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The Developmental Toxicity of Bisphenol A in Rats and Mice[1]

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The Developmental Toxicity of Bisphenol A in Rats and Mice. MORRISSEY, R. E., GEORGE, J. D., PRICE, C. J., TYL, R. W., MARR, M. C., AND KIMMEL, C. A. (1987). *Fundam. Appl. Toxicol.* 8, 571–582. Bisphenol A (BPA) was evaluated for developmental toxicity in CD rats (0, 160, 320, or 640 mg/kg/day) and CD-1 mice (0, 500, 750, 1000, or 1250 mg/kg/day) dosed daily by gastric intubation on Gestational Days 6 through 15. Timed-pregnant dams were sacrificed 1 day prior to parturition, the uterine contents were examined, and all fetuses were examined for external, visceral, and skeletal malformations. In rats, maternal weight gain during gestation, weight gain corrected for gravid uterine weight, and weight gain during treatment were significantly reduced at all BPA doses. Gravid uterine weight and average fetal body weight per litter were not affected by BPA. No increase in percentage resorptions per litter or percentage fetuses malformed per litter was detected. In mice, maternal mortality occurred at all BPA doses, reaching 18% at the high dose, which also produced a significant decrease in maternal body weight gain during gestation and treatment. Weight gain corrected for gravid uterine weight was not affected by BPA. Reductions in gravid uterine weight and average fetal body weight were observed with the 1250 mg/kg dose of BPA. Relative maternal liver weight was increased at all doses of BPA. There was a significant increase in the percentage of resorptions per litter with 1250 mg BPA/kg/day. Malformation incidence was not altered by BPA. Thus, BPA treatment at maternally toxic dose levels during organogenesis produced fetal toxicity in mice but not in rats and did not alter fetal morphologic development in either species.

Bisphenol A (BPA) is a monomer used in the manufacture of epoxy, polycarbonate, and corrosion-resistant unsaturated polyester–styrene resins used in interior coatings of cans and drums, reinforced pipes, adhesives, flooring, water main filters, artificial teeth, nail polish, and food packaging materials (Knaak and Sullivan, 1966; Patents, 1974, 1975, and 1978; Chemical and Engineering news, 1979; Kirk-Othmer, 1978, 1979). Estimated average annual U.S. production of BPA for 1980 was 530 million pounds (USITC, 1980) and has been projected to increase to meet an estimated annual demand of 770 million pounds.

Most human exposure to BPA occurs in industry during the manufacture of resins (Fregert, 1981). Hardened BPA-containing resins are nonallergenic. However, when cured at room temperature, 5 to 25% of the resin remains unhardened for months. Contact with the unhardened resins can cause contact dermatitis (Romaguera *et al.*, 1981; Pegum, 1979; Fregert, 1981), consisting of redness and edema with weeping, followed by crusting and scaling usually confined to the

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area of contact. Since the face is frequently affected, this may indicate that exposure can occur by inhalation. Absorption through the skin has resulted in extensive damage to kidneys, liver, spleen, pancreas, and lungs (Sax, 1975). NIOSH (1979) estimates that approximately 200,000 individuals are exposed to BPA during resin manufacture or formulation.

Exposure to unhardened epoxy resins in the nonworking environment occurs mainly from coated household objects and hobby glues (Fregert, 1981; Fregert *et al.*, 1980). Unhardened epoxy resin oligomers containing BPA have been detected on twist-off covers, film cassettes, metal packages, and door knobs (Fregert *et al.*, 1979, 1980). The amount of unhardened resin is sufficient to elicit dermatitis in already sensitized individuals (Fregert, 1981). In addition, unreacted BPA can migrate from resins used in food packaging to food surfaces (Knaak and Sullivan, 1966).

When administered as a single dose by gavage to male CFE rats, 28% of the ^{14}C-labeled BPA was excreted in the urine (primarily as the glucuronide) and 56% in the feces (35% as free bisphenol A, 35% as hydroxylated bisphenol A, and the remaining portion as an unidentified conjugate) over the 8-day observation period (Knaak and Sullivan, 1966). No carbon-labeled residues were detected in the animals killed 8 days after exposure to the compound.

Due to widespread occupational and consumer exposure to BPA, additional toxicology and carcinogenesis studies were undertaken by the National Toxicology Program (NTP, 1982; Huff, 1984). In Fischer 344 rats, the single oral LD50 was 4.1 g/kg (males) and 3.3 g/kg (females). Oral LD50 values for bisphenol A from previous investigations were 4.24 g/kg for rats and 2.5 g/kg for mice (AIHA, 1967; NIOSH, 1978). For $B6C3F_1$ mice, the LD50 was 5.2 g/kg (males) and 4.1 g/kg (females). In NTP 2-year studies, BPA was not considered carcinogenic to either species (NTP, 1982; Huff, 1984). BPA administered in feed (0.25, 0.5, or 1.0%) over an 18-week period in a fertility assessment by continuous breeding mouse study caused a decrease in mean number of litters per pair and mean number of live pups per litter in the 0.5 and 1.0% treatment groups (Reel *et al.*, 1985). A crossover mating trial between the control and 1.0% groups provided evidence that reproductive processes of both male and female mice were adversely affected. BPA has also been screened for teratogenic potential (Hardin *et al.*, 1981) following intraperitoneal injection of BPA in corn oil into pregnant Sprague–Dawley rats on Gestational Days 1 through 15 at doses of 85 and 125 mg/kg. The high dose significantly impaired the establishment of pregnancy, while both doses caused a significant reduction in the number of live fetuses per litter. There was a significant reduction in fetal body weight and crown–rump length. There was an increased number of fetuses with imperforate anus (125 mg/kg), incomplete ossification (85 and 125 mg/kg), and enlarged cerebral ventricles (125 mg/kg). Because of the small number of litters available in the treated groups ($n = 3$ or 4), only the incomplete skeletal ossification (85 mg/kg) and enlarged cerebral ventricles (125 mg/kg) occurred at a statistically increased rate when these data were analyzed by litter. Another study conducted by NIOSH (Hardin, personal communication) suggested that BPA exerted an estrogenic effect (as measured by percentage uterine water in ovariectomized rats) at various times after exposure to a single dose of BPA by inhalation (156 mg/m^3/6 hr), gavage (1250 mg/kg), dermal (8000 mg/kg), or ip (130 mg/kg) routes. These investigators suggested that a teratologic evaluation using a larger number of pregnant rats was indicated by the preliminary data.

Because of the potential for exposure to BPA, in both the workplace and the home environment, and the preliminary results cited above, the present studies in rats and mice were done to more completely characterize

TABLE I

MATERNAL TOXICITY IN CD RATS EXPOSED TO BISPHENOL A BY GAVAGE ON GESTATIONAL DAYS 6 THROUGH 15

	Bisphenol A (mg/kg/day, po)			
	0	160	320	640
Subjects (Dams)				
Total treated	27	27	27	29
Removed	2[a]	0	1[b]	0
No. pregnant (%) at sacrifice	23 (92)[c]	26 (96)	24 (92)	29 (100)
Maternal weight gain (g)[d]				
Gestation period (GD 0–20)	122.2 ± 4.4**	108.4 ± 3.2*	106.2 ± 3.4*	104.6 ± 2.9*
Treatment period (GD 6–15)	42.0 ± 1.8**	27.2 ± 2.3*	19.6 ± 2.9*	19.4 ± 2.4*
Corrected weight gain[e]	50.3 ± 2.5**	36.4 ± 2.6*	37.4 ± 2.1*	33.4 ± 2.5*
Gravid uterine weight (g)[d]	71.9 ± 3.7	72.0 ± 2.5[f]	68.8 ± 2.6	71.2 ± 2.7
Maternal liver weight[d]				
Absolute (g)	15.4 ± 0.3	14.5 ± 0.2	15.0 ± 0.3	14.7 ± 0.3
Relative (% body wt)	4.3 ± 0.1	4.2 ± 0.1	4.4 ± 0.1	4.3 ± 0.1

[a] One dam was removed due to punctured esophagus; one dam was removed due to preexisting pathological conditions found at sacrifice including large bladder, calculi, and small left kidney.

[b] One dam received 1/2 the appropriate dose on GD 13, due to a malfunction of the gavage syringe, and was removed.

[c] One dam had all resorptions.

[d] Includes all dams pregnant at sacrifice; mean ± SE.

[e] Weight gain during gestation minus gravid uterine weight.

[f] One gravid uterine weight was not recorded.

* Dunnett's test or Williams' test, $p < 0.05$.

** Linear trend test, $p < 0.05$.

the maternal and fetal responses to BPA during periods of major organogenesis.

MATERIALS AND METHODS[4]

Animals and environmental conditions. The experimental animals were CD rats [(COBS) Crl:CD (SD)BR] and CD-1 mice [(COBS) Crl:CD-1 (ICR)BR].[5] Female rats weighed 200–275 g and female mice weighed 20–35 g on Gestational Day (GD) 0 (i.e., day of sperm or vaginal plug detection).

Animals were individually identified by ear tags during their 7-day quarantine period. During this study, animals were housed on Ab-Sorb-Dri cage litter[6] in solid-bottom polypropylene or polycarbonate cages with stainless-steel wire lids and molded filter tops.[7] Feed[8] and deionized/filtered water were available *ad libitum* throughout the study. Animal rooms were equipped with automatic light cycles (lights on 7:00 AM to 7:00 PM). Temperature and relative humidity were maintained at 21–23°C and approximately 40%, respectively, for the rat study, and 20–21°C and 47%, respectively, for the mouse study. Air in each animal room was exchanged 12 to 14 times per hour.

Animal husbandry. A female rat in estrus or proestrus was placed overnight in the home cage of a singly housed male of the same strain. On the following morning, vaginal smears were examined for the presence of sperm. Fe-

[4] All studies were conducted in accordance with Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (FDA, 1978). Copies of the final study reports (George *et al.*, 1985a,b) are available from the National Technical Information Service, Springfield, VA 22161.

[5] Charles River Breeding Laboratories, Inc., Kingston, NY.

[6] Laboratory Products, Garfield, NJ.

[7] Ancare Corp., Manhasset, NY.

[8] Purina Certified Rodent Chow (5002), Ralston Purina Co., St. Louis, MO.

TABLE 2

DEVELOPMENTAL TOXICITY IN PREGNANT CD RATS FOLLOWING MATERNAL EXPOSURE TO BISPHENOL A BY GAVAGE ON GESTATIONAL DAYS 6 THROUGH 15

	Bisphenol A (mg/kg/day, po)			
	0	160	320	640
All litters[a]	23	26	24	29
No. implantation sites per litter[b]	13.5 ± 0.6	13.6 ± 0.4	13.7 ± 0.5	14.1 ± 0.4
% Resorptions per litter	7.5 ± 4.4	3.1 ± 0.9	6.5 ± 1.3	6.8 ± 2.1
% Litters with resorptions	30	35	54	45
Live litters[c]	22	26	24	29
No. live fetuses per litter	13.5 ± 0.4	13.2 ± 0.4	12.8 ± 0.5	13.1 ± 0.5
% Males per litter	45.4 ± 2.8	48.6 ± 3.2	43.2 ± 2.1	47.8 ± 2.8
Average fetal body weight (g) per litter	3.45 ± 0.06	3.45 ± 0.05	3.39 ± 0.04	3.44 ± 0.06
% Fetuses malformed per litter	7.5 ± 1.8	4.9 ± 1.5	8.5 ± 2.4	5.0 ± 1.3
% Litters with malformed fetuses	54	35	54	41

[a] Includes all dams pregnant at sacrifice; litter size = No. implantation sites per dam.
[b] Values are reported as means ± SE.
[c] Includes only dams with live fetuses; litter size = No. life fetuses per dam.

male mice were "primed" prior to breeding (Whitten, 1956) by placing a single male mouse in a small wire mesh cage inside the home cage of 10 females. Forty-eight hours later, each female was placed overnight in the cage of one male for mating and then examined the next morning for the presence of a vaginal plug. Sperm-positive rats or plug-positive mice were group-housed (maximum of four per cage).

Chemicals. Bisphenol A[9] (CAS No. 80-05-7) of greater than 95% purity[10] (3.4% of 2,4'-bisphenol A, an impurity formed during chemical synthesis, was present as a contaminant) was suspended in commercially available food-grade corn oil. The corn oil used in this study was analyzed for peroxides as an indicator of rancidity prior to first use. Dosing solutions were verified to be 88–110% of the theoretical concentration by gas chromatography both prior to and following completion of dosing. Each dosing solution was coded so that treatment and examination of animals were performed without knowledge of the dose concentrations.

Study design and treatment. Two replicates of the developmental toxicity evaluation were conducted for each species. Approximately equal numbers of sperm- or plug-positive females were assigned to each of five dose groups in each replicate (minimum of 10 per dose group per replicate) for a total of at least 20 confirmed-pregnant females per dose group. A replicate was defined as a substudy involving sperm- or plug-positive females mated on up to seven consecutive breeding days and distributed across dose groups. The last breeding day of the first replicate and the first breeding day of the second replicate were separated by 3 (mice) or 7 weeks (rats). Females of each species were assigned to dose groups by the method of stratified randomization so that body weight on GD 0 was not significantly different across dose groups within individual replicates or for each completed study (Tables 1 and 4).

Animals were dosed by gavage with BPA solutions or corn oil (vehicle) between 8:30 AM and 10:00 AM on GD 6 through 15. The volume administered (5.0 ml/kg for rats; 10.0 ml/kg for mice) was based on body weight recorded on each day of the dosing period. The doses selected were 0, 160, 320, 640, and 1280 mg/kg/day for rats and 0, 500, 750, 1000, and 1250 mg/kg/day for mice based on preliminary toxicity studies performed using timed-pregnant rats and mice (8 per dose group) dosed

[9] Obtained for Research Triangle Institute by Midwest Research Institute, Kansas City, MO. Manufactured by Alfa Products (Lot No. 011681).

[10] Purity determinations were conducted by Midwest Research Institute and involved filtration, thin-layer and high-performance liquid chromatography, and infrared, uv/visible, and NMR spectroscopy. See George *et al.* (1985a,b) for details.

TABLE 3

MORPHOLOGIC DEFECTS IN CD RAT FETUSES FOLLOWING MATERNAL EXPOSURE TO BISPHENOL A BY GAVAGE ON GESTATIONAL DAYS 6 THROUGH 15[a]

	Bisphenol A (mg/kg/day, po)			
	0	160	320	640
All malformations				
Malformed fetuses/total examined[b]	23/297	18/342	22/307	20/379
Litters with malformed fetuses/total litters examined[c]	12/22	9/26	13/24	12/29
External malformations				
No. fetuses with defects[d]	2	1	1	0
No. litters with defects[e]	2	1	1	0
Small right ear				
Anophthalmia (bilateral)	1			
Constriction of the tail	1		1	
Edema		1		
Absent oral and nasal orifices	1			
Microdactyly	1			
Anal atresia			1	
Visceral malformations				
No. fetuses with defects[d]	16	15	10	12
No. litters with defects[e]	8	9	7	8
Hydroureter (uni- or bilateral)	12	9	7	9
Hydronephrosis (uni- or bilateral)	5	7	2	4
Abnormal vessel (long innominate)		1	1	
Abnormally small organ[f]		1	1	
Reversed aorta	1	1		
Aorta behind trachea and/or esophagus		1		
Missing kidney (right)				1
Skeletal malformations				
No. fetuses with defects[d]	7	3	12	8
No. litters with defects[e]	5	2	6	4
Short rib	5	1	12	8
Missing thoracic arch		2		
Thoracic centra misaligned	1	1		
Lumbar centra misaligned	1			
Lumbar centra off center		1		
Thoracic centra off center	1			
Variations				
No. fetuses with defects[g]	88	94	74	89
No. litters with defects[h]	18	26	24	29
Bipartite centra	46	44	33	28
Distended ureter(s)	25	21	16	26
Hematoma	23	28	23	28
Wavy rib	1		5	7
Incomplete ossification (parietals and/or interparietals)	1		3	6
Misaligned sternebrae	3	1	2	2
Hemorrhagic adrenal(s)	1	3		1
Extra ossification site(s)		2		
Frontals and parietals fused on lateral edges		1		
Globular heart	1			
Small or missing pubis			1	
Very soft tissue (kidney)		1		

[a] Fetuses may have more than one malformation or variation.
[b] Only live fetuses were examined for malformations.
[c] Includes only litters with live fetuses.
[d] Fetuses with one or more malformations.
[e] Litters with one or more malformed fetuses.
[f] 160 mg/kg group, auricular flaps; 320 mg/kg group, kidney.
[g] Fetuses with one or more variations.
[h] Litters with one or more fetuses with variations.

TABLE 4

MATERNAL TOXICITY IN CD-1 MICE EXPOSED TO BISPHENOL A BY GAVAGE ON GESTATIONAL DAYS 6 THROUGH 15

	Bisphenol A (mg/kg/day, po)				
	0	500	750	1000	1250
Subjects (Dams)					
Total treated	29	29	29	34	33
Removed	0	1[a]	1[a]	2[b]	0
Dead	0	2	1	2	6
No. pregnant (%) at sacrifice	26 (90)	23 (88)	21 (78)	23 (77)	21 (78)
Maternal weight gain (g)[c]					
Gestation period (GD 0–17)	19.8 ± 0.9**	21.8 ± 1.2	20.7 ± 1.0	18.1 ± 1.6	13.5 ± 2.1*
Treatment period (GD 6–15)	11.6 ± 0.4**	12.2 ± 0.8	12.5 ± 0.7	9.4 ± 1.1	6.6 ± 1.2*
Corrected weight gain[d]	4.9 ± 0.4	6.2 ± 0.5	6.0 ± 0.8	4.6 ± 0.8	3.5 ± 0.7
Gravid uterine weight (g)[e]	14.7 ± 0.8**	15.6 ± 0.9	14.8 ± 0.9[e]	13.4 ± 1.2	10.0 ± 1.5*
Maternal liver weight[c]					
Absolute (g)	2.6 ± 0.0	3.0 ± 0.1*[f]	3.1 ± 0.1*	3.0 ± 0.1*	2.8 ± 0.1[f]
Relative (% body wt)	5.4 ± 0.1**	5.9 ± 0.1*	6.1 ± 0.1*	6.3 ± 0.2*	6.8 ± 0.2*

[a] One dam was removed due to punctured esophagus.
[b] Two dams were removed due to delivery before scheduled sacrifice.
[c] Includes all dams pregnant at sacrifice; means ± SE.
[d] Weight gain during gestation minus gravid uterine weight.
[e] Gravid uterine weight for one dam was inadvertently not recorded.
[f] One liver weight was incorrectly recorded and was not included.
* Dunnett's test or Williams' test, $p < 0.05$.
** Linear trend test, $p < 0.05$.

on GD 6 through 15 with 0, 120, 250, 500, 750, 1000, 1500, 2000, 2500, or 3000 mg/kg/day on GD 6 through 15 (George *et al.*, 1985a,b). Study doses were expected to cause no more than 10% maternal mortality at the high dose, gradations of maternal and fetal toxicity at intermediate doses, and no effect on maternal or fetal parameters at the low dose.

Observations. Rats were weighed on GD 0, 6 through 15, and 20; mice were weighed on GD 0, 6 through 15, and 17. Dams were observed daily during treatment for clinical signs of toxicity. On GD 20, all mated rats were anesthetized with carbon dioxide and killed by cervical dislocation; mice were killed by cervical dislocation on GD 17. Maternal liver weight, gravid uterine weight, and number of corpora lutea were recorded. The uteri of dams with no apparent implantations were treated with a solution of 10% ammonium sulfide in order to visualize possible implantation sites (Salewski, 1964). Uterine contents (i.e., number of implantation sites, resorptions, dead fetuses, and live fetuses) were evaluated, and live fetuses were dissected from the uterus and anesthetized by placing on ice. Each live fetus was weighed and examined for external morphological abnormalities, and the viscera were examined by a fresh tissue technique (Staples, 1974). Half of the fetuses were decapitated prior to dissection and the heads were fixed in Bouin's solution for free-hand sectioning and examination (Wilson, 1965). All fetal carcasses were prepared with Alizarin Red S stain and examined for skeletal malformations, as modified from Peltzer and Schardein (1966) and Crary (1962).

Statistical analyses. Analyses of data were carried out by the General Linear Model (GLM) procedure in the SAS software library (SAS Institute, 1982a,b). Prior to analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) and Bartlett's test for homogeneity of variance (α level = 0.001) was performed on all data to be analyzed by GLM. Dose–response relationships for selected measures were evaluated with a test for linear trend. Analysis of variance (ANOVA) was used to determine whether significant dose effects, replicate effects, or dose × replicate interactions had occurred. When ANOVA revealed significant differences among groups, then Williams' multiple comparison test (Williams, 1971, 1972) and Dunnett's test (Dunnett, 1955, 1964) were used to compare BPA-treated groups with the vehicle control group (α level = 0.05). A one-tailed test was used

TABLE 5

DEVELOPMENTAL TOXICITY IN PREGNANT CD-1 MICE FOLLOWING MATERNAL EXPOSURE TO BISPHENOL A BY GAVAGE ON GESTATIONAL DAYS 6 THROUGH 15

	Bisphenol A (mg/kg/day, po)				
	0	500	750	1000	1250
All litters[a]	26	23	21	23	21
No. implantation sites per litter[b]	11.9 ± 0.4	12.3 ± 0.5	11.7 ± 0.7	12.1 ± 0.4	11.8 ± 0.5
% Resorptions per litter	14.1 ± 4.2**	12.8 ± 4.5	7.5 ± 1.6	18.4 ± 6.9	40.1 ± 9.6*
% Litters with resorptions	69	61	62	52	81
Live litters[c]	25	22	21	20	14
No. Live fetuses per litter[b]	10.8 ± 0.5	11.6 ± 0.5	10.7 ± 0.7	11.2 ± 0.5	10.9 ± 0.8
% Males per litter	51.4 ± 3.3	51.4 ± 3.4	47.9 ± 3.0	52.5 ± 3.7	50.0 ± 3.5
Average fetal body weight (g) per litter	0.96 ± 0.02**	0.94 ± 0.02	0.94 ± 0.04	0.87 ± 0.04	0.82 ± 0.03*
% Fetuses malformed per litter	2.7 ± 1.0	2.4 ± 1.0	1.3 ± 0.7	5.7 ± 4.3	5.8 ± 2.2
% Litters with malformed fetuses	24	27	14	15	43

[a] Includes all dams pregnant at sacrifice; litter size = No. implantation sites per dam.
[b] Values are reported as means ± SE.
[c] Includes only dams with live fetuses; litter size = No. live fetuses per dam.
* Dunnett's test, $p < 0.05$.
** Linear trend test, $p < 0.05$.

for all parameters except measures of maternal body and organ weight, fetal body weight, and percentage males per litter for which a two-tailed test was used. The absence of significant (i.e., $p > 0.05$) replicate effects or dose × replicate interactions on selected measures was considered as evidence that pooling data across replicates for nonparametric analysis was justified for related nominal scale data. Nominal scale measures were analyzed by a test for linear trend on proportions and a χ^2 test for independence among groups (Siegel, 1956). When χ^2 revealed significant ($p < 0.05$) differences among groups, then a one-tailed Fisher exact probability test (α level = 0.05) was used for pairwise comparisons between each BPA-treated group and the vehicle control group.

RESULTS

Rats. Dams in the high-dose group (1280 mg/kg/day) exhibited an unexpectedly high mortality rate of 26% (7/27 animals). This is in contrast to the 10% mortality rate that was predicted for this dose group on the basis of a probit analysis conducted on data from the preliminary toxicity study (predicted LD10 = 1276 mg/kg/day). In the first replicate, 10% (1/10) maternal mortality occurred as predicted, but in the second replicate, 35% (6/17) of the maternal animals died. There was no clear indication in the study records of the cause of this replicate-dependent increase in maternal mortality; no dose-related deaths were observed in the other dose groups, and dosing solutions in the 1280 mg/kg/day group (second replicate) were analyzed at 88–98% of the nominal value. Due to the unexpected replicate-dependent toxicity observed only in the 1280 mg/kg/day dose group, only the data from the 0, 160, 320, and 640 mg/kg/day dose groups are reported. Full details are available elsewhere (George *et al.*, 1985a); there was no increase in the incidence or percentage of fetuses malformed per litter in the 1280 mg/kg/day dose group.

TABLE 6

MORPHOLOGIC DEFECTS IN CD-1 MOUSE FETUSES FOLLOWING MATERNAL EXPOSURE TO BISPHENOL A BY GAVAGE ON GESTATIONAL DAYS 6 THROUGH 15[a]

	Bisphenol A (mg/kg/day, po)				
	0	500	750	1000	1250
All malformations					
Malformed fetuses/total examined[b]	7/269[c]	7/254	3/224	15/224	11/153
Litters with malformed fetuses/total litters examined	6/25	6/22	3/21	3/20	6/14
External malformations					
No. fetuses with defects[e]	0	2	2	11	4
No. litters with defects[f]	0	2	2	1	3
Cleft palate			1	11	4
Exencephaly		2			
Gastroschisis			1		
Heart outside body cavity (ectocardia)			1		
Visceral malformations					
No. fetuses with defects[e]	5	3	1	4	1
No litters with defects[f]	4[g]	2	1	2	1
Enlarged epididymis	2	2	1	2	
Enlarged uterine horn	1			1	
Enlarged auricular flaps	1				
Extra vessel	1[h]				1[i]
Kidneys 1/2 normal size		1			
Hydrocephaly				1	
Skeletal malformations					
No. fetuses with defects[e]	2	2	1	3	7
No. litters with defects[f]	2	2	1	2	3
Missing rib		1		1	2
Short rib		1		1	
Ribs fused to each other				1	3
Thoracic centra off center					4
Fused sternebrae	2				
Misshapen skull bone—frontals, parietals			1		1
Missing thoracic arch				1	1
Fused thoracic arches					1
Missing centra—thoracic					1
Thoracic arch smaller than normal					1
Thoracic centra misaligned				1	
Variations					
No. fetuses with defects[j]	44	86	53	66	48
No. litters with defects[k]	19	20	17	16	12
Misaligned sternebrae	38	78	48	57	40
Hematoma (tail)	6	5	5	7	5
Bipartite centra		1			2
Open eye		3	1		
Extra ossification site(s)—sternum		2			
Incomplete ossification—pelvic girdle					2
Very soft tissue (kidney)				2	
Wavy rib				2	
Blood in amniotic sac (live fetus only)		1			

TABLE 6—*Continued*

	Bisphenol A (mg/kg/day, po)				
	0	500	750	1000	1250
Variations					
No. litters with defects[k]					
Cream colored liver lobe(s)		1			
Distended ureter(s)		1			
Frontals and parietals fused on lateral edges		1			
White spots in ventricle (left)					1

[a] Fetuses may have more than one malformation or variation.
[b] Only live fetuses were examined for malformations.
[c] One fetus was lost during the staining process and was not examined for skeletal defects.
[d] Includes only litters with live fetuses.
[e] Fetuses with one or more malformations.
[f] Litters with one or more malformed fetuses.
[g] Data for fetal head exams, dam 162, were not recorded.
[h] Extra vessel stemming from the aorta.
[i] Extra vessel between left carotid artery and left subclavian artery.
[j] Fetuses with one or more variations.
[k] Litters with one or more fetuses with variations.

In the 27 to 29 treated rats in each dose group, pregnancy was confirmed in 92 to 100% of the treated females (Table 1). No dams were removed from the study for treatment-related causes. Dams did not differ significantly among treatment groups with respect to maternal body weight on Gestation Days 0 or 6, the day of initiation of treatment (data not shown). No significant differences were observed among treatment groups with respect to the total number of corpora lutea per dam, the number of implantation sites per dam, or the percentage of preimplantation loss (data not shown), indicating that the animals used in this study were simlar in reproductive capacity. Maternal weight gains (gestation, treatment, and gestational corrected for gravid uterine weight) were reduced in a dose-related manner. Statistically significant pairwise differences between the control group and each BPA dose group were observed for all three measures. Gravid uterine weight and absolute and relative maternal liver weight were unaffected by BPA treatment.

The primary clinical signs associated with BPA treatment were lethargy, piloerection, pica, rough coat, wet urogenital area, weight loss, and alopecia. These effects occurred infrequently in control group rats. They occurred more frequently in the 640 mg/kg/day groups than in other BPA dose groups, but most noticeably as a result of BPA treatment contrasted with the control group (data not shown).

There was no significant effect of BPA treatment on GD 6 through 15 on any observed measure of developmental toxicity (Table 2). This included no effect on percentage resorptions per litter, percentage litters with resorptions, number of live fetuses per litter, sex ratio, average fetal body weight per litter, percentage fetuses malformed per litter, and percentage litters with malformed fetuses. There were no effects of BPA on the incidence of external, visceral, or skeletal malformations per litter (Table 3). BPA treatment was not associated with a particular malformation or group of malformations in any dose group.

Mice. Approximately equal numbers of CD-1 females were pregnant in each treatment group (Table 4). BPA-treated females exhibited clinical signs of toxicity including arched back, lethargy, piloerection, rough coat, vaginal bleeding, vocalization, alopecia, weight loss, and wheezing (data not shown). Few mice in the control group showed these signs. Maternal deaths occurred in all BPA dose groups, reaching 18% in the 1250 mg/kg/day group. There was a trend toward reduced maternal weight gain during both gestation and treatment, with mice in the 1250 mg/kg/day group gaining significantly less weight than those in the control group. Corrected weight gain (weight gain during gestation minus gravid uterine weight) was not significantly different among dose groups. There was a trend toward reduced gravid uterine weight with increasing BPA dose; the mean high-dose weight was significantly less than that of the control group. Maternal liver weight was significantly increased for the 500, 750, and 1000 mg/kg/day dose groups compared to the control group. Relative maternal liver weight, i.e., liver weight as a function of body weight, was significantly increased in all BPA-dosed groups in a dose-dependent manner.

There was no difference among treatment groups in the number of implantation sites per litter (Table 5), but the number of corpora lutea per dam decreased in relation to increasing BPA dose (data not shown). There was no effect of BPA dose on percentage preimplantation loss (data not shown). The percentage resorptions per litter increased in the two highest dose groups, with the 1250 mg/kg/day group significantly different from the control group. There were seven litters in the high-dose group that were totally resorbed. BPA had no effect on the number of live fetuses per litter or on sex ratio (Table 5). A dose-dependent decrease in average fetal body weight per litter was observed with increasing BPA dose; the 1250 mg/kg/day average fetal body weight was significantly less than that of the control group. There was no significant effect of BPA on percentage of fetuses malformed per litter or on percentage of litters with malformed fetuses.

There were no significant treatment effects on any measure of teratogenic response (Table 6). BPA treatment on GD 6 through 15 had no effect on the incidence of external, visceral, or skeletal malformations. Cleft palate in the 1000 mg/kg dose group occurred in one litter, rather than over several litters. Treatment was not associated with any particular malformation or group of malformations in any dose group.

DISCUSSION

There are few experimental data, either clinical or nonclinical, concerning the teratogenic potential of BPA. Hardin *et al.* (1981) administered BPA by intraperitoneal injection to Sprague–Dawley rats on Gestational Days 1 through 15 at doses of 0, 85, or 125 mg/kg/day. The 125 mg/kg dose caused a significant reduction in the number of pregnant rats as a function of the number of sperm-positive rats started on the study, and both doses caused a significant reduction in the number of live fetuses per litter. Dose-related decreases in fetal body weight and crown–rump length were also reported. Maternal toxicity (histiocytosis, intraalveolar pigmented macrophages, and peritonitis) was observed in the high-dose group. Litter-based statistical analyses indicated that there was a significant increase in the number of litters with incomplete ossification in the 85 mg/kg/day group, and an increase in litters with fetuses having enlarged cerebral ventricles or hydrocephaly in the high-dose group. In the present rat study, BPA doses of 640 mg/kg/day by gavage on Gestation Days 6–15 had no effect on the percentage resorptions, number of live fetuses per litter, average fetal body weight per litter, or incidence of malformations, even in the presence of significant maternal toxicity (clinical signs and significant reduction in maternal weight gains). The

difference in the teratogenic effect of BPA on the offspring of pregnant rats between these two studies may be due to differences in route of exposure, time and duration of treatment, and dose of BPA. In CD-1 mice, 1250 mg/kg/day BPA administered on GD 6 through 15 caused decreased maternal weight gain during treatment and gestation periods, principally as a result of the 40% resorption rate in this group. Increased relative maternal liver weight in all BPA groups may be an indication of hepatotoxicity, although the high-dose group value is also influenced by the high resorption rate and attendant decreased body weight. In the present study, BPA at the high dose (1250 mg/kg/day) significantly increased the percentage of resorptions per litter and caused a significant decrease in average fetal body weight. BPA did not significantly affect the number of live fetuses per litter or cause an increase in the proportion of fetuses malformed per litter. In summary, postimplantation exposure to BPA (gavage) did not cause external, visceral, or skeletal malformations at doses that caused significant maternal toxicity (rats) or mortality (mice).

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