 µg/cm²/h) Exposure duration : triangular (3h/d – 6.5h/d – 10h/d) Surface in contact : 12 cm² BW (pregnant women) : specific distribution from EDEN study Systemic bioavailability : 100%		Min 0.020 P5 0.064 P25 0.118 P50 0.171 P75 0.241 P90 0.316 P95 0.366 Max 0.630 Mean 0.187 Geometric mean 0.165

Response to point n°4: It was asked why we use a value of 100% for the systemic bioavailability.

As indicated in the first supplement document provided, in view of the results, a sensitivity analysis was carried out:

- To identify the influence of the input variables variability on the variability of the internal exposure dose (IED) calculated at the output;
- **To test the influence of different systemic bioavailability values after the cutaneous absorption.**

The model includes the percutaneous absorption but does not involve the systemic bioavailability factor after the absorption. Indeed, **there is no data to determine this bioavailability factor in the scientific literature, thus it was considered by default that BPA absorbed through the skin was then bioavailable in the body, with a systemic bioavailability factor of 100%.**

That's why we've conducted two different exercises to test the influence of the bioavailability factor *f* on the daily intake: we've tested a uniform distribution (0.01% - 100%) for this parameter rather than a deterministic value of 100% ("1st exercise"), and we've tested too other different deterministic values for this parameter (5%, 10%, 30%, 50% and 75%) ("2nd exercise"). Please, refer to the first supplement document provided, and see more particularly page 6 to 9.

Response to point n°5: It was asked to provide geometric means.

The tables below present the geometric means calculated for all the IED distributions presented in the first supplement document provided.

Worker scenario	
Input parameters	**Geometric Mean (µg/kgBW/d)**
Absorption flow: uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 100%	0.172
Absorption flow: uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: Uniform]0.01% - 100%] (eg sensitivity analysis)	0.064
Absorption flow: uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 5% (eg sensitivity analysis)	0.009
Absorption flow : uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 10% (eg sensitivity analysis)	0.017

Absorption flow: uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 30% (eg sensitivity analysis)	0.052
Absorption flow: uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 50% (eg sensitivity analysis)	0.086
Absorption flow: uniform (0.026 - 0.331 µg/cm^2/h) Exposure duration: triangular (3h/d - 6.5h/d - 10h/d) Surface in contact: 12 cm^2 BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 75% (eg sensitivity analysis)	0.129

Consumer scenario	
Input parameters	**Geometric Means**
Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 100%	0.012

Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: Uniform]0.01% - 100%] (eg sensitivity analysis)	0.005
Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 5% (eg sensitivity analysis)	0.001
Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 10% (eg sensitivity analysis)	0.001
Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 30% (eg sensitivity analysis)	0.004

Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 50% (eg sensitivity analysis)	0.006
Absorption rate: triangular (10% - 27% - 60%) BPA quantity deposited by contact: uniform (0.035 – 3.75 µg/finger) Number of finger in contact: uniform (1 – 10) BW (pregnant women): specific distribution from EDEN study Systemic bioavailability: 75% (eg sensitivity analysis)	0.009

Response to point n°6: The RAC co-rapporteur stressed the importance of a correct assumption for the absorption flow as it is the most sensitive input parameter in addition to the dermal bioavailability assumption. He stressed that the members had been very critical to the assumptions during the plenary meeting.

On this subject, the RAC rapporteur makes a specific comment:

" Absorption flow input data taken from Marquet et al (2011) experiments with acetone as a vehicle is the weakest point in this modeling as acetone even in short contact with the skin could have influenced the lipid layer of the skin and following the flow of percutaneous absorption through the skin. The study of Biedermann et al. (2010) suggesting a maximum absorption rate of 60 % estimated by 2 hours after the immersion of the finger in a BPA/acetone solution is in favour for the rather great role of vehicle used. Besides, it is the most influential parameter in this model according to sensitivity analysis performed by DS. Water used as vehicle in the experiments of Demierre et al. (2012) is more appropriate not changing the skin properties. The same can be said with respect to water – ethanol solutions used by a number of other authors. Both water and alcohol are polar protic solvents, but acetone – polar aprotic solvent(1).

(1): In general terms, any solvent that contains labile H+ is called a protic solvent. The molecules of such solvents readily donate protons (H+) to reagents. Conversely, aprotic solvents cannot donate hydrogen."

In response, we would like to bring the following items:

- It is true that the percutaneous absorption flow input data are the more sensitive in the model and that is what our sensitivity analysis has showed (see page 6 in the first supplement document provided).
- The choice of absorption flow data from the Marquet *et al* (2011) study results from an analysis of the different scientific papers related to percutaneous absorption of BPA available in the literature. In our first supplement document, we've provided descriptions of these different studies wich were not numerous.
- The study of Biedermann et al. (2010) suggests a maximum absorption rate of 60 % estimated by 2 hours after the immersion of the finger **in a BPA/ethanol solution, and not in a BPA/acetone solution**. This confusion may come from the mistake in p.10 of the document previously sent to the RAC (Thermal Papers containing BPA, 22th september 2014), even if it is well written in p.20 of the same document.
  Note: The publication of Bidermann et al. (2010) is provided to permit the RAC rapporteur to verify.
- **The weaknesses from the use of the absorption flow input data taken from Demierre et al (2012) should be mentioned**. Some limits from the use of the Demierre *et al* study (2012) have been described in Annex 5 of the opinion of the ANSES in response to the consultation of the EFSA *https://www.anses.fr/sites/default/files/documents/SUBSTANCES2014sa0033EN.pdf* (Annex 5 still in French), as mentioned in the document previously sent to the RAC (Thermal Papers containing BPA: BPA Restriction, EXPOSURE ASSESSMENT : EXPLANATIONS & ARGUMENTS, 22th september 2014). The limits identified are summarized below :
  - the limited number of donors (n = 2)
  - the absence of donor information (age, gender, race)
  - verification of the thickness of the dermatomed skin samples is not indicated; the dermatome settings give only a first indication of the final thickness of the skin sample
  - skin sample integrity and BPA absorption were determined by measuring the Radioactivity of the tritiated water and of the [^{14}C] BPA; no details are provided to explain the measures taken to reduce the radioactive cross-contamination
  - both skin samples give very similar results, which does not reflect the high inter-donor variation observed by Marquet et al. with BPA, or reported ex vivo on human skin with other molecules (Van de Sandt et al, 2004)
  - using an aqueous solution of BPA does not correspond to exposure conditions related to the deposition of BPA on the fingers from thermal receipts
  - no details are provided as to the conditions of the deposition of 10 µL/cm^2 of an aqueous solution containing BPA to ensure an homogeneous deposition on the entire surface of the sample. A volume of 10 µL per cm^2 of skin of an aqueous solution does not ensure a homogeneous distribution by simple spreading
  - unlike the study of Zalko et al. (2011), only one concentration was tested (193 µg / mL)
  - the authors estimate that only 9.3% of the applied dose is bioavailable after 24 h of exposure. This estimate is based on the percentage of the deposited dose that is present in the receptor fluid (8.6%) and in the skin (0.6% excluding the *stratum corneum*). The amount present in the *stratum corneum* (considered non-bioavailable by the authors) represents 34.9% of the deposited dose. For Kaddard et al. (2008), in a study with pigskin, 10 h of exposure, 0.7 µg, 10

µg/mL, aqueous solution, and for Morck et al. (2010) (with human skin, 259 µg/cm^2, 4000 µg/ml, hydroalcoholic solution), the rate of BPA in the dermis (8.8% and 17.2%, respectively) is greater than that in the epidermis (5.4% and 7.4%, respectively)

- it may be noted that the Kp calculated from the flow reported by the authors of 0.022 µg/cm^2/h and for a BPA concentration in aqueous solution of 193.6 µg/mL is 1.1 10^{-4} cm/h. This Kp value is similar to that derived from the data of Zalko et al. (2011) or Morck et al. (2010) with hydroalcoholic alcoholic solutions, 0.9 10^{-4} cm/h and 1.75 10^{-4} cm/h respectively.

- **Even if acetone is a polar aprotic solvent, it could seem speculative to mention that "*acetone even in short contact with the skin could have influenced the lipid layer of the skin and following the flow of percutaneous absorption through the skin*"** as no study compared BPA percutaneous absorption with acetone and with other vehicles such as water or ethanol. As mentioned in the document previously sent to the RAC (Thermal Papers containing BPA, 22th September 2014), the absorption flux of BPA (0.12 µg/cm^2/h) applied at a rate of 200 µg/cm^2 of skin (after evaporation of acetone) (Marquet et al, 2011) was approximately 6-7 times smaller than the BPA flux of 0.70 µg/cm^2/h (13%/48h X 259 µg/cm^2) obtained after applying BPA in a hydro-alcoholic solution at a rate of 259 µg BPA/cm^2. This difference in flux can be attributed to the need to first dissolve solid BPA before it penetrates the skin in the Marquet et al study.
- In the frame of an HRA, if the choice of the input values cannot be based on the data quality/representativity, one should use the most protective available data or combine the data from the various key studies if possible.

D – Results from re-runs of the models required by RAC rapporteur

D.1 - Percutaneous absorption flow model

↳ Exposure assessment: workers

$$IED = \frac{F \times D \times S}{BW} \times f\ systemic\ bioavailability$$

Where:

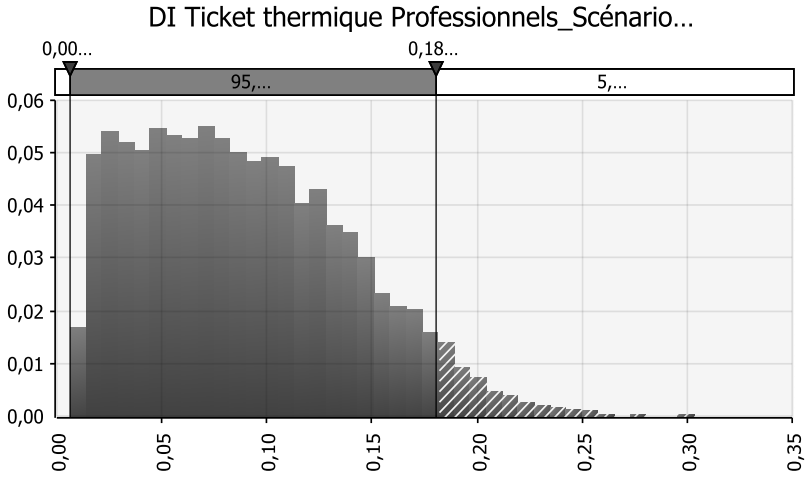
IED: Internal (exposure) daily dose	[µg/kg$_{BW}$/d]
F: Absorption flow	[µg/cm^2/h]
D: Duration of exposure to the till receipt	[h/d]
S: Surface in contact with the till receipt	[cm^2]
BW: Body weight	[kg$_{BW}$]
$f_{systemic\ bioavailability}$	[%]

RAC proposal for new input data (**scenario I**)

Input parameter	**Value**	**Comments**
F: Absorption flow	Uniform distribution within the range **0.026 – 0.331** µg.cm-2.h-1	Unchanged
D: Duration of exposure to the till receipt	Triangular distribution with min, mean and max values **3, 5.5, 8** h/d	**Changed**
S: Surface in contact with the till receipt	**6** cm^2	**Changed** (pads of the 5 fingers of one hand)
BW: Body weight	Discrete distribution of probabilities illustrating the body weight for the pregnant woman	Unchanged
$f_{systemic\ bioavailability}$	100 %	Unchanged

→ **Result:**

IED distribution [µg/kgBW/d]	**Descriptive statistics**



Min	0.006
P5	0.019
P25	0.048
P50	0.084
P75	0.125
P90	0.160
P95	0.181
Max	0.311
Mean	0.090
Geo. Mean	0.073

RAC proposal for new input data (**scenario II**)

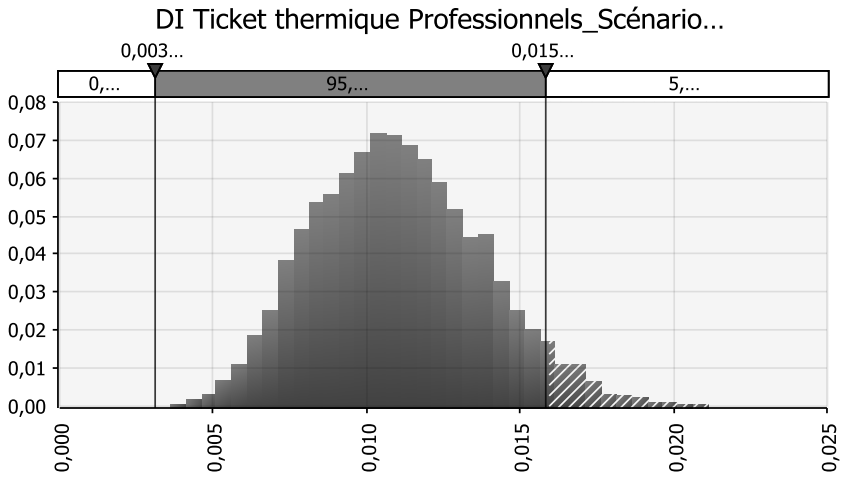
Input parameter	**Value**	**Comments**
F: Absorption flow	~~Max~~ Mean value of **0.022** µg.cm-2.h-1	**Changed** acc. to Demierre *et al.* (2012) using water as a vehicle
D: Duration of exposure to the till receipt	Triangular distribution with min, mean and max values **3, 5.5, 8** h/d	**Changed**
S: Surface in contact with the till receipt	**6** cm^2	**Changed** (pads of the 5 fingers of one hand)
BW: Body weight	Discrete distribution of probabilities illustrating the body weight for the pregnant woman	Unchanged
$f_{systemic\ bioavailability}$	100 %	Unchanged

Note to the RAC rapporteur: Indeed, both the Demierre and Marquet results are expressed with the maximum flow, reflecting the penetration rate under steady state conditions (linear phase). The range of input values in the scenario I corresponds to the range of the Marquet results values, and the value of 0.022 µg.cm-2.h-1 in this second scenario **corresponds to the mean of the Demierre results values.**

In this scenario, the use of the mean value 0.022 µg.cm-2.h-1 from the Demierre study (2 donors) could underestimate the absorption flow variability, which could be high according to the higher inter-donor variation observed in the Marquet study (6 donors)

→ Result:

IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.003
P5	0.007
P25	0.009
P50	0.011
P75	0.013
P90	0.015
P95	0.016
Max	0.023
Mean	0.011
Geo. Mean	0.011

↳ Exposure assessment: consumers

$$IED = \frac{F \times D \times S}{BW} \times f\ systemic\ bioavailability$$

Where:

IED: Internal (exposure) daily dose [$\mu g/kg_{BW}/d$]

F: Absorption flow [$\mu g/cm^2/h$]

D: Duration of exposure to the till receipt [h/d]

S: Surface in contact with the till receipt [cm^2]

BW: Body weight [kg_{BW}]

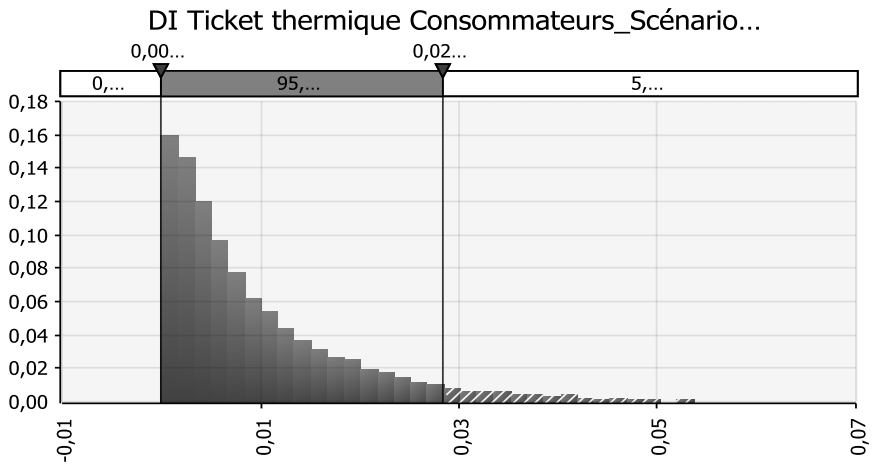
$f_{systemic\ bioavailability}$ [%]

RAC proposal for new input data (**scenario I**)

Input parameter	**Value**	**Comments**
F: Absorption flow	Uniform distribution within the range **0.026 – 0.331**µg.cm-2.h-1	Unchanged*
D: Duration of exposure to the till receipt	Uniform distribution up to **2** h/d as a maximum	Unchanged
S: Surface in contact with the till receipt	Uniform distribution within the range **1-6** cm^2	**Changed** (pads of the 5 fingers of one hand)
BW: Body weight	Discrete distribution of probabilities illustrating the body weight for the pregnant woman	Unchanged
$f_{systemic\ bioavailability}$	100 %	Unchanged

→ Result:

IED distribution [µg/kgBW/d]	Descriptive statistics



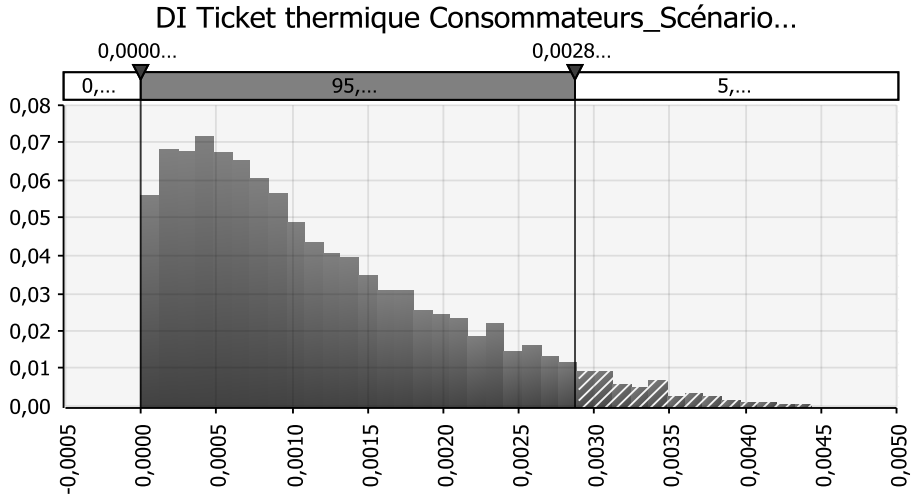
Min	0.000
P5	0.001
P25	0.003
P50	0.006
P75	0.013
P90	0.022
P95	0.028
Max	0.067
Mean	0.009
Geo. Mean	0.005

RAC proposal for new input data (**scenario II**)

Input parameter	Value	Comments
F: Absorption flow	~~Max~~ Mean value of **0.022** µg.cm-2.h-1	**Changed** acc. to Demierre *et al.* (2012) using water as a vehicle
D: Duration of exposure to the till receipt	Uniform distribution up to **2** h/d as a maximum	Unchanged
S: Surface in contact with the till receipt	Uniform distribution within the range **1-6** cm^2	**Changed** (pads of the 5 fingers of one hand)
BW: Body weight	Discrete distribution of probabilities illustrating the body weight for the pregnant woman	Unchanged
$f_{systemic\ bioavailability}$	100 %	Unchanged

→ Result:

IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0000
P5	0.0001
P25	0.0005
P50	0.0009
P75	0.0017
P90	0.0024
P95	0.0029
Max	0.0048
Mean	0.0012
Geo. Mean	0.0008

D.2 – Absorption rate model

↳ Exposure assessment: consumers

$$IED = \frac{Rabs \times Qsubs \times N \times D}{2 \times BW} \times f\ systemic\ bioavailability$$

Where:

IED: Internal (exposure) daily dose [$\mu g/kg_{BW}/d$]

R_{abs}: Level of absorption (absorption rate)

established for absorption duration of 2 hours [%]

Q_{subs}: Quantity of the substance deposited by contact [µg/finger]

N: Number of fingers in contact with the till receipt [finger]

D: Absorption duration [h/d]

BW: Body weight [kg_{BW}]

$f_{systemic\ bioavailability}$ [%]

RAC proposal for new input data (**scenario I**)

Input parameter	**Value**	**Comments**
R_{abs}: Level of absorption (absorption rate) established for absorption duration of 2 hours	Discrete values **10** and **27** % (the factor 2 must not be used in the equation with the 10% absorption rate. Factor 2 is useful only for the 27 % the equation with the 10% absorption rate modelling in order to take into account the BPA/skin contact duration of 2 hours in the Biedermann study)	**Changed**
Q_{subs}: Quantity of the substance deposited by contact	Uniform distribution within the range **0.035-3.75** µg/finger	Unchanged

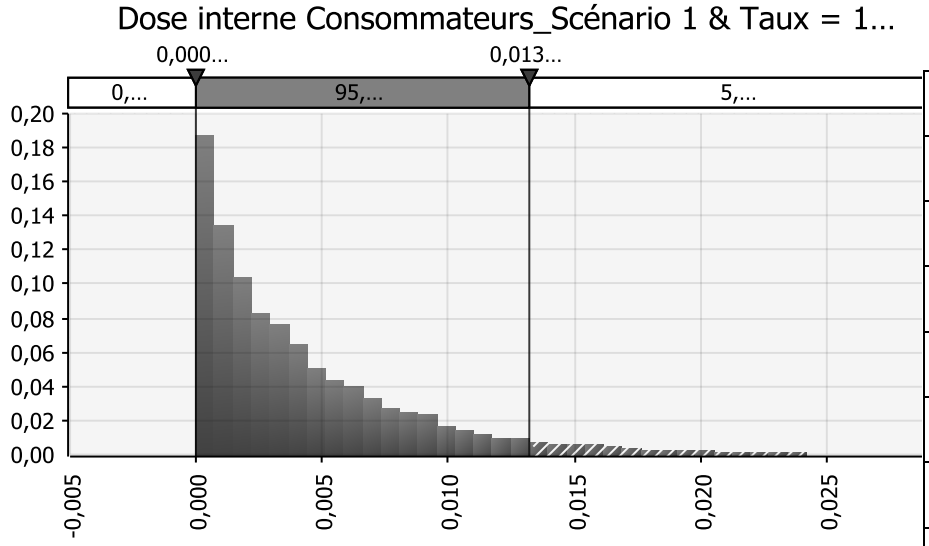
N: Number of fingers in contact with the till receipt	Uniform distribution within the range **1-5** fingers	**Changed**
D: Absorption duration	Uniform distribution up to **2** h/d as a maximum	Unchanged
BW: Body weight	Discrete distribution of probabilities illustrating the body weight for the pregnant woman	Unchanged
$f_{systemic\ bioavailability}$	100 %	Unchanged

Note: for these new inputs, we'll obtain two results (two distributions):

- Result 1: R_{abs} of 10% combined with the new distribution of N specified (the other inputs parameters are unchanged);
- Result 2: R_{abs} of 27% combined with the new distribution of N specified (the other inputs parameters are unchanged).

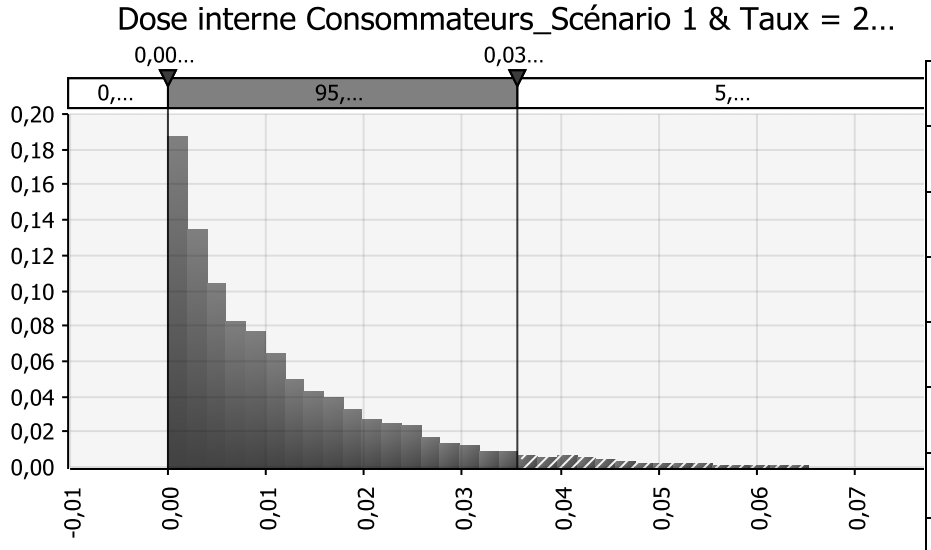
They are presented below.

→ Result 1 (R_{abs} of 10% combined with the new distribution of N specified; the other inputs parameters are unchanged) (Results must be multiplied by 2, see note into previous table).

IED distribution [µg/kgBW/d]	Descriptive statistics	
	Min	0.00000
	P5	0.00018
	P25	0.00104
	P50	0.00283
	P75	0.00601
	P90	0.00998
	P95	0.01319
	Max	0.02936
	Mean	0.00422
	Geo. Mean	0.00225

→ Result 2 (R_{abs} of 27% combined with the new distribution of N specified; the other inputs parameters are unchanged)

IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0000
P5	0.0005
P25	0.0028
P50	0.0076
P75	0.0162
P90	0.0270
P95	0.0356
Max	0.0793
Mean	0.0114
Geo. Mean	0.0061

RAC proposal for new input data (**scenario II**)

Input parameter	**Value**	**Comments**
R_{abs}: Level of absorption (absorption rate) established for absorption duration of 2 hours	Discrete values **10** and **27** %	**Changed**
Q_{subs}: Quantity of the substance deposited by contact	Discrete values **11**, **28**, **103** µg/finger (dry, greasy and wet finger, respectively)	**Changed**
N: Number of fingers in contact with the till receipt	Uniform distribution within the range **1-5** fingers	**Changed**
D: Absorption duration	Uniform distribution up to **2** h/d as a maximum	Unchanged
BW: Body weight	Discrete distribution of probabilities illustrating the body weight for the pregnant woman	Unchanged
$f_{systemic\ bioavailability}$	100 %	Unchanged

Note 1 to the RAC rapporteur: In this scenario II, the quantity of BPA deposited by contact (11, 28, 103 µg/finger for dry, greasy and wet finger, respectively) is extremely higher than the range 0.035-3.75 µg/finger used in the RAC scenario I. Biedermann et al (2010) show that the quantity of BPA deposited on the skin is not correlated with the repetition of contacts with thermal papers, but it is rather constant.

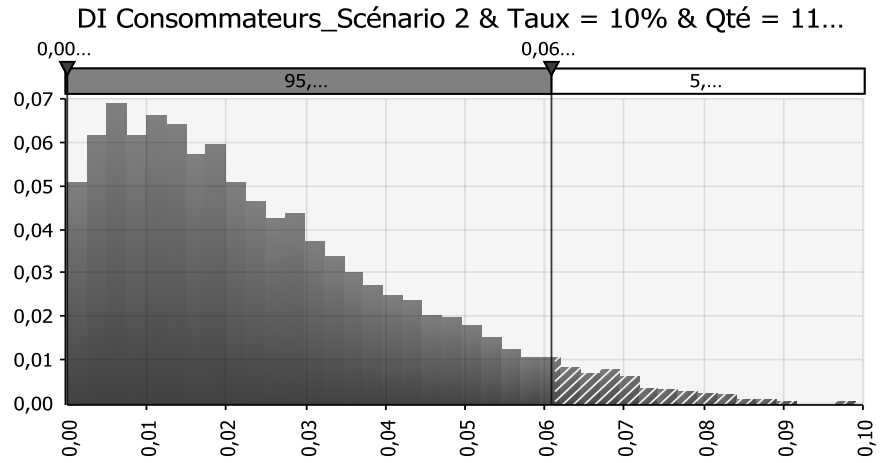
Note 2: for these new inputs, we'll obtain 6 results (6 distributions):

- Result 1: R_{abs} = 10%, Q_{subs} = 11 µg/finger, new distribution of N specified (the other inputs parameters are unchanged);
- Result 2: R_{abs} = 10%, Q_{subs} = 28 µg/finger, new distribution of N specified (the other inputs parameters are unchanged);
- Result 3: R_{abs} = 10%, Q_{subs} = 103 µg/finger, new distribution of N specified (the other inputs parameters are unchanged);
- Result 4: R_{abs} = 27%, Q_{subs} = 11 µg/finger, new distribution of N specified (the other inputs parameters are unchanged);
- Result 5: R_{abs} = 27%, Q_{subs} = 28 µg/finger, new distribution of N specified (the other inputs parameters are unchanged);
- Result 6: R_{abs} = 27%, Q_{subs} = 103 µg/finger, new distribution of N specified (the other inputs parameters are unchanged);

They are presented below.

→ Result 1: R_{abs} = 10%, Q_{subs} = 11 µg/finger, new distribution of N specified (the other inputs parameters are unchanged)

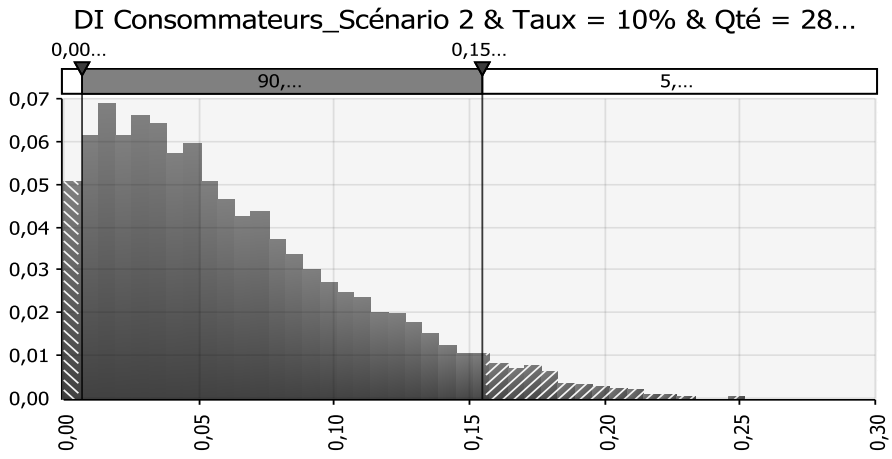
IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0001
P5	0.0026
P25	0.0103
P50	0.0204
P75	0.0352
P90	0.0511
P95	0.0607
Max	0.0990
Mean	0.0246
Geo. Mean	0.0172

→ Result 2: R_{abs} = 10%, Q_{subs} = 28 µg/finger, new distribution of N specified (the other inputs parameters are unchanged)

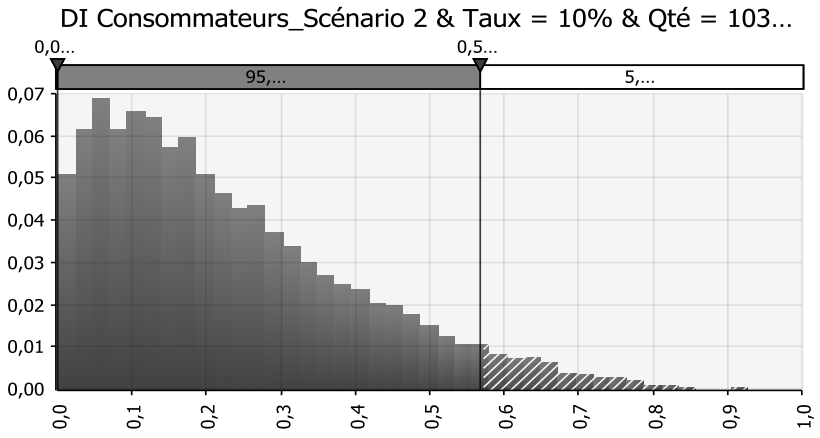
IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0003
P5	0.0065
P25	0.0262
P50	0.0518
P75	0.0896
P90	0.1301
P95	0.1546
Max	0.2521
Mean	0.0626
Geo. Mean	0.0438

→ Result 3: R_{abs} = 10%, Q_{subs} = 103 µg/finger, new distribution of N specified (the other inputs parameters are unchanged)

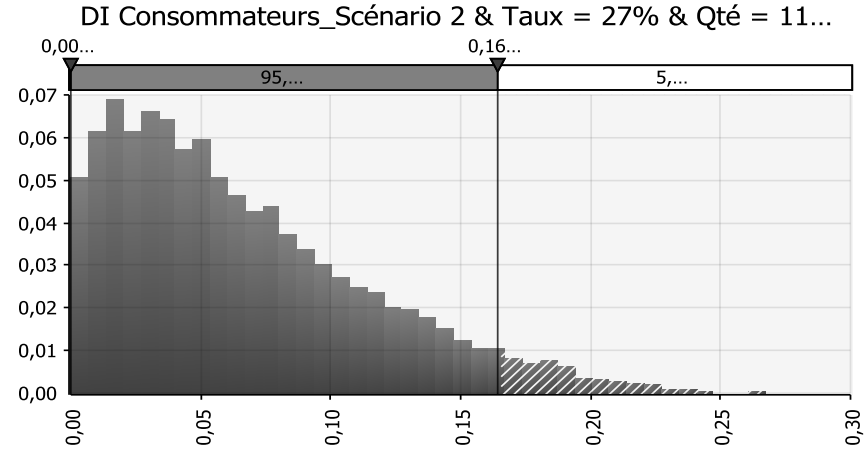
IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0009
P5	0.0240
P25	0.0964
P50	0.1907
P75	0.3296
P90	0.4784
P95	0.5688
Max	0.9274
Mean	0.2303
Geo. Mean	0.1610

→ Result 4: R_{abs} = 27%, Q_{subs} = 11 µg/finger, new distribution of N specified (the other inputs parameters are unchanged)

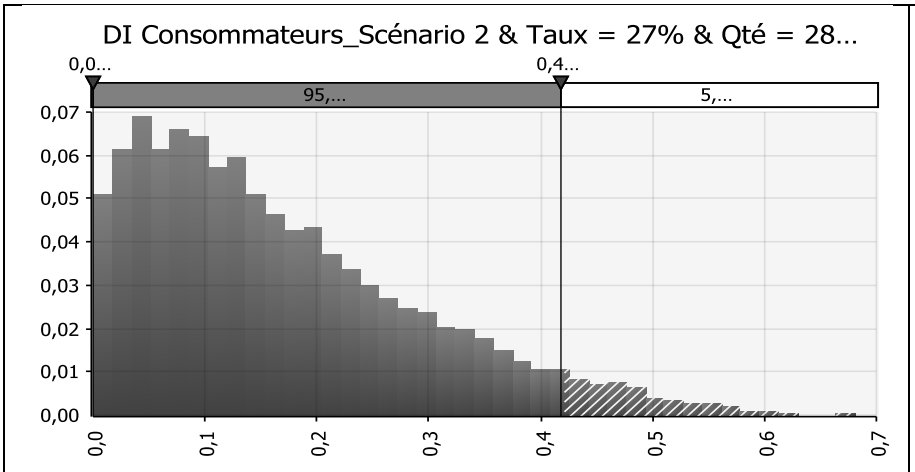
IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0003
P5	0.0069
P25	0.0278
P50	0.0550
P75	0.0950
P90	0.1379
P95	0.1640
Max	0.2674
Mean	0.0664
Geo. Mean	0.0464

→ Result 5: R_{abs} = 27%, Q_{subs} = 28 µg/finger, new distribution of N specified (the other inputs parameters are unchanged)

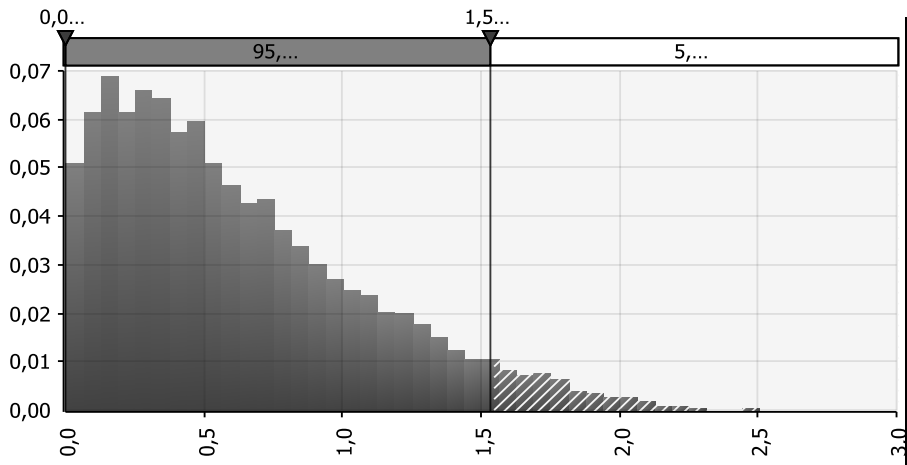
IED distribution [µg/kgBW/d]	Descriptive statistics



Min	0.0007
P5	0.0176
P25	0.0707
P50	0.1400
P75	0.2419
P90	0.3511
P95	0.4175
Max	0.6807
Mean	0.1691
Geo. Mean	0.1182

→ Result 6: R_{abs} = 27%, Q_{subs} = 103 µg/finger, new distribution of N specified (the other inputs parameters are unchanged)

IED distribution [µg/kgBW/d]	**Descriptive statistics**



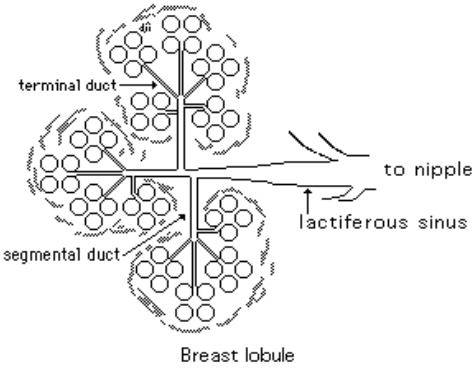
Min	0.003
P5	0.065
P25	0.260
P50	0.515
P75	0,890
P90	1.292
P95	1.536
Max	2.504
Mean	0.622
Geo. Mean	0.435

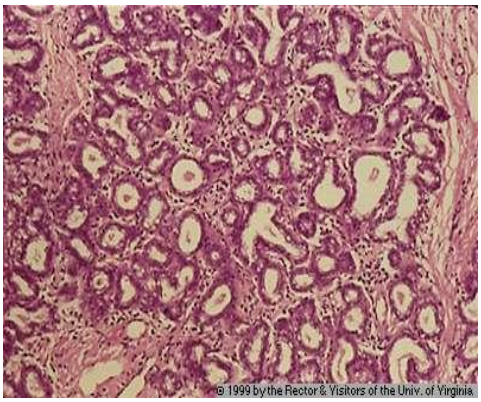
Annex 8: Mammary gland changes - State of knowledge concerning preneoplastic lesions of the breast - Discussion Points

Extract from Anses 2011 report *Health Health effects of Bisphenol A*, Annexes 8 and 9.

The frequency with which pathologists and clinicians are faced with preneoplastic or precancerous lesions of the breast has increased significantly with the widespread use of mammographic screening and, to a lesser extent, with the improvement in the management of pathological surgical specimens.

Precancerous lesions of the breast correspond to atypical epithelial proliferations that develop within the lactiferous tree and are of two types: ductal and lobular. These two types are distinguished not by their location but by their constituent cell type. Indeed, in 1994, Wellings *et al.* demonstrated that most pre-invasive breast lesions begin in the ***terminal duct lobular unit*** (TDLU), very sensitive to hormonal factors and located at the termination of the lactiferous ducts and their junction with the lobules (Wellings and Alpers, 1994).





TDLU: High power view showing the two-cell layer epithelium

Figure 1: anatomy of the breast and terminal duct lobular unit

Both types, ductal and lobular, are characterised at the molecular level by the presence at the cytoplasmic membranes of an intercellular junction complex protein: E-cadherin.

Histological diagnosis of precancerous lesions is difficult, and **inter-pathologist reproducibility** is **poor**, as evidenced by a number of studies. The diagnosis seems slightly improved with the contribution of immunohistochemistry in lesions that have a specific profile (MacGrogan *et al.*, 2008).

To facilitate the diagnosis and management of precancerous lesions, new terminology was proposed in the early 2000s and adopted by the WHO in 2003. It is superimposed on the traditional terminology and is **based on morphological,** non-molecular criteria.

2003 WHO classification of precancerous lesions

It is based on the terms describing *intraepithelial neoplasia*: ductal or DIN: lobular or LIN. The ductal epithelial proliferations are divided into five categories and lobular proliferations into three categories.

Intraepithelial neoplasia, ductal type

1. Atypical cylindrical metaplasia (ACM): DIN-1A
2. Atypical ductal hyperplasia (ADH): DIN-1B
3. Ductal carcinoma *in situ* (DCIS) low-grade: DIN-1C
4. DCIS intermediate grade: DIN-2
5. DCIS high-grade: DIN-3

The first two categories correspond to precancerous lesions.

Ductal carcinoma *in situ* (DCIS) is not a **precancerous lesion** but a **pre-invasive** cancerous lesion. In the US, DCIS accounts for nearly 20% of detected cancers (1 case of DCIS for 1300 screening mammograms) (Ernster *et al.*, 2002).

Mammary intraepithelial neoplasia, lobular type

1. Atypical lobular hyperplasia (ALH): LIN-1
2. ALH/LCIS: LIN-2
3. Lobular carcinoma *in situ* (LCIS): LIN-3, which includes three different types

This new terminology has led to improved management of precancerous lesions. For example, for lobular lesions, the recommendations of the International Agency for Research on Cancer (IARC), published in November 2009, advocate simple monitoring for LIN-1 and LIN-2, and when the histological examination reveals LIN-3, the initial treatment is based on surgical excision.

Preneoplastic breast lesions: risk of transformation to invasive cancer

When left in place, preneoplastic or precancerous lesions may transform into pre-invasive carcinoma or carcinoma *in situ*, which can itself progress to invasive carcinoma (Figure 2).

The introduction of the concept of the terminal duct lobular unit has led to the concept of malignant transformation – non-obligatory – passing through various stages, similar to colon cancer. Normal cells located in the terminal duct lobular unit are transformed to atypical hyperplasia, and ductal carcinoma *in situ* to invasive cancer (Figure 2 below).

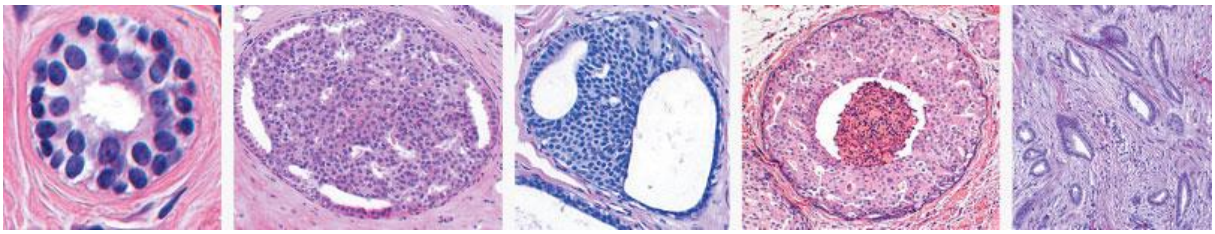


Figure 2: Pathological slides illustrating the transformation of the normal mammary gland to invasive cancer (Figure taken from Burstein *et al.*, 2004)

The hypothesis of the existence of a continuum between the normal mammary gland and invasive breast cancer, although it may seem too simplistic, is based on direct and indirect arguments (Antoine *et al.*, 2010).

Historical studies since the early 19th century have observed that benign lesions were more frequently found in patients with breast cancer. More recent epidemiological studies have shown that women with a history of benign breast lesions have an increased risk of breast cancer.

Until the early 1990s, pathologists and clinicians referred to earlier works, notably those of Dupont and Page (Dupont and Page, 1985) who calculated the risk of developing subsequent breast cancer in patients with benign lesions, often found as palpable lesions before the era of mammography screening.

The natural history of low-grade ductal carcinoma *in situ* (DCIS) was determined by long-term monitoring studies in women who underwent diagnostic biopsy without further treatment before the era of organised mass screening. After 10 years of follow-up, 14 to 60% of these women were diagnosed with invasive cancer in the same breast (Page *et al.*, 1995). The demonstration of this risk of invasion has also led to the present attitude of actively treating these lesions. The natural history of high-grade DCIS or clinically palpable DCIS is, however, not well characterised, because in most cases, the tumour is removed completely by surgery, which is also the case for atypical ductal hyperplasia (ADH) lesions.

The significant increase in biopsies done on the basis of subclinical images and recent data provided by the molecular study of lesions have shed new light on the transformation risk of hyperplastic lesions to cancer.

During the transformation of hyperplastic lesions to carcinoma *in situ* and then invasive cancer, imbalances are observed at the chromosomal level, with a loss of heterozygosity in 70% of high-grade carcinomas *in situ*, compared to nearly 40% cases of atypical hyperplasia and 0% in healthy breast tissue (Aubele *et al.*, 2000). Molecular markers of breast tumour transformation have been identified. The oestrogen receptor expressed by normal epithelial breast cells is expressed by more than 70% of ductal carcinoma *in situ*. The HER2/neu proto-oncogene is overexpressed in half of DCIS but not in atypical hyperplasia (Allred *et al.*, 1992).

Published work from the early 2000s on the molecular pathways involved have shown that breast cancer is not a single disease, but rather a set of different diseases occurring in the same anatomical structures (TDLU). These molecular biology techniques have also shown that **precancerous and pre-invasive lesions** are as heterogeneous as invasive cancers.

Different models of progression according to the **histological grade** (low-grade or high-grade) and the presence or absence of **oestrogen receptors** have been proposed (see figures below taken from the publication of (Lopez-Garcia *et al.*, 2010)).

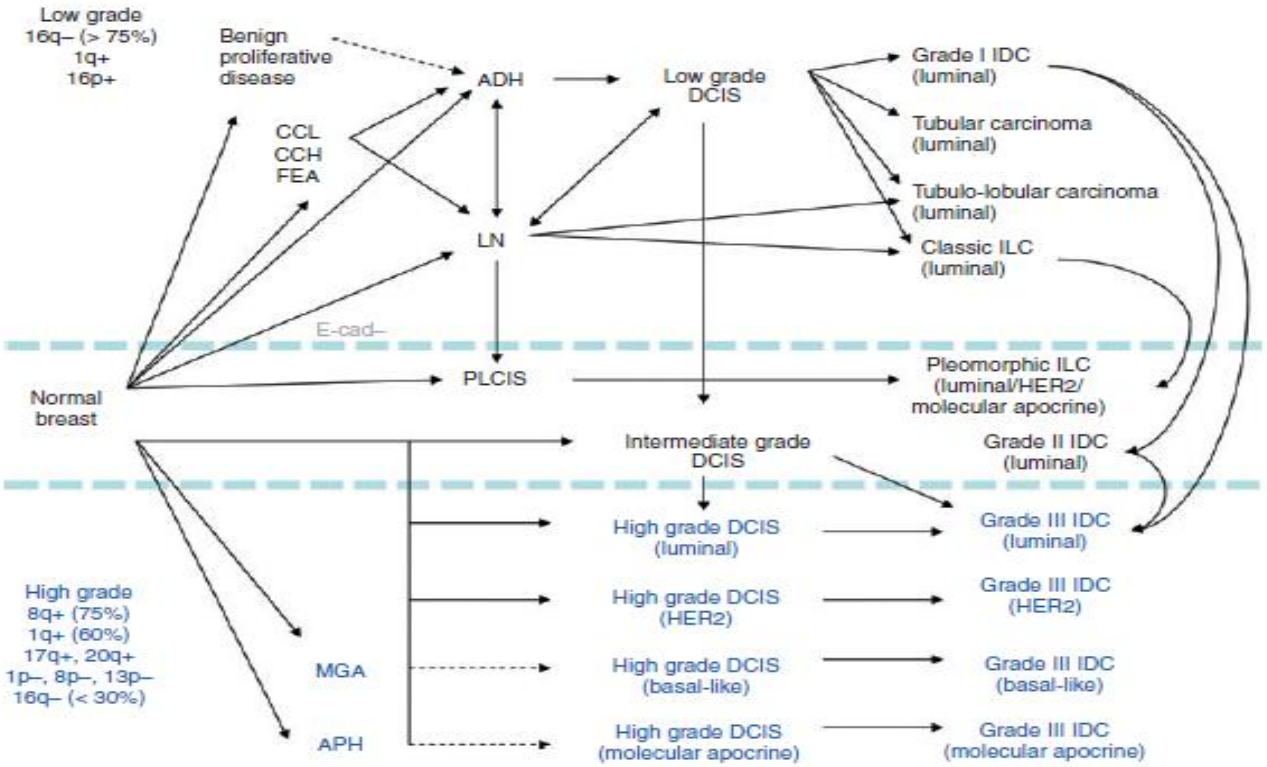


Figure 3. Low- and high-grade multistep model of breast cancer progression based on morphological, immunophenotypical and molecular features. Connectors drawn with continuous lines represent links between morphological entities which are demonstrated by morphological and/or molecular data. Connectors drawn with discontinuous lines represent hypothetical links yet to be demonstrated. ADH: atypical ductal hyperplasia; APH: atypical apocrine hyperplasia; CCH: columnar cell hyperplasia; CCL: columnar cell lesion; DCIS: ductal carcinoma *in situ*; E-cad: E-cadherin; FEA: flat epithelial atypia; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; LN: lobular neoplasia; MGA: microglandular adenosis; PLCIS: pleomorphic lobular carcinoma *in situ*.

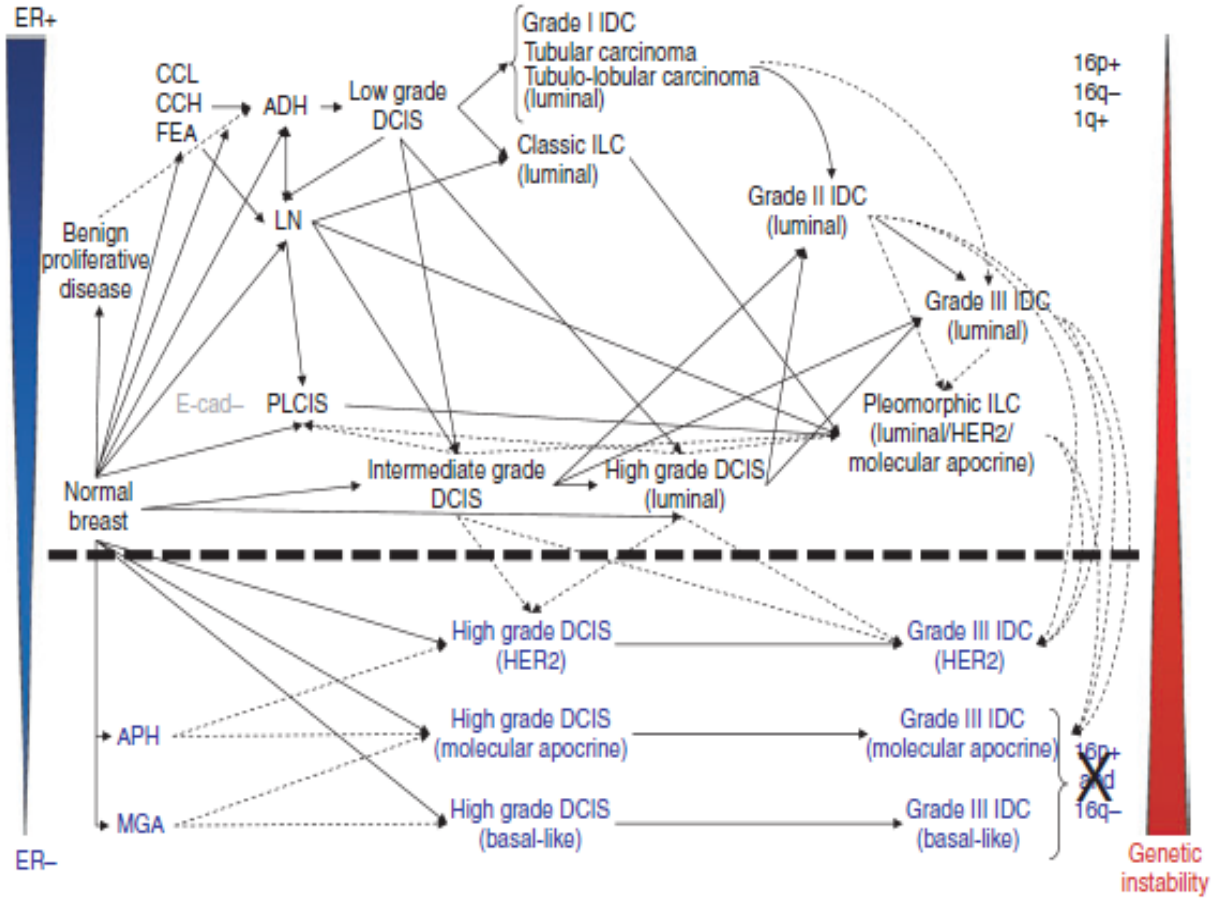


Figure 4. Revised multistep model of breast cancer evolution based on oestrogen receptor (ER)-status. Note that the two main pathways are defined by the expression of ER and ER-regulated genes. In this model, the ER-positive arm encompasses most of the precursor lesions and a range of invasive lesions which may progress from low to high grade due to the acquisition of genetic instability and accumulation of stochastic genetic events. The ER-negative arm includes ER-negative DCIS and invasive tumours; MGA and APH are proposed as non-obligate precursors of these lesions. ER and genetic instability bars on either side of the image represent the levels of ER expression and genetic instability, respectively. ADH: atypical ductal hyperplasia; APH: atypical apocrine hyperplasia; CCH: columnar cell hyperplasia; CCL: columnar cell lesion; DCIS: ductal carcinoma *in situ*; E-cad: E-cadherin; FEA: flat epithelial atypia; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; LN: lobular neoplasia; MGA: microglandular adenosis; PLCIS: pleomorphic lobular carcinoma *in situ*.

Moreover, recent data show that the epithelial atypias represent not only a **risk factor for secondary carcinogenesis**, but also a **risk marker for concomitant cancer in the surrounding area** (from Mascarel *et al.*, 2007).

Conclusion

Improved knowledge provided by immunohistochemistry, cytogenetics, and molecular biology has improved the classification of precancerous lesions and the understanding of the relationship between epithelial atypias and breast cancer. These elements have helped to refine the diagnosis, to better determine the risk of progression to invasive cancer, and thus to optimise patient treatment.

Annex 9: Substitution from BPA to Pergafast 201

Subject

ECHA, ANSES and INERIS conducted a number of telephone discussions with specific stakeholders in the paper industry (paper producers, paper distributors, customers, and chemical suppliers) to establish the state of play with substitution of BPA in thermal paper, finding out the main cost drivers and establishing a view on the impacts of substitution on thermal paper users (e.g., shops).

Main findings

Pergafast 201 is considered to be an alternative for BPA. BPS is by some stakeholders seen as a non-suitable alternative as regulatory pressure on this substance is mounting. Other sources indicated that some thermal paper manufacturers would however consider switching to BPS in the immediate short term. Most stakeholders indicated that in the long term a phenol-free solution would make more sense.

The main driver of the cost appears to be the difference in price between the raw materials used for making Pergafast 201. This implies that by scaling up production, little economies of scale would be achieved and hence there would be little downward pressure on the price of Pergafast 201.

According to estimates of one of the main European producers of thermal paper, the production costs between of Pergafast 201 containing paper are about 10 % higher than of BPA containing paper once the market has stabilised.

This was corroborated by paper converters who indicated that the whole price for a cashier roll is about 50 cents per roll, meaning that the final price will increase to about 55-60 cents per roll. In the long run this cost might decrease somewhat (towards 55 cents per roll). In the operating of a cashier the costs for thermal paper are not recognised as a major cost-item, e.g. labour costs are, in absolute and relative terms, a lot higher.

The pieces of information collected from other (wholesale and retail) sources are consistent. Although some actors indicated values as high as 35%, the price/cost increase of Pergafast-containing thermal paper compared with BPA containing thermal paper seems to be between 10% and 20%.

Most actors indicated that should a restriction on BPA come into force, time is needed to adjust production of phenol free paper to an increase in demand. This would take 2-3 years.

From the interviews it became evident thatmany large retailers have, substituted to phenol free thermal paper in their points of sale. These were Lidl, Carrefour, S-market andK-market. The reasons varied to some extent but overall, the additional cost of thermal paper was considered relatively low compared to their operation costs. Actually, in one company the phenol free alternative was cheaper. This was probably because the company carried out a competitive bidding and thus was able to get a lower price than it had paid for PBA containing thermal paper. According to Chemical Sensitivity Network[93] Aldi Nord, Rewe and German Railways also use phenol free thermal paper.

[93] http://www.csn-deutschland.de/blog/2012/08/16/edeka-und-kaisers-verwenden-noch-immer-giftige-kassenbons/

The following companies were interviewed:

Company	Interviewed on
Koehler Paper, Germany	23 March 2015
BASF, Germany	16 April 2015
Schades, France	31 March 2015
R S Group	31 March 2015
Carrefour, France	9 April 2015
Lidl, France	15 April 2015
S-group, Finland	14 April 2015
Kesko, Finland	23 April 2015
Hankkija Oy, Finland	24 April 2015
PK Systema, Finland	15 April 2015

Annex 10: Consideration of societal impact

Reply of RAC to the request of SEAC Rapporteurs on BPA

In response to the "Request from SEAC Rapporteurs to RAC on BPA (corrected 02/06/2015)", RAC replies as follows:

1) RAC is of the view that the available data on the mammary gland, reproductive, metabolic, immunotoxicity and neuro-behavioural effects from BPA does not allow a quantification of the dose-response relationship in experimental animals. In the absence of a dose-relationship, RAC is therefore unable to estimate the incidences of the above mentioned effects in the population at risk (i.e., the unborn child of workers handling BPA-containing thermal paper).
2) RAC takes note of the annual incidence rates of disease calculated by SEAC in the table below. RAC understands that these incidence rates reflect only the monetised disease burden derived from and equal to the costs of the proposed restriction on BPA. RAC has no exact knowledge of the underlying assumptions resulting in the incidence rates presented in the table below. This applies both to the monetisation as well as the diseases represented by the effects.
3) In general, RAC considers concurrent incidences of such high magnitude for these types of effect exceptionally unlikely for any substance.
4) **In response to the question posed by SEAC, RAC emphasises that it is exceptionally unlikely that all of the incidence rates in the table below would occur concurrently in the population at risk due to exposure of workers to BPA from thermal paper.**

Request from SEAC Rapporteurs to RAC on BPA (corrected 02/06/2015)

As a result of the lack of robust dose-response relationships necessary to perform a health impact assessment and corresponding cost-benefit assessment of the proposed restriction, SEAC would like to ask RAC for assistance in order to aid their proportionality assessment. The approach taken by SEAC to assessing proportionality (a break-even analysis) requires one to identify the likelihood of observing annual incidence rates of a certain size of the identified adverse effects arising as a result of exposure to BPA from thermal paper. Adverse effects are in this case only effects that are recognized and considered as a disability, disease or illness.

In accordance with RAC's assessment, SEAC uses the multiple risk characterisation endpoints encompassed in the composite DNEL for workers (as described in the RAC opinion). The table below indicates the minimum annual incidence rates of effects associated with each endpoint that would be required to be observed in the population at risk (i.e. the offspring of female cashiers with RCR greater than or equal to 1).

The incidence rates are expressed as the percentage of the population that would have to experience adverse effects associated with the different endpoints, **as a result of exposure to BPA from thermal paper.**

In order to be able to correctly interpret the table below, it is necessary to look not just at the individual incidence rates for each endpoint, but also for all of the endpoints together, i.e., **all** of the incidence rates would have to be observed **concurrently** across the population at risk.

In other words, SEAC would like RAC's views on the likelihood of observing **all** the following incidence rates in the offspring of female cashiers at risk as a result of exposure to BPA in thermal paper.

- at least around 17% having mammary gland changes
- at least around 13% having adverse immunotox effects

- at least around 7% having adverse reprotoxic effects
- at least around 4% getting adverse metabolic effects

Moreover, note that the incidence rates (due to the exposure to BPA in thermal paper) would come in addition to the baseline incidence rate (due to other causes). For example if the incidence rate for the general population for endometrial hyperplasia (as an example of an adverse reprotoxic effects) would be 0.2%, one would observe this disease in 0.2%+7%=7.2%[94] of the population at risk from BPA from thermal paper.

Another way of interpreting these incidence rates is thus: Each of the unborn female children at risk has an additional 17% chance of getting mammary gland changes and an additional 13% chance of experiencing adverse immunotox effects and an additional 7% chance of having adverse reprotoxic effects and an additional 4% chance of adverse metabolic effects. As mentioned above, adverse effects are only effects that are recognized and considered as a disability, disease or illness.

PLEASE USE THE TABLE AT THE END TO INDICATE YOUR RESPONSES USING THE FOLLOWING PROTOCOL

Virtually certain (VC)	99-100 % probability
Very likely (VL)	90-100 % probability
Likely (L)	66-100 % probability
As likely as not (ALAN)	33-66 % probability
Unlikely (U)	0-33 % probability
Very unlikely (VU)	0-10 % probability
Exceptionally unlikely (EU)	0-1 % probability

RAC ASSESSMENT OF LIKELIHOOD OF EFFECTS AND INCIDENCE RATES

Endpoint	**Annual incidence rate in population at risk, due to BPA in thermal paper as provided by SEAC rapporteurs**	**RACs assessment of the likelihood of the incidence rates**
Mammary Gland	17%	RAC finds it is *[insert choice]* that all of these incident rates for the given effects would occur *concurrently* in the population at risk, due to exposure to BPA from thermal paper.
Immunotox	13%	
Neurobehaviour	N/A[3]	
Reprotox	7%	

[94] Note that this is a simplification, as the incidence rate of the population at risk would affect the average incidence rate. However, given that the size of the population at risk is small compared to the general population this effect is, for the purpose of this example, assumed to be negligible.

Metabolic – cholesterol	2%	
Metabolic – obesity	2%	

[3] Unable to estimate incidence rate due to lack of necessary information