Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Bisphenol A;
4,4'-isopropylidenediphenol

EC number: 201-245-8
CAS number: 80-05-7

CLH-O-0000004110-93-03/F

Adopted

14 March 2014

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14 March 2014

CLH-O-0000004110-93-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: **Bisphenol A; 4,4'-isopropylidenediphenol**
EC number: **201-245-8**
CAS number: **80-05-7**

The proposal was submitted by **France** and received by the RAC on **18 July 2013**. All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **27 August 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 October 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Christine Bjørge**

Co-rapporteur, appointed by the RAC: **Marianne van der Hagen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **14 March 2014** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on **Bisphenol A** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	604-030-00-0	bisphenol A; 4,4'-isopropylidenediphenol	201-245-8	80-05-7	Repr. 2 STOT SE 3 Eye Dam. 1 Skin Sens. 1	H361f*** H335 H318 H317	GHS08 GHS07 GHS05 Dgr	H361f H335 H318 H317		
Dossier submitters proposal	604-030-00-0	bisphenol A; 4,4'-isopropylidenediphenol	201-245-8	80-05-7	**Modify:** Repr. 1B	**Modify:** H360F		**Modify:** H360F		
RAC opinion	604-030-00-0	bisphenol A; 4,4'-isopropylidenediphenol	201-245-8	80-05-7	Repr. 1B	H360F		**Modify:** H360F		
Resulting Annex VI entry if agreed by COM	604-030-00-0	bisphenol A; 4,4'-isopropylidenediphenol	201-245-8	80-05-7	Repr. 1B STOT SE 3 Eye Dam. 1 Skin Sens. 1	H360F H335 H318 H317	GHS08 GHS07 GHS05 Dgr	H360F H335 H318 H317		

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of reproductive toxicity: adverse effect on sexual function and fertility

Contents

1 SUMMARY OF THE DOSSIER SUBMITTER'S PROPOSAL

1.1 Introduction

BPA is currently classified as reproductive toxicant Cat. 2 for adverse effects on sexual function and fertility based on the classification by TC C&L (Technical Committee of Classification and Labelling) in 2002.

The current CLH proposal was restricted by the Dossier Submitter (DS) to adverse effects on sexual function and fertility, for which classification in category 1B was proposed. The CLH proposal was based on the key studies from the TC C&L assessment (2002), the EU RAR (2003), and additional studies presented in the ANSES (The French Agency for Food, Environmental and Occupational Health and Safety, 2011) report together with new data on fertility published since the assessment of the EU RAR i.e. from 2002 to 31/12/2012. In the CLH report there were four guideline studies: a two-generation (OECD 416) study in mice (that was performed in 2008 and had not been considered by TC C&L) and in rats, a multigeneration study in rats and a continuous breeding study in mice. In addition, the CLH report contained very many non-guideline studies on animals (mice, rats, sheep and monkeys) and epidemiological studies on humans, which investigated effects of BPA on sexual function and fertility via different routes of exposure and over different exposure periods in males and females.

1.2 Toxicokinetics

According to the DS, BPA was rapidly and nearly completely absorbed from the gastrointestinal tract, consistent with its substantial solubility and lipophilicity. Following oral administration, BPA underwent substantial pre-systemic metabolism primarily to the glucuronide conjugate (BPA-G) mainly in the liver, but also in the intestine to a lesser extent. Glucuronide conjugates of BPA, unlike the unconjugated BPA, did not bind to the estrogen receptor. In rodents, BPA-G was subject to biliary excretion, enterohepatic recirculation and principally faecal excretion with half-lives between 20 and 80 hours, whereas humans excreted conjugated forms of BPA in urine. Because of an effective first-pass metabolism, there was extremely low systemic availability of unconjugated BPA in humans after absorption from the GI-tract following oral exposure. The enterohepatic cycling and decreased first pass metabolism of BPA in rats was reported to result in higher plasma levels of unconjugated BPA in rats compared to humans given the same dose. On the other hand, recent studies combining the use of tritium/deuterium-labeled BPA and specific and sensitive detection techniques indicated that the existence of an enterohepatic cycle in rodents (unlike in primates) had very limited consequences on the clearance of BPA. The DS also noted that although there were pharmacokinetic differences in the elimination of BPA and BPA-G between 5 species (mice, rats, dogs, sheep and pigs), they do not impact the BPA plasma concentrations which reflect the systemic exposure to BPA (Farbos thesis, 2012).

Transmucosal absorption sublingually bypassed the first-pass hepatic metabolism and led to a much higher systemic exposure to unconjugated BPA (relative to the administered dose) than after BPA absorption from the GI tract following oral administration in dogs. Dermal absorption occurred to a lesser degree than absorption from the gastro-intestinal tract after oral intake, and showed variability in the presented human, pig and rat *in vitro* and/or *in vivo* studies. There was no data on the toxicokinetics of BPA after exposure via inhalation, but the results from inhalation studies in animals indicated that absorption through the lungs occurred.

Overall, due to the effective first-pass metabolism, the systemic availability of unconjugated BPA after absorption from the GI-tract following oral exposure was considered to be extremely low in humans but internal exposure to unconjugated BPA was reported to be similar for adult rodents, non-human primates and humans. Transmucosal absorption sublingually bypassed the first-pass hepatic metabolism and was expected to lead to a much higher systemic exposure to unconjugated BPA than after absorption from the GI-tract. Also absorption via dermal and inhalation routes was concluded to occur.

1.3 Sexual function and fertility - females

1.3.1 Animals

1.3.1.1 Effects on reproductive tract and ovaries

Ovarian cysts - exposure via subcutaneous route

The DS concluded that BPA caused ovarian cysts in mice and rats. An increased occurrence of ovarian cysts was found in the available non-guideline studies investigating the influence of subcutaneously injected BPA on ovarian morphology. In Newbold *et al*. (2009), prenatal exposure (GD9 (gestation day 9)-GD16) of female CD-1 mice to 1 µg/kg bw/day BPA led to benign ovarian cysts and to more severe ovarian lesions at 10, 100 and 1000 µg/kg bw/d. In Signorile *et al*. (2010), cysts were reported in the offspring of Balbc mice exposed to 100–1000 µg/kg bw/d BPA during GD1–PND7. In Fernandez *et al*. (2010), exposure of Sprague Dawley rats during PND1 (post natal day 1)-PND10 resulted in cysts at 25-62.5 mg/kg bw/d BPA. In Adewale *et al*. (2009), exposure of Long Evans rats during PND1-PND3 resulted in cysts at 50µg/kg bw/d and at 50 mg/kg bw/d BPA. In Newbold *et al*. (2007), cysts and also more severe lesions were found in ovaries after exposure of female CD-1 mice to 100 µg/kg bw/d BPA during PND1-PND5.

Oocyte development - exposure via oral and subcutaneous routes

Concerning oocyte development, meiotic abnormalities leading to aneuploidy were demonstrated in three studies in rodents with different treatment conditions: in mice treated with 20, 40 or 100 µg/kg bw/d BPA via oral gavage during PND20-PND22 (Hunt *et al*., 2003); in mice implanted with BPA pellets designed to leach a dose of 20µg/kg bw/d from GD11.5 to GD18.5 (Susiarjo *et al*., 2007); and in rats injected subcutaneously with 20 mg/kg bw/d BPA on PND1, 3, 5 and 7 (Rodriguez *et al*., 2010). However, the DS could not draw conclusions on the significance of these findings because too few studies showing the effect were available.

Uterus morphology - exposure via subcutaneous route

BPA also induced changes in the uterus morphology in several studies in mice. Benign lesions, like endometrial hyperplasia or atypical hyperplasia, were revealed following daily subcutaneous injections in three studies in mice: during GD9 to GD16 (Newbold *et al*., 2009), GD1 to PND7 (Signorile *et al*., 2010) and PND1 to PND5 (Newbold *et al*., 2007). Malignant invasions (squamous metaplasia or polyps) were also described in the studies performed by Newbold *et al*. (2007 and 2009). Although these effects were significant, the DS considered it difficult to conclude on the importance of these findings because of a small number of studies showing the effect.

1.3.1.2 Effects on the onset of puberty

Exposure via oral route

In Howdeshell *et al.* (1999), mice were prenatally treated with 2.4 µg/kg bw/d BPA via oral gavage from GD11 to GD17. There was no effect on the onset of vaginal opening but the number of days between the vaginal opening and the onset of first estrus was reduced by 2.5 days. Delayed puberty was observed in the high dose group of the multigeneration study in Tyl *et al*. (2002), in which rats were exposed via diet to BPA at 0, 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg/day for three generations from 10 weeks before mating until PND21. Vaginal patency was delayed in all three generations at high dose only and the effect was associated with a reduced body weight.

Oral BPA exposure (0.002-320 mg/kg bw/d) of rats during the second half of gestation and during the lactation period did not reveal any effect on the onset of puberty determined by vaginal opening and/or the first estrus. (Yoshida *et al*., 2004; Rubin *et al*., 2001; Ryan *et al*., 2010; Kwon *et al*., 2000).

Exposure via the subcutaneous route

In Honma *et al*. (2002), mice were subcutaneously exposed to 2 or 20 µg/kg bw/d of BPA from GD11 to GD17. At 20 µg/kg bw/d an earlier vaginal opening (ca. 1 day) ($p < 0.01$) and an earlier first estrus (ca. 1 day) ($p < 0.05$) were reported. In Nikaido *et al*. (2004), mice were subcutaneously exposed to 0.5 or 10 mg/kg/day of BPA from GD15 to GD19. There was an earlier vaginal opening (1 or 2 days) at 10 mg/kg/day ($p < 0.01$).

In Adewale *et al*. (2009), rats were subcutaneously exposed to 50 µg/kg bw/d or 50 mg/kg bw/d BPA from PND1 to PND3. In comparison with controls, the vaginal opening occurred two days earlier in the lower dose group ($p < 0.01$), but not in the higher dose group. In Fernandez *et al*. (2009), female Sprague Dawley rats were injected subcutaneously with BPA doses ranging from 0.25 to 62.5 mg/kg bw/d from PND1 to PND10. The vaginal opening was early by 2 days at doses ranging from 2.5 to 6.2 mg/kg and by 4 days at doses ranging from 25 to 62.5 mg/kg ($p < 0.05$). In Nah *et al*. (2011), mice were subcutaneously exposed to 0.1, 1, 10, 100 mg/kg bw BPA on PND8. The vaginal opening occurred one or two days earlier at all doses ($p < 0.05$) as compared to controls.

The DS conclusion on effects on the onset of puberty

Overall, there were contradictory results regarding BPA effects on the onset of puberty. The DS concluded that results from rodent studies on the effect were not easy to interpret and the effect was dependent on the time window and duration of BPA exposure.

1.3.1.3 Effects on the estrous cycle

Exposure via oral route

In Mendoza-Rodriguez *et al*. (2011), an oral exposure of rats to 1.2 mg/kg bw/d of BPA via drinking water from GD6 to PND21 induced in 79.2% of the 4-month-old female offspring irregular estrous cycles characterized by predominant persistent estrus or persistent diestrus. Down-regulation of the protein Estrogen Receptor α (ERα) in uterine cells on the estrus day was also reported. In Rubin *et al*. (2001), rats were exposed to 0.1 or 1.2 mg BPA/kg bw/day via drinking water from GD6 through the period of lactation. The offspring exposed to the higher dose of BPA exhibited extended diestrus, proestrus and/or estrus periods.

Sprague Dawley rats perinatally exposed to 3.2-320 mg/kg bw/day via oral gavage during GD11-PND20 failed to reveal an effect of BPA on the estrous cycle (Kwon *et al*., 2000).

Exposure via subcutaneous route

In Nikaido *et al*. (2004), the estrous cycle length and diestrus were prolonged in the offspring of CD-1 mice after subcutaneous exposure of dams to 0.5 and 10 mg/kg/day of BPA from GD15 to GD19. In Honma *et al*. (2002), the length of the estrous cycle was increased in the offspring by 1 day after subcutaneous exposure of the dams to 2 and 20 µg/kg bw/d of BPA from GD11 to GD17.

In Adewale *et al*. (2009), 14% of the rats that were subcutaneously exposed to 50 µg/kg bw/d BPA from PND1 to PND3 were no longer cycling by 15 weeks after vaginal opening and 67% of females subcutaneously exposed to 50 mg/kg bw/d BPA from PND1 to PND3 were no longer cycling by 15 weeks after vaginal opening. In Fernandez *et al*. (2009), rats subcutaneously injected with BPA doses ranging from 25 to 62.5 mg/kg during PND1-PND10 had an irregular estrous cycle with an extended estrus period.

ICR mice subcutaneously exposed to 10 mg/kg bw/day of BPA during PND1-PND3 failed to reveal an effect of BPA on the estrous cycle (Nikaido *et al*., 2005).

The DS conclusion on effects on the estrous cycle

Overall, altered patterns of estrous cycle in the female offspring of mouse and rat dams exposed during gestation and/or lactation were reported in several studies. In most cases, BPA treatment induced significantly longer estrous cycle. Two studies did not reveal significant differences in patterns of estrous cycle. The DS concluded that there may be differences in the sensitivities between rat and mouse strains to endocrine system-mediated toxicity.

1.3.1.4 Effects on the hypothalamic-hypophyseal axis

Exposure via oral route

In Rubin *et al*. (2001), female rats were exposed via drinking water to 1.2 mg BPA/kg bw/day from GD6 through the period of lactation. The offspring exhibited decreased plasma LH levels (19% relative to controls) three months after ovariectomy in adulthood.

Exposure via subcutaneous route

In Savabieasfahani *et al*. (2006), pregnant ewes were subcutaneously injected with 5 mg/kg/d BPA from GD30 to GD90. In the 1-month-old offspring, there was a two-fold increase in the plasma LH concentration, a 1-month prolonged first breeding season and a reduced magnitude of LH surge after estrous cycle synchronization with prostaglandine F2α at 10 months of age.

Exposure via the intramuscular route

In Evans *et al*. (2004), female sheep were intramuscularly exposed to 3.5 mg/kg twice a week for 7 weeks from the 4th week of life. Their basal LH concentration, pulse amplitude and frequency were decreased. In Collet *et al*. (2010), ewes were intramuscularly exposed to 3.5 mg/kg BPA twice a week during 8 weeks from the 5th month of life. They had a decreased mean LH pulse frequency and basal concentrations, but not the amplitude 6 weeks after the treatment.

Exposure via intravenous route

Ewes of 3-4 months of age were intravenously exposed to 0.5, 1, 2.5, 5, 10, 20, 40 or 80 mg/kg bw/d for 54 h. Their LH pulse was abolished at 40 and 80 mg/kg bw/d and they had a decreased LH pulse frequency at 2.5, 5 and 20 mg/kg bw/d (Collet et al., 2010).

The DS conclusion on effects on the hypothalamic-hypophyseal axis

The DS concluded that an alteration in the secretion of hypothalamic-pituitary hormones may have been responsible for any long-term disturbance in reproductive function. According to the DS, the available studies investigating the effects of BPA on the hypothalamic-hypophyseal axis on rodents and sheep showed that BPA could influence the pattern of GnRH (gonadotropin-releasing hormone) or LH (luteinizing hormone) secretion. As the feedback loop regulated the hormonal secretion which was considered to be dependent on the exposure and observation periods, the DS considered it difficult to demonstrate the subtle effect of BPA on GnRH and LH secretion. However, the DS concluded that BPA caused a decrease in the concentration and pulse amplitude of LH.

1.3.1.5 Effects on the reproductive capacities

Exposure via oral route

In Tyl *et al*. (2002), Sprague-Dawley rats were orally exposed to 0.001, 0.02, 0.3, 5, 50 or 500 mg/kg bw/d from 10 weeks before mating until PND21 for 3 generations. The numbers of total and live pups per litter were reduced at birth and on PND4 at 500 mg/kg/d in all three generations. There were reduced body weights and body weight gains in the F1 males at 500 mg/kg/d. However, feed consumption did not show clear treatment-related effects. The DS considered the slight to mild renal tubular degeneration and chronic hepatic inflammation in females at 50 and

500 mg/kg/d to be a strong and direct effect of BPA on these organs rather than a sign of systemic toxicity.

In the continuous breeding study (NTP, 1985b) CD-1 mice were orally exposed to 0, 300 or 325, 600 or 650 or 1,200 or 1300 mg/kg bw/day of BPA for males or females, respectively. The number of litters produced/pair, litter size and the number of live pups per litter were decreased in the mid- and high-dose groups. The litter size reductions occurred across all matings and they were dose-related. No effects on fertility were observed in the low-dose group. A statistically significant decrease in the litter size and in the number of live pups per litter was also observed in the cross-over mating. In the continuous breeding phase, a statistically significant decrease in the live pup weight (6%) on PND0 was observed in females at the top dose after adjustment for litter size, including live and still births. In the F1 generation, BPA treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or on the mean pup weights at birth. Regarding the parental toxicity, there were no clinical signs of toxicity among the F0 generation animals. In the continuous breeding phase, a statistically significant decrease in the maternal body weight (between 6 and 9%) was observed after each litter at the top dose on PND0 as compared to controls. No effect was observed on maternal body weight on PND0 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. At necropsy of the F0 generation (controls and top dose group only), treatment-related effects were seen at the highest dose; in both sexes relative liver weight was increased by 28% and the relative combined kidney/adrenal weight was increased by 10-16% as compared to controls. No histological changes were observed in female reproductive organs and no effect was observed on the oestrous cycle. 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 (by 6-29%) and kidney/adrenal weights (by 13-20%) were observed in all treated groups. Overall, the signs of systemic toxicity were not considered to be marked in this study and therefore the effects on fertility were not considered to be a consequence of parental toxicity.

Intragastric exposure of mice to 5, 25 or 100 µg/kg bw/d of BPA for 28 days from PND60 increased the ratio of number of resorptions to total number of implantations at 25 and 100 µg/kg and the number of animals with resorptions at all doses (Al Hiyasat *et al*., 2004).

Ryan *et al*. (2010), did not find any effect on the fecundity of the rat offspring when the dams were exposed via oral gavage to only low doses of BPA (2, 20 or 200 µg/kg bw/d) during GD7-PND18. However, the authors had emphasized that the sample sizes were very small and therefore the results could not be considered as very conclusive. In Tyl *et al*. (2008), the oral exposure of mice to 0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d BPA from 8 weeks before mating until PND21 for 2 generations did not affect mating or fertility indices or litter size or cause post-implantation loss. No effect on fertility was observed in the rat oral two-generation study (Ema *et al*., 2001) either, but only low doses were employed in that study (0.2-200 µg/kg/day).

Exposure via subcutaneous route

In mice, the offspring of dams exposed to 0.025, 0.25 or 25 µg/kg bw/d of BPA via a subcutaneous implant from GD8 to day 16 of lactation had a decreased number of pregnancies at 25µg/kg and a decreased number of pups born per litter at 0.25 and 25µg/kg (Cabaton *et al*., 2010).

In Fernandez *et al*. (2010), rats subcutaneously exposed to BPA from 0.25 to 62.5 mg/kg bw/d during PND1-PND10 showed infertility at doses ranging from 25 to 62.5 mg/kg/d and reduced fertility at doses ranging from 2.5 to 6.2 mg/kg/d. In Varayoud *et al*. (2011), rats were subcutaneously injected with 0.05 or 20 mg/kg/d BPA during PND1, 3, 5 and 7. On GD18, there were a decreased number of implantation sites at 20 mg/kg/d ($p < 0.05$) and an increased number of resorption sites in 80-d old females at both doses.

Berger *et al*. (2007, 2008 and 2010) studied the effects of adulthood BPA exposure on female reproductive capacity in mice. In Berger *et al*. (2007), subcutaneous BPA injections of 0.0005, 0.0015, 0.0046, 0.0143, 0.0416, 0.125, 0.375, 1.125, 3.375 or 10.125 mg/mouse/d during GD1-GD4 decreased the number of pups born at 3.375 and 10.125 mg/mouse/d, and, at 10.125

mg/mouse/d, the percent of females giving birth and the number of implantation sites. In Berger *et al*. (2008), subcutaneous injections of 0.0005, 0.0045, 0.05, 0.125, 1.125, 3.375, 6.75, or 10.125 mg/mouse/d of BPA during GD1-GD4 decreased implantation sites at 6.75 and 10.125 mg/d on GD6. In Berger *et al*. (2010), subcutaneous injections of 100, 200 or 300 mg/kg bw/d during GD1-GD4 decreased the number of implantation sites and caused histological modifications to the wall of the uterine cavity at 200 and 300 mg/kg.

In Honma *et al*. (2002), a subcutaneous exposure of mice to 2 or 20 µg/kg/d of BPA during GD11-GD17 did not affect the total number of pups born. The observations in this study were limited to the first pregnancy of the offspring exposed during gestation, and the DS highlighted the importance of including a repetitive breeding protocol in a study in order to get a more reliable result.

The DS conclusion on the effects on the female reproductive capacities

Overall, the DS concluded that BPA induced an increase in the total number of resorptions or a decrease in the number of pregnancies and implantations as a result of at the least postnatal and adulthood exposure. The DS considered it likely that the decrease in litter size was mediated by a mechanism involving a disruption of intrauterine blastocyst implantation rather than by a post-implantational mechanism.

1.3.2 Humans

1.3.2.1 Effects on ovaries

In a prospective study by Mok-Lin *et al*. (2010), BPA was detected in the urine of the majority of women in a group (n=84) undergoing *in vitro* fertilization (IVF) treatment. The geometric mean BPA urine level of specific-gravity adjusted BPA (SG-BPA) was calculated from two urine samples collected during each of 91 IVF cycles and from one urine sample collected during each of 21 IVF cycles. The number of oocytes recovered was decreased on average by 12% per cycle and the amplitude of the preovulatory oestradiol peak was decreased on average by 213 pg/ml for each log unit increase in urinary SG-BPA. According to the DS, the urine BPA concentration reflected the exposure at the time of urine collection and not the exposure during the period of follicular maturation several months earlier. In addition, the DS noted that it was difficult to apply the results obtained from infertile women undergoing IVF to the general population. However, according to the DS, the results were consistent with the observations in 58 women undergoing IVF treatment, reported in Fujimoto *et al*. (2011), in which a reduced likelihood of a successful *in vitro* fertilization (fertilization rate) attributed to impaired oocyte quality was associated with an increased serum concentration of unconjugated BPA.

In a preliminary study on 44 women undergoing IVF, serum concentrations of unconjugated BPA were inversely associated with an oestradiol peak response during the IVF, but no association was indicated with the number of oocytes retrieved (Bloom *et al*., 2011).

In a Japanese cross-sectional study (Takeuchi *et al*., 2004), the serum BPA level (measured by a non-validated immunoassay method) was higher in non-obese (n=13) and obese (n=6) women with polycystic ovary syndrome (PCOS) and in obese non-PCOS women (n=7) as compared to non-obese non-PCOS women (n=19). Because of some limitations in the study, the DS considered that the results could not be considered conclusive.

The results of Takeuchi *et al*. (2004) were consistent with the results of a study by Kandari *et al*. (2011), in which women with PCOS (n=71) had higher serum BPA concentrations than the control women (n=100). According to the DS, the main limitation of the study was the analytical method (ELISA), which did not discriminate between different forms of BPA. On the other hand, the DS noted that the concentration obtained could be considered as a systemic indicator of the exposure to BPA.

The DS concluded that although rodent ovarian cysts, observed in the animal studies, were not the ideal model for PCOS, it was interesting to note that in women, the two available

epidemiological studies showed that a higher incidence of polycystic ovaries correlated with higher serum BPA concentrations (Kandaraki *et al*., 2011 and Takeuchi *et al*., 2004).

1.3.2.2 Effects on uterus: endometriosis and endometrial hyperplasia

The DS noted that epidemiological studies had reported contradictory effects of BPA on the human endometrium:

- In a population of women from an Italian gynecological-obstetric clinic, BPA (determined by HPLC) was more commonly detected in the serum of women with endometriosis (n=58) than in their age-matched control women (n=11) who had no detectable BPA in their serum (Cobellis *et al*., 2009). The DS noted that the methodology used in this study was questionable.

- In a cross-sectional study in 140 Japanese women consulting a physician for infertility problems, urinary BPA concentrations (measured by HPLC) were not significantly associated with the stage of endometriosis (Itoh *et al*., 2007). According to the DS the two main limitations with the study were that urinary BPA levels (single sample) did not reflect long-term exposure but instead the most recent exposure to BPA (4 to 6 hours prior to the assay) and that a valid control group was lacking.

- In a prospective cross-sectional study, reduced levels of serum BPA were associated with endometrium hyperplasia (Hiroi *et al*., 2004). However, the DS noted the low number of subjects studied (16 cases and 21 controls), the non-optimal analytical method (ELISA) and the uncertainty associated with a single BPA plasma sample per subject.

1.3.2.3 Effects on implantation and pregnancy

In a group of 137 women undergoing IVF treatment in the US, there was an increased chance of implantation failure in the higher quartile of urinary BPA concentrations (325 urine samples, specific gravity adjusted; HPLC measured) in a study by Ehrlich *et al.* (2012). There was a positive linear dose-response relationship resulting in almost double chance of implantation failure in the group of women with highest urinary BPA levels (fourth quartile of exposure) as compared to the group of women with the lowest urinary BPA levels (first quartile of exposure).

In a case-control study of Sugiura-Ogasawara *et al*. (2005), a higher serum level of BPA was reported in women with a history of three or more miscarriages (n=45) compared to control women (n=32; no history of live birth or infertility). There was also some evidence that the 35 women who subsequently had a successful pregnancy had lower serum BPA levels than those miscarrying. According to the DS the results from this study could not be considered as totally proven (ELISA method used for analyzing BPA, comparability of groups, confounding factors, statistical tools, limited size population, median serum BPA levels identical in both groups).

Higher urine BPA concentrations in single spot samples taken between pregnancy weeks 30 and 37 (specific-gravity adjusted; HPLC analysis) were found in women who delivered prematurely (before week 37) (n=12) as compared to women who delivered later than week 37 (n=48) in a nested case-control study by Cantonwine *et al*., (2010). However, the DS considered that there were many limitations in the study, such as the time of urine collection in relation to the pregnancy stage and food intake.

1.3.2.4 Effects on onset of puberty

No relationship was demonstrated between urine concentration of BPA and the onset of puberty in 186 nine-year-old girls in a cross-sectional study by Wolff *et al.* (2008). The result was later confirmed in a cohort of 1151 girls between the ages of 6 to 8 years by Wolff *et al*. (2010).

1.3.2.5 The DS's summary of effects on sexual function and fertility in women

In the summary of the data on fertility effects of BPA in women, the DS concluded that several of the epidemiological studies performed in women had methodological limitations related to small sample sizes, limited details on the selection criteria for subjects, and in many cases a cross-sectional design with a limited control for potential confounding factors. These limitations in the study design reduced the DS's ability to make clear conclusions on the weight that the data derived from the epidemiological studies should have in a weight of evidence analysis leading to the final conclusions on the potential health hazards of BPA in women. Nevertheless, the DS was of the opinion that the reported associations between urine/serum BPA concentrations and the adverse effects on sexual function and fertility in the epidemiological studies in women confirmed the effects of BPA observed in female animals and showed the human relevance of the hazards demonstrated in animal studies. The DS also pointed out that all epidemiological studies in women in the CLH report related to environmental (low level) BPA exposures.

1.4 Sexual function and fertility – males

1.4.1 Animal studies

1.4.1.1 Effects on reproductive tract

Exposure via oral route

BPA exposure *in utero* and/or during lactation caused effects on sperm production or quality or abnormalities in the seminiferous tubules.

In Tinwell *et al*. (2002), Sprague Dawley and Alderley Park (AP) rats were exposed daily to 20 µg/kg, 100 µg/kg bw or 50 mg/kg of BPA via oral route during GD6 – GD21. A significant decrease in sperm production was observed at 50 mg/kg in 90-day-old AP rats. In Salian *et al*., 2009c, Holtzman rats were exposed via oral route to 1.2 or 2.4 µg/kg bw/d of BPA during GD12 – PND21. There was a decrease in sperm count and motility at both doses. In Iida *et al*., 2002, ddY mice were exposed via oral gavage to 0, 1, 10 or 100 mg/kg bw/day of BPA during GD10 – GD17. Histological abnormalities in seminiferous tubules were observed in 60- and 90-day-old mice at all doses.

Oral postnatal exposure affected sex hormone levels and other fertility parameters in rats.

In Della Seta *et al*. (2006), circulating testosterone was reduced in Sprague-Dawley rats on PND37 by one-third after oral exposure to 40 µg/kg bw/d of BPA during PND23-PND30. Long-Evans weanling male rats were exposed via oral gavage to 0 – 2.4 µg/kg bw/day of BPA during PND21-PND90. On PND35, there was a decrease in the serum luteinizing hormone and testosterone levels, and on PND91 there was a decrease in the sex hormone levels, specifically the testosterone levels produced by Leydig cells (Akingbemi *et al*., 2004). In Chitra *et al*. (2003), 45-day-old Wistar adult rats were exposed via oral gavage to 0, 0.2, 2 or 20 µg/kg bw/day of BPA for 45 days. There was a dose-dependent decrease in the testes and epididymides weight by 6% and 12-25%, respectively. Ventral prostate weight was increased by 12-30%. There was also a dose-dependent decrease in the epididymal sperm motility and sperm count. In Tan *et al*. (2003), Sprague Dawley rats were exposed via gavage to 100 mg/kg bw/d of BPA during PND23-PND53. Of the treated rats, 66.7% reached a complete preputial separation compared to 100% in control rats and most of the rats in the treatment group showed some evidence of morphological changes or differences in the testicular histology when compared to the control group. Of the BPA-treated rats, 33% did not show any form of spermatogenic cycle and multinucleated giant cells were present in some of the lumen of the seminiferous tubules. The rest of the BPA-treated rats showed spermatogenic cycle in some of the seminiferous tubules but giant cells were also present in these tubules.

There were also studies in which no effects on reproductive parameters were reported in pups after oral exposure of dams to BPA during pregnancy and/or lactation or in young rats exposed postnatally to BPA.

No effects were observed in Dawley and Alderley Park rats exposed via gavage to 20 µg, 100 µg or 50 mg /kg bw/d of BPA during GD6-GD21 (Tinwell *et al*., 2001), in Evans rats exposed via the oral route to 2, 20 or 200 µg/kg bw/d of BPA during GD7-PND18 (Howdeshell *et al*., 2008) and in C57/BL6 mice exposed to 0, 50 or 1000 µg/kg via oral gavage during GD10-GD16 (La Rocca *et al*., 2011). Kobayashi *et al*. (2012) did not observe effects on reproductive development in the offspring of Sprague-Dawley dams exposed via diet to 0, 0.05, 0.5 or 5 mg/kg/d of BPA during GD6-PND21. Oral exposure of 4-week-old Jcl:Wistar rats to 0.25% (200 mg/kg bw/d) BPA for 2 months caused no effects on the weights of the reproductive organs or on the daily sperm production or efficiency (Takahashi and Oishi, 2003).

Exposure via subcutaneous route

Subcutaneous BPA exposure of ICR mouse dams to 100 µg or 5 mg BPA three days before mating and to 1.2 or 60 µg/day during gestation and lactation caused a decrease in the percentage of seminiferous tubules with mature spermatids in 4-week-old pups of the high dose group (Okada and Kai, 2008).

Subcutaneous neonatal BPA exposure induced effects on fertility, sperm parameters, reproductive organ weights, histology of the seminiferous tubules or plasma concentrations of testosterone. In Salian *et al*. (2009b), Holtzman rats were exposed subcutaneously to 100, 200, 400, 800 or 1600 µg/kg bw/d of BPA during PND1-5. Sperm count and motility were significantly decreased at ≥100 and 200 µg/kg bw/d, respectively. In Aikawa *et al*. (2004), SHN mice were exposed subcutaneously to 175 or 17 500 µg/kg bw/day of BPA on PND1-PND5. The percentage of moving sperm was decreased at the higher dose, and malformed sperm in the epididymides was increased at 10 weeks of age at both 175 (approximatively 45%; $p < 0.05$); and 17 500 µg/kg bw/day (78.2%; $p < 0.001$) groups compared to the vehicle control group (6.8%). In Nakamura *et al*. (2010), Wistar/ST 4-week-old rats were exposed subcutaneously to 11.4, 57.1 and 114.2 mg/kg bw/day of BPA for 4 days a week during 6 weeks. The testis weight decreased by 10% at the highest dose, and both mid- and high-dose exposure reduced the epididymis weight by 10 to 18%, the seminal vesicle weight by 35 to 48% and the prostate weight by 30% as compared to controls. BPA at mid and high dose decreased the plasma testosterone levels to one-third of the controls. The testicular testosterone levels were also decreased at these doses. There was a 20% decrease in the number of Leydig cells per seminiferous tubule. In Toyama and Yuasa, 2004b, new-born Wistar rats and ICR (CD-1) mice (n=5) were injected subcutaneously with BPA ranging from 0.1 to 10 µg BPA/injection/mouse and from 1 to 600 µg BPA/injection/rat. Animals were treated on PND1, 3, 5, 7, 9 and 11, and they were subsequently terminated weekly at the age of 2-10 weeks. There were abnormalities in spermatids in juvenile-adult animals at all doses but the effect was not dose-dependent.

Subcutaneous exposure of 4-week old Jcl:Wistar rats to 200 mg/kg bw/day BPA for 4 weeks significantly decreased the weights of testis, epididymis, prostate and seminal vesicle and the testicular daily sperm production (Takahashi and Oishi, 2003). Wistar Rats that were subcutaneously exposed to 3000 µg/kg bw/d of BPA during PND52-PND87, had a significant decrease in plasma testosterone levels and in the epididymal sperm count, and an increase in ventral prostate weight with high IGF-1 level (Herath *et al*., 2004). In Toyama *et al*. (2004), ICR mice (3-month-old) and Wistar rats (4-month-old) were exposed subcutaneously to 20 or 200 µg/kg bw of BPA during 6 consecutive days. In both treated groups, the acrosomal vesicles, acrosomal caps, acrosomes and nuclei of the spermatids were severely deformed. Sertoli cells were not affected except the ectoplasmic specialization between them and around the spermatids (incomplete specialization, redundant ectopic specialization and aplasia). The adverse effects of BPA observed in rats and mice were reversible since the fertility of the treated males was not affected 2 months after the end of the treatment.

In contrast, no effects on reproductive parameters were reported in Sprague-Dawley rats that were subcutaneously exposed to 2, 11, 56, 277 or 97000 µg/kg bw/d of BPA during PND1-PND9 (Kato *et al*., 2002).

Exposure via intraperitoneal route

4-week-old Wistar rats were exposed intraperitoneally to 2 or 20 mg/kg bw/day of BPA for 4 days a week during one month. A decrease in the prostate and seminal vesicle weight was statistically significant only for the ventral prostate at 20 mg/kg. Serum testosterone level was decreased by almost 70% at 20 mg/kg ($p < 0.05$) (Takahashi and Oishi, 2003).

The DS conclusion on the effects on the male reproductive tract

The DS concluded that considering the complexity of the fertility homeostasis, it was difficult to explain the discrepancies between the available studies. The studies were generally performed according to different protocols and different animal strains might have had different sensitivity to the effects. The DS pointed out that Sprague-Dawley rats may be insensitive (NTP, 2001) or may have a low sensitivity (Yamasaki *et al*., 2002) to estrogenic compounds. The DS also considered that the route and window of exposure may be important factors for the manifestation of effects. The DS concluded that the oral route of exposure during a specific period of time may be more sensitive than the subcutaneous route of exposure.

1.4.2 Humans

1.4.2.1. Effects on sex hormone levels

Environmental BPA exposure

According to the DS there were three studies on BPA effects on male sex hormone levels that were of an acceptable quality.

In a cross-sectional study by Meeker *et al*. (2010a), 167 men were recruited from an infertility clinic and their single spot urinary BPA levels were found to be inversely associated with serum levels of inhibin B and with the oestradiol:testosterone (E_2:T) ratio (considered as a marker for reduced aromatase activity) and to be directly correlated with FSH levels. The hormone levels were measured on the same day as the urine was sampled. Furthermore, for about half of the men (n=75), a second (or further) urinary BPA level was determined in samples collected weeks or months after the serum sampling. When these additional samples were taken into consideration in the analysis, the urinary BPA association with FSH and E_2:T ratio remained, but it was somewhat weakened with inhibin B. The free androgen index (FAI; ratio of testosterone to sex hormone binding globulin) was inversely associated with urinary BPA levels in the group of 75 men with repeated BPA sampling. The DS was of the opinion that the study had several limitations, as there were only single hormone and BPA measurements in more than half of the men and a high temporal variability in BPA exposure within individuals and because the men were recruited through an infertility clinic. The DS pointed out that the value of generalising the results to the general population may have been limited, although according to authors of the study (Meeker *et al*, 2010a), there was no available evidence that men from an infertility clinic were differently affected by BPA exposure compared to men in the general population.

Mendiola *et al.* (2010) found that urinary BPA levels (single sample) were inversely associated with the free androgen index (FAI; ratio of testosterone to sex hormone binding globulin (SHPG)) and the FAI/LH ratio and a weakly positive association with SHBG (sex hormone binding globulin) in serum (single samples) of fertile men (n=375, partners to pregnant women).

A higher 24-hour urine excretion of BPA in Italian men in the general population (n=715, of which 533 were 65-74 years old) was associated with a higher total serum testosterone concentration, after adjustment for various parameters (Galloway *et al*., 2010). No correlation was found with serum estrogen or SHBG levels.

Considering the different sample sizes in the above mentioned studies, the DS regarded the results of the studies as consistent where the same parameters had been studied.

1.4.2.2. Effects on sexual function, sperm parameters and offspring

Occupational BPA exposure

Sexual function was estimated via questionnaires in interviews of male workers exposed to BPA and to controls (workers not occupationally exposed to BPA). Participation rates were 62% (i.e. 230 out of 373 eligible) and 55% (i.e. 284 out of 515 eligible), respectively (Li *et al*., 2010a). There was a dose-response relationship between high levels of cumulative exposure to BPA (time-weighted-average air levels x duration; for each individual) and increased risk of impaired sexual function. The air exposure assessments were consistent with the result of spot urine BPA measurements. The association remained also when persons with a history of exposure to other chemicals were excluded from the analysis. The DS raised some questions related to uncertainties with the interviews (self-reporting) and noted the lack of clinical data.

In a second study by Li *et al*. (2010b), urine samples were collected from BPA exposed workers before and after a work shift. Increasing levels of urine BPA correlated with worsening sexual function (as was self-reported in interviews). Thus, the results of the previous study were supported. A similar trend was found among workers exposed only via the environment, not occupationally.

In Li *et al*. (2011), high levels of urine BPA in exposed workers were associated with decreased sperm count, mobility and vitality, but not with semen volume or sperm morphology in another study (n=218 out of 888 eligible). Only men with semen specimens meeting the WHO's collection guidelines were included in the analysis. A similar association was found among a smaller group of workers (n=87-88) only exposed to BPA via the environment, not occupationally.

In a retrospective study by Miao *et al*. (2011), parental occupational exposure to BPA (based on air measurements and urine levels, see Li *et al*., 2010a, above) during pregnancy was associated with a shortened anogenital distance (AGD) in the sons, after controlling for the age and weight of the boys. The association was stronger for maternal BPA exposure. The number of boys with parental occupational BPA exposure was 56, and 97 without parental BPA exposure, and the ages of the boys ranged from 0 to 17 years.

Environmental BPA exposure

No correlation was found between sperm parameters (seminal volume, sperm count, concentration, motility or morphology) and urinary concentration of BPA (single samples) in fertile men (n= 375, partners to pregnant women) in the study by Mendiola *et al*. (2010).

However, in 190 men recruited from an infertility clinic, single spot urinary BPA levels correlated with declines in sperm concentration, motility and morphology, though the effects were not statistically significant (Meeker *et al*., 2010b). An increase in urinary BPA concentration was associated with a decline in sperm concentration by 23%, in motility by 7.5% and in morphology by 13%. Multiple BPA measures were available for a subset of participants. The DS noted that some of the semen analysis parameters were not performed according to WHO recommendations.

In a preliminary study on 27 couples for whom embryos were generated during an *in vitro* fertilization (IVF), a 30% decrease in the adjusted chance of a higher embryo cell number (ECN) and a 46% decrease in the adjusted chance of a higher embryo fragmentation score (EFS) for each log unit increase in serum BPA of the men was reported, suggesting an inverse relation between paternal BPA concentration and impaired embryo quality (Bloom *et al.,* 2011). The DS underlined the need to confirm the results especially with regard to the small number of participating couples.

The level of unconjugated BPA was measured in the cord blood of 152 boys born after 34 weeks of gestation with cryptorchid or descended testes in a study by Fenichel *et al*. (2012). No difference was found between the BPA levels in cord blood from boys with cryptorchidism as compared to control boys (without cryptorchidism). The DS pointed out that the presence of unconjugated BPA in all cord blood samples suggested a placental transfer and fetal exposure to unconjugated BPA.

1.4.2.3. The Dossier Submitter's summary

In a summary of the data on fertility effects of BPA on men, the DS concluded that despite the limitations noted in the epidemiological studies, the DS had confidence in the data on the dose-effect relations identified in these various studies. The effects, such as effects on sexual hormones and sexual function, including sperm parameters, observed in men were consistent with the findings in animal studies.

1.5 Final conclusions of the Dossier Submitter

The DS concluded that BPA acted as a weak oestrogen mimick. It had a much lower affinity for the estrogen receptors (ERα and ERβ) than endogenous oestrogen and it was rapidly metabolized to BPA-glucuronide which was not hormonally active. More recently, BPA had been shown to bind with high affinity to the estrogen-related receptor (ERR-γ), which may be related to BPA's ability to act as an endocrine disruptor on fertility. According to the available *in vitro* and *in vivo* studies, BPA had not demonstrated either androgenic or anti-androgenic activity.

Based on the evidence from numerous animal studies, the DS concluded that BPA impacted the male reproductive system with effects on seminiferous tubules, reproductive hormones levels and the quantity and quality of sperm following an *in utero* exposure at doses that did not cause major toxicity and in specific models that could predict human toxicity. When the exposure occurred neonatally, effects on fertility and on the organs of the reproductive tract were observed. The exposure during puberty caused effects on the levels of the reproductive hormones, on seminal vesicles, on prostate, testis and epididymis weights, and on sperm quality. The BPA exposure in adulthood induced effects on plasma testosterone levels, on the organs of the reproductive tract and on the sperm production and quality. Based on human studies, the DS concluded that exposure to BPA affected male fertility and reproductive hormone levels in specific populations.

In female animals, following a pre- and post-natal exposure, an increased occurrence of ovarian cysts or disturbance of estrous cycles were observed in the animal studies presented in the CLH report (exhaustive literature search from 2002 to 2011). According to the DS, these observations supported the risks identified in human epidemiological studies. When the exposure occurred postnatally or during adulthood there was a systematic decrease in the number of pregnancies and implantations. This seemed to be contradicted by multigeneration studies, although pre-implantation losses were not assessed. The DS concluded that in rodents the pre-implantation loss seemed to be responsible for the effect of BPA on fecundity.

Implantation failures were reported in women undergoing a medically-assisted procreation with higher urinary levels of BPA than control women. Also the pregnancy outcome was considered to be affected by the exposure to BPA because miscarriages and premature birth were observed in different studies. In a few animal studies, endometrial hyperplasia was observed. Endometriosis and hyperplasia were reported also in epidemiological studies in women. Finally, an earlier onset of puberty or changes in the sex hormones levels were observed in animal studies but these findings were contradicted or not corroborated by epidemiological studies.

The DS concluded that many, but not necessarily all findings in animal studies were corroborated or validated by human data. The DS therefore proposed a classification as Repr. 1B (Repr. Cat. 2; R60 according to Directive 67/548/EEC) but welcomed a discussion on the uncertainties in the human data and on a potential classification for Repr. 1A; H360F. The DS did not consider classification for Repr. 2 as appropriate since there were more than "some evidence" of adverse effects in animals or humans", and according to the DS the observed effects were sufficiently convincing to classify BPA at least in category 1B for adverse effects on sexual function and fertility.

2 COMMENTS RECEIVED DURING PUBLIC CONSULTATION

Seventeen comments were received during the public consultation. Among commenting parties were Member State Competent Authorities (MSCAs), Industry or Trade Associations, Company-Manufacturers, International NGOs, and one private individual.

Comments were received from eight MSCAs, four in agreement with the proposal and three not in agreement with the proposal. One MSCA did not indicate any preference for Repr. 1B or Repr. 2, and recommended the DS to improve their justification of the animal evidence in support of Repr. 1B for fertility. One MSCA that agreed on the proposal also indicated that based on the epidemiological data, BPA could have adverse effects on human reproduction and therefore welcomed a discussion within RAC on whether the data from these studies was sufficiently robust to support a Repr. 1A classification. Another MSCA considered that the reported animal data confirmed the data obtained from various human studies. However, it was uncertain whether the human data were strong enough to justify a Repr. 1A classification.

The MSCAs that did not agree with the CLH proposal questioned the use of studies using subcutaneous or other parenteral routes of exposure for classification, and considered that these studies could only be used to indicate the potential of BPA to induce reproductive toxicity. These MSCAs also considered it important to assess whether the reported reproductive effects observed in animal studies were a direct effect of BPA or a secondary non-specific consequence of other toxic effects. One MSCA thought that there was some evidence of adverse effects on fertility. However, due to the inconsistencies across the effects and discrepancies between the studies, the MSCA considered the present classification of BPA as Repr. 2 as the most appropriate. Another MSCA proposed to consider a classification also for developmental effects. As a response, the DS encouraged other MSCAs to prepare a proposal for a harmonized classification and labelling for the adverse effects of BPA on development. Another MSCA considered that it would have been better to address the effects on chromosome segregation in oocytes under germ cell mutagenicity. One MSCA stated that several human studies had serious methodological flaws with regard to confounders, population size, etc.

One MSCA considered that there was a large inconsistency in the studies included in the proposal, and was of the opinion that there was no evidence of a direct effect of BPA on human reproduction. The human data showed some correlations but no causation of effects, and a classification for Repr. 2 was considered as more appropriate. Based on the effects on liver and kidney following the exposure to BPA also a classification for repeated dose toxicity was considered as appropriate.

The BPA REACH Consortium representing more than 30 producers, importers and users of BPA in Europe did not agree with the proposal to classify BPA as Repr. 1B. They considered that the proposal should have been rejected since it failed to consider the quality of the data and it failed in the weight of evidence analysis. Their main arguments were as follows:

- Effects on animal fertility were only reported at higher doses of BPA and rather than being specific reproductive effects, they were merely related to systemic toxicity.

- The proposal was not in accordance with ECHA's Guidance on the preparation of CLH dossiers (ECHA 2010) which includes the use of a weight of evidence approach for compounds with a large database, such as BPA. The CLH dossier did not follow the CLP Regulation regarding the request that "both positive and negative results shall be assembled together in a weight of evidence determination" and regarding that "the quality of the data shall be given appropriate weight".
- The CLH proposal selectively relied only on studies, assessments, and the 1 out of 1409 self-classifications that supported its proposal and, therefore, portrayed an inaccurate and incomplete picture of the state of the science on BPA. The information was not comprehensive and it was inconsistent throughout the report. Statements related to the value of regulatory guideline studies as compared to the value of exploratory studies were biased. Furthermore, statements on multi-generation animal studies upon which regulators had relied (Tyl *et al*., 2002 and 2008) were inconsistent, incorrect and incomprehensible.

- Recent (after December 31, 2012) and important scientific research from government agencies was not considered. These studies did not support a classification of BPA as a Category 1B Reproductive Toxicant.

The DS responded that due to the existing very large dataset for BPA it was possible that some studies were unintentionally omitted from the CLH report. The criteria for the selection of the studies included in the CLH proposal were described in the RCOM.

The Can Manufacturers Institute (United States) opposed the proposal to classify BPA as Repr. 1B since in studies where reproductive toxicity was reported, these effects occurred at dose levels above those where systemic toxicity was reported. Thus, the criteria for the Category 1B were not met.

An international NGO, ChemSec, commented that the recent epidemiological review (Rochester, 2013) outlined the body of literature showing a clear association between BPA exposure and adverse prenatal, childhood, and adult health outcomes, including reproductive and developmental effects, metabolic disease, and other health effects. ChemSec made a summary of the conclusions in Rochester (2013), and also compiled the studies into four tables indicating that 94, 77, 78 and 86 % of the studies showed strong evidence of a capacity to interfere with reproduction, development, metabolic disease and other effects, respectively, in humans. ChemSec concluded that the strong evidence available today and the recent human studies corroborating the animal findings confirmed a strong presumption that BPA had the capacity to interfere with reproduction in humans, suggesting a CLH as Repr. 1A.

Another international NGO, European Environmental Bureau (EEB), also requested that the recent review conducted by Rochester (2013) should be taken into account in the hazard assessment. In the review, associations were revealed between BPA exposure and adverse perinatal, childhood and adult health outcomes in humans, including reproductive and developmental effects, metabolic disease and other health outcomes, particularly behavioral effects in children. According to the review and as supported by EEB, these studies confirmed that BPA could be harmful to humans at exposure levels of the general population.

Exponent (a private consultant company) submitted a review of the epidemiological studies in the CLH report. They heavily criticized nearly all the studies regarding sampling of serum and blood for the determination of exposure, and especially the use of single spot samples. They also commented that the studies lacked information on factors such as BPA via diet, and that most of the studies were cross sectionally designed, and thus they could be used only for correlations but not for causality. They concluded that the inconsistent results, null findings, doubtful results and contradictory findings in the 20 epidemiological studies were best compatible with a situation where no biologically plausible relationships could be established. Consequently, the presence of any robust adverse health effects caused by BPA could be excluded.

All comments as well as the responses by the DS and RAC are compiled in the RCOM in Annex 2 to the RAC Opinion.

3 ADDITIONAL KEY STUDIES SUBMITTED DURING PUBLIC CONSULTATION

US National Centre for Toxicological Research (NCTR) study: NCTR Evaluation of the toxicity of BPA in male and female Sprague-Dawley rats exposed orally from gestation day 6 through postnatal day 90.

In this subchronic (90 day) oral gavage study in Sprague-Dawley (SD) rats, a broad range of in-life endpoints and organ histopathology was studied. Background BPA levels in the study material and BPA levels in serum were determined, and the groups consisted of naïve controls, vehicle controls, concurrent reference substance estrogen (ethinyl estradiol, EE2) dose groups and BPA dose groups. The litter was used as the unit for data analysis. Test substances were orally administered daily in 0.3% carboxymethylcellulose to NCTR SD rats from gestation day 6 until parturition. Pups were directly dosed from PND 1 since the lactational transfer of the test substances was reported to be minimal. BPA doses were 2.5, 8, 25, 80, 260, 840, 2700, 100 000, or 300 000 µg/kg bw/day. EE2 doses were 0.5 or 5.0 µg/kg bw/day. On PND4, 21, and 80, serum samples were taken at the approximate time of maximal concentration after dosing to measure the BPA and EE2 levels. Unconjugated (bioactive) BPA levels on PND4 ranged from 0.4 nM at the lowest dose to 49 µM at the highest dose. On PND21 and PND80, the ranges were 0.05 nM - 13.9 µM and 0.02 nM – 0.8 µM, respectively. Unconjugated BPA was ≤ 5% of the total BPA except at the two highest doses on PND4. Clear adverse effects of BPA were confined to the two highest

dose groups. BPA affected gestational weight gain at 100 000 µg/kg body weight/day and had multiple reproductive organ and serum clinical chemistry effects in both sexes at 300 000 µg/kg bw/day (delayed testicular descent, decreased testicular size and degeneration of seminiferous tubular epithelium in males, and an increase in ovarian cysts, small ovaries, decreased ovary weight, increased corpus luteum depletion and antral follicles depletion as well as abnormal estrus cycles in females). These effects overlapped with EE2 effects, although not all effects induced by BPA at high doses overlapped with the effects induced by EE2. Female mammary gland ductal hyperplasia was observed only in BPA-treated animals on PND21 and PND90 but not in EE2-treated animals, although this effect was considered as treatment related only by the study authors but not by the original study pathologists or by CFSAN Pathology during the subsequent review of the study report. It was concluded by the reviewers of the NCTR study that this effect was weak and it was considered as an equivocal finding.

Rochester, 2013: BPA and human health: A review of the literature.

Rochester (2013) identified 91 studies examining BPA effects on human health. Four studies in Chinese and one case study were not reviewed. The rest of the studies were analysed for quality based on the National Toxicology Program Office of Health Assessment and Translation (OHAT) approach, and the strength of evidence was discussed. Only the fertility part of the review is summarised below: the author concluded that there was some evidence that BPA may have contributed to infertility in humans, mainly based on the studies from fertility clinics. The author further concluded that the link between BPA exposure and male sexual function reported in the Li studies (Li *et al*., 2010a and b), which she descibed as excellent cohort studies, would be strengthened if replicated in another cohort. According to the author, consistent results showing correlations between higher urinary BPA and lower semen quality had been observed in different populations (Li *et al*., 2011; Meeker *et al*., 2010; Mendiola *et al*., 2010). The author also concluded that the studies related to sex hormone concentrations and BPA exposure were strong and supported the hypothesis that BPA had activational effects on circulating levels of sex hormones. The role of BPA in causing polycystic ovary syndrome (PCOS) was considered unclear by the author. The author also stated that the literature did not seem to support the relationship between BPA and endometrial disorders. The author concluded that there was some (but preliminary) evidence of a relationship between recurrent miscarriage and BPA exposure in women, and some evidence of an association between BPA and premature deliveries. Finally, the author concluded the review stating that the current literature-to-date indicated BPA in the environment may pose a health risk to humans.

Cantonwine et al., (2013); BPA and human reproductive health.

The purpose of this review was to summarize the current epidemiological literature regarding fertility and pregnancy risks associated with BPA exposure. The authors commented that although there had been some evidence of altered semen quality and hormone levels in males which were associated with urinary BPA concentrations, the results had not been consistently significant and they were drawn from cross sectional designs. Therefore, there was a need for additional longitudinal studies with repeated biomarker measures of exposure and with sufficient statistical power to better understand the potential associations of BPA exposure with male fertility and reproductive function. Consistent findings from two cohorts of reduced peak estradiol (E2) and oocyte yield suggested that BPA may have altered reproductive function of women undergoing an IVF and therefore further examination among larger study populations was warranted.

Additionally, the observation of an association between BPA exposure and increased odds of implantation failure by Ehrlich *et al*. (2012), provided intriguing evidence of adverse effects, which, if replicated, may provide a plausible explanation for BPA effects on female infertility. The authors concluded that while there was a growing body of literature suggesting that BPA exposure had an adverse relationship with fertility and birth outcomes, the human studies remained extremely limited and highlighted the need for more epidemiological research. Furthermore, it was noted that the often contradictory findings for the adverse effects of BPA on fertility, pregnancy and birth outcomes may have reflected analytical differences, study populations and methodological issues related to the exposure assessment or study design. The majority of the epidemiological studies reviewed here relied upon a single time point measurement of BPA which failed to address the temporal relationship between toxicant exposure and pregnancy outcomes.

4 ASSESSMENT AND COMPARISON WITH THE CLASSIFICATION CRITERIA

The CLP 3.7.1.3 defines adverse effects on sexual function and fertility as follows:

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

4.1 Toxicokinetics (absorption, distribution, metabolism and elimination)

BPA was rapidly absorbed from the gastrointestinal (GI) tract in mammals including humans. Toxicokinetic analysis of the area under the curve (AUC) showed that the GI absorption was greater than 85% in rats and monkeys. The studies conducted in human adults at relatively low doses (0.025 to 5 mg total) showed that BPA was rapidly and completely absorbed from the GI tract. Transmucosal absorption from the oral cavity, as sublingual absorption, bypassed the first-pass hepatic metabolism (first pass effect) and led to a much higher internal exposure to unconjugated BPA relative to the administered dose than after absorption from the GI tract. A recent dog study demonstrated 70-90% systemic bioavailability of BPA after sublingual absorption. Dermal absorption occurred to a lesser degree than absorption from the GI tract after oral intake and varied in the *in vitro* and *in vivo* studies presented in the CLH report, but it was shown to occur in all presented studies. There was no data on the toxicokinetics of BPA after exposure via inhalation, but results from inhalation studies in animals indicated that absorption through the lungs occurred.

Once absorbed from the GI tract after oral exposure, BPA entered the liver via the portal vein. In rodents, the highest concentrations of BPA were found in the liver and kidney. In all mammalian species studied the major metabolic pathway was glucuronidation of BPA via a first pass effect in the liver, catalyzed by UGT2B1 in rats and UGT2B15 and UGT2B7 isoforms in humans**.** Also BPA sulfate was identified as a metabolite in humans and rodents.

In rodents, conjugated and unconjugated BPA were subject to biliary excretion, enterohepatic recirculation and principally to fecal excretion. Humans and other primates were not capable of biliary elimination of BPA-G. They excreted systemically available forms of BPA (conjugated and unconjugated) primarily in urine. Both BPA-G and BPA sulfate represented detoxification pathways of BPA, as they were not active on the estrogen receptor, though deconjugation could occur at specific tissue sites by glucuronidases and/or sulfatases.

The absorption and elimination of BPA in human studies showed that spot urine samples of total BPA reflected exposure in the preceding 4 – 6 hours, but not the full day exposure.

BPA was reported to pass through the human placenta mainly as unconjugated BPA, and the capacity of human fetal liver to conjugate free BPA was limited. In addition, the placenta was reported to contain glucuronidase capable of producing unconjugated BPA from BPA-G, but the recent data in monkeys argued against the hypothesis that BPA was selectively deconjugated by the placenta or fetus.

Human neonates had an immature renal excretory function and glucuronidation capacity, but the presence of sulfo-transferases was reported to compensate for the immature glucuronidation capacity. In monkeys the Phase II metabolism (conjugation) of BPA, the internal exposure to and elimination of unconjugated BPA was reported to be similar in neonatal and adult monkeys, although there was some evidence of immature renal function in neonatal monkeys with respect to excretion of conjugated BPA. In neonatal rats the internal exposure to unconjugated and total BPA was reported to be considerably higher than in adult rats, possibly as a result of immature metabolism, enterohepatic circulation as well as renal and/or biliary excretory function. In neonatal rats the internal exposure to unconjugated BPA was reported to be much lower after oral exposure than after subcutaneous exposure indicating the presence of first-pass metabolism despite the evidence for diminished Phase II metabolic capacity.

Taken together, the bioavailability of conjugated BPA was reported to be low in rodents and primates after oral exposure to low doses of BPA due to an extensive first-pass metabolism. RAC concluded that the data from experimental oral animal studies were relevant to humans and for the classification of BPA for adverse effects on sexual function and fertility. Also other routes of exposure, such as sublingual, buccal, dermal and inhalation route, were potential routes of human exposure which led to a bypass of the extensive first-pass hepatic metabolism (first-pass effect). Subcutaneously administered BPA was not subject to the first pass effect that was shown to efficiently reduce the bioavailability of the active, aglycone BPA. The doses used in several of these studies were as a consequence relatively low. The subcutaneous route of exposure did not relate to a potential route of human exposure, but these studies provided supplementary information in the WoE evaluation as it was expected by RAC that reproductive organs were not exposed to unrealistically high levels of the active aglycone BPA as no signs of systemic toxicity were reported in these studies.

4.2 Animal studies on sexual function and fertility

In the weight of evidence analysis, RAC evaluated the two- and multi-generation guideline studies, conformed to internationally agreed test guidelines that were given the most weight and that were considered as key studies. The additional non-guideline studies on female and male reproductive toxicity were considered by RAC as supplementary studies.

4.2.1 Two- and multi-generation studies

A fertility assessment by continuous breeding was studied in NTP, 1985b. CD-1 mice were exposed via oral route (n= 20/ treated group/ sex, n= 40/ control group/ sex) with the following doses in diet: 0, 0.25, 0.5 or 1.0% (daily intakes of BPA were estimated to be 0, 300 or 325, 600 or 650 or 1,200 or 1300 mg/kg for males and females, respectively). Four tasks were performed in the study; dose range-finding study (Task 1), continuous breeding phase (Task 2), cross over mating (only the high-dose group was tested) (Task 3) and offspring assessment and assessment of reproductive capacity (Task 4). The premating period was 1 week followed by a 14 week mating trial (Task 2). The litters remained with their mothers until weaning on PND21.

The results of the continuous breeding phase (Task 2) showed that here was a statistically significant decrease in litters/pair at the two highest dose levels (4.5 and 4.7 compared to 5.0 for controls), in the litter size (6.5 and 9.8 compared to 12.2 for controls) and in the number of live pups per litter (6.3 and 9.7 compared to 12.1 for controls). Litter size reductions were reported across all matings, and they were dose-related. No effects on fertility were reported in the low-dose group.

The results of the cross over mating (Task 3) showed a statistically significant decrease in litter size, (controls: 11.4, treated males: 9.1 and treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5). This result indicated that in this study the most significant effects were reported in exposed females.

The results of the offspring assessment and assessment of reproductive capacity (Task 4) showed a statistically significant decrease in the live pup weight (6%) in females on PND0 in the high-dose group after the adjustment for litter size (including live and still births). In the litters selected for mating, death was reported up to PND74 in 6%, 4%, 14% and 38% in controls, low-, mid- and high-dose groups, receptively. This may have indicated a possible effect on pups due to their exposure to BPA during lactation. In the high dose group, there were only 8 litters that had at least one male and one female for the mating phase. Therefore only 11 breeding pairs were included in the high-dose group compared to 19-20 in the control, low- and mid-dose groups. No effects on fertility index, litter size, number of live pups per litter or pup weight were reported in the F1 generation. Nevertheless, the absence of effects in the single F mating did not detract from the reproducible results across the 4-5 litters produced by each F generation pair in the continuous breeding phase.

In male mice, a statistically significant dose-related decrease in the right epididymis weight was reported (11%, 16% and 18% as compared to the controls in the low-, mid- and high-dose groups, respectively). Left testis/epididymis weights were statistically significantly decreased, by 10% at

the mid dose and by 9% at the high dose, and the seminal vesicle weight was statistically significantly decreased, by 28%, in the high-dose group.

General toxicity was evident in the continuous breeding study as a statistically significant decrease in F0 maternal body weight on PND0 after each litter (6% - 9%) in the high-dose group. No effects on maternal body weight on PND0 were reported in the cross-over mating study. No effects on body weight gain were reported in males. At necropsy of the F0 generation (controls and top dose group only), relative liver weight was increased 28% and relative combined kidney/adrenal weight increased 10-16% compared to controls in both sexes at the high dose. No histological changes were observed in female reproductive organs and no effect was observed on the oestrous cycle. 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. RAC considers that the general toxicity was not marked and the effects on fertility are therefore not considered to be a consequence of parental toxicity.

A two-generation reproductive toxicity study in CD-1 mice by Tyl *et al*. (2008) was performed according to the OECD TG 416 and in compliance with GLP. CD-1 mice (28 mice/dose group) were administered BPA in the diet at 0, 0.018, 0.18, 1.8, 30, 300 or 3500 ppm corresponding to 0, 0.003, 0.03, 0.3, 5, 50 or 600 mg/kg bw/day. A positive control group exposed to 0.5 ppm of 17β estradiol (0.08 mg/kg bw/day) was included in the study. The exposure period started 8 weeks before mating and last until PND21 in the F2 generation. In females, the vaginal patency was accelerated at 600 mg/kg bw/day when adjusted to the body weight on PND21. F0 treated females were twice more frequently in estrus as compared to controls. An increase in the length of the gestation period by 0.3 days in F0 and F1 generations and a decrease in the body weight of the F1 pups during lactation were reported. In F0 males, there was a statistically significant decrease in the epididymal sperm concentration at 600 mg/kg bw/day. In F1 males, a statistically significant reduction in anogenital distance (AGD) when adjusted to the body weight was reported on PND21 at 50 and 600 mg/kg bw/day. In males, a statistically significant reduction in testis weight (17%) with histopathology findings including an increased incidence of minimal to mild hypoplasia of the seminiferous tubules in F1 (12% vs. 1% in control animals) and F2 (35% vs. 4% in control animals) weanlings at 600 mg/kg bw/day was reported. Furthermore, an increase in the incidence of undescended testes was reported in F1 and F2 weanlings at 600 mg/kg bw/day. Mild parental toxicity including increased kidney and liver weight as well as reduced body weight gain was reported. The general toxicity was not marked and the adverse effects on sexual function and fertility are therefore not considered to be a consequence of parental toxicity

After the publication of the Tyl *et al*. (2008) study, questions were raised by Myers *et al*. (2009) about the procedures and/or animals used by Tyl *et al.* (2008), since an abnormally high prostate weight was reported in the control animals as compared to the prostate weight reported in other studies performed with the same strain of mice at similar age. It was suggested that the technique used for dissecting the prostate resulted in nonprostatic tissue being weighed along with prostate and therefore rendering the result on unchanged prostate weight unreliable. Alternatively, it was noted by Myers *et al*. (2009) that as male rodents age, they are prone to develop prostatitis leading to an increase in prostate size and could thus account for the very large prostate weights reported by Tyl et al. (2008a). Prostatitis was reported to be rare in young-adult mice or rats, and the size of the prostates in the Tyl et al. (2008a) study were considered to be similar to those for middle-aged and old male mice. The age of the F0 and F1 males was reported to be ~19 and ~14 weeks at the sacrifice in the publication by Tyl *et al*. (2008). However, the age of the male animals at sacrifice has been discussed in the scientific comunity, and therefore RAC agreed that the results of the prostate weight of the study by Tyl *et al*. (2008) were unreliable.

In a two-generation study by Ema *et al*. (2001), IGS Sprague Dawley rats were exposed to BPA orally via gavage. 25 rats /sex /group were dosed with 0, 0.2, 2, 20 and 200 µg/kg bw/day for two generations. The study protocol was similar to the OECD 416 guideline, but females were treated for only two weeks before mating whereas males were exposed during a 10-week premating period. The only effect reported was a statistically significant decrease in the absolute (by 17%) and relative (by 20%) weight of seminal vesicles in F2 males only at the lowest dose. No parental toxicity or effects on fertility were reported in this study testing only low doses of BPA.

In a multi-generation study by Tyl *et al*. (2002), Sprague Dawley rats (30 rats/sex/group) were exposed to BPA via the oral route in diet at 0, 0.15, 0.3, 4.5, 75, 750 and 7500 ppm corresponding to approximately 0, 0.001, 0.02, 0.3, 5, 50 and 500 mg/kg bw/day during a 10-week premating period until PND2 for three generations. The study was performed in compliance with GLP. General toxicity was reported as a 13% reduction in body weight gain in all exposed generations and as effects in kidney, evident as renal tubular degeneration in females (not in F3) at 500 mg/kg bw/day. There was a statistically significant reduction in the average number of live pups per litter at 500 mg/kg bw/day in all generations on PND0 (F1: 11.5 compared to 14.3 in controls, F2: 10.8 compared to 14.6 in controls and F3: 10.9 compared to 14.8 in controls). The decrease was reported without a statistically significant effect on post-implantation loss or on the number of dead pups per litter. The absolute and relative paired ovary weights were statistically significantly decreased by 16-34% and 15-34%, respectively, in adult F1, F2 and F3 females at 500 mg/kg bw/day.

In the F1, F2 and F3 offspring, a statistically significant decrease in the body weight per litter (12-27%) was reported at 500 mg/kg bw/day in males and females on PND7-21 as compared to controls. A statistically significant increase in the AGD of F2 females (measured only in F2 and F3 offspring) was reported on PND0 at 0.01 (3%), 0.02 (3%), 0.3 (3%) and 50 mg/kg bw/day (4%). Furthermore, the onset of puberty (evaluated as the age of vaginal patency) was statistically significantly delayed at 500 mg/kg bw/day in F1 (PND33.0 compared to PND30.5 in controls), F2 (PND34.5 compared to PND31.0 in controls) and F3 (PND33.8 compared to PND31.3 in controls) females. In males, a statistically significant delay in preputial separation was reported in F1 (PND45.8 compared to PND 41.9 in controls) in F2 (PND47.9 compared to PND42.1 in controls) and F3 (PND45.2 compared to PND42.1 in controls) generations.

4.2.1.1 Conclusions on animal two- and multi-generation studies

Four two- or multi-generation studies were included in the CLH proposal for BPA submitted by France; two in rats and two in mice. The first rat two-generation study included very low doses of BPA (0.2 to 200 µg/kg bw/day), and no effects on fertility were reported (Ema *et al*., 2001). In the second rat study, a multi-generation study, rats were exposed to doses from 0.001 up to 500 mg/kg bw/day of BPA (Tyl *et al*., 2002). General toxicity was reported as a 13% reduction in body weight gain in all generations and as effects on kidney, evident as renal tubular degeneration in females (not in F3) at 500 mg/kg bw/day. In this study, at 500 mg/kg bw/day, a statistically significant reduction in the average number of live pups per litter in all generations was reported. The effect was reported without effects on post-implantation loss or on the number of dead pups per litter. Furthermore, the absolute and relative paired ovary weights were statistically significantly decreased in adult F1, F2 and F3 females. Information on the BPA effects on pre- and postimplantation was provided by Berger *et al*. (2007, 2008 and 2010). Their results indicated that the decrease in litter size in mice was a result of a disrupted intrauterine blastocyst implantation associated with an alteration of uterine morphology. Therefore RAC was of the opinion that the reported reduced litter size at birth may have been related to a pre-implantational (fertility) effect rather than a post-implantational effect.

In a continuous breeding study in mice (NTP, 1985), a statistically significant decrease compared to control animals was reported in litters/pair, litter size and the number of live pups per litter at 600 and 1200 mg/kg bw/day. Litter size reductions were reported across all matings and they were dose-related. The results from the cross-over matings indicated that the most severe effects on fertility were reported following exposure to F0 females.

In the 2-generation study in mice (Tyl *et al*., 2008), the F0 females were twice more in estrus and the F0 and F1 females had a lengthened gestation period by 0.3 days at 600 mg/kg bw/day as compared to controls. In F0 males, a statistically significant decrease in epididymal sperm concentration was reported at 600 mg/kg bw/day. Furthermore, a statistically significant reduction in testis weight with histopathology including increased incidence of minimal to mild hypoplasia of the seminiferous tubules were reported in F1 and F2 weanlings at 600 mg/kg bw/day. Mild parental toxicity including increased kidney and liver weight as well as reduced body weight gain was reported.

4.2.2 Effects on the female reproductive capacity from supplementary non-guideline studies

Not all relevant studies were included in Table 10 of the CLH report (Summary table of the BPA effects on the fertility in female animals), i.e. several of the studies in Table 8 of the CLH report (Summary table of the BPA effects on the estrous cycle in female animals) were relevant for assessing female reproductive capacity and they were hence included in a new more comprehensive table (Table 1 at the end of this document).

In total, there were 15 studies covering female reproductive capacity, of which 4 guideline studies were considered as the key studies by RAC. One of the 11 remaining studies (Varayoud *et al*., 2008) did not investigate relevant effects on female reproductive capacity, and it was therefore considered of low relevance for this evaluation and it was not included in Table 1. The remaining studies were considered by RAC as relevant and supplementary to the guideline studies. In 5 studies, the animals were exposed orally (Ryan *et al*., 2010; Kwon *et al*., 2000; Kobayashi, 2012; NCTR, 2013; Al-Hiyasat *et al*., 2004), whereas in 4 studies the animals were exposed subcutaneously (Honma *et al*., 2002; Cabaton *et al*., 2010; Varayoud *et al*., 2011; Fernandez *et al*., 2010) and in one study (Berger *et al*., 2007) the animals were exposed both orally and subcutaneously.

Of the oral non-guideline studies, most studies (4 of 6) reported negative findings following doses ranging from 2 µg/ kg bw/day to 320 mg/kg bw/day. One study (Al-Hiyasat *et al*., 2004) reported positive findings with increased number of resorptions, but this study was considered inadequate by the Centre of Evaluation of Human Reproduction (CERHR, 2008) due to small sample sizes. In another study (Berger *et al*., 2007) none of the animals exposed to 68 mg BPA/animal/day in the diet was parturient although, in control animals, 11 of 12 were parturient ($p<0.001$). However, very high doses of BPA were used in this study therefore the study was considered inadequate by CERHR.

Of the 5 subcutaneous studies, 4 reported adverse effects on reproductive capacity (Cabaton *et al*., 2010; Varayoud *et al*., 2011; Fernandez *et al*., 2010; Berger *et al*., 2007).

The study by Cabaton *et al.* (2010) was considered by RAC to be a reliable and important study to understand effects induced by BPA. In the study, BPA purity had been verified by analytical measurements, the estrogenicity in food, cages, water and bedding was determined as negligible, and the choice of experimental design was described (forced breeding with a prolonged breeding (32 weeks) period for the F1 females and a sufficient number of animals giving satisfactory statistical power in order to be able to identify the anticipated effects. The effect on female reproductive capacity was not evident immediately, but it was significantly reduced after 16 weeks of continuous forced breeding. The statistically significant effects increased with time. RAC noted that this study provided unique information, since the standard reproductive experimental protocols did not include such prolonged breeding and therefore they may not have identified such late adverse effects on the female reproductive capacity induced by BPA.

In two of the other subcutaneous studies showing adverse effects on reproductive capacity (Varayoud *et al*., 2011; Fernandez *et al*., 2010), animals were exposed neonatally and significantly higher doses were used than in Cabaton *et al*. (2010). In Varayoud *et al*. (2011), neonatally exposed F1 females were analysed for reproductive capacity at ca 10 weeks of age, and they had a tendency to an increased number of resorptions at both tested doses (0.05 and 20 mg/kg bw/day) and a reduced number of implantations (25%; $p < 0.05$) at the high dose (20 mg/kg bw/day). In the study of Fernandez et al. (2010), female Sprague Dawley rats delivered significantly fewer pups ($p < 0.05$) at doses ranging from 2.5 to 6.2 mg/kg, indicating subfertility and at the highest doses (25 to 62.5 mg/kg) animals were infertile. In the study by Berger *et al*. (2007) a significant decrease in the number of pups born was observed, however the subcutaneous doses of BPA were very high in this study (3.375 and 10.125 mg/animal/day) and the study was therefore considered inadequate by CERHR and by RAC.

In conclusion, the assessment of the supplementary research studies with oral exposure to BPA showed negative results or some effects on female reproductive capacity in studies which had limitations. The subcutaneous supplementary studies supported the existence of an adverse effect on female reproductive capacity. The effects were observed at lower doses in the subcutaneous studies than after oral exposure in the guideline studies which may be explained by

the extensive first pass metabolism having been bypassed when the subcutaneous route of exposure was used.

4.3 Sexual function and fertility - females

4.3.1 Animals

4.3.1.1 Ovarian toxicity

From the available studies, it is clear to RAC that BPA may exert toxic effects on the ovaries, either indirectly via effects on the HPO-axis or as a direct effect on the ovaries. Ovarian toxicity in the form of reduced absolute and relative ovarian weight was reported at the two highest doses (statistically significant at the highest dose) in the rat multi-generation study (Tyl *et al*., 2002) as a consequence of exposure in adulthood only (F0) and in the F1-F3 females exposed also during gestation and post-natally. In the rat NCTR-study with exposure from GD6 to PND80, reduced ovarian weights as well as an increase in ovarian follicular cysts with a high severity profile were observed in the F1 females at the high dose (300 mg/kg bw/day) of BPA in 14 out of 19 animals. No cysts were observed at the lower doses. In addition, a depletion of corpora lutea and antral follicles was observed at the high dose level. Similar ovarian effects were either not observed or not reported in the other two- or multi-generation studies (NTP 1985, Tyl *et al*., 2008 and Ema *et al*., 2001). However, ovarian toxicity was reported in several supplementary subcutaneous exposure studies. Most of the oral non-guideline studies examined effects at low doses (e.g. Yoshida *et al*., 2004, Ryan *et al*., 2010, Kobayashi *et al*., 2012) and a lack of effect was thus not inconsistent with the positive findings of the Tyl *et al*. (2002) and NCTR-study. The only non-guideline oral study that reported ovarian toxicity following high oral doses was the study by Kwon *et al*. (2000) in SD rats exposed from GD11 to PND20.

In the studies using subcutaneous route of administration, reduced ovarian weight, an increase in follicular cysts, and a reduced number of corpora lutea (CL) were findings reported in the majority of the mouse and rat studies (Kato 2003, Adewale *et al*., 2009; Newbold *et al*., 2007, 2009; Signorile *et al*., 2010 ; Fernandez *et al*., 2010). None of the presented subcutaneous studies reported normal ovaries after BPA exposure.

In the studies by Tyl *et al*. (2002 and 2008), the number of primordial follicles was counted and no changes were observed. In contrast, a decrease in the number of primordial follicles was reported in Wistar rats following early postnatal subcutaneous exposure (Rodrigues, 2010). A study by Karavan *et al*. (2012) reported effects on the processes leading to the formation of primordial follicles and follicular development on PND5 following early postnatal subcutaneous BPA-exposure. Furthermore, a series of studies (Hunt *et al*., 2003; Susiarjo *et al*., 2007; Hunt *et al*., 2012) suggested that gestational and adult exposure to BPA may induce disturbances in the meiotic processes leading to more subtle functional defects. However, it was pointed out during the PC that the aneuploidy associated with BPA exposure in the studies by Hunt *et al*. (2003 and 2012) had not been reproduced by a later study, which was not included in the CLH report. Thus, while RAC could not rule out the possibility that BPA had an effect on the number and functionality of the primordial follicles, possibly contributing to a premature ovarian ageing the evidence was not complete.

4.3.1.2 Uterus morphology

Regarding the effects on uterus morphology, the majority of the studies that investigated this endpoint reported hyperplasia of the endometrium or no effects. A few studies reported a thinner endometrium.

In the NCTR study, a dose-dependent increase in cystic endometrial hyperplasia was observed in the uterus at the highest doses of BPA (statistically significant at 300 mg/kg bw/day only). This effect was observed also in other studies (Newbold *et al*., 2007 and 2009; Signorile at al., 2010).

4.3.1.3 Oestrous cycle disturbances

Three of the guideline studies (NTP, 1985, Tyl *et al*. 2002, 2008, Ema *et al*., 2001) did not report any significant effects on the oestrous cyclicity. However, in Tyl *et al*., 2008, a higher percentage of the high-dose females were in oestrus as compared to controls.

Furthermore, in the NCTR (2013) study, in which SD rats were exposed during GD6-PND90, 63% of the animals in the high-dose group had an asynchronous oestrous cyclicity versus 12% in the vehicle control. It was noted by RAC that the control vehicle group was also affected as compared to the naïve controls (0% asynchronous estrous cyclicity). Based on vaginal cytology, disruption of the oestrous cycle at the highest BPA dose was reported on PND69-90 and at the two highest doses on PND150-170 in a similar manner as for the positive control (EE2). The increase of the proportion of animals showing asynchronous estrous cycle on PND150-170 was statistically significant at 100 mg/kg bw/day (n=14), but not at 300 mg/kg bw/day (n=7). Maternal toxicity in this study included a significant reduction in body weight gain (6-13% with an average at 10%) at PND4 and beyond in the two BPA high dose groups. No effect on body weight gain was observed in low dose groups.

Several of the remaining non-guideline studies reported BPA-induced irregularities in the oestrus cycle (Mendoza-Rodríguez *et al*., 2011, Kato *et al*., 2003; Fernandez *et al*., 2009).

In contrast, in the study by Kwon *et al*. (2000), in which SD rats were exposed via oral gavage to 3.2, 32 or 320 mg BPA/kg bw/day between GD11 and PND20, no effects were reported on the oestrous cycle.

RAC concluded that BPA-treated F0 females were twice more in estrus as compared to controls at 600 mg/kg in Tyl et al. (2008), and that BPA induced irregularities in the oestrus cycle also in the NCTR (2013) study and in most of the studies using subcutaneous dosing (Mendoza-Rodríguez 2011, Kato et al. 2003; Fernandez et al. 2009).

4.3.1.4 Onset of puberty

The vaginal opening and the first vaginal oestrus were considered as markers of puberty which was studied in several of the BPA studies. The CLH report showed contradicting results on the effect of BPA on the onset of puberty.

In the key studies, Tyl *et al*. (2002) reported delayed puberty in rats whereas Tyl *et al*. (2008) observed an accelerated puberty onset in mice on PND21 when adjusted to bw, but the finding was not significant when adjusted to body weight at the time of puberty onset. In NCTR (2013), BPA did not significantly affect the onset of puberty, although the positive control (EE2) significantly delayed it. In the study by Tinwell *et al*. (2002), there was a 1.6-day delay in vaginal opening in Alderley-Park rats in the high-dose BPA group whereas no effect was noted in Sprague Dawley rats following the same treatment.

4.3.1.5 Steroidogenesis/serum hormone levels

Serum levels of progesterone, oestrogen and luteinizing hormone (LH) were measured in several of the studies in the CLH report which are discussed below.

In the study by Ema *et al*. (2001), there were significant decreases in serum LH levels at 0.2, 2, and 20 mg/kg bw/d in F0 females. No treatment related changes were observed in the measured serum hormone levels in any other group.

In the NCTR (2013) study, the hormone levels were measured on PND80. Serum oestradiol levels were increased in both 100 and 300 mg/kg bw/d BPA groups, but LH concentration was not affected. Cholesterol level was reduced in the 100 mg/kg bw/d BPA group and progesterone and prolactin levels were reduced in the 300 mg/kg bw/d BPA group.

Cholesterol and prolactin levels of Wistar rats were not affected in the Mendoza-Rodriguez *et al*. (2011) study. In the study of Fernandez *et al*. (2010), an accelerated GnRH pulse frequency in hypothalamic explants from female offspring of Sprague Dawley rats was associated with increased serum testosterone and oestradiol levels and with reduced progesterone levels.

Several studies had been performed in ewes with a focus on hormonal responses. Prenatal treatment of Suffolk ewes with subcutaneous injections of BPA caused an increase in LH concentration and prolonged the first breeding season (Savabieasfahani *et al*., 2006). A study with a long term exposure during the prepubertal period demonstrated that BPA could suppress LH secretion in ovariectomized animals (Evans *et al*., 2004). In mature animals of 4-5 months of age, the mean LH pulse frequency and basal concentrations, but not the amplitude, were slightly decreased from the sixth week of treatment (Collet *et al*., 2010). Collet *et al*. (2010) also investigated the impact of acute BPA exposure by dosing prepubertal female sheep for 54h hours with BPA by IV infusion. At the lower doses, BPA inhibited the LH pulse frequency after a 48-h latency period. Similar qualitative events were observed with the 17-β oestradiol used as positive control. The lowest plasma concentrations of 17-β oestradiol and BPA associated with the inhibition of pulsatile secretion of LH were 2 pg/mL and 38 ng/mL, respectively (Collet *et al*., 2010).

4.3.1.6 Summary on female sexual function and fertility - animals

In the CLH-report it was concluded that there was strong evidence (considered as proven) for the effects on ovarian morphology and disturbances of the HPO-axis. The DS also concluded that the observed oocyte aberrations and the effects on uterus morphology were supported by too few studies. Furthermore, the DS could not form a clear conclusion on BPA effects on puberty onset and on the estrous cyclicity.

The RAC evaluation is based on the studies presented in the CLH report and on the NCTR-study that was introduced during the PC. In addition to the four guideline studies, there were several supplementary oral and subcutaneous studies investigating female reproduction. Among these non-guideline supplementary studies, the new NCTR-study (2013) was of particular relevance for the evaluation of ovarian toxicity.

From the studies presented in the CLH report as well as from the recent NCTR study, it was clear to RAC that BPA may exert toxic effects on the ovaries, either indirectly via effects on the HPO-axis or as a direct effect on the ovaries. Regarding the effects on uterus morphology, the majority of these studies reported hyperplasia of the endometrium or no effects. A few studies reported a thinner endometrium.

The effect of BPA on the onset of puberty seemed to vary according to the experimental design such as exposure period, species, strain and dose. Similar variation in the onset of puberty was also induced by EE2, a positive oestrogenic control included in some of the studies, e.g. the onset of puberty was markedly delayed by EE2 in SD rats in the oral NCTR (2013) study, whereas it was accelerated it in Long Evans rats in the study by Ryan *et al*. (2010) after orally administered EE2.

The guideline multi-generation studies did not report any significant effects on oestrous cyclicity. However, in the NCTR (2013) study and in most of the studies using subcutaneous dosing, BPA induced irregularities in the oestrus cycle (Mendoza-Rodríguez 2011, Kato *et al*. 2003; Fernandez *et al*. 2009). On the other hand, relatively few of the studies referred to oestrus cyclicity.

4.3.2 Humans

According to the review of Cantonwine *et al*. (2013), consistent findings from two prospective cohort studies (Boston and San Francisco cohorts) of reduced peak estradiol (E2) levels and oocyte yield suggested that BPA may alter reproductive function in women undergoing IVF and warranted further examination among larger study populations.

In RAC's view the most relevant results in women came from the prospective cohort study (Boston cohort, n=137) in women receiving IVF treatment (Ehrlich *et al*., 2012). An increased chance of implantation failure correlated with higher urinary BPA concentrations. The results were considered as preliminary by the authors.

No clear picture emerged from the studies on effects during pregnancy. None of the studies with positive associations between urinary/serum BPA and the effects were considered to be very reliable by RAC. Several shortcomings existed such as using an ELISA method that did not enable to discriminate between different forms of BPA, using non-validated methods, poor comparability

of groups, small groups, non-normalisation of urinary BPA, poorly detailed statistical analysis and other confounding factors.

The studies of effects on ovaries also did not allow RAC to make firm conclusions, due to small groups, application of the ELISA method and other shortcomings.

In conclusion, RAC could not identify a clear causal relationship between BPA exposure and the adverse effects on sexual function and fertility from the available studies in women, but an effect of BPA on female fertility could not be totally ruled out.

4.4 Sexual function and fertility – males

Approximately 26 non-guideline supplementary studies related to male sexual function and fertility were referred to in the CLH report.

4.4.1 Animals

The effects of BPA on the male reproductive system following exposure during gestation/lactation, neonatal phase, pre-pubertal phase or adulthood in different strains of mice and rats, with great diversities in doses, durations and routes of exposure (i.e. gavage, diet, sub-cutaneous injection or sub-cutaneous implant) were included in the CLH report. RAC focused on the studies reporting effects on male reproductive system following oral exposure to BPA (Table 2 at the end of this document). However, effects observed in sub-cutaneous studies were also included in order to extend the evaluation of the different effects.

4.4.1.1 Hormone balance

Male exposure to BPA decreased the levels of testosterone. The BPA exposure-mediated decrease in sexual hormone levels was observed in animals exposed *in utero* (Akingbemi *et al*., 2004), during puberty (Della Seta *et al*., 2006; Nakamura *et al*., 2010; Takahaski and Oshi, 2003; Akingbemi *et al*., 2004) and in adult animals (Herath *et al*., 2004). In a recent guideline study (NCTR, 2013), the serum testosterone level first increased from 2.5 to 25 µg/kg bw/day of BPA and then the level decreased from 80 µg/kg bw/day to 300 mg/kg bw/day.

4.4.1.2 Sperm production

Effects on sperm production were observed when animals were exposed prenatally. In the Tinwell *et al*. (2002) study, a statistically significant decrease in total and daily sperm production was observed in Alderley Park rats exposed by gavage to 50 mg/kg bw/day BPA (i.e., the highest BPA dose evaluated) during GD6 – GD21, and in the Salian *et al*. (2009c) study, in which a decrease in sperm count was observed in the F1 male offspring of pregnant Holzman female rats exposed via gavage to 1.2 and 2.4 µg/kg bw of BPA during GD12 – PND21. The sperm count was also significantly decreased in the Salian *et al*. (2009b) study, in which male Holzman rats were exposed subcutaneously to various doses of BPA from PND1 to PND5, and the effects were observed in rats exposed to doses of 100 µg/kg bw/day and higher. In animals exposed to BPA before puberty, a statistically significant decrease in the daily sperm production was observed in Jcl:Wistar rats exposed to 200 mg/kg bw/day for 4 weeks via subcutaneous administration (Takahashi and Oishi, 2003). Reductions in sperm production were also observed in adult rats exposed to BPA for 5 weeks via two different routes of exposure (Chitra *et al*., 2003; Herath *et al*. 2004). In Chitra *et al*. (2003), rats were exposed orally, while in Herath *et al*. (2004), they were exposed via subcutaneous injections. RAC concluded that these studies supported the effects of BPA on sperm parameters reported in the guideline studies (Tyl *et al*., 2002; Tyl *et al*., 2008). In Tyl *et al*. (2002), a decrease in the epididymal sperm concentration and in the daily sperm production was observed at 7500 ppm in F1 and F3 males, respectively.

4.4.1.3 Other parameters

When rats or mice were exposed to BPA, effects on sperm morphology were generally observed (Aikawa *et al*., 2004; Toyama and Yuasa, 2004b; Okada and kai, 2008) with effects on the

weights of the reproductive organs (testis, epididymis, seminal vesicle, prostate), with abnormalities in the seminiferous tubules (Iida *et al*., 2002; Okada and Kai, 2008; Nakamura *et al*., 2010; Toyama and Yuasa, 2004b; Tan *et al*. 2003) or sperm motility (Chitra *et al*., 2003; Salian *et al*., 2009c). These studies supported some of the effects observed in the guideline studies (NTP, 1985b and Tyl *et al*., 2002) and in the recent NCTR (2013) study. In the NTP (1985b) study, a statistically significant decrease in the relative right epididymis weight was observed in all treated groups, testis/epididymis weights were significantly decreased by 10% at the mid-dose and by 9% at the high dose, and seminal vesicle weight was significantly decreased by 28% at the top dose. In the guideline study by Tyl *et al*. (2002), a decreased paired testis weight was observed in F1 male pups at 3500 ppm, and the paired epididymal weight was decreased in F1 parental males at 3500 ppm. In F2 male pups, the seminal vesicle coagulating gland weight was decreased at all doses and statistically significantly at 3500 ppm. In the NCTR (2013) study, a dose dependent decrease in the seminal vesicle weight occurred at BPA doses of 2.7 mg/kg bw/day and higher, and a decrease in the ventral prostate weight (~ 20 %) occurred in the highest dose group, but these changes were not statistically significant.

4.4.1.4 Conclusions on male sexual function and fertility - animals

Around 26 non-guideline supplementary studies were referred to in the CLH report. These studies demonstrated effects of BPA on male reproductive function. These non-guideline studies were original research studies with various shortcomings e.g. small sample sizes, few or single dose groups, non-oral routes of administration and lack of details of the methodologies used, which sometimes limited the usefulness of the findings in them. The differences in the strains, doses, routes of exposure or windows of exposure made a direct comparison between the studies difficult. Despite these limitations, they were considered useful as supplemental information in a weight of evidence analysis. More than 2/3 of the supplemental non-guideline oral route studies included in the CLH report reported effects on male sexual parameters (either on sperm quality, spermatogenesis, sex hormones, or on sexual function). In several studies, the exposure to BPA (with various doses or periods of exposure) led to a decrease in the serum testosterone level, to some effects in reproductive organs, and to a decrease in sperm production. RAC concluded that these findings generally support observations reported in the guideline studies (Tyl *et al*. 2002; Tyl *et al*. 2008; NTP 1985b) and in the recent NCTR (2013) study that was conducted in compliance with FDA GLP.

4.4.2 Humans

4.4.2.1 Effects on male sex hormones

Three studies were presented by the DS (Meeker *et al*., 2010a; Mendiola *et al*., 2010; Galloway *et al*., 2010).

The BPA level in single spot urine samples (n=167) and in repeated (repeated once n=75, repeated twice n=4) urine samples from men recruited through an infertility clinic correlated with altered blood serum hormone levels in the Meeker *et al*. (2010a) study. The FSH level was increased and the ratio estradiol/testosterone was decreased with increasing levels of urine BPA. However, the hormone level findings and associations were not statistically significant. Mendiola *et al*. (2010), found a small but significant inverse association between urinary BPA concentration and Free Androgen Index (FAI) levels and the FAI/LH (lutenizing hormone) ratio, as well as a small but significant positive association between BPA and sex hormone–binding globulin (SHBG) in 375 partners of pregnant women. The authors indicated that environmental levels of BPA may have been associated with a modest reduction in markers of free testosterone, but questioned any clinical significance of this. Galloway *et al*. (2010) reported an association between higher daily urinary excretion (24 h, single day) of BPA and total testosterone concentration in serum among men in a large study (n=715, 533 were 65-74 years old). No associations with serum estradiol or SHBG measures were found in the study.

In RACs view the findings in male sex hormones in these studies may indicate an endocrine effect as a result of exposure to BPA, but this was not confirmed.

4.4.2.2 Effects on sexual function, sperm parameters and offspring

In male workers occupationally exposed to high levels of BPA there was a dose-response relationship between the increasing level of cumulative BPA exposure (work duration x time weighted average for 8 hours (TWA8i) and increased risk of impaired sexual function (self-reported in personal interviews) (Li *et al*., 2010a). The high cumulative exposure estimated was supported by spot urine BPA levels, in sub-groups of exposed and unexposed workers.

In a second paper by Li *et al*. (2010b), which completed the first study, a correlation between BPA exposure (measured in 2 spot urine samples – before and after the shift) and sexual dysfunction was found, supporting the previous findings. However, the result was less significant when workers with previous exposure to other chemicals and heavy metals were excluded.

The Li studies (2010a, b) were described as excellent cohort studies by Rochester (2013), but they were heavily criticized by industry during the public consultation (see section on comments received from Exponent). These studies were considered relevant by RAC because of the high exposure in the occupational settings. However, in RACs view their significance may be questioned.

In the study by Li *et al*. (2011), 218 men provided a semen specimen according to WHO guidelines. A significant association between increasing urinary BPA level and reduced semen quality (sperm concentration and sperm count) was found. In sub-groups of men exposed only via the environment, similar significant associations were observed.

No correlations between sperm parameters and urinary concentrations of BPA were observed among 375 partners of pregnant women. For changes in male sex hormones, see the section above (Mendiola *et al*., 2010). In the study by Meeker *et al*. (2010b), urinary BPA was associated with semen quality, but without statistical significance. A small study (n=27) by Bloom *et al*. (2011a) showed some findings on the association between paternal serum BPA and embryo quality, but the results were considered preliminary by the authors and a causal relationship remains to be confirmed.

A reduction of AGD in sons of male and female workers occupationally exposed to BPA was observed in a study by Miao *et al*. (2011). The effect was statistically significant when the mothers had been exposed during pregnancy. IND had pointed to study design flaws as this study had used the same study population as the studies by Li *et al*. IND also commented that the authors did not take exposure to other compounds into account. RAC agrees with IND on these comments and considers especially that Miao *et al*. should have used the anogenital index (AGI) instead of AGD and controlled for weight as recommended by Swan *et al*. (2008).

Equal levels of fetal exposure to BPA, measured as BPA in cord blood, were found in boys with and without cryptorchidism. The presence of BPA in cord blood indicated that placental transfer and foetal exposure took place in humans (Fenichel *et al*., 2012).

A list of epidemiological studies on fertility not referenced by the DS (references received from IND during the PC, and references reviewed by Rochester, 2013) can be found at the end of this document.

4.5 Conclusions on human information on sexual function and fertility

Multiple statistical analyses had been performed in most of the epidemiological studies on BPA, using a vast range of statistical methods. Some, but not all, of the tested parameters were presented in the papers. The scientific question that initiated the studies was often not described well and the outcome of the studies tended to focus on the associations with the highest statistical significance. Taken together, the studies gave indications that BPA could become systemically available and may have an effect on fertility in men and women. The scientific documentation is regarded by RAC as weak supporting evidence for the classification of reproductive toxicity, and it is therefore not considered robust enough to justify classification as Repr. 1A.

4.6 Mode of Action and human relevance

Unconjugated, aglycone BPA was considered to be the endocrine active form of BPA which acted as a weak oestrogen mimick but with a significantly lower affinity for the nuclear forms of oestrogen receptors ERα and ERβ than oestrogen. However, BPA has more recently been shown to bind with higher affinity to other oestrogen binding receptors, including oestrogen-related receptor (ERR-γ), and membrane-bound forms of oestrogen receptors (mERα, mERβ, and GPER/GPR30ERα). The action of BPA on oestrogen signalling is thus complex and effects are likely to depend on organ and cell type specific interactions as well as on the timing of exposures.

In general, RAC considers that the mode of action for disruption of the reproductive tract may be caused by a direct or an indirect disruption of the HPG-axis, or by a direct organ specific toxicity.

The OECD (2008) guidance document entitled "*Guidance document for histologic evaluation of endocrine and reproductive tests, Morphological patterns of endocrine disruption" (Part 3, Section 5*), reported morphological patterns that may be observed in female rodent reproductive tract following endocrine disruption. This scheme comprised three types of morphological responses, based on the combined histological appearance of the vagina, uterus and ovary:

- Type I atrophic vagina, uterus and ovary
- Type II atrophic ovary with hyperplastic/hypertrophic uterus and vagina
- Type III hyperplastic/hypertrophic ovary, uterus and vagina

Based on all the data on female fertility that was presented in the CLH report and including data from the recent NCTR-study it is clear to RAC that the pattern of effects observed following BPA exposure in the majority of the positive studies fall into category II supporting an overall oestrogen-like effect of BPA, although data on vaginal morphology were scarce.

Effects on the HPO axis:

RAC concluded that exposure to exogenous oestrogens may directly influence the HPO axis due to a negative feedback at the hypothalamic-pituitary level resulting in reduced gonadotropin release and in ovarian atrophy. However, hyperplasia and hypertrophy of the uterus and vagina are expected to be direct effects of oestrogen on these organs.

As described in the CLH report, early BPA exposure during the period of brain sexual differentiation may exert indirect effects on reproductive tract tissue by altering the function of the HPG axis, an effect that would become apparent after puberty. In a study with ovariectomised sheep, BPA suppressed LH secretion and thus this result supported the hypothesis that BPA has direct negative effects on gonadotropin secretion (Evans *et al*., 2004). In some of the studies with exposure of rodents to BPA during gestation and/or early postnatal age, an effect on the pattern of the hypothalamic-pituitary hormones was reported (Fernandez *et al*., 2010; Rubin *et al*., 2001; Navarro *et al*., 2009; Patisaul *et al*., 2009; Brannick *et al*., 2012).

RAC concluded that distinguishing the BPA effects on ovaries to direct effects and to indirect effects (via the effects on the HPO-axis) was not easily made in most of the available studies, and the effects were expected to occur both directly and indirectly. Serum measurements revealed both decreases and increases in serum estradiol (E2) levels as a result of BPA exposure. The variability in responses may have been related to the degree of development of hyperactive ovaries/cysts.

The impairment of sperm production as a result of BPA exposure was accompanied by a decrease in testosterone levels. RAC considered that the effects observed on the testosterone levels might explain the decrease in sperm production.

Conclusion:

BPA was shown to influence the female reproductive tract. The associated alterations in pituitary signalling, serum hormone concentrations and reproductive organ morphology were likely causes of the reduced female fertility effects reported in the NTP (1985b) and Tyl *et al*. (2002) oral multi-generation studies and in several non-guideline research studies. Both oestrogenic and

anti-oestrogenic effects of BPA were described and not all expected oestrogenic effects were observed. However, RAC concluded that the observed pattern of effects on the female reproductive tract suggest an overall oestrogen-like response *in vivo*.

Effects on the male reproductive tract, evident as impaired sperm production following BPA exposure, were observed in several studies. The decrease in sperm production was accompanied by lower testosterone levels. The effects observed on the testosterone levels may be the cause of the decreased sperm production.

RAC concluded that the classification of BPA for adverse effects on sexual function and fertility should be based mainly on the results from rodent studies. Disruption of oestrogenic signalling was considered to be the main mode of action for the effects of BPA on fertility, based on current knowledge. The hormonal systems are well conserved between mammalian species, and the effects observed in rodents are therefore also relevant for humans. Detection of the active form of BPA (aglycone/unconjugated BPA) has been reported in humans (serum, cord blood and in placenta), but the credibility of these low-concentration measurements has been questioned due to the analytical techniques applied and potential contamination of the samples. However, after oral administration of low doses of stable isotope-labelled BPA (to exclude confounding sample contamination) and using sensitive and specific methodology, low systemic concentrations of aglycone/unconjugated BPA have been reported in rodents and in non-human primates, suggesting that unconjugated BPA becomes bioavailable in primates and rodents after oral exposure. Additionally, RAC noted that other routes of exposure such as sublingual, buccal, dermal and inhalation exposure are potential routes of human exposure and they bypass the extensive first-pass hepatic metabolism (first-pass effect).

Taken together, RAC considered the MoA to be relevant to humans.

4.7 General toxicity

In the oral toxicity studies, the effects of BPA exposure on fertility parameters were in general observed at higher doses. As female reproduction was sensitive to stress and to reduced food consumption, a careful evaluation of the pattern of systemic and reproductive effects and their dose response relationships was important in the Weight of Evidence analysis.

In section 3.7.2.2.1.1 of the CLP Guidance, the following is stated with regard to parental toxicity:

> *Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes.*
>
> *There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity. However, mating behavior can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification.*

Following exposure to BPA, parental toxicity was reported as reduced body weight and effects on the liver and kidney. These effects were reported in several of the studies following high dose BPA exposure. RAC considered the effects on liver and kidney as primary effects of BPA and not as secondary effects caused by a reduction in body weight. The following is a short description of the data on general toxicity in the key studies:

NTP (1985): General toxicity was evident in the continuous breeding study as a statistically significant decrease in F0 maternal body weight by 6% - 9% on PND0 after each litter in the high dose group. No effects on maternal body weight on PND0 were reported in the cross-over mating study. No effects on body weight gain were reported in males. At necropsy of the F0 and F1 generation in the high dose group, the relative liver weight was increased by 6-29% and the relative combined kidney/adrenal weight was increased by 10-20% as compared to controls. In females, no histological changes were reported in the reproductive organs or in the oestrus cycle. RAC considers that the general toxicity was not marked and the effects on fertility are therefore not considered to be a consequence of parental toxicity.

Tyl *et al*. (2008): Mild parental toxicity was reported including increased kidney and liver weight as well as reduced body weight gain.

Ema *et al*. (2001): No parental toxicity or effects on fertility was reported in this study investigating only low doses of BPA.

Tyl et a. (2002): General toxicity was reported as a 13% reduction in body weight gain in all exposed generations and as effects in the kidney, evident as renal tubular degeneration in females (not in F3) at 500 mg/kg bw/day. NCTR (2013): Reduced gestational body weight gain in dams was observed at the two highest doses (11% and 16%, respectively), but the pup weight was not affected. In F1 male and female adults, the body weight had decreased (~ 10%) and the absolute or relative liver weight had increased at the highest dose. Renal cysts were increased in both naive and BPA treated animals.

These studies showed that at the oral doses causing reduced fertility and/or reproductive organ toxicity there were also signs of systemic toxicity including slight to moderate reductions in the body weight gain of dams and offspring and an increase in liver weight and renal effects. Based on the CLP guidance on maternal toxicity, the reductions in body weight observed in the BPA studies were not large enough to possibly explain the observed fertility-related effects. This conclusion by RAC was supported by the available data on thymus weights. A reduction in thymus weight was considered as a sensitive marker of high dose stress responses (Haschek and Rousseaux's handbook of toxicologic pathology, third addition 2013, chapter 60), but it was not observed in Tyl *et al*. (2002) or in NCTR (2013), and it was not reported in the CLH report description of the NTP (1985) study. Neither the slight increase in liver weight nor the effects on the kidney were expected by RAC to cause adverse effects on fertility.

The pattern of adverse effects on fertility and the reproductive tract observed in the oral studies on which the classification proposal was based, was supported by findings in a range of studies using subcutaneous administration. Subcutaneously administered BPA was not subject to the first pass effect that was shown to efficiently reduce the bioavailability of the active, aglycone BPA. The doses used in several of these studies were as a consequence quite low. In particular, in the subcutaneous study by Cabaton *et al*. (2010), effects on fertility were seen in response to doses as low as 0.025 and 25 µg/kg bw/day. Furthermore, ovarian toxicity was observed following gestational or postnatal exposures to doses in the µg/kg bw/day range. No signs of systemic toxicity were reported.

RAC concluded after an overall evaluation of the data presented here related to systemic toxicity that effects on fertility observed following exposure to BPA are not secondary non-specific consequences of other toxic effects.

4.8 Comparison with the CLP criteria

According to Annex 1, part 3 of CLP, a substance should be classified in Category 1 for reproductive toxicity when it is known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Repr. 1A: The classification of a substance in Category 1A is largely based on evidence from humans.

Multiple statistical analyses had been performed in most of the epidemiological studies on BPA, using a vast range of statistical methods. Some, but not all, of the tested parameters were presented in the original papers. The scientific question that initiated the studies was often not

described well and the outcome of the studies tended to focus on the associations with highest statistical significance. Taken together, the studies gave indications that BPA could become systemically available and may possibly have an effect on fertility in men and women. The scientific documentation in humans was regarded by RAC as weak supportive evidence for classification for adverse effects on sexual function and fertility, but it was not considered robust enough to justify classification in category 1A.

RAC considered that the human data available for BPA is not sufficient to justify classification of BPA as Repr. 1A for adverse effects on sexual function and fertility.

Repr. 1B: The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an **adverse effect** on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**. However, when **there is mechanistic information that raises doubt about the relevance of the effect for humans**, classification in Category 2 may be more appropriate

Adverse effect on fertility following exposure to BPA was reported in multi-generation studies in rats and mice. In a mouse continuous breeding study, a statistically significant decrease was reported in the number of litters/pair, litter size and the number of live pups per litter at 600 and 1200 mg/kg bw/day. Litter size reductions were reported across all matings and they were dose-related. Results from cross-over mating (i.e. only one of the parents exposed to BPA) indicated that the most severe effects on fertility occurred in female mice following an exposure to BPA. An effect on female reproductive organs evident as ovarian toxicity was supported by several supplementary non-guideline studies. General toxicity in the mouse multi-generation study was not considered as marked and the effects on fertility were therefore not considered to be a consequence of parental toxicity. In a multi-generation study in rats exposed to doses up to 500 mg/kg bw/day of BPA, a statistically significant reduction in the average number of live pups per litter was reported in all generations. The effect was reported without statistically significant effects on post-implantation loss or on the number of dead pups per litter. Furthermore, the absolute and relative paired ovary weights were statistically significantly decreased in F1, F2 and F3 adult females. General toxicity in this study was not considered as marked and the effects on fertility were therefore not considered to be a consequence of parental toxicity.

Further data from the non-guideline supplementary studies were used in a weight of evidence approach. RAC concluded that many of these studies supported the findings in the guideline studies (e.g. effects on female reproductive capacity, on sperm parameters and on male and female reproductive organs) and support the classification of BPA as Repr. 1B for adverse effects on sexual function and fertility.

Effects on female reproductive capacity:

In one of the supplementary studies (Cabaton *et al*., 2011), using a forced breeding design enabling the identification of effects that became apparent with time, a reduction in the cumulative number of pups from F1 females exposed *in utero* to BPA in the highest dose group was observed. This finding was evident in the absence of systemic toxicity in the exposed F0 dams and the F1 generation. The effect on the number of pups was therefore not considered to be a secondary non-specific consequence of other toxic effects. The effect reported in the Cabaton *et al*. (2011) study supported the effects on fertility reported in the NTP (1985) continuous breeding study and in the multi-generation study by Tyl *et al*. (2002). There were also other subcutaneous non-guideline supplementary studies with higher doses but with shorter exposure periods than in the study Cabaton *et al*. (2011), providing some support to the fertility effects in the NTP (1985) and Tyl *et al*. (2002) studies.

Effects on female reproductive organs:

RAC concludes that BPA exerts its toxic effects on the ovaries, either due to direct effects on the ovaries or indirectly via effects on the HPO-axis. Regarding the effects on uterus morphology, the majority of the studies reported hyperplasia of the endometrium or no effects. The effect of BPA on the onset of puberty seemed to vary according to experimental design such as exposure period, species, strain and dose. This variation in the onset of puberty was also seen in animals in the

positive control group, e.g. orally administered 17α-ethinylestradiol (EE2) markedly delayed the onset of puberty in SD rats in the NCTR (2013) study, whereas it accelerated it in Long Evans rats in the study by Ryan *et al*. (2010). The guideline studies did not report any significant effects on the oestrous cyclicity. However, in the NCTR (2013) study and in most of the studies using subcutaneous dosing, BPA induced irregularities in the oestrus cycle (Mendoza-Rodríguez *et al*., 2011, Kato *et al*., 2003; Fernandez *et al*., 2009).

Effects on male reproductive organs:

Several non-guideline supplementary studies included in the CLH report demonstrated effects of BPA on male reproductive function. The original studies had variable shortcomings e.g. small sample sizes, a few or single dose groups, non-oral routes of administration and/or lack of details on the methodologies used, which in some cases limited the usefulness of the findings. RAC notes that differences in strains, doses, routes of exposure or windows of exposure made a direct comparison between the studies sometimes difficult. Despite the limitations in these studies, they were considered acceptable to be used in a weight of evidence approach. More than 2/3 of the supplementary oral route studies included in the CLH report reported effects on male sexual parameters (either on sperm quality, spermatogenesis, sex hormones, or on sexual function). In several studies, exposure to BPA (at various doses or periods of exposure) led to a decrease in the serum testosterone level, to some effects in reproductive organs, and/or to a decrease in sperm production.

RAC concluded that these findings supported the observations reported in the test-guideline studies (Tyl *et al*. 2002; Tyl *et al*. 2008; NTP 1985b) and in the recent GLP-compliant NCTR 2013 study.

Mode of Action: BPA was shown to influence the female reproductive tract. The associated alterations in pituitary signaling, serum hormone concentrations and in reproductive organ morphology were considered by RAC as the likely causes of the reduced female fertility effects reported in the NTP (1985b) and Tyl *et al*. (2002) oral multi-generation studies and in several non-guideline studies. Both oestrogenic and anti-oestrogenic effects of BPA were described, and not all expected oestrogenic effects were observed. However, according to RAC the observed pattern of effects on the female reproductive tract suggested an overall oestrogen-like response *in vivo*.

Effects on the male reproductive tract, evident as an impaired sperm production following BPA exposure, were observed in several studies. The decrease in sperm production was accompanied by lower testosterone levels. RAC noted that the observed effects on the testosterone levels may have been the cause of the decreased sperm production.

RAC concluded that the mode of action was considered to be relevant to humans.

General toxicity: The overall evaluation of the data presented on BPA-related systemic toxicity support the conclusion that the adverse effects on sexual function and fertility observed following the exposure to BPA are not secondary non-specific consequences of other toxic effects.

High dose effects: RAC recognized that the adverse effects on sexual function and fertility were observed in the oral guideline studies only at mid- and/or high-doses. A specific dose as a limit has deliberately not been included in the CLP criteria for reproductive toxicity, even though some guidelines for test methods recommend a specific limit dose. The CLP (3.7.2.5.8.) states that "*In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate*".

RAC concluded that as the adverse effects on sexual function and fertility at the mid and/or high-doses were not co-occurring with marked systemic toxicity in any of the two- or multi-generation guideline studies, they were relevant for classification in accordance with the CLP criteria and the CLP guidance.

Toxicokinetics: In rodents, conjugated and unconjugated BPA were subject to biliary excretion, enterohepatic recirculation and principally to fecal excretion. Humans and other primates were not capable of biliary elimination of BPA-G. They excreted systemically available forms of BPA (conjugated and unconjugated) primarily in urine. Both BPA-G and BPA sulfate represented detoxification pathways of BPA as they were not active on the estrogen receptor, though deconjugation could have occurred at specific tissue sites by glucuronidases and/or sulfatases.

The bioavailability of conjugated BPA was reported to be similarly low in rodents and primates after oral exposure to low doses of BPA due to an extensive first-pass metabolism. RAC concluded that the data from experimental oral animal studies were relevant to humans and for the classification of BPA for adverse effects on sexual function and fertility. Also other routes of exposure, such as sublingual, buccal, dermal and inhalation route, were potential routes of human exposure and they bypassed the extensive first-pass hepatic metabolism (first-pass effect). Subcutaneously administered BPA was not subject to the first pass effect that was shown to efficiently reduce the bioavailability of the active, aglycone BPA. The doses used in several of these studies were as a consequence relatively low. The subcutaneous route of exposure did not relate to a potential route of human exposure, but these studies provided supplementary information in the WoE evaluation as it was expected by RAC that reproductive organs were not exposed to unrealistically high levels of the active aglycone BPA as no signs systemic toxicity were reported in these studies.

Repr. 2: *CLP Annex 1 part 3: Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.*

The animal data provided clear evidence of adverse effects on sexual function and fertility and they were not considered to be secondary non-specific consequences of other toxic effects. The mode of action of BPA indicated that the effects were relevant to humans, supported by some findings in humans. RAC concluded therefore that the criteria for Repr. 2 for adverse effects on sexual function and fertility were not appropriate in the case of BPA.

4.9 RAC Conclusion on classification and labelling

In conclusion, RAC agrees with the Dossier Submitter that the findings represent clear evidence of an adverse effect on sexual function and fertility, which is not considered to be secondary non-specific concequence of other toxic effects. Therefore, RAC concludes that the available data on BPA support classification of BPA as Repr. 1B; H360F.

TABLE 1. FEMALE ANIMAL STUDIES ON ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

Species (n)	Routes	Dose Exposure Period Experimental details	Effects	References	Evaluation according to CERHR (2008)
			Gestational exposure		
Mouse ICR Jcl 10 females/dose	Subcutaneous	2, 20 µg/kg bw/d GD11- GD17	- No effect on the total number of pups per F2-litter or F2 sex ratio - No data on the GLP/OECD guideline compliance	Honma *et al*., 2002	adequate for inclusion but of limited utility
			Perinatal exposure		
Rat Long Evans 13 - 29 females / dose in block 1 6 - 14 females / dose in block 2	**Oral (gavage)**	2 – 20 - 200 µg/kg bw/day GD7 – PND18	No effect in any parameters observed (F0 and F1 weight, anogenital distance, age at puberty, reproductive tract morphology, fertility, fecundity, or sexual dimorphic behaviors) - No effect on the total number of pups per mother - No data on the GLP/OECD guideline compliance	Ryan *et al*., 2010	
Mouse CD-1 18-20 females /dose	Subcutaneous implant	0.025, 0.25, 25 µg/kg bw/d based of dam at GD6 Purity of BPA analysed GD8 – PND16	- Reduced fertility: ↘ the number of pregnancies at 25µg/kg (p = 0.024) - Reduced fecundity: ↘ of the number of pups born per litter at 0.25 and 25µg/kg - No data on the GLP/OECD guideline compliance	Cabaton *et al*., 2010	
Rat Sprague-Dawley 8 females/dose	**Oral (gavage)**	3.2, 32 or 320 mg/kg/d (n = 8/dose) GD11 – PND20	<u>Observations in F1 females:</u> No effect on : - the number of live pups per litter and body weights of live pups on PND1or 7. - the age at vaginal opening and the age at	Kwon *et al*., 2000	Adequate

			first estrus. - the estrous cycle - the lordosis behavior - the volume of the SDN-POA - the morphology of ovaries and uteri. No data on the GLP/OECD guideline compliance		
Rats Sprague-Dawley 10 females/dose	**Oral (diet)**	0-33 ppm (0-~5mg/kg/d) GD6-PND21	<u>Observations in F1 females:</u> -no sign effects on live pups/litter -ovarian weights slightly increased at 5 weeks, no effect at 3 month -slight, non-significant decrease in E2 and increase in progesteron -slight shorthening of AGD at the 5 week time point; no effect at 3 month	Kobayashi 2012	
			Gestational to post-pubertal exposure		
Rats Sprague-Dawley 18-23 females/dose	**Oral (gavage)**	2.5, 8, 25, 80, 260, 840, 2,700, 100,000, and 300,000 µg/kg bw/day GD6-PND90, observation at PND??	No effects on number of live pups born, the number of dead pups born, resorptions, litter body weight, sex ratio Number of implantation sites measured in the uteri of the F0 daMSCA was not affected by treatment Increased cystic follicles, depleted corpora lutea and antral follicles at highest dose level Reduced ovarian weight at two highest doses (sign at highest) Disruption of the estrous cycle at 2 highest doses (PND69-90: high dose; PND 150-170: slight effects and sign at 100 mg dose) No effect on timing of puberty as measured by VO and time to first oestrus Serum hormonal levels at PND80: increased	NCTR 2013	

			oestradiol and prolactin, decreased progesterone at two highest doses. Reduced leptin at highest dose. Increased TSH at 2 highest doses. Hyperplastic and cyctic endometrium at highest dose No effect on AGD Systemic toxicity: 100,000 and 300,000 µg BPA/kg bw/day doses depressed gestational body weight gain in daMSCA. 300,000 µg BPA/kg bw/day dose depress pre- and post-wean body weight gain in pups in both sexes, Increased liver weight, doses??. Increased renal cysts in naive and BPA treated animals.doses??		
			Postnatal exposure		
Rat Wistar BPA at 0.05mg/kg: n=17 BPA at 20 mg/kg: n=20	Subcutaneous	0.05 and 20 mg/kg/day Purity>=99% Neonatal exposure. PND1, 3, 5 and 7	<u>Observations in adult females (80-d old)</u> ↘ in the number of implantation sites on day 18 of pregnancy at 20 mg/kg ($p < 0.05$) ↗ of the number of resorptions site on day 18 of pregnancy at both doses ↘ in the ERα and PR mRNA levels on day 5 of pregnancy at both doses ($p < 0.05$) ↘ in Hoxa10 uterine expression on day 5 of pregnancy at both doses ($p < 0.05$) No effect on the number of Corpora Lutea and on E2 and P serum levels No data on GLP/OECD guideline compliance	Varayoud *et al*., 2011	
Rat Sprague Dawley No data on # animals/ dose	Subcutaneous	Ranging from 0.25 to 62.5 mg/kg bw/day Neonatal exposure. PND1- PND10	- Infertility at doses ranging from 25 to 62.5 mg - Subfertility at doses ranging from 2.5 to 6.2 mg - No effect at doses ranging from 0.25 to 0.62 mg	Fernandez *et al*., 2010	

			- No data on GLP/OECD guideline compliance		
			Adult exposure		
Mice Swiss 10 females/dose mated	**Oral (gavage)**	5, 25 or 100 µg/kg bw/day 28 days from 60 days of age	<u>Observations in unmated females (n=5):</u> Body weights were decreased at all dose levels. Increased uterine weight in the mid-and high-dose Ovarian weight was increased in mice of the high-dose group <u>Observations in mated females (n=10):</u> ↗ in the number of resorptions out of the total number of implantations at 25 ($p < 0.01$) and 100 µg/kg ($p < 0.05$) ↗ in the number of animals with resorptions at all doses No effect on the number of pregnancies, implantations or in the number of viable foetuses No data on the GLP/OECD guideline compliance	Al-Hiyasat *et al*., 2004	Inadequate due to small sample sizes
Mice CF-1 No data on the nb of animals / dose	Sc and **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	The dose of 68.84 mg of BPA/day/animal (corresponding to a BPA supplementation at 6%) causes the abortion of all gestations. If one assume that each mouse weighs approx. 30 g, the doses are 1147,33 and 2294,67 mg/kg bw/day No modification of litter size, percentage of pups surviving after birth, or in sex ratio of pups No data on the GLP/OECD guideline compliance	Berger *et al.*, 2007	Inadequate
			Multigenerational exposure		

Rat **IGS (SD) rats** 25 rats /sex /group administered	**Oral (gavage)**	0, 0.2, 2, 20 and 200 µg/kg/day 2 generation study similar to OECD 416 (Deviations: *Female treated for 2 weeks only before mating. *Low doses used)	No effect on behaviour (i.e. performance in learning tests), oestrus cycle, fertility index and the number of implantations in F0 and F1 females were not affected by treatment with BPA. Absolute AGD decreased but no more relevant when correlated with BW (decreased). - Overall no effect.	Ema *et al*., 2001	
Rat Sprague-Dawley 30 males/ dose 30 females/dose	**Oral**	0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/day Exposure from 10 weeks before mating until PND21 (3 generations)	The absolute age at puberty (evaluated by the age at vaginal patency) was delayed in the F2 generation at 50mg/kg and in the F1, F2 and F3 generations at 500 mg/kg. **Reduced fecundity i.e. number of total and live pups per litter at birth and on PND4 at 500 mg/kg for F1, F2 and F3 ($p < 0.001$).** 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) **No effect on** estrous cycle length, paired ovarian primordial follicle counts, reproductive organs histology, mating, **fertility**, pregnancy, dead pups per litter or percent post-implantation loss. According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl *et al*., 2002	
Mice CD-1 N=28 animals/dose	**Oral**	0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/day Exposure from 8 weeks before mating until PND21	No effect on the absolute age at puberty at any dose (evaluated by the age at vaginal patency) at any dose. Vaginal patency was accelerated when adjusted for the PND21 body weight at	Tyl *et al*., 2008	

		(2 generations)	600mg/kg. F0 treated females were twice more in estrous as compared to controls at 600 mg/kg. ↗ the length of the gestation by 0.3 days at 600mg/kg ↘ the body weight of the pups during lactation at 600mg/kg **No effect on** 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.** Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study		
Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex)	**Oral** **(diet)**	0, 0.25, 0.5 or 1.0% (daily intakes BPA estimated 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg for males or females respectively. Continuous breeding study: (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.)	**Adverse effect on fertility**: statistically significant ↘/ ctrl in the number of litters produced / 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 = f (dose-related). No effects on fertility were observed in the low-dose group. A statistically significant ↘ 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	NTP, 1985b	

			cross-over mating. In the continuous breeding phase, a statistically significant ↘ 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 ↘ in body weight gain (4%) was only observed in treated females at study termination. No effect on the sex ratio in the F1 generation. Possibility that there may be potential effects on pups due to exposure to BPA via the milk. In the F1 generation, BPA treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or mean pup weights at birth.		

TABLE 2. MALE ANIMAL STUDIES ON ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

Method: Species/Routes/Dose/Exposure Period	Effects	Reference	Evaluation *according to CERHR (2008)
Exposure during gestation/lactation			
Sprague Dawley and Alderley park (derived from Wistar) Rats Oral route 20 µg/kg, 100 µg/kg bw, 50 mg/kg GD6 - GD21	Observations made in adults (90 days) : Significant ↘ sperm production at 50 mg/kg only in AP rats	Tinwell *et al.*, 2002	*Adequate
ddY mice oral route by gavage 0, 1, 10 and 100 mg/kg bw/day GD10 - GD17	Observations made at 60 and 120 days (adults): Histological abnormalities in seminiferous tubules	Iida *et al*., 2002	*Inadequate
Crj: CD (SD) IGS strain Rats Oral route 4 - 40 and 400 mg/kg bw/d GD6 - PND20	Observations made at PND63 and PND252: significant ↗ of the plasma testosterone concentration at 9 weeks only, with no alterations of LH or FSH concentrations from 4mg/kg bw/d and onward.	Watanabe *et al.*, 2003	NA?
ICR mice Oral route 5 or 10 µg BPA/mL in drinking water throughout embryonic/fetal life and during lactation	Observations made in 4-weeks old pups: ↗ thiobarbituric acid-reactive substances in testis ↘ wet weight of testis	Kabuto *et al.*, 2004	*Inadequate
Long-evans female rats Oral route – gavage 0 – 2.4 µg/kg bw/day GD12-PND21 (Exp. 2)	Observations made at 90-day old: ↘ testis and seminal vesicles weight. Unchanged prostate weight. ↘ specific Leydig cells testosterone production	Akingbemi *et al.*, 2004	*Inadequate
CF-1 mice Oral route 0 – 10µg/ kg bw/d GD14-18	Abnormal growth of the prostate since primitive prostate gland duct epithelial proliferation was found at birth	Timms *et al*., 2005	*Adequate
Holtzman Rats Oral route 1.2 – 2.4 µg/kg bw/d GD12 - PND21	**F1, F2, F3** 1.2 et 2.4µg/kg bw/d : ↘ litter size significant ↗ post-implantation loss at both doses in F3. ↗ bodyweight (except F1 for 2.4µg/kg bw/d) ↘ sperm count and sperm motility at both doses. ↗ copulation delay. ↘ expression profile of testicular ER β□e↘ expression profile testicular AR (except for F2 and F3 à 2.4µg/kg bw/d).	Salian *et al.*, 2009c	NA
Pre-/Pubertal exposure			

Long-evans weanling male rats Oral route – gavage 0 – 2.4 µg/kg bw/day PND21-35 (Exp. 1)	<u>Observations made at PND35:</u> Decrease in the serum LH and T levels	Akingbemi *et al.*, 2004	*Adequate
Sprague Dawley rats Oral -gavage 40 µg/kg bw/d PND23 - PND30	<u>Observations made at pubertal and adult age</u>: ↘ Testosterone levels in juveniles, lasting until adult age. Decrease of sexual performances in adult animals.	Della Seta *et al.*, 2006	*Adequate
Sprague Dawley rats Oral route – gavage 100 mg/kg bw/d PND23-53	<u>Observations made at PND53:</u> Only 66.7% of the treated rats reached a complete preputial separation. No significant effects were seen on the testis, epididymis or adrenal weight but morphological changes or differences in testicular histology.	Tan *et al*., 2003	*Adequate
Long-Evans weanling male rats Oral route – gavage 0 – 2.4 µg/kg bw/day PND21-90 (Exp. 3)	<u>Observations made at 91days:</u> No effect on the body weight or the testis weight. Decrease of the sex hormone levels, specifically the T levels produced by the Leydig cells.	Akingbemi *et al.*, 2004	*Adequate
Juvenile male Sprague-Dawley rats, PND 22, Oral route – gavage. 3 weeks of treatment (until PND 43); 100 mg/kg bw	The results of the present study showed that, soya extract, BPA, and 17β-estradiol can alter the histological structure of the testes and influence circulating steroidal hormone levels.	Norazit *et al*., 2012	NA
Exposure during adulthood			
Wistar Rat Oral route 0,2 - 2 - 20 µg/kg bw/d PND45 – PND90	<u>Observations at adult age</u>: Significant ↘ relative weights of testis and epididymis Significant ↗ of the relative weight of the ventral prostate. Significant ↘ epididymal sperm motility and sperm count. Effects on levels of enzymes related to oxydative stress.	Chitra *et al.*, 2003	*Adequate
Sprague-Dawley Rats Oral route 0.02 – 0.2 - 2 – 20-200 mg/kg bw/d Exposure from Day 6 to adult age (11 weeks)	No significant effect on the sperm production.	Sakaue *et al.*, 2001	*Adequate
NCTR 2013 study			
Rats Sprague-Dawley 18-23 pregnant females/dose Oral (gavage) 2.5, 8, 25, 80, 260, 840, 2.700, 100.000, and 300.000 µg/kg bw/day GD 6 – PND 90	Testicular descent was delayed compared to control (23.6 ± 0.2 days) at 260 µg/kg bw/day (24.7 ± 0.2) and 300,000 µg/kg bw/day (25.7 ± 0.4). Increased incidence of seminiferous tubule giant cells at a single dose (2.5 µg/kg bw/day). Increased T3 level at PND15 and decrease cholesterol level at PND90 following an exposure to 100mg BPA/kg bw/day.	NTCR, 2013	

	Trend: first increased serum testosterone level from 2.5 to 25 µg/kg bw/day compared to the vehicle control: then decreased serum testosterone level from 80 µg/kg bw/day to 300 mg/kg. A decrease in the weight of the epidydimal fat pad for both high doses (only significant for the 300 mg BPA group); A dose dependent decrease of the seminal vesicles weight from 2.7 mg BPA onward (not statistically significant). A decrease in the ventral prostate weight (~ 20 %) occurred in the highest dose group (not statistically significant).		
Multi-generations exposure			
Mice Oral route 0 – 0.015 – 0.3 – 4.5 – 75 – 750 – 7500 ppm corresponding 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 Exposure from 10 weeks before mating until adult age.	No effect on reproduction has been seen except at the highest dose (7500 ppm): Effect on reproductive organ weights, on DSP and epididymal sperm concentration.	Tyl *et al.*, 2008	
Rat Oral route 0 – 0.018 – 0.18 – 1.8 – 30 – 300 and 3500 ppm Exposure from 10 weeks before mating until adult age.	No effect on reproduction has been seen except at the highest dose (3500 ppm):	Tyl *et al.*, 2002	
Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex) 0, 0.25, 0.5 or 1.0% (daily intakes BPA estimated 0, 300 or 325, 600 or 650 and 1,200 or 1300 mg/kg for males or females resp. In diet	Relative seminal vesicle weight and proportion of motile sperm ↘19% and 39% / ctrls. No histological changes were observed in male reproductive organs. Left testis/epididymis weights were significantly ↘ by 10% at the mid dose and 9% at the high dose, and seminal vesicle weight was significantly ↘ by 28% at the top dose. Continuous breeding study (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.)	NTP, 1985b	

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ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).