Proposal for harmonised classification and labelling :

Bisphenol A

We welcome the FRENCH proposal on the BPA classification.

The Dossier Submitter proposed a classification Rep. 1B-H360 F based on animals and human evidences. The classification of a substance in the Category 1B-H360 "*…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…*"The hazard statement H360F "May damage fertility" is assigned "*a substance classified in Rep. Cat. 1A/1B on the basis of* ***fertility effects*** *and effects on* ***developmental toxicity*** *can be* ***excluded*** *according to* ***reliable and adequate data***."

The CLH proposal sums up various studies in animals and also epidemiological studies without giving a clear argumentation, based on a weight of evidence approach, to justify this upgrading in the classification. Some findings can justify discussion for a possible upgrading of the current classification We think that we need to reconsider all the data available, the three good quality studies: the multi-generation studies in the rat and the continuous breeding study in the mouse, and adding the new information, considering the quality of the different studies. Here below, some studies chosen that could be considered as relevant for the classification (but there are also quite limited).

For the effects observed in male, Lida et al. (2002) observed histological abnormalities in seminiferous tubules in males mice exposed in utero. However, information is lacking regarding the number of animals affected, the link between the dose and the effects, the age of the animals affected (60 or 120 days?). Here below, the table with the sum up of the mentioned studies. Two studies were also conducted on adult males. Chitra et al (2003) demonstrated a statistically significant dose-dependant decrease of the testes weight in rats by approximately 6% ($p < 0.05$) and of the epididymides weight by 12-25% ($p < 0.05$). A very important increase of the ventral prostate weight (by 12-30%) and a decrease of the epididymal sperm motility and sperm count in a dose-dependent manner ($p < 0.05$) were also observed. Sakaue et al (2001) demonstrated a statistically decrease in daily sperm production in males exposed during 6 days. (!!!The DS should revise the table on p 102 regarding this study because some inconsistencies are observed: 1) the table indicate an exposure of 11 weeks while 6 days of exposure are reported in the text on p 90 , 2) the conclusion in table p.102 states "no significant effects on the sperm production" while significant daily sperm production decrease is reported) . We need to compare these effects with the effects observed in mice by Tyl et al (2008).

Exposure during gestation		
ddY mice oral route by gavage	Observations made at 60 and 120 days (adults): **Histological abnormalities in seminiferous**	Iida et al. 2002

0, 1, 10 and 100 mg/kg bw/day GD10 - GD17	**tubules**	
Adult exposure		
Wistar rats Oral gavage 0.2, 2, 20 µg/kg bw/d PND45-90	Observation at adult age : Significant decrease relative weights of testis and epididymis Significant increase of the relative weight of the ventral prostate Significant decrease of epididymal sperm motility and sperm count Effects on levels of enzymes related to oxidative stress	Chitra et al., 2003
13-weeks old Sprague-Dawley rats Oral gavage Exp.1: 0, 0.02, 0.2, 2, 20, 200 mg/kg bw/d Exp.2: 0, 0.002, 0.02, 0.2, 2, 200, 2000 µg/kg bw/d 6 days exposure Observation at PNW14 & PNW18	From 0.02 mg/kg: - Statistically decrease in daily sperm production - Statistically decrease in daily sperm production per gram testis (efficiency of spermatogenesis)	Sakaue et al (2001)

Multi-generations exposure		
Mice Oral route in diet 0 – 0.018 – 0.18 – 1.8 – 30 – 300 - 3500 ppm corresponding to 0, 0.003, 0.03, 0.3, 5, 50 or 600 mg/kg bw/day Exposure from 8 weeks before mating until adult age. N = 28 animals/dose	**No effect on reproduction** has been seen at any dose **except at the highest dose** where only a slightly increased gestional length was observed (3500 ppm): A statistically significant decrease on epididymal sperm concentration was observed at this dose in F0 but was not considered as treatment-related by the authors, based on no effect in the F1 generation, no effects for any other andrological endpoint, and lack of any effects on male fertility. At this highest dose, testicular findings (delayed descent, decreased weight and histopathology) were also observed in F1/F2 but were considered as transient effects from systemic toxicity as these effects were not observed on adult testis structure or function (F1 retained male). Follow OECD guideline 416 (two generation reproduction toxicity study), TG 416 enhanced GLP compliant study	Tyl et al. (2008)

Other studies indicate no effects on the male reproductive toxicity.

Exposure during gestation		
C57/BL6 mice Oral gavage 0 – 50 – 1000 µg/kg GD10-16	Observations made at PND56: No significant changes in male body weight in F1 exposed males. No differences detected in seminiferous tubules diameters. No effect on serum testosterone level. No changes in sperm production observed in adult testis after exposure to BPA.	LaRocca *et al.*, 2011
Neonatal exposure		
Sprague-Dawley male rats Sub-cutaneous injections 0 – 2 – 11 – 56 – 277 – 97,000 µg BPA/kg bw/day Birth – PND9	Observations made at PND10, 35 and 150: Normal reproductive parameters.	Kato *et al*. 2006

This difference of findings can be explained by the different way of exposure, the different strains or the different species, the quality of the studies, the different doses,…and can not be compared. We would strongly recommend the DS to explain those differences in the CLH proposal in order to constitute a supportive evidence for male reproductive toxicity.

The female studies were less convincing due to the lack of data: some studies lack data on the number of animals tested and in a lot of them, there are no control. This is difficult to assess the findings of such study. Tyl et al (2002) reported a decrease in the absolute and relative organ paired ovary weights observed in F1, F2 and F3 offspring and adults which is statistically significant but this was observed only at 7500 ppm, a dose where systemic toxicity was also observed. Ema et al. (2001) carried out a study in rat similar to OECD 416 and they concluded that there were no effects at low doses.

Rat **IGS (SD) rats** 25 rats /sex /group administered 0, 0.2, 2, 20 and 200 µg/kg/day Oral by gavage 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

Tyl et al. (2008) carried out a study in mice (similar to OECD 416) where no effects were reported except the increase of the length of the gestation by 0,3 days at the highest dose but the DS did not provide if the findings are statistically significant and dose related which could be an good evidence for female toxicity. A decrease in pups BW is also reported at the highest dose during lactation. We highlight that this effect could be considered as a developmental effect or effect via lactation.

Mice CD-1 N=28 animals/dose Exposure from 8 weeks before mating until PND21 (2 generations) 0.003, 0.03, 0.3, 5, 50 or 600 mg/kg/d Oral	No effect on the absolute age at puberty at any dose (evaluated by the age at vaginal patency).Vaginal patency was accelerated when adjusted for the PND21 body weight at 600mg/kg. F0 treated females were twice more in estrus 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	Tyl *et al*., 2008

Two other studies reported effects that can be considered as **developmental toxicity if observed with no maternal toxicity.** In mice (NTP 1985) and in rat (Tyl et al. 2002), a reduced in the number of live pups/litter were observed. We draw the attention that those effects are reported in the NTP study as effect on fertility but we considered as developmental toxicity. Besides, the NTP study concluded *on the possibility of potential effects on pups due to exposure to BPA via the milk.*

Mice CD-1 (n= 20/ treated group/ sex, n= 40/ control group/ sex) Continuous breeding study (Task 1, dose-finding; Task 2, continuous breeding phase; Task 3, identification of the affected sex, and Task 4, offspring assessment.) 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	Adverse effect on fertility (developmental effects?) : statistically significant ↘/ ctrl in the number of litters produced / pair (4.5 and 4.7 compared to 5.0 for ctrls), 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 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.	NTP, 1985b

Rat Sprague-Dawley 30 males/ dose 30 females/dose Exposure from 10 weeks before mating until PND21 (3 generations) 0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/d Oral	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. 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. However, at 500 mg/kg reduced number of total and live pups per litter at birth and on PND4 for F1, F2 and F3 ($p < 0.001$) . According to EPA OPPTS 837.38000, 1998 GLP compliant study	Tyl *et al*., 2002

All in all, we acknowledge that some effects can be considered as relevant for the reproductive toxicity but we question the interpretation of the DS on the clear evidence fulfilling the criteria for Rep. 1**B.** Studies present limitations : a clear description of the dose related effects, the SS effects, the way of exposure Oral(µg/kg/d), Subcutaneous, intra-peritoneally, implantation, intramuscular, IV infusion, how to interpretate when no OECD guideline, (different results due to different strains?species?way of exposure?), no control (in female studies) , Number of animals tested (too low, sometimes no data),the windows of observation (a delay in development could be missed if observed too late for example), the windows of exposure for the GD (p 57-58: GD 30-60, GD 6 to lactation period), the dosages not so clear (P61: 2 **to** 200 ug/kg bw/ day?), no info on maternal toxicity.

We considered also that the epidemiological studies are not so strong enough to constitute a weight of evidence: some studies are contradictory in females (sometimes the controls had more BPA than the women with an endometrial carcinoma, no significant association between urinary BPA levels and the stage of endometriosis,…). In males, we question the subjects observed (their age, analysis of sperm (only questionnaire),…). We therefore have a strong reservation for classification Rep. 1A where the classification is ***largely based on evidence from humans***.

We strongly recommend the DS to improve their justification for the clear evidence observed in animals studies to support Rep. 1B for fertility. In our opinion, the development should be assessed regarding available data. Did the DS assess the potential developmental effects after in utero exposure like effects on brain and/or sex difference in behavior?

Editorial comments:

We also recommend to the DS to reword the text in 4.1.5 on the toxicokinetic of BPA during gestation and in fetuses, because it is misleading. In the ex vivo study (Balakrishnan et al), it is effectively shown that BPA crosses the placental barrier but this study can not be used to conclude that the transfer across the human placenta is mainly in active unconjugated form because only free BPA was perfused. Moreover, studies in rodents show that the major part of the BPA in the foetal compartment is the conjugated form (ANSES report, p.77). A second remark concerns the first reference cited in this paragraph. (Doerge et al, 2010). Only Doerge et al, 2010a should be cited for the lactational transfer. It would be great to add a reference for the placental transfer.

In § 1.4.6 on toxicokinetic of BPA for newborns, the difference between the rat and the monkey is just cited by the phrase “it does not seem to be the same in monkeys.” It would be nice to give a little more explanation by saying that “In monkeys, even the youngest animal tested showed a highly developed capacity in the gut and liver for first-pass Phase II metabolism of BPA.”

We were also surprised to see on page 30 that “Sheep is a good model for examining this critical issue related to reproduction because their ovarian cycle, steroid, and GnRH secretion patterns are very similar to that of women” Could we have a short explanation for this?In the tables, when BPA is given in the diet, it should be better to specify it or to give the corresponding doses in ppm for the clarity (i.e. in the studies of Tyl et al, 2002 and 2008). We found also errors in the table reporting these two studies on page 102 and 103. In the 2-generation study on mice (Tyl et al, 2008), BPA was administered at doses ranging from 0.018 to 3500 ppm (corresponding to 0.03 to 600 mg/kg bw) and in the 3-generation study on rats (Tyl et al, 2002), BPA was administered at doses ranging from 0.015 ppm to 7500 ppm (corresponding to 0.01 to 500 mg/kg). Please correct these two entries.

Moreover, there are two tables 8, two tables 9 and two tables 10.

In the second table 8 (page 54), it is said that F0 treated females (Tyl et al, 2008) were twice more in estrus as compared to controls at 600 mg/kg. It was not reported in the paper. Where is this information coming from?