ANNEX D: OVERVIEW OF RELEVANT STUDIES FOR CLASSIFICATION OF BISPHENOL A

BPA is not a selective reproductive toxicant as indicated by the following guideline studies that cover a very wide dose range, from very low up to high doses. As the studies show, effects on animal fertility only occur at high doses of BPA and are a consequence of systemic toxicity Criteria for classification of BPA as a Category 1B Reproductive Toxicant are not met.

Rat Studies showed no selective reproductive or developmental effects:

Key study:

Tyl et al. 2002: Three Generation Reproductive Toxicity Study

SD Rat, GLP, according OECD 416 with additional assessments
(e.g. histopathological examination of all groups, andrological assessment in all males)

30 rats/group/sex received BPA in the diet at levels of 0, 0.015; 0.30; 4.5; 75; 750; 7500 ppm over 3 generations = 0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/day

Adult systemic toxicity at 500 mg/kg bw: reduced bodyweight gain (reduction of body weight up to 25%), female renal and hepatic pathology

Adult systemic toxicity at 50 mg/kg bw: reduced body weight gain (reduction of body weight up to 12 %), reduced liver and kidney weights in males

No effect on mating and fertility

Reproductive toxicity: at 500 mg/kg bw reduced litter size and reduced ovarian weights

Offspring toxicity: at 500 mg/kg bw reduced offspring body weight and organ weights, delayed preputial separation and delayed vaginal patency due to reduced body weights.

NOAEL adult systemic 5 mg/kg bw, NOAEL reproductive and postnatal 50 mg/kg

Supporting studies:

Stump et al. 2010: Developmental neurotoxicity study in rats

SD Rat, GLP, OECD 426 and U.S. EPA guidelines for the study of developmental neurotoxicity

24 rats/group/sex received dietary concentrations of 0, 0.15, 1.5, 75, 750, and 2250 ppm daily from gestation day (GD) 0 through lactation day (LD) 21 = ca. 0,01, 0,1, 5, 50 and 150 mg/kg bw/day

Adult systemic toxicity at 50 and 150 mg/kg bw/day: reduced body weight gain and reduced food consumption

No effect on reproductive parameters (litter size, number of pups born alive, postnatal survival) and on developmental landmarks (age at preputial separation and vaginal patency), reduced gestational body weight gain (F0); offspring body weight gain reduced PND 7-14 only

Remark: There was no evidence that BPA is a developmental neurotoxicant in rats and the NOAEL for developmental neurotoxicity was 2250 ppm, the highest dose tested

NCTR 2013: Subchronic toxicity study with exposure from gestation day 6 through postnatal day 90

SD Rat, GLP, subchronic toxicity study in male and female rats exposed orally from gestation day 6 through postnatal day 90 (dose-finding study for subsequent chronic study; concurrent two reference estrogen groups (EE2) as well as vehicle and naïve control groups)

28-36 rats/group received dietary concentrations of 0, 0.0025, 0.008, 0.025, 0.26, 0.84, 2.7, 100 or 300 mg/kg bw/day daily from gestation day (GD) 6 through postnatal day 90 (target litter number 20 per dose group)

Systemic toxicity at 100 and 300 mg/kg bw/day: reduced body weight gain during gestation (-16% and -10%) and in offspring

No effect on reproductive parameters (gestation lengths, implants, resorptions, litter size, number of pups born alive, sperm parameters in offspring)

With 100 and 300 mg/kg bw/day some evidence of organ toxicity and effects on estrus cycle as well as reduced prewean survival at 300 mg/kg bw/day

NOAEL systemic 2.7 mg/kg bw/day, LOAEL systemic 100 mg/kg (remark: large spacing between NOAEL and LOAEL), no specific reproductive effects

Mouse studies showed no selective reproductive or developmental effects:

Key study:

Tyl et al. 2008: Two Generation Reproductive Toxicity Study

CD Mouse, GLP, according OECD TG 416 with additional assessments (e.g. additional histopathological, two negative controls, concurrent positive control (E2)); the study was conducted as part of the European Union (EU) Risk Assessment,in cooperation and with oversight of a Bisphenol A Steering Group (a group of expert reproductive/developmental toxicologists from EU member countries).

28 mice/group/sex received BPA in the diet at levels of 0, 0.018, 0.18, 1.8, 30, 300, and 3500 ppm = 0, 0.003, 0.03, 0.3, 5, 50, 600 mg/kg body weight

Adult systemic toxicity at 600 mg/kg bw: reduced bodyweight gain (reduction of body weight up to 25%), increased liver and kidney weights, renal and hepatic pathology

Adult systemic toxicity at 50 mg/kg bw: centrilobular hepatocyte hypertrophy

No effect on mating and fertility, ovarian primordial follicle counts, estrus cyclicity and sperm parameters. Slight delay in gestation lengths at 600 mg/kg bw/day (19.3 vs. 19.0 days for the control groups); however, the toxicological significance of this minor difference, if any, is unknown.

Offspring toxicity: at 600 mg/kg bw slight reduction in weanling body weights and delay of preputial separation and testes descent

NOAEL adult systemic 5 mg/kg, NOAEL reproductive and postnatal 50 mg/kg bw

Supporting studies:

NTP 1985: early Continuous Breeding Study

Test was done under NTP test development and validation process: evaluation and refinement of the continuous breeding protocol 1984

Standard Protocol: 4 Tasks (Task 1 = range finding test, Task 2 = continuous breeding phase F0, Task 3 = Cross-over mating phase F0, Task 4 = offspring assessment and breeding F1)

20-40 mice/group/sex received BPA in the diet at levels of 0; 2500, 5000 or 10 000 ppm = 0, 300, 600 or 1200 mg/kg bw/day (according to RAR) or 438, 875 or 1750 mg/kg bw/day (according to NTP study report)

Reduced litter size and number of live pups per litter with 600 and 1200 mg/kg bw in the F0 generation (or 875 and 1750 mg/kg according to NTP-report)

No effect on litter size and live pups per litter in the F1 generation

Ample evidence of systemic toxicity in the maternal/paternal animals with 600 mg/kg bw and 1200 mg/kg bw (or 875 and 1750 mg/kg according to NTP-report)

Tyl 2002: abbreviated One-Generation Study

This study was performed in 2001 to address the uncertainties related to parental toxicity in the Mouse Continuous Breeding Study during discussion of classification in Europa

Maternal toxicity was assessed on the day of delivery after dietary application of 5000 or 10,000 ppm of BPA during 2 weeks of prebreeding, mating and gestation and effects on reproduction and offspring were evaluated:

20 mice/group/sex received BPA in the diet at levels of 0; 5000 or 10 000 ppm = 0, 750-950 or 1400 – 1800 mg/kg bw/day

Adult systemic toxicity: decrease of body weight gain during pregnancy (both dose groups, > 10% in the 10 000 ppm group), increase of kidney and liver weight in both dose groups, increase of Blood Urea Nitrogen (10 000 ppm group). Histopathology liver and kidney: hepatocyte hypertrophy, renal tubular degeneration and regeneration in both dose groups

No effect on fertility indices and implantation, reduced litter size in the 10 000 ppm group (11.6 vs. 13.7)

The study showed in general a similar pattern of results as the Mouse Continuous Breeding Study (NTP 1985), but provides broader and more detailed information on maternal toxicity.

- Dose-dependent maternal toxicity was clearly demonstrated in both dose groups (5000 or 10 000 ppm)
- The study confirmed that effects on reproduction parameters in mice were observed only at doses of significant toxicity, whereas mild parental toxicity led to no effects on reproduction.

Comprehensive studies show no low-dose / non-monotonic dose responses

The studies mentioned above cover low- and high doses (with the exception of the Continuous Breeding Study and the abbreviated One-Generation Study). All treatment-related effects exhibited monotonic dose response curves, with no evidence of effects at low doses. Due to the discussion in the context of the “low dose hypothesis” comprehensive studies sponsored by independent government agencies investigated doses below the overall No Adverse Effect Level for general systemic toxicity (5 mg/kg bw/day, oral dosing) and concluded that large, robust studies of BPA do not show low-dose effects and do not exhibit non-monotonic dose response curves.

- **Ema (2001)** sponsored by the National Institute of Health Sciences, Osaka, Japan: The authors completed a two generation guideline developmental study in rats and concluded that: *"The data indicate that oral doses of BPA of between 0.2 and 200 µg/kg over 2 generations did not cause significant compound related changes in reproductive or developmental parameters in rats."*

- **Kobayashi (2010, 2012)** sponsored by the National Institute of Occupational Safety and Health, Kawasaki, Japan: In the first study Kobayashi (2010) examined the impact of dietery exposure in utero and during lactataion to mice and concluded that *"These findings indicate that dietary exposure to bisphenol A between 0.33 and 33 ppm does not adversely affect reproduction or development as assessed in two generations of mice."*
- The second study (2012) was specifically designed to examine whethere lowdose effects would be seen in rats after dietary exposure to BPA to dams from gestational day 6 to lactational day 21. The authors concluded that *"These outcomes indicate that low-dose exposure to BPA in the diet does not adversely affect reproductive development in F1 rat offspring".*

- **Howdeshell (2008) and Ryan (2010)** sponsored by the United States Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, North Carolina, USA: Howdeshell examined the impact of BPA exposure to male rat offspring and found a *"lack of nonmonotonic dose response for the endpoints evaluated in the male offspring reported here."*

- In a similar experiment **Ryan (2010)** found no effect from BPA on female rat offspring, and conclude that *"The lack of effect of BPA on female and male rat offspring after oral exposure to low doses in our studies is consistent with …(Cagen et al., 1999; Ema et al., 2001; Tinwell et al., 2002; Tyl et al., 2002)."*

Overall summary

- Guideline generation studies of high regulatory relevance are available in rats and mice that cover a wide range of doses, from the very low up to high doses.
- NOAEL for systemic toxicity 5 mg/kg bw/day in rats and mice; LOAEL >= 50 mg/kg bw/day.
- In rats no adverse effects on oestrus cycle, fertility, litter sizes, pre- and post-natal survival, growth and development at doses at doses up to ca. 200 mg/kg bw/day. With 500 mg/kg bw/day reduced litter size (at systemic toxic dose) in the Three Generation Reproduction Study.
- In mice no adverse effects on fertility, litter sizes, pre- and post-natal survival at doses up to ca. 600 mg/kg bw/day. With 600 mg/kg bw/day delayed offspring development in the Two Generation Reproduction Study.

Therefore:

- **Reduction of litter size or delay in development at toxic doses only (>= 500 mg/kg bw/day)**
- **BPA is not a selective reproductive toxicant**
- **Recent valid and regulatory relevant data are in line with the overall assessment re classification from 2002; there is no need to change the existing classification**

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