ANNEX C: "NCTR EVALUATION OF THE TOXICITY OF BISPHENOL A (BPA) IN MALE AND FEMALE SPRAGUE-DAWLEY RATS EXPOSED ORALLY FROM GESTATION DAY 6 THROUGH POSTNATAL DAY 90"

A study entitled "NCTR Evaluation of the toxicity of Bisphenol A (BPA) in Male and Female Sprague-Dawley Rats Exposed Orally from Gestation Day 6 through Postnatal Day 90" was conducted by the US National Center for Toxicological Research (NCTR); Jefferson, AR; Study director Barry Delcos. The following information is based on the Technical Report of this study (Report Number: 2176.01; dated March 4, 2013) and became recently available.

Here in brief the most important aspects of relevance for the CLH proposal:

BPA was administered by oral gavage from gestation day 6 through the start of labor and then directly to pups from postnatal day (PND) 1 (day of birth = PND 0) until termination at PND 90 ± 5 to Sprague-Dawley rats from the NCTR breeding colony (Sprague-Dawley/CD23/NCTR BR). BPA doses were 2.5, 8, 25, 80, 260, 840, 2,700, 100,000, and 300,000 µg/kg body weight (bw)/day.

Vehicle (0.3% carboxymethylcellulose) and naïve control groups were included to assess any effects of the gavage procedure on the endpoints measured. Two doses (0.5 and 5.0 µg/kg bw/day) of the synthetic estrogenic substance ethinyl estradiol (EE2) were also included.

The litter was the unit of statistical analysis and the target litter number was 20 per dose group (actual n = 18 – 23). Rats were fed soy- and alfalfa-free diet and caged in polysulfone cages with hardwood chip bedding, glass water bottles with food-grade silicone stoppers. Prior to mating female rats were randomized to treatment groups stratified by body weight to give approximately equivalent mean body weights in each group. During mating females were examined daily for presence of an *in situ* vaginal plug or sperm-positive smear.

Data collected included body weights, weekly feed consumption, litter parameters, anogenital distances at PND 1 and PND 90, measures of sexual development (vaginal opening and time to first estrus for females; nipple retention, testicular descent, and preputial separation for males), vaginal cytology, clinical chemistry, organ weights, and histopathology. Vaginal cytologies were collected from PND 69 until termination in animals utilized for histopathology; the cytology data were used to attempt to terminate the females in estrus. A subset of females was also removed from dosing at PND 90 ± 5 and assessed for cyclicity from PND 150 to PND 170. For clinical chemistry, blood was taken from subsets of pups at PND 15, PND 80 and at terminal necropsy on PND 90 ± 5. Sperm motility, testicular spermatid head count, caudal epididymal sperm count, and sperm morphology were assessed at terminal necropsy. Organ weights collected at terminal necropsy included adrenals, brain, dorsolateral and ventral prostate, epididymides, heart, kidneys, liver, ovaries, pituitary (after fixation), seminal vesicles with coagulating gland, spleen, testes, thymus, thyroid (after fixation), uterus, epididymal, ovarian and parametrial (combined), and retroperitoneal fat pads. Organs evaluated microscopically included adrenals, aorta (thoracic), bone marrow (femur), brain, right epididymis, heart, kidneys, liver, 5th left mammary gland (inguinal) from both sexes, ovaries, oviduct, pancreas, pituitary, prostate (dorsolateral and ventral), seminal vesicles with coagulating gland, spleen, right testis, thymus, thyroid, uterus, and vagina.

BPA in the dose region between 2.5 and 2,700 µg/kg bw/day did not exhibit clear adverse effects in either sex under the conditions of this study. BPA exhibited adverse effects in females at 100,000 and 300,000 µg/kg bw/day and in males at 300,000 µg/kgbw/day. Both the 100,000 and 300,000 µg BPA/kg bw/day doses statistically depressed gestational body weight gain by 11% and 16%, respectively. The reported summary tables are shown as back up information at the end of this document.

This NCTR study measured parameters mentioned in the CLH proposal in chapters “Conclusion on male reproductive system in animals” on page 97-98 and/or chapters 4.11.5 “Summary and/or discussion of reproductive toxicity” and/or 4.11.6 “Comparison with [CLP] criteria”. Only those endpoints mentioned in the CLH proposal are discussed in detail in the following comments. Additional information is available as back up information at the end of this document.

Males:

Sperm production or quality

At necropsy on PND 90 ± 5, the left testis and epididymis were removed and used for determination of testicular spermatid head counts, cauda sperm counts, percent sperm motility, and sperm morphology evaluation. No dose of BPA significantly affected any measured sperm parameter.

Testes weight and histology

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). There were no differences in body weights at the time of testicular descent in these groups.

At necropsy on PND 90 ± 5 tests weight was not significantly affected by BPA. Increased incidence of seminiferous tubule giant cells was reported at a single dose (2.5 µg/kg bw/day).

Testosterone concentrations (not mentioned in the chapter conclusion on male reproductive system on page 97-98 but in chapter summary and discussion of reproductive toxicity on page 112)

Male serum hormone data from PND 80 ± 3 serum samples indicated no significant treatment related differences between any treatment group and vehicle control for the measured hormones FSH, LH, testosterone, and prolactin.

Prostate and epididymis weight and histology (not mentioned in the chapter conclusion on male reproductive system on page 97-98 but in chapter summary and discussion of reproductive toxicity on page 112)

At necropsy on PND 90 ± 5 prostate and epididymis weight and histopathology was not significantly affected by BPA.

Females:

Ovarian cysts:

At necropsy on PND 90 ± 5 organ weights are affected in females only at the highest BPA dose tested (300,000µg/kg bw/day): decrease: ovary, fat pad (retroperitoneal and ovarian parametrial); increase: liver.

Table 44. Female histopathology, PND 90 ± 5: vehicle and EE_2 dose groups[a]

	0.3 % CMC (Vehicle)	0.5 EE_2 (µg/kg bwday)	5.0 EE_2 (µg/kg bwday)
Ovary			
Follicle, Cyst	***[b]	***	***
Incidence[c]	4/20	16/19	19/20
Severity profile[d]	0/4/0/0	0/2/4/10	1/1/5/12
Corpus luteum, depletion	***	***	***
Incidence	0/20	17/19	18/20
Severity profile	0/0/0/0	0/5/5/7	0/0/1/17
Antral follicles, depletion	***	***	***
Incidence	3/20	16/19	19/20
Severity profile	N/A	N/A	N/A

High dose BPA			
	BPA Dose (µg/kg body weight/day)		
	Vehicle	100, 000	300,000
Ovary			
Cyst	***[b]	-	***
Incidence[c]	4/20	0/21	14/19
Severity profile[d]	0/4/0/0	0/0/0/0	1/5/4/4
Corpus luteum, depletion	***	-	***
Incidence	0/20	1/21	14/19
Severity profile	0/0/0/0	0/1/0/0	3/6/4/1
Antral follicles, depletion	**	-	*
Incidence	3/20	2/21	10/19
Severity profile	N/A	N/A	N/A

Estrus cycle:
The protocol defined an abnormal cycle as a cycle with 3 or more days of estrus (E) or 4 or more days of diestrus (D). The presence of many intermediate or transition stage cells (P/E, E/D, and D/P, where "P" indicates proestrus) were reported in this study, and according to the study report necessitated the use of broader definitions of abnormal. In addition, because runs of 4 consecutive D smears were common, an alternate definition of abnormal that required at least 5 consecutive days of D was evaluated. Regardless of which definition of abnormal was used in the report, between 20 and 35% of vehicle control animals showed at least one abnormal cycle over the evaluation period, and there was no significant difference between naïve and vehicle controls. The majority of the abnormal cycles were due to extended D. Low BPA doses had no effect on the percentage of animals showing abnormal cycles (Table 11). Similarly, 100,000 µg BPA/kg bw/day did not affect the proportion of animals showing abnormal cycles, while 300,000 µg BPA/kg bw/day significantly increased the proportion of animals showing abnormal cycles in a manner similar to that of EE2.

Table 45. Aberrant estrous cyclicity based on the morphology of the ovary, uterus, and vagina as a functional reproductive unit[a]

Condition	Treatment												
	Control		EE2 (µg/kg bw/day)		BPA (µg/kg bw/day)								
	Naïve	Vehicle	0.5	5.0	2.5	8	25	80	260	840	2,700	100,000	300,000
Asynchrony[b]	0/18 (0%)	2/17 (12%)	16/17[c] (94%)	19/19[c] (100%)	1/21 (5%)	0/18 (0%)	0/20 (0%)	0/18[d] (0%)	0/18[c] (0%)	0/17 (0%)	1/17 (6%)	1/20 (5%)	12/19 (63%)

Advanced age of puberty:
No dose of BPA affected the time of vaginal opening or the body weight at which the landmark was achieved. Time to first estrus was not affected by BPA.

Decline in reproductive capacity:
The number of implantation sites measured in the uteri of the F0 dams was not affected by treatment. Other endpoints evaluated for the litters included number of live pups born (total, male, female, and unsexed), the number of dead pups born, resorptions (calculated from the number of implants minus the number of pups born), litter body weight (total and by sex), sex ratio, and anogenital distance (AGD) and AGD index (AGDI, AGD divided by the cube root of body weight). No dose of BPA these endpoints.

Overall, the conclusions drawn in the CLH proposal **on male and female fertility endpoints are not supported by data derived in this comprehensive NCTR study:**

- No effects were reported on **male** sperm production, serum testosterone concentration, reproductive organ weight or histopathology (testes, prostate or epididymis). The absence of findings on serum testosterone levels in this robust study challenges the reported findings such as in section 4.11.2.1.1 as well as the use of such data in section 4.11.5 as mechanistic support of various reproductive and developmental effects. Testicular descent was delayed compared to control at 260 µg/kg bw/day and 300,000 µg/kg bw/day and there were no differences in body weights at the time of testicular descent in these groups.

- In **females** there were no effects reported on time of vaginal opening, time to first estrus or any litter parameter. Effects on the estrus cycle, ovary weight and histology were reported only at the highest BPA dose investigated, 300 mg/kg/day. Systemic toxicity expressed as significantly reduced gestational body weight gain was reported at 100 mg/kg/day, a dose were none of the above mentioned observations were reported, and 300 mg/kg/day.

Relevance of the NCTR study for classification and labeling: in conclusion, this comprehensive study supports that BPA is not a selective reproductive toxicant and that a re-classification of BPA is unwarranted.

Back up information

NCTR E2176.01 Technical Report (Amendment #2, 7/11/2013)

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Gestational Weight Gain	-	-	-	-	-	-	-	-	↓	↓	↓	↓
Anogenital Distance/Anogenital Distance Index, Males	↑/↑	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/↑	-/-	-/-
Prewean Body Weight, Males	-	-	-	-	-	-	-	-	-	↓	-	-
Prewean Body Weight, Females	-	-	-	-	-	-	-	-	-	↓	-	-
Prewean Survival, Males	↓	-	-	-	-	-	-	-	-	↓	-	-
Prewean Survival, Females	-	-	-	-	-	-	-	-	-	↓	-	-
T3, PND 15 (Only Males Evaluated)	-	-	-	-	-	-	-	-	↑	↑	-	-
TSH, PND 15 (Only Males Evaluated)	-	-	-	-	-	-	-	-	-	↓	-	-
Postwean Body Weight, Males	-	-	-	-	-	-	-	-	-	↓	-	-
Postwean Body Weight, Females	-	-	-	-	-	-	-	-	-	↓	-	↑
Vaginal Opening, Age	-	-	-	-	-	-	-	-	-	-	↑	↑

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Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Vaginal Opening, Body Weight	-	-	-	-	-	-	-	-	-	-	↑	↑
Time to First Estrus	-	-	-	-	-	-	-	-	-	-	-	↑
Testicular Descent, Age	-	-	-	-	-	↑	-	-	-	↑	-	↑
Testicular Descent, Body Weight	-	-	-	-	-	-	-	-	-	-	↑	↑
Preputial Separation, Age	-	-	-	-	-	-	-	-	-	-	-	↑
Preputial Separation, Body Weight	-	-	-	-	-	-	-	-	-	-	-	↑
Abnormal Estrous Cycles, PND 69 - 90	-	-	-	-	-	-	-	-	-	↑	↑	↑
Abnormal Estrous Cycles, PND 150 - 170	-	-	-	-	-	-	-	-	↑	-	↑	↑
PND 80, Clinical Chemistry, Females												
Estradiol	-	-	-	-	-	-	-	-	↑	↑	↑	↑
Progesterone	-	-	-	-	-	-	-	-	-	↓	↓	↓
Prolactin	-	-	-	-	-	-	-	-	-	↑	↑	↑

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
PND 90 Organ Weights, Females												
Adrenal	-	-	-	-	-	-	-	-	-	-	↑	↑
Fat pad, ovarian parametrial	-	-	-	-	-	-	-	-	-	↓	↓	↓
Fat pad, retroperitoneal	-	-	-	-	-	-	-	-	-	↓	↓	↓
Heart	↑	-	-	-	-	-	-	-	-	-	↑	↑
Kidney	-	-	-	-	-	-	-	-	-	-	↑	↑
Liver	-	-	-	-	-	-	-	-	-	↑	↑	↑
Ovary	-	-	-	-	-	-	-	-	-	↓	↓	↓
Pituitary	-	-	-	-	-	-	-	-	-	-	↑	-
Spleen	-	-	-	-	-	-	-	-	↓	-	-	↑
Uterus	-	-	-	-	-	-	-	-	-	-	-	↓
PND 90 Organ Weights, Males												
Adrenal	-	-	-	-	-	-	-	-	-	-	-	↑
Epididymis	-	-	-	-	-	-	-	-	-	-	-	↓

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Fat pad, epididymal	-	-	-	-	-	-	-	-	-	-	-	↓
Heart	-	-	-	-	-	-	-	↓	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-	-	↑
Pituitary	-	-	-	-	-	-	-	-	-	-	-	↑
Prostate, dorsolateral	-	-	-	-	-	-	-	-	-	-	-	↓
Prostate, ventral	-	-	-	-	-	-	-	-	-	-	-	↓
Seminal vesicles with coagulating gland	-	-	-	-	-	-	-	-	-	-	-	↓
Spleen	-	-	-	-	-	-	-	-	-	-	-	↑
Testis	-	-	-	-	-	-	-	-	-	-	-	↓
PND 90, Clinical Chemistry, Females												
Alkaline phosphatase	-	-	-	-	-	-	-	-	-	-	-	↑
Alanine transferase	-	-	-	-	-	-	-	-	-	-	-	↑
Aspartate aminotransferase	-	-	-	-	-	-	-	↑	-	-	-	-

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Blood urea nitrogen	-	-	-	-	-	-	-	-	-	-	-	↑
Cholesterol	-	-	-	-	-	-	-	-	↓	-	↑	↓
Leptin	-	-	-	-	-	-	-	-	-	↓	-	-
Sorbitol dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	↑
T4	-	-	-	-	-	-	-	-	-	-	↓	-
TSH	-	-	-	-	-	-	-	-	↑	↑	↑	↑
Total protein	-	-	-	-	-	-	-	-	-	-	↑	-
Triglycerides	-	-	-	-	-	-	-	-	↓	-	-	-
PND 90, Clinical Chemistry, Males												
Cholesterol	-	-	-	-	-	-	-	-	↓	↓	-	↓
Creatinine	-	-	-	-	-	-	-	-	-	↑	-	↑
Glucose	-	-	-	-	-	-	-	-	↓	-	-	-
Leptin	-	-	-	-	-	-	-	-	-	↓	-	-

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
T3	-	-	-	-	-	-	-	-	-	-	-	↑
T4	-	-	-	-	-	-	-	-	-	↑	-	-
Total bile acids	-	-	-	-	-	-	-	-	-	↓	-	-
Cauda sperm count	-	-	-	-	-	-	-	-	-	-	-	↓
Histopathology, PND 21												
Female Mammary Gland												
Duct, hyperplasia	-	-	-	-	-	-	-	↑	↑	-	-	-
Male Mammary Gland												
Duct, hyperplasia	-	-	-	-	-	-	-	-	-	-	↑	↑
Histopathology, PND 90, Females												
Ovary												
Follicle, cyst	-	-	-	-	-	-	-	-	-	↑	↑	↑
Corpus luteum, depletion	-	-	-	-	-	-	-	-	-	↑	↑	↑
Antral follicles, depletion	-	-	-	-	-	-	-	-	-	↑	↑	↑

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Uterus												
Endometrium, hyperplasia, cystic	-	-	-	-	-	-	-	-	-	↑	-	-
Mammary Gland												
Duct, hyperplasia	-	-	-	-	-	-	-	-	-	↑	-	-
Kidney												
Cyst[b]	↑	-	-	↑	↑	↑	↑	↑	↑	↑	↑	-
Renal tubule mineralization	-	-	-	-	↑	-	-	-	-	-	-	↑
Nephropathy	-	-	-	↑	-	-	-	↑	-	-	-	↑
Thyroid												
Cyst	-	-	-	-	-	-	-	-	-	-	↑	-
Histopathology, PND 90, Males												
Mammary Gland												
Duct, hyperplasia	-	-	-	-	-	-	-	-	-	-	↑	↑
Alveolus, hyperplasia	-	-	-	-	-	-	-	-	-	-	↑	↑

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Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Testis												
Germinal epithelium, degeneration	-	-	-	-	-	-	-	-	-	-	-	↑
Seminiferous tubule, giant cell	-	↑	-	-	-	-	-	-	-	-	-	-
Epididymis												
Hypospermia	-	-	-	-	-	-	-	-	-	-	-	↑
Exfoliated germ cells	-	-	-	-	-	-	-	-	-	-	-	↑
Coagulating gland												
Epithelium, hyperplasia	-	-	-	-	-	-	-	-	-	-	-	↑
Kidney												
Renal tubule dilatation	-	-	-	-	-	-	-	-	-	-	-	↑
Renal tubule mineralization	-	-	-	-	-	-	-	-	-	-	-	↑
Cyst	-	-	-	-	-	-	-	-	-	↑	-	-
Nephropathy	-	-	-	-	-	-	-	-	↑	-	-	-

Table S-1. Summary of Treatment Effects Relative to Vehicle Controls[a]

Endpoint		BPA (µg/kg bw/day)									EE_2 (µg/kg bw/day)	
	Naïve	2.5	8	25	80	260	840	2,700	100,000	300,000	0.5	5.0
Pancreas												
Acinus, degeneration	↑	-	-	-	-	-	-	-	-	-	-	-
Infiltration, cellular	↑	-	-	-	-	-	-	-	-	-	-	-

[a] Endpoints affected by any treatment are summarized, with the exception of food consumption and metabolic efficiency. Food consumption and metabolic efficiency data are presented in the body of the report. An up arrow (↑) indicates a statistically significant increase or delay in the treatment group relative to the vehicle control, a down arrow (↓) indicates a statistically significant decrease or acceleration in the treatment group relative to the vehicle control, and a dash (-) indicates no statistically significant increase, decrease, delay, or acceleration in the treatment group relative to the vehicle control. Details of the specific statistical analyses used for each group of endpoints are described in the body of the report. As discussed in the report, pairwise comparisons were conducted and adjusted for multiple comparisons in the following subgroups: 1) naïve versus vehicle; 2) low dose BPA (2.5 – 2,700 µg/kg bw/day) versus vehicle; 3) high dose BPA (100,000 and 300,000 µg/kg bw/day) versus vehicle; and 4) EE_2 dose groups versus vehicle. For organ weights, results are shown for the organ weight adjusted for body weight. Results for absolute organ weight and organ weight adjusted for brain weight are presented in the body of the report. For histopathology, the results of the Poly-3 tests are shown.

[b] The low incidence of background kidney cysts in the vehicle control confounded the interpretation of this endpoint. The incidences of cysts (number of affected animals/total examined) in all dose groups were as follows: vehicle, 1/20; naïve, 6/20; 2.5 µg BPA/kg bw/day, 5/23; 8 µg BPA/kg bw/day, 5/18; 25 µg BPA/kg bw/day, 8/21; 80 µg BPA/kg bw/day, 6/20; 260 µg BPA/kg bw/day, 6/21; 840 µg BPA/kg bw/day, 5/20; 2,700 µg BPA/kg bw/day, 6/20; 100,000 µg BPA/kg bw/day, 8/21; 300,000 µg BPA/kg bw/day, 12/19; 0.5 µg EE_2/kg bw/day, 7/20; 5.0 µg EE_2/kg bw/day, 2/20.