DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

AD

Date July 24, 2007

From Mary E. Shackelford, Ph D.
Division of Food Contact Notifications, Toxicology Group 2

Subject Acceptance of Final TDERs for studies reviewed under contract with ICF Consulting (Contract No. 223-96-2302) for Food Additive Master File No. 580 under Work Assignment 2000-19 (ICF 419), Task Numbers 3, 4, 5, and 6

To Food Additive Master File 580

Through Michelle Twaroski, Ph.D.
Team Leader, Toxicology Group 1, DFCN

Following the Agency's instructions, ICF Consulting, the contractor, has generated comprehensive Toxicological Data Evaluation Reports (TDERs) for the studies which are listed below. Dr. Mary E. Shackelford has reviewed and accepted the final TDERs for Work Assignment Number 2000-19, Task Number 3, 4, 5, and 6. The final TDERs for Work Assignment Number 2000-19 for Task Number 3, 4, 5, and 6 may be incorporated into the file for Food Additive Master File 580

1. Teratologic Evaluation of Bisphenol A (Cas No. 80-05-7) Administered to CD® Rats on Gestational Days 6 through 15 (NTP-85-089) (Work Assignment Number 2000-19, Task Number 3)

2. Bisphenol A A Reproduction and Fertility Assessment in CD-1 Mice When Administered in the Feed (NTP 85-192) (Work Assignment Number 2000-19, Task Number 4)

3. Bisphenol A Reproduction and Fertility Assessment in CD-1 Mice When Administered Via Subcutaneous Silastic Implants (NTP 84-105) (Work Assignment Number 2000-19, Task Number 5)

4 Teratologic Evaluation of Bisphenol A (Cas No. 80-05-7) Administered to CD-1 Mice on Gestational Days 6 through 15 (NTP 85-088) (Work Assignment Number 2000-19, Task Number 6)

/s/
/s/
Mary E. Shackelford, Ph.D.

Attachments. Copy of final TDERs for Work Assignment Number 2000-19, Tasks Number 3, 4, 5, and 6 under Contract Number 223-96-2302

MAY 1 7

/s/

/s/

Contract Number

223-96-2302

Work Assignment Number

2000-19
(ICF 419)

Task Number

03

FDA Study Identification Number

NTP-85-089

Signature of Program Manager

/s/

/s/

Date

5-10-01

STUDY TITLE

Teratologic Evaluation of Bisphenol A (CAS No. 80-05-7) Administered to CD(R) Rats on Gestational Days 6 through 15 (NTP-85-089)

TESTING LABORATORY

Research Triangle Institute (RTI)
Post Office Box 12194
Research Triangle Park, NC 27709

COMPLETION DATE OF STUDY

Report Date. February 15, 1985

SPONSORS OF STUDY

The National Toxicology Program
Research Triangle Park, NC 27709

and

National Center for Toxicological Research
Division of Teratogenesis
Jefferson, AR 72079

LABORATORY STUDY NUMBER

RTI Study Code No Rt82-BPA
RTI Project No 31U-2077

STUDY SUMMARY

GLP Compliance/Quality Assurance

The protocol (Appendix II, page 29) indicated that the testing facility was in compliance with Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies (1978) and that the laboratory was accredited by the American Association for Accreditation of Laboratory Animal Care The protocol further stated that the study would be conducted in compliance with GLP guidelines. However, a statement confirming that the study complied with GLP guidelines was not included in the study report.

The study report included a signed and dated (August 6, 1984) Quality Assurance Statement (page 5), which reported a list of eight Quality Assurance Unit (QAU) inspections conducted during the study as well as a Final Report Audit and Data Record Audit. A footnote to the list of inspections, however, indicated that several of the inspections were performed on a comparable study rather than on the present

study The study report was considered by the QAU to be an accurate account of the study procedures and findings

Protocol(s)

Two protocols were provided in the study report an original protocol dated 7/19/92 (date of Study Director signature) and an amended protocol dated 10/26/92. The study designs were very similar in both the original protocol and the amended protocol However, the amended protocol differed in the following areas· (1) elimination of a preliminary toxicity study using nonpregnant animals, (2) alterations in the acceptable age and weight range for female rats at study initiation, (3) wider ranges of test article doses to be examined, and (4) modified statistical evaluation Our reviewers noted that these protocol changes did not have a negative impact on the study.

Objective

The objective of this study was to evaluate the toxic and teratogenic effects of the test material (bisphenol A) administered orally to pregnant rats during the period of organogenesis (gestation days 6-15)

Test Material

Test Article

The test article was identified as Bisphenol A (CAS No 80-05-7, molecular formula $C_{15}H_{16}O_2$, molecular weight 228 28) and was obtained from Midwest Research Institute (MRI), Kansas City, MO. MRI obtained the test article from Alfa Products (Lot No 011681, Batch No 01) (Appendix IV, page 10) MRI described the test article as a white crystalline powder (Appendix IV, page 15) Although the product label indicated 98% purity (Appendix IV, page 10), MRI estimated a purity of approximately 95% by high performance liquid chromatography (HPLC), which identified two impurities comprising approximately 3 9% and 1 0% of the sample (Appendix IV, page 11). Nonaqueous titration of the phenol groups indicated a purity of approximately 99%, however, this method could not detect any phenolic or acidic impurities in the sample (Appendix IV, page 11) The major impurity was later identified as the 2,4' isomer of Bisphenol A by capillary gas chromatography/mass spectrometry, which estimated the impurity to be about 3 4% of the sample (Appendix IV, page 48) According to MRI, the test article was stable when stored for two weeks protected from light at temperatures up to 60°C (Appendix IV, page 20) At MRI, the test article was stored sealed in plastic bags inside a 10-gallon metal can at 5°C (Appendix IV, page 14). the storage conditions at RTI were not provided in the study report The expiration date of the test article also was not provided in the study report There were no significant differences, however, in Bisphenol A samples returned to MRI from RTI before the present study (9/14/82) and after the present study (1/27/83) compared to frozen reference samples maintained by MRI (Appendix IV, pages 41, 59, and 65).

Vehicle

The vehicle was Mazola® Corn Oil (commercial food grade) The corn oil was stored at 4°C The lot/batch number, purity, stability, and expiration date of the vehicle were not provided in the study report Prior to use, however, each bottle of corn oil was tested for the presence of peroxides as an

indication of rancidity (a sample with peroxide content > 10 meq/kg would have been considered rancid) Analysis of samples of the corn oil used to formulate each dosing suspension did not reveal any peroxides

Dose Preparation

Prior to the study, dosing formulations were prepared by combining appropriate amounts of the test substance with corn oil in flasks and shaking (chemical/vehicle mixing protocol described in detail in Appendix IV, page 31) A sufficient quantity of each formulation was prepared to be used throughout each replicate of the study For replicate I of the study, one batch of dosing solutions was prepared, whereas, two batches of dosing formulations were prepared for replicate II of the study All dosing formulations were stored at room temperature in screw cap vials To ensure that the study was conducted blind for dose, random 5-digit number codes and color codes were used to identify the concentration in each vial. The suspensions were thoroughly agitated prior to dosing and were stirred continuously on a magnetic stir plate during dosing to maintain homogeneity

Formulation Analysis

Both pre-dosing and post-dosing analyses of the concentrations of the dosing formulations were conducted All pre-dosing analyses for replicate I and replicate II of the study yielded values within 90-110% of the nominal concentration for each formulation In replicate I of the study, post-dosing analyses yielded values within 90-110% of the nominal concentration for each formulation No post-dosing analyses were conducted on the first batch of dosing formulations in replicate II, however, post-dosing analyses of the second batch revealed two formulations outside the 90-110% nominal concentration range The actual concentrations of the 320-mg/kg/day and 1280-mg/kg/day formulations were 88% of the nominal values

Homogeneity and stability of Bisphenol A in corn oil were evaluated by MRI (Appendix IV, pages 20-26) The results of the homogeneity analyses indicated that by using appropriate mixing procedures, Bisphenol A could be suspended in corn oil formulations suitable for gavage administration Furthermore, suspensions stored for five days could be rehomogenized by stirring. The stability analyses revealed that bisphenol A suspended in corn oil was stable for three weeks when at room temperature sealed in the dark Bisphenol A suspended in corn oil was stable for three hours when open to air in the light Homogeneity and stability analyses of the actual dosing solutions used by RTI do not appear to have been conducted.

Test Animals

Receipt

A total of 166 female and 96 male caesarean-originated, barrier-sustained (COBS) CD® (SD)BR outbred albino rats were received from the Charles River Breeding Laboratory, Inc (Kingston, NY) Upon arrival, females and males were 8-10 weeks and 10-12 weeks old, respectively.

Species selection was justified in the study report (page 20)

Housing

Upon receipt, animals were randomly assigned to cages for a seven day quarantine period Individually coded identification tags were affixed to the ear of each rat during this time Males were housed singly, and females were group housed (four per cage). The cages were solid bottomed polypropylene or polycarbonate with stainless steel wire lids and molded filter tops Ad-Sorb-Dri® cage bedding was used Cages were organized on galvanized-steel racks with adjustable shelves Following mating. females were again group-housed (four per cage) with other females assigned to the same treatment group

Environment

The animals were housed in rooms that received 12 hours of continuous light and 12 hours of continuous dark each day The rooms were air-conditioned, and at least 12-14 air changes were provided per hour The rooms were monitored continuously for temperature and humidity The temperature range was 69 0-73 0 °F, which was slightly outside the range specified in the protocol (70 ± 2 °F) The relative humidity range was 20-62%, a target range was not specified in the protocol

Diet

Purina Certified Rodent Chow® (5002) and deionized/filtered water were available, *ad libitum* Analyses of the feed and water were not included in the study report

Mating

No male breeder was used more than twice in any given dose group Individual females in estrus or proestrus (estrous cycle stage determined by vaginal smear) were caged overnight with individually caged males (i e , only one male and one female per cage) The females were considered to be fertilized if a vaginal smear was positive for sperm on the following morning The day on which sperm were found was designated gestational day zero (gd 0) In addition to the individually coded ear tags previously affixed to each rat, sperm-positive females were assigned a dam number on gd 0 According to the study report, all 137 sperm-positive females were within the weight range 200-275 g on gd 0, however, individual animal data were not provided Actual weight ranges on gd 0 were provided only for the 120 females confirmed pregnant at sacrifice

Study Design

Dose Selection

Dose levels were selected based on a preliminary teratology study in which bisphenol A suspended in corn oil was administered by gavage in a volume of 5 0 mL/kg to 56 time-mated female (COBS) CD® (SD)BR rats (8/dose) at dose levels of 0, 500, 1000, 1500, 2000, 2500, or 3000 mg/kg/day on gestation days 6-15 The dams were observed for mortality or clinical signs at least once daily during the treatment period, and body weights were determined on gd 0, gd 6-15, and gd 20 Animals that died on study were necropsied to determine the cause of death Following sacrifice on gd 20, dams were assessed for clinical signs of toxicity, body weight, liver weight, gravid uterine weight, and uterine contents (i e., implantation sites, resorptions, dead fetuses, and live fetuses) Live fetuses were weighed and examined

for gross malformations Statistical analysis of the preliminary study data included nonparametric methods to discern dose-response trends

Two dams were removed from the study one 2500-mg/kg/day dam was missing from her cage and one 3000-mg/kg/day dam received only half the intended dose on gd 14 There was excessive treatment- and dose-related maternal toxicity, beginning on gd 12 The rates of maternal mortality were 0% (0/8), 0% (0/8), 0% (0/8), 12 5% (1/8), 62.5% (5/8), 42 8% (3/7), and 71.4% (5/7) for the vehicle control through high dose group, respectively The most common clinical signs observed at daily observation were diarrhea (with or without a mucoid discharge), lacrimation (tearing), lethargy, rough coat, wheezing, wet urogenital area, arched back, clinical weight loss, and death Other signs observed sporadically included dyspnea and/or wheezing, bloody nose, generalized weakness, disorientation, bloody urethra, alopecia, piloerection, and vaginal bleeding Maternal body weight was significantly decreased on gd 11 and 15 in all treatment groups and on gd 20 at ≥1500 mg/kg/day All surviving dams were pregnant when sacrificed on gd 20. Maternal weight gain during gestation (gd 0-20) was significantly decreased at ≥1000 mg/kg/day, and maternal weight gain during the treatment period (gd 6-15) and absolute weight gain (i e , weight gain during gestation minus gravid uterine weight) were significantly decreased in all treatment groups. Negative relative weight gain (i e , weight[1] decreases compared to gd 0 values) occurred at ≥1500 mg/kg/day, and negative absolute weight gain occurred at 3000 mg/kg/day Gravid uterine weight was significantly decreased at 2000 and 2500 mg/kg/day (but not at 3000 mg/kg/day) Maternal body weight, maternal body weight gain (gestation period, treatment period, and absolute weight gain), and gravid uterine weight also showed dose-related decreasing trends. There were no significant changes in maternal liver weight

The percent of resorptions per litter was significantly increased at ≥1500 mg/kg/day The percent of nonlive implants (resorptions plus dead fetuses) and adversely affected fetuses (resorptions, dead fetuses, and malformed live fetuses) per litter were significantly increased at 2000 and 2500 mg/kg/day (but not at 3000 mg/kg/day) The percent of resorptions per litter, the percent of nonlive implants per litter, and the percent of adversely affected fetuses per litter also showed a dose-related upward trend. Although no significant dose effects were found, the number of live fetuses per litter exhibited a dose-related downward trend There were no effects on the percent of dead fetuses per litter or the percent of litters with resorptions, dead fetuses, nonlive implants, or adversely affected fetuses.

Average fetal body weight per litter exhibited a dose-related downward trend, and was significantly decreased at ≥1000 mg/kg/day compared to controls. There was no significant difference in the incidence of gross malformations in offspring of treated dams In the 2000-mg/kg/day group, one fetus out of a total of 412 live fetuses examined exhibited a malformation (meningoencephalocele).

Probit analysis of the maternal mortality data yielded an LD_{10} of 1276 mg/kg/day for bisphenol A administered to pregnant CD rats on gd 6-15 Therefore, 1280 mg/kg/day was chosen as the high dose for the definitive teratology study In addition to the vehicle control, 160, 320, and 640 mg/kg/day were chosen as dose levels Based on the results of the preliminary study, the study author expected the 1280-mg/kg/day and the 640-mg/kg/day to cause maternal and/or fetal toxicity, whereas the

---

[1]Our reviewers noted that only two samples were available for evaluation in the 3000 mg/kg group due to excessive maternal mortality.

160-mg/kg/day dose was expected to be without detectable maternal and/or fetal toxicity. Toxicity at 320-mg/kg/day was considered possible but less likely.

Animal Assignment

Sperm-positive females were randomly assigned to the dose groups indicated in Table 1 using a procedure based on stratified body weight to ensure comparable group mean body weights for each treatment group. The experimental design involved two replicates A replicate was identified as a substudy which included sperm-positive females bred on up to seven consecutive mating days with the last mating day of one replicate separated from the first mating day of another replicate by at least two weeks

Table 1. Animal Assignment

Dose (mg/kg/day)	Number of Sperm-Positive Females		
	Replicate I [a]	Replicate II [b]	Total
0	10	17	27
160	10	17	27
320	10	17	27
640	11	18	29
1280	10	17	27

[a]Gestational day 0 for Replicate I 11/10-11/12, 1982
[b]Gestational day 0 for Replicate II 1/4-1/6, 1983

Administration

All doses were administered once daily by gavage, on gestation days 6 through 15, at a constant dose volume of 5 mL/kg of body weight/day. Although the dams were weighed daily prior to dosing on gd 6-15, the study report does not specify that dosing was based on the most recent body weight determination.

Maternal Observations

Mortality/Clinical Observations

The animals were checked for mortality or clinical signs at least once daily during the treatment period (at the time of daily dosing) The study report indicates that animals were also observed for mortality or clinical signs four hours post-dosing, when staff scheduling allowed Clinical signs were also recorded prior to sacrifice on gd 20 (p VIII-2)

Body Weight/Food Consumption

Body weight data were recorded on gestation days 0, 6 through 15, and 20 (immediately following sacrifice) Non-pregnant animals were excluded from the analyses Individual food consumption data were not determined.

Gross Pathology/Cesarean Examinations/Organ Weights

Animals that died between gd 0 and gd 20 were subjected to a gross necropsy to determine the cause of death According to the study report (p. 29), surviving dams were sacrificed on gd 20 (cervical dislocation under carbon dioxide anesthesia) and evaluated for clinical signs of toxicity, body weight, liver weight, gravid uterine weight, total number of corpora lutea, and status of uterine implantations (i e , implantation site, resorptions, dead fetuses, and live fetuses) Appendix VIII further indicated that any internal abnormalities observed during dissection were noted, and affected tissues, if any, were removed, labeled, preserved in 10% neutral-buffered formalin, and placed in storage (p VIII-2) The uteri of dams with no apparent implants were treated with a solution of 10% ammonium sulfide in order to visualize possible implantation sites. The viable fetuses were dissected from the uterus and immediately placed on a moist paper towel on a tray of ice According to the study report, this procedure induces anesthesia by lowering core body temperature below 25 °C Individual live fetuses were weighed and sexed

Histopathology

Histopathological examinations were not conducted in this study

Fetal Observations

External Examinations

All viable fetuses were examined macroscopically for any external abnormalities

Visceral Examinations

Using the Staples' fresh tissue dissection method, all live fetuses were examined for visceral abnormalities. Prior to dissection, half of the fetuses were decapitated, the heads were fixed in Bouin's solution for free hand sectioning and examination.

Skeletal Examinations

All fetal carcasses (50% without heads) were examined for skeletal abnormalities following fixation with Alizarin Red S stain Rare or difficult to describe malformations were photographed or sketched

Data Analysis

Indices

No indices were calculated

Statistical Evaluation

Data for all measures were pooled across replicates and analyzed parametrically or non-parametrically, as appropriate. Maternal weight data (body weight, body weight gain, gravid uterine weight, and absolute and relative liver weight) and per litter data (implantation sites per litter, % resorptions per litter, % dead fetuses per litter, % nonlive implants per litter, % affected fetuses per litter, live fetuses per litter, average fetal weight per litter, average male fetal weight per litter, average female fetal weight per litter, % males per litter, % male fetuses malformed per litter, % female fetuses malformed per litter, and % fetuses malformed per litter) were evaluated using parametric techniques Relative maternal liver weight and litter derived percentage data (% resorptions per litter, % dead fetuses per litter, % nonlive implants per litter, % affected fetuses per litter, % males per litter, % male fetuses malformed per litter, % female fetuses malformed per litter, and % fetuses malformed per litter) were first subjected to arcsine-square root transformation. Bartlett's test for homogeneity of variance was conducted on all data to be analyzed using parametric techniques The study report indicates, however, that Bartlett's test was not used to decide whether analysis of variance (ANOVA) procedures should be used on selected data (page 39) A two-way ANOVA (dose x replicate) was used to determine whether significant dose effects, replicate effects, or dose x replicate interactions had occurred in any of the parameters listed above For maternal body weights or weight gain measures, a three-way ANOVA (dose x replicate x day) was used if no significant dose effect was observed in the two-way ANOVA In addition, average male and female fetal body weight per litter and percent males and females malformed per litter were evaluated using a three-way ANOVA (dose x replicate x sex) to test for significant effects of sex The Linear Trends Test also was conducted on all data analyzed by ANOVA to determine the significance of the dose-response relationship When ANOVA indicated significant ($p<0.05$) effects for dose, Williams' and/or Dunnett's Multiple Comparison Test was used to compare individual treatment groups to the control group A one-tailed test was used for all parameters except measures of maternal body and organ weight, fetal body weight, and percent males per litter for which a two-tailed test was used When significant ($p<0.05$) replicate effects or dose x replicate effects were noted, data were further analyzed for dose effects separately for each replicate of the study using a one-way ANOVA, Linear Trend Test, and Williams' and/or Dunnett's Multiple Comparison Test Nominal scale measures (% litters with resorptions, % litters with dead fetuses, % litters with nonlive implants, % litters with affected fetuses, % litters with gross malformations, % litters with visceral malformations, % litters with skeletal malformations, and % litters with malformations) were analyzed using non-parametric techniques. The Chi-Square Test for Independence was used to assess differences among treatment groups. When Chi-Square revealed significant ($p<0.05$) differences among treatment groups, a one-tailed Fisher's Exact Test was used to compare individual treatment groups to the control group When significant ($p<0.05$) replicate effects or dose x replicate effects were noted for parameters analyzed by ANOVA, associated nominal scale data were further analyzed for dose effects separately for each replicate of the study

Archive

Copies of the final study report, the original study data sheets, work sheets, and computer printouts generated in the statistical analysis of data, and all biological samples were stored at RTI Copies of the final report also were provided to NTP The study report indicates that records were to be maintained in accordance with the FDA GLP regulations for Nonclinical Laboratory Studies (1978) The data and reports were to be retained in storage for a minimum of two years The chemical and biological samples were to be retained in storage for a minimum of two years or for as long as the quality of the preparation affords evaluation, whichever was less

Critical Appraisal of the Protocol and Study Design

A protocol was included in the study report Overall, the study was adequately designed, and the study personnel were clearly indicated in the protocol (page 26) and study report (page 33).

Our reviewers noted several deficiencies in the study design with respect to the test article and vehicle Specifically, the storage conditions at RTI and the expiration date of the test article were not provided In addition, the lot/batch number, purity, stability, and expiration date of the vehicle were not provided Although the homogeneity and stability of sample dosing solutions were evaluated by the supplier (MRI), the actual dosing solutions were not analyzed for homogeneity or stability under the storage conditions used at RTI Degradation of the test article or the vehicle prior to preparation of the dosing solutions or degradation of the dosing solutions prior to administration could have affected the study results However, test articles samples from RTI were comparable to frozen reference samples maintained by the supplier (MRI) In addition, analysis of samples of the corn oil used to formulate each dosing suspension did not reveal any peroxides (an indicator of rancidity). Finally, pre-dosing and post-dosing analyses of the concentrations of the dosing formulations yielded values that were predominantly within the range of 90-110% of nominal. It should be noted, however, that the concentrations were not determined for the first batch of dosing solutions for replicate II of the study. Overall, however, our reviewers concluded that the integrity of the study was not affected by these deficiencies

Our reviewers also noted several deficiencies in the study design with respect to the test animals. For example, the age of the animals at mating was not specified in the study report The use of animals that are too young or too old could alter reproductive parameters The study report indicated that males were 10-12 weeks of age and females were 8-10 weeks of age upon arrival at the testing facility; however, the protocol indicated that both males and females were to be 8-10 weeks old upon arrival The age at mating could not be determined despite the fact that gd 0 dates were provided because the arrival date of the animals was not provided The lack of information on the exact age of the animals at mating is particularly problematic due to the use of two replicates for the definitive teratology study Unless two separate shipments of animals were received, the animals in replicate II would have been about two months older than the animals in replicate I Although the study report did not specify that two shipments were received, this possibility seems likely due to the number of animals in the initial shipment compared to the number of animals required. The study report indicated that 166 female CD rats were received from the breeder and used for both the preliminary range-finding study and the definitive teratology study, however, according to the data tables, 56 sperm-positive females were assigned to the preliminary study and 137 sperm-positive females were assigned to the definitive teratology study (i e , a total of 193 dams) A second group of animals could have been used for replicate II of the study These deficiencies and discrepancies may be an indication of incomplete data audit, incomplete quality control, or incomplete quality assurance review Nevertheless, they are considered by our reviewers to not have affected the interpretation of the study results

Our reviewers also noted several deficiencies in the study design with respect to animal husbandry In particular, the females were group-housed rather than housed individually. Standard toxicity testing guidelines suggest that females should be single-caged except during mating In addition, the temperature range was 69 0-73 0 °F, which was slightly outside the range specified in the protocol (70 ± 2 °F) The relative humidity range was 20-62% Although a target range was not specified in the protocol, the reported relative humidity value range is slightly outside the normal range Our reviewers were slightly more concerned with the low values for relative humidity due to the potential for increased

insensible water losses We noted that the lowest decreases in relative humidity were observed during Replicate II As detailed in the Results section of this TDER, Replicate II had higher treatment-related mortality compared to Replicate I, and no explanation for the increased mortality was provided by the study authors Increased insensible water loss is generally not a problem for healthy animals with adequate hydration, but may become a problem for high-dose animals on toxicity studies The observed fluctuations in temperature would not be expected to alter the outcome of the study. Finally, the study report did not include analyses of the animal diet and water for levels of contaminants. Although control and treatment animals presumably would have been exposed equally to any contaminants present in the diet and water, there is a potential for synergistic effects between potential contaminants and the test article

One deficiency was noted by our reviewers regarding administration of the dosing solutions. The body weight determination on which dosing was based was not specified Dosing is assumed to have been based on the most recent available body weight, and the animals were weighed daily during the treatment period

Our reviewers also noted several deficiencies in the study design regarding maternal toxicity parameters. Specifically, dams were checked for mortality and clinical signs of toxicity at least once daily prior to dosing The study report indicates that animals were also observed for mortality or clinical signs four hours post-dosing, when staff scheduling allowed. The study results did not indicate when, or if, this second daily check was made Nevertheless, standard toxicity testing guidelines suggest that dams should be observed a minimum of twice daily for mortality and clinical signs In addition, food consumption data were not recorded in this study Potential effects on maternal body weight could result from either a direct toxic effect of bisphenol A or from a decrease in food consumption due to the irritant properties of bisphenol A. According to the study report, weight depression in previously conducted chronic studies was attributed to significantly reduced food consumption (page 16).

Finally, our reviewers noted deficiencies in data reporting Specifically, individual animal data were not included in the study report for the following parameters· daily observations of dams for mortality and clinical signs, maternal body weight measurements, maternal organ weight determinations (gravid uterine weight and liver weight), gross necropsy findings, and all reproductive parameters (total number of corpora lutea, implantation sites, resorptions, dead fetuses, and live fetuses as well as fetal sex and fetal body weight). This lack of individual animal data for these parameters precludes verification/validation of the corresponding results reported.

STUDY RESULTS

Maternal Observations

Mortality and Clinical Observations

Two vehicle control dams were removed from the study· one following accidental death (punctured esophagus during dosing) and one following the discovery of a preexisting clinical condition (large urinary bladder stones and a shrunken left kidney) on gd 20 In addition, one 320-mg/kg/day dam was removed from the study because she received only one half the appropriate dose on gd 13 due to a malfunction of the gavage syringe

There were no treatment-related deaths in the 0, 160, 320, or 640 mg/kg/day groups Dams in the high-dose group (1280 mg/kg/day), however, had excessive mortality (26% or 7/27) Further analysis revealed that mortality in the high-dose group was replicate-dependent 10% (1/10) maternal mortality occurred in replicate I of the study, whereas 35% (6/17) maternal mortality occurred in replicate II of the study The study author noted that the cause of this replicate effect was not evident All subsequent study data were analyzed with and without the 1280-mg/kg/day dose group

Clinical signs for the 1280-mg/kg/day dose group were not included in the study report The predominant clinical signs observed in the 160, 320, and 640 mg/kg/day groups during the treatment period were lethargy, piloerection, pica, rough coat, wet urogenital area, clinical weight loss, and alopecia (320 and 640 mg/kg/day groups only) Other signs observed sporadically in the 160, 320, or 640 mg/kg/day groups included tremors, red-stained coat, dyspnea, chromodacryorrhea (bloody tears), vocalization, wheezing, and excess salivation Alopecia, piloerection, and weight loss also occurred sporadically in the vehicle control group

Body Weight

Maternal body weight data, analyzed without and with the high-dose group, are provided in Tables 2a and 2b, respectively In both analyses, dams did not differ significantly among treatment groups with respect to maternal body weight on gd 0 or on gd 6. Maternal body weight on gd 11 and on gd 15 exhibited a significant downward trend with respect to increasing dose when data were analyzed both with and without the 1280-mg/kg/day dose group Significant differences from the control group were noted in the 160-mg/kg/day, 320-mg/kg/day, 640-mg/kg/day, and 1280-mg/kg/day groups When analyzed without the 1280-mg/kg/day group, maternal body weight on gd 20 exhibited a significant dose-related decrease, but there was no significant effect for dose nor pairwise differences in treatment groups compared to the control. The results for maternal body weight on gd 20 differed, however, when the 1280-mg/kg/day group was included in the analysis A significant dose-related decrease in body weight occurred, and both the 640- and 1280-mg/kg/day groups were significantly below the control group.

Table 2a Mean Maternal Body Weight During Gestation (g)[a]

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day
GD 0	MEAN	234 37	238 69	238 24	236.64
	S E M	2 70	2 53	2 86	2.60
	N	23	26	24	29
GD 6	MEAN	260 61	261.82	260.91	258.73
	S E M.	2 62	2 64	3 11	2.94
	N	23	26	24	29
GD 11	MEAN	281 35†††	269.02*#	262 70**##	260.92**##
	S E M	3 05	3 41	3 85	3 20
	N	23	26	24	29
GD 15	MEAN	302 66†††	289 07*#	280 50**##	278 13**##
	S.E.M.	3 36	3 67	4 38	3.55
	N	23	26	24	29
GD 20	MEAN	356 55†	347.06	344 43	341.24
	S E M	5 32	4 19	4 48	3 76
	N	23	26	24	29

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included in body weight calculations
†Significant dose-related downward trend (p<0 05)
†††Significant dose-related downward trend (p<0 001)
#Significantly different from control, Williams' Test (p<0 05)
##Significantly different from control, Williams' Test (p<0 01)
*Significantly different from control, Dunnett's Test (P<0 05)
**Significantly different from control, Dunnett's Test (P<0 01)

Table 2b Mean Maternal Body Weight During Gestation (g)[a]

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
GD 0	MEAN S E M N	234 37 2 70 23	238 69 2 53 26	238 24 2 86 24	236 64 2 60 29	235 31 3 17 18
GD 6	MEAN S E M. N	260 61 2 62 23	261 82 2 64 26	260 91 3 11 24	258 73 2 94 29	256.91 3 21 18
GD 11	MEAN S.E.M N	281 35††† 3 05 23	269 02*# 3 41 26	262.70**## 3 85 24	260 92**## 3 20 29	240 76**## 4 20 18
GD 15	MEAN S E M N	302 66††† 3 36 23	289 07# 3 67 26	280.50**## 4 38 24	278 13**## 3 55 29	245.20**## 7 78 18
GD 20	MEAN S E.M N	356 55††† 5 32 23	347 06 4 19 26	344 43 4.48 24	341 24# 3 76 29	306 70**## 9.76 18

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included in body weight calculations

†††Significant dose-related downward trend (p<0 001)

#Significantly different from control, Williams' Test (p<0 05)

##Significantly different from control, Williams' Test (p<0 01)

*Significantly different from control, Dunnett's Test (P<0.05)

**Significantly different from control, Dunnett's Test (P<0.01)

Maternal body weight gain data, analyzed without and with the high-dose group, are provided in Tables 3a and 3b The two analyses yielded comparable results with slight differences in p-levels as noted in the tables A significant dose-related downward trend was noted for maternal weight gain during gestation, maternal weight gain during treatment, and absolute maternal weight gain (i e , weight gain during gestation minus gravid uterine weight) in both analyses All treatment groups were significantly different from the control group for all three parameters. In the 1280-mg/kg/day group, negative weight gain (i e., overall loss of weight with respect to gd 0 values) was observed during the treatment period

Table 3a Mean Maternal Body Weight Gain During Gestation (g)[a]

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day
Gestation (gd 0-20)	MEA N S.E M N	122 17†† 4 36 23	108 37*## 3 25 26	106.20**## 3.39 24	104 60**## 2 87 29
Treatment (gd 6-15)	MEA N S E M N	42 05††† 1 78 23	27 25**## 2 27 26	19 60**## 2 91 24	19.40**## 2 42 29
Absolute[b]	MEA N S E M N	50 32††† 2 49 23	36 36**## 2 58 26	37.44**## 2 13 24	33.37**## 2.49 29

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included in body weight calculations

[b]Weight gain during gestation minus gravid uterine weight

††Significant dose-related downward trend (p<0 01)

†††Significant dose-related downward trend (p<0 001)

##Significantly different from control, Williams' Test (p<0.01)

*Significantly different from control, Dunnett's Test (P<0 05)

**Significantly different from control, Dunnett's Test (P<0 01)

Table 3b. Mean Maternal Body Weight Gain During Gestation (g)[a]

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Gestation (gd 0-20)	MEAN SEM N	122 17††† 4 36 23	108 37# 3 25 26	106 20# 3 39 24	104 60*## 2 87 29	71 39**## 9 95 18
Treatment (gd 6-15)	MEAN SEM N	42.05††† 1 78 23	27.25*## 2 27 26	19 60**## 2 91 24	19 40**## 2 42 29	-11 71**## 8 20 18
Absolute[b]	MEAN SEM N	50 32††† 2 49 23	36.36**## 2 58 26	37 44*## 2 13 24	33 37**## 2.49 29	12 56**## 5 49 18

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included in body weight calculations

[b]Weight gain during gestation minus gravid uterine weight

†††Significant dose-related downward trend (p<0 001)

#Significantly different from control, Williams' Test (p<0 05)

##Significantly different from control, Williams' Test (p<0 01)

*Significantly different from control, Dunnett's Test (P<0.05)

**Significantly different from control, Dunnett's Test (P<0.01)

The following statistically significant replicate-dependent body weight results were observed only when the 1280 mg/kg/day group was included (1) significant differences between replicates for maternal body weight on gd 0 and absolute maternal weight gain and (2) significant dose by replicate interactions for maternal body weight on gd 20, maternal weight gain during gestation, and maternal weight gain during treatment The parameters that exhibited dose by replicate interactions were reexamined by replicate. These results are presented in Table 4 For maternal body weight on gd 20 and maternal weight gain during gestation, significant dose effects were noted in replicate II only. Specifically, significant dose related downward trends and significant differences among dose groups were observed for both parameters with the 640-mg/kg/day and 1280-mg/kg/day groups significantly lower than the control group in pairwise comparisons For maternal body weight gain during treatment, significant dose effects were noted in both replicate I and replicate II. Significant dose related downward trends and significant differences among dose groups were observed in both replicates with all treatment groups significantly lower than the control group in pairwise comparisons The statistical significance levels varied slightly between replicate I and replicate II for this parameter In addition, negative weight gain was observed during the treatment period in the 1280-mg/kg/day group for replicate II only

Table 4. Analysis by Replicate of Body Weight Parameters that Exhibited Dose by Replicate Interactions

Replicate		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Maternal Body Weight on gd 20 (g)						
I	MEA N S.E.M. N	349.61 12 07 9	342 62 7 98 10	333 66 9 21 8	340 56 5 73 11	324 92 7 19 8
II	MEA N S.E.M N	361 01††† 4 14‡‡‡ 14	349 83 4 72 16	349 82 4 53 16	341 66# 5 06 18	292 11**## 15 44 10
Maternal Weight Gain During Gestation (g)						
I	MEA N S E M. N	121 25 8 99 9	104 56 5 67 10	98.23 7 86 8	107 87 3 62 11	95.11 5.53 8
II	MEA N S E M. N	122 77††† 4 56‡‡‡ 14	110 75 3 95 16	110 18 2 98 16	102 60*# 4 07 18	52 41**## 15.09 10
Maternal Weight Gain During Treatment (g)						
I	MEA N S E M N	40 70††† 1 60‡‡ 9	22.92# 2 92 10	23 30# 4 68 8	21.79*# 4.86 11	5.15**## 9.87 8
II	MEA N S E M N	42 92††† 2 77‡‡‡ 14	29 96# 3.07 16	17.75**## 3 69 16	17 93**## 2 58 18	-25 19**## 11 08 10

†††Significant dose-related downward trend (p<0 001)
‡‡Significant difference among dose groups (p<0 01)
‡‡‡Significant difference among dose groups (p<0.001)
#Significantly different from control, Williams' Test (p<0 05)
##Significantly different from control, Williams' Test (p<0 01)
*Significantly different from control, Dunnett's Test (P<0 05)
**Significantly different from control, Dunnett's Test (P<0 01)

Food Consumption

Food consumption data were not recorded in this study

Maternal Necropsy Findings

Pinworms, fat deposits fused to kidneys, and mottled kidneys were reported as clinical signs on gd 20 in the study report, there was no relationship to treatment The nature of these reported clinical signs, however, suggests that they were observed during the dissection following sacrifice Animals that died on study were to have been necropsied, however, these findings were not included in the study report

Reproductive Parameters/Organ Weights

Data on reproductive parameters and organ weights, analyzed without and with the high-dose group, are provided in Tables 5a and 5b, respectively When analyzed without the high-dose group, there were no significant effects on any of the reproductive parameters or organ weights Inclusion of the 1280-mg/kg/day group resulted in a significant dose-related upward trend in the per litter percent of resorptions, dead fetuses, and nonlive implants, however, there were no significant differences among treatment groups in the ANOVA and no significant pairwise differences between treatment groups and the control group. In addition, the average live fetal body weight (males and females combined) per litter and the average live male fetal body weight per litter exhibited a significant dose-related downward trend. Again, there were no significant differences among treatment groups in the ANOVA and no significant pairwise differences between treatment groups and the control group Average live female fetal body weight per litter was not significantly affected by treatment. Gravid uterus weight exhibited a significant dose-related downward trend although there were no significant differences among treatment groups in the ANOVA and no significant pairwise differences between treatment groups and the control group. Finally, relative liver weight exhibited a significant dose-related upward trend with the 1280-mg/kg/day group significantly higher than the control group

Table 5a. Reproductive Parameters and Organ Weights[a]

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day
Subjects					
Number Treated		27	27[b]	27	29[b]
Number Removed		2[c]	0	1[d]	0
Number Dead		0	0	0	0
Number (%) Pregnant at Sacrifice		23 (92.0)	26 (96 3)	24 (92.3)	29 (100 0)
All Litters					
Number of Litters		23	26	24	29

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day
Number of Corpora Lutea per Dam	Mean S E M	16 74 0 77	16 12[e] 0 78	15 83 0 59	16 00[e] 0 77
Number of Implantation Sites per Litter	Mean S E M	13 48 0 63	13 58 0 44	13.71 0 52	14 07 0 45
Preimplantation Loss Percentage	Mean S E.M	16 56 4 27	13.99 3.02	13 72 3 68	12 03 2 75
Percent Resorptions per Litter	Mean S E.M	7 54 4 39	3 13 0 93	6 48 1 33	6 85 2 06
Percent Dead per Litter	Mean S E.M	0 00 0 00	0 00 0 00	0 00 0 00	0 00 0 00
Percent Nonlive per Litter[f]	Mean S E M	7 54 4 39	3 13 0 93	6 48 1 33	6 85 2 06
Live Litters					
Number of Litters with Live Fetuses		22	26	24	29
Number of Live Fetuses per Litter[g]	Mean S E M	13 50 0 36	13 15 0 45	12 79 0 50	13 07 0 50
Percent Male Fetuses per Litter	Mean S.E.M.	45 45 2 81	48 61 3.23	43.22 2 10	47.83 2.78
Average Fetal Weight (g) per Litter[g]	Mean S E M	3 495 0.56	3 453 0.052	3.386 0 045	3 444 0 061
Average Male Fetal Weight (g) per Litter[g]	Mean S E M	3 590 0 062	3 538 0 054	3 458 0 049	3.516 0 064
Average Female Fetal Weight (g) per Litter[g]	Mean S E M	3 421 0 053	3 387 0 052	3 331 0 050	3 366 0 058

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day
Organ Weights					
Gravid Uterus Weight (g)	Mean S E M N	71 86 3 70 23	71.98[h] 2 51 26	68 76 2 61 24	71.23 2 70 29
Maternal Liver Weight (g)	Mean S E.M. N	15 38 0.27 23	14 52 0 22 26	15 03 0 30 24	14.69 0 30 29
Relative Maternal Liver Weight (% body weight)	Mean S.E M N	4.32 0.06 23	4.20 0.08 26	4 36 0 07 24	4 31 0.08 29

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included

[b]One dam observed to have dosing formulation running out of the mouth and/or nose just after dosing on gd 9

[c]One dam removed following accidental death (punctured esophagus during dosing); one dam removed following the discovery of a preexisting urinary tract condition (large urinary bladder stones and a shrunken left kidney) at sacrifice

[d]Dam removed after receiving half the appropriate dose on gd 13 due to a malfunction of the gavage syringe.

[e]Number of corpora lutea for one dam was not recorded

[f]Nonlive includes both dead and resorbed fetuses

[g]Includes only dams with live fetuses.

[h]Gravid uterine weight for one dam was not recorded

Table 5b. Reproductive Parameters and Organ Weights[a]

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Subjects						
Number Treated		27	27[b]	27	29[b]	27
Number Removed		2[c]	0	1[d]	0	0
Number Dead		0	0	0	0	7
Number (%) Pregnant at Sacrifice		23 (92.0)	26 (96 3)	24 (92 3)	29 (100 0)	18 (90.0)

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
All Litters						
Number of Litters		23	26	24	29	18
Number of Corpora Lutea per Dam	Mean S E M	16 74 0 77	16 12[e] 0 78	15 83 0 59	16.00[e] 0 77	15.06 0.76
Number of Implantation Sites per Litter	Mean S.E.M	13 48 0 63	13 58 0 44	13 71 0 52	14 07 0 45	13.67 0 48
Preimplantation Loss Percentage	Mean S E M	16 56 4.27	13 99 3.02	13.72 3 68	12.03 2 75	8.83 2 52
Percent Resorptions per Litter	Mean S E.M	7.54† 4.39	3.13 0 93	6 48 1 33	6 85 2.06	17 86 7 34
Percent Dead per Litter	Mean S E M	0.00† 0.00	0.00 0 00	0 00 0 00	0 00 0 00	0 43 0 43
Percent Nonlive per Litter[f]	Mean S E.M	7 54†† 4 39	3 13 0 93	6.48 1.33	6 85 2.06	18 28 7.29
Live Litters						
Number of Litters with Live Fetuses		22	26	24	29	16
Number of Live Fetuses per Litter[g]	Mean S E M.	13 50 0 36	13.15 0.45	12 79 0 50	13 07 0 50	12.50 0 58
Percent Male Fetuses per Litter	Mean S E M	45 45 2.81	48 61 3 23	43.22 2 10	47.83 2 78	48 50 3 34
Average Fetal Weight (g) per Litter[g]	Mean S.E.M	3 495† 0 56	3.453 0.052	3 386 0 045	3 444 0.061	3 259 0 102
Average Male Fetal Weight (g) per Litter[g]	Mean S.E.M	3 590† 0.062	3 538 0 054	3.458 0 049	3 516 0.064	3.314 0 096
Average Female Fetal Weight (g) per Litter[g]	Mean S E M	3 421 0 053	3.387 0.052	3 331 0.050	3.366 0 058	3 225 0 103

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Organ Weights						
Gravid Uterus Weight (g)	Mean S E.M N	71 86†† 3.70 23	71 98[h] 2 51 26	68 76 2 61 24	71 23 2 70 29	58.83 5 53 18
Maternal Liver Weight (g)	Mean S E M N	15 38 0 27 23	14.52 0 22 26	15.03 0.30 24	14.69 0.30 29	14 44 0 34 18
Relative Maternal Liver Weight (% body weight)	Mean S E.M N	4 32††† 0.06 23	4 20 0 08 26	4.36 0.07 24	4.31 0 08 29	4 77** 0 16 18

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included

[b]One dam observed to have dosing formulation running out of the mouth and/or nose just after dosing on gd 9

[c]One dam removed following accidental death (punctured esophagus during dosing); one dam removed following the discovery of a preexisting urinary tract condition (large urinary bladder stones and a shrunken left kidney) at sacrifice

[d]Dam removed after receiving half the appropriate dose on gd 13 due to a malfunction of the gavage syringe

[e]Number of corpora lutea for one dam was not recorded

[f]Nonlive includes both dead and resorbed fetuses.

[g]Includes only dams with live fetuses

[h]Gravid uterine weight for one dam was not recorded

†Significant dose-related downward trend (p<0 05)

††Significant dose-related downward trend (p<0 01)

†††Significant dose-related downward trend (p<0 001)

**Significantly different from control, Dunnett's Test (P<0 01)

The following statistically significant replicate-dependent reproductive and organ weight results were observed only when the 1280 mg/kg/day group was included: (1) significant differences between replicates for number of corpora lutea per dam and (2) significant dose by replicate interactions for percent of nonlive implants per litter, number of live fetuses per litter, percent affected fetuses per litter, and gravid uterus weight The parameters that exhibited dose by replicate interactions were reexamined by replicate These results are presented in Table 6 For percent of nonlive implants per litter and gravid uterus weight, significant dose effects were noted in replicate II only Specifically, a significant dose related upward trend in the percent of nonlive implants per litter was observed with the 1280-mg/kg/day group significantly higher than the control group in pairwise comparisons. For gravid uterus weight, there was a significant dose-related downward trend, significant differences among treatment groups, and a significant decrease in the 1280-mg/kg/day group compared to the control group There were no significant dose effects on the number of live fetuses per litter when analyzed by replicate

Table 6 Analysis by Replicate of Reproductive and Organ Weight Parameters that Exhibited Dose by Replicate Interactions

Replicate		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Percent of Nonlive Implants per Litter						
I	MEA N S E M N	13 83 10 92 9	3 07 1 29 10	5 54 2.22 8	5 40 2 10 11	5 13 2 85 8
II	MEA N S E.M. N	3 50††† 1 77 14	3 17 1 31 16	6 95 1 70 16	7.74 3 10 18	28 80**## 12.17 10
Number of Live Fetuses per Litter						
I	MEA N S E M N	14 13 0.52 8	12 70 0 82 10	11 38 1.13 8	12.27 0 92 11	13 75 0 53 8
II	MEA N S.E M N	13 14 0 47 14	13 44 0 52 16	13 50 0.43 16	13 56 0 56 18	11 25 0.84 8
Percent of Affected Fetuses per Litter						
I	MEA N S E.M N	24 70 10.11 9	6 56 3 30 10	15.74 5.80 8	9 53 2.36 11	7 63 3.27 8
II	MEA N S E M N	8 00††† 1 82‡‡ 14	8 62 2.17 16	13.96 2 62 16	12.91 3 20 18	34.16** 11 80 10

Replicate		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Gravid Uterus Weight (g)						
I	MEA N S E M. N	69 29 8 83 9	68 17 4 75 10	62.02 6 17 8	67.60 4 50 11	71 75 2 89 8
II	MEA N S.E.M. N	73 51††† 2.57‡‡‡ 14	74 51 2 69 15	72 13 2 12 16	73 44 3 36 18	48 49** 8 50

†††Significant dose-related downward trend (p<0.001)
‡‡Significant difference among dose groups (p<0.01)
‡‡‡Significant difference among dose groups (p<0 001)
##Significantly different from control, Williams' Test (p<0 01)
**Significantly different from control, Dunnett's Test (P<0 01)

Histopathology

Tissues were not examined histologically

Fetal Observations

The results of analyses of fetal parameters were comparable with and without the 1280-mg/kg/day group, and therefore, are not presented separately These results are shown in Table 7. The percent of fetuses malformed per litter, percent of male fetuses malformed per litter, and percent of female fetuses malformed per litter were comparable among treatment groups. There were no significant replicate effects for these parameters

Table 7. Summary of Fetal Observations

		0 mg/kg/day	160 mg/kg/day	320 mg/kg/day	640 mg/kg/day	1280 mg/kg/day
Number of Fetuses Examined[a]		297	342	307	379	200
Number of Litters Examined[b]		22	26	24	29	16
Percent Fetuses Malformed per Litter	MEAN S E M	7 47 1 85	4 90 1 54	8 51 2 45	4 98 1.26	5.37 2 11
Percent Male Fetuses Malformed per Litter	MEAN S E.M.	6 50 2 11	5 30 1 84	7 58 2 67	4 69 1 70	5 47 3 11
Percent Female Fetuses Malformed per Litter	MEAN S E.M	7 71 2.08	5.00 2 33	9 38 2 92	4 02 1 31	3 26 1 96

[a]Only live fetuses were examined for malformations.
[b]Only litters with live fetuses were included

Fetal External Examination

The occurrence of external malformations was not evaluated statistically In total, external malformations were observed in two control fetuses (two litters), one 160-mg/kg/day fetus, and one 320-mg/kg/day fetus, there were no external malformations in 640-mg/kg/day and 1280-mg/kg/day fetuses Grossly observed changes occurred sporadically and included small right ear, bilateral anophthalmia, constriction of the tail, edema, absent oral and nasal orifices, microdactyly (small digits), and anal atresia (no anal opening). The total fetal and litter incidences of external variations were not provided, nor were the variations reported divided into external, visceral, and skeletal categories External variations appear to have consisted of hematomas on the back, jaw, lower limb, face, head, neck, abdomen, tail, and upper limb

Fetal Visceral Examination

The occurrence of visceral malformations was not evaluated statistically In total, visceral malformations were observed in 16 control fetuses (8 litters), 15 160-mg/kg/day fetuses (9 litters), 10 320-mg/kg/day fetuses (7 litters), 12 640-mg/kg/day fetuses (8 litters), and 5 1280-mg/kg/day fetuses (3 litters). Hydroureter and hydronephrosis were the most common findings. The total fetal and litter incidences of visceral variations were not provided, nor were the variations reported divided into external, visceral, and skeletal categories Visceral variations appear to have consisted of distended ureter(s), hemorrhagic adrenals, globular heart, and very soft kidney tissue.

Fetal Skeletal Examination

The occurrence of skeletal malformations was not evaluated statistically In total, skeletal malformations were observed in seven control fetuses (five litters), three 160-mg/kg/day fetuses (two litters), twelve 320-mg/kg/day fetuses (six litters), eight 640-mg/kg/day fetuses (four litters), and five 1280-mg/kg/day fetuses (four litters) Short rib was the most common finding The total fetal and litter incidences of skeletal variations were not provided, nor were the variations reported divided into external, visceral, and skeletal categories. Skeletal variations appear to have consisted of bipartite centra, wavy rib, incomplete ossification of the parietals and/or interparietals, misaligned sternebrae, extra ossification site(s), frontals and parietals fused on lateral edges, and small or missing pubis

ASSESSMENT

The purpose of this study was to evaluate the toxic and teratogenic effects of the test material (bisphenol A) administered orally to pregnant rats during the period of organogenesis (gestation days 6-15) The deficiencies and deviations in experimental design were discussed above. The lack of food consumption data and the low relative humidity are the major deficiencies noted by our reviewers The effects on body weight observed in the dams may have been due to a treatment-related decrease in food consumption or increased insensible water losses However, our reviewers comment that the study data clearly indicate that high doses of bisphenol A are toxic and are associated with increases in mortality ranging from 10% (preliminary study) to 26% (definitive teratology study) In contrast, at lower doses of the test article, decreases in food consumption and insensible water loss might explain decreases in body weight Our reviewers noted that other study design deficiencies were not likely to have affected the study results

CONCLUSIONS

The administration of the test article, bisphenol A, in corn oil resulted in 26% mortality in the high-dose group (1280 mg/kg/day), but no treatment-related deaths in other dose groups Statistical analysis of these data indicated that this effect was replicate-dependent, other maternal toxicity parameters also exhibited replicate-dependent effects in the high-dose group. Therefore, the study author decided to exclude all high-dose group data from their conclusions to avoid making erroneous determinations regarding the teratogenic/toxic potential of bisphenol A Accordingly, the study author concluded that bisphenol A doses of 160, 320, or 640 mg/kg/day caused maternal toxicity as evidenced by significant dose-related effects on maternal body weight and body weight gain The study author further concluded that bisphenol A doses of 160, 320, or 640 mg/kg/day had no embryotoxic, fetotoxic, or teratogenic effects Our reviewers agree with the study author's conclusions but note, however, that the maternal body weight effects can not be clearly attributed to the direct toxic effect of bisphenol A in the absence of food consumption data and given the low relative humidity Our reviewers also note that the study conclusions would not be altered by inclusion of the high-dose group results Therefore, the LOAEL for maternal toxicity is 160 mg/kg/day based on body weight effects, no maternal NOAEL can be established The NOAEL for developmental effects is 1280 mg/kg/day, no developmental LOAEL could be established

EXECUTIVE SUMMARY

In a developmental toxicity study, bisphenol A in corn oil was administered to mated female COBS CD® (SD)BR outbred albino rats (27-29/dose) by gavage at dose levels of 0, 160, 320, 640, or 1280 mg/kg/day from days 6 through 15 of gestation. The high-dose group exhibited 26% mortality, there were no treatment-related deaths in other dose groups Bisphenol A doses of 160, 320, 640, or 1280 mg/kg/day resulted in significant dose-related effects on maternal body weight (gd 11 and gd 15) and body weight gain (gestation, treatment, and absolute) Because food consumption data were not recorded, it is unclear whether these body weight effects are the result of direct bisphenol A toxicity or the result of treatment-related decreases in food consumption In addition, insensible water loss resulting from the low relative humidity could have contributed to body weight effects. There were no embryotoxic, fetotoxic, or teratogenic effects at any dose level Therefore, the LOAEL for maternal toxicity is 160 mg/kg/day based on body weight effects, no maternal NOAEL can be established. The NOAEL for developmental effects is 1280 mg/kg/day, no developmental LOAEL could be established

Teratologic Evaluation of Bisphenol A (CAS No 80-05-7) Administered to CD(R) Rats on Gestational Days 6 through 15 (NTP-85-089)

Technical Reviewer:

/s/

/s/

Date. 4-26-01

Jennifer Welham, M S

QA/QC Reviewer:

/s/

/s/

Date: 4-30-01

Jennifer Rojko, D V.M , PhD , D A.C V P

Appendix A. Data Validation

Teratologic Evaluation of Bisphenol A (CAS No. 80-05-7) Administered to CD® Rats on Gestational Days 6 through 15 (NTP-85-089)

I. ANIMALS FOLLOWED THROUGHOUT THE STUDY

The limited nature of the data provided for individual animals in the study precluded the traditional random review throughout the study. Observed fetal malformations and variations were provided in Table 14 (also in Table I-5, Appendix I), as well as the maternal weight gain during treatment and gestation (Appendix VI), but there were no means by which to validate these data

II. CRITICAL EFFECTS AND SPOTCHECKS

A comparison of the study Materials and Methods section to the study Protocol was done. The initial Protocol was amended as noted in Appendix II of the study. The amended Protocol called for males and females, approximately 8-10 weeks of age upon arrival. The Materials and Methods section noted males to arrive between 10-12 weeks of age and females arrived at 8-10 weeks of age. The protocol also called for a 5ml aliquot of the dosing formulations to be analyzed to verify concentration The study used a 1ml aliquot to verify concentrations. In addition, the amended Protocol said that Duncan's Multiple Range Test would be used for intergroup comparison when significant main effects occurred and the Materials and Methods section noted that Williams' and/or Dunnett's Multiple Comparison Test was used.

The limited nature of the data provided for individual animals in the study restricted the validation of mean values, incidence rates, and statistical analyses. A limited number of checks were made when data were available. Using the fetal malformation and variation data provided (Table 14), a spotcheck of the summary incidence rates (Table 13) showed no inconsistencies. The summary data (Table 9) for Maternal Weight Gain during gestation and treatment (data found in Appendix VI), were checked for all four dosing groups (0, 160, 320, and 640 mg/kg/day). The one minor issue noted was that data was listed for 25 dams (in the 320 mg/kg/day group) in Appendix VI. This differed from the 24 pregnant dams on which the mean value and all other calculations were made (as noted in Table 9). The data in Table 9 did not seem to be compromised, as the mean value appeared to exclude that extra data point.

III. FINDINGS

The stated findings of the study appear to be accurately reflected in the data provided. The deviations from the protocol do not appear to effect the validity of the study results. The limited amount of data actually presented did not allow for a complete and random validation effort. Even so, there appears to be no reason to doubt the validity of the study results.

/S/

/s/

Siddha Parker, B.A

8/1/00

Date

AUG 16 2001

Contract Number

223-96-2302

Work Assignment Number

20000-19
(ICF 419)

Task Number

04

FDA Study Identification Number

NTP-85-192

Signature of Project Manager

/s/

/s/

Date

8-7-01

STUDY TITLE

Bisphenol A: Reproduction and Fertility Assessment in CD-1 Mice
When Administered in the Feed

TESTING LABORATORY

Research Triangle Institute
Chemistry and Life Sciences Group
Life Sciences and Toxicology Division
P.O Box 12194
Research Triangle Park, NC 27709

COMPLETION DATE OF STUDY

May 31, 1985

SPONSOR OF STUDY

National Toxicology Program
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

LABORATORY STUDY NUMBER

RTI #31U-2344

STUDY SUMMARY

Compliance Statement

The study protocol indicated that the study would be conducted according to FDA GLP standards The study report was signed by the Study Director on May 29, 1985, but did not contain a specific compliance statement.

Quality Assurance Statement

A quality assurance (QA) statement was included with the study and was signed by the Quality Assurance Unit on August 7, 1985 The QA statement indicated that the final report and final data audits were performed August 1-2, 1985 [1]

Study Objective

The study objective was to evaluate the effects of bisphenol administered in the diet to CD-1 mice on mating performance, fertility, and pregnancy in the F0 generation The study was conducted according to NTP's "Fertility Assessment by Continuous Breeding" which consists of four tasks. Task 1 is a 14-day repeat dose range-finding study. Task 2 is the continuous breeding phase to ascertain effects on reproduction in the F0 animals. Task 3 is only conducted when positive findings are noted in Task 2, and consists of cross-over breeding of the control and high-dose F0 animals to determine the affected sex. Task 4 is only conducted if no effects are noted in Task 2, and evaluates reproduction in the F1 animals from the control and high-dose groups. In this study, Tasks 1-4 were conducted

MATERIALS AND METHODS

Reviewer's Note: There was little information regarding materials and methods in the report narrative. The information presented in the TDER was inferred from the materials and methods described in the study protocol and report appendices.

Test Article

Description The study protocol (Section 5 of the NTP Report) stated that the source of the Test Material (Bisphenol A) was Alpha Products (Lot No 011681) The Quality Assurance Statement (page 8 of the NTP Report) stated that the bisphenol A was obtained from Dow Chemical Company (Lot No. DC 6-24-75, MRI Batch No. 02) via Midwest Research Institute (MRI) The NTP Report No. 85-192 does not state whether the Protocol was amended. Therefore, the source and Lot Number of the Bisphenol A that was used to prepare the test diets is not clearly stated in the NTP Report No. 85-192.

Analysis of Purity, Homogeneity and Stability. In the NTP Report there is a MRI report of analysis, homogeneity, and stability on bisphenol A (Lot No DC 6-24-75) which was obtained from Dow Chemical Company. The NTP Report did not provide a similar analysis for bisphenol A from Alpha

---

[1] Our reviewers note that it is unusual to have the data and the report audited after the report was issued (i.e , date of the Study Director's signature). There was no explanation provided in the report

Products MRI conducted spectrophotometric and chromatographic analyses of the compound, and determined that the test article was approximately 95% pure. For the analysis of heat stability, samples of bisphenol A were stored for 2 weeks at -20°C, 5°C, 25°C, and 60°C. Analysis of these samples by vapor-phase chromatography showed that bisphenol A was stable for 2 weeks at temperatures up to 60°C. For analysis of homogeneity, bisphenol A was mixed with Wayne Lab-Blox Rodent Feed in a blender for one hour. During the blending period, a sample from the top of each shell and one from the bottom center trap were removed at 15 minute intervals and analyzed by vapor-phase chromatography. MRI concluded that the mixture of 10% bisphenol A in feed is homogeneous after 15-30 minutes in the blender For analysis of storage, another batch of 10% bisphenol in feed was produced with 30 minutes of mixing in the blender. Samples were removed and stored for 2 weeks at -20°C, 5°C, 25°C, and 45°C Analysis of these samples by vapor-phase chromatography showed that bisphenol A mixed with feed was stable for 2 weeks at temperatures up to 45°C The certificate of analysis is provided in Section 6 of the study report.

Diet Preparation. The basal diet was NIH-07 open formula ground rodent chow supplied by Zeigler Brothers, Gardners, PA. The test article was mixed with an aliquot of the ground rodent chow in a beaker with a spatula This aliquot was then added to the remainder of the rodent chow and blended for 20 minutes in an eight quart Patterson-Kelley Liquid-Solids Twin Shell Blender. The diets were prepared weekly and stored at 4°C. Diets were color- and number-coded to ensure that all personnel engaged in in-life data collection were blinded to dose.

Reserve Samples A sample of the test and control diets were taken prior to the initiation of Task 1 and at weeks 1, 6, 12 and 18 of Task 2. The samples were shipped to MRI to analyze the concentrations and stability of the test article in the diets. The results are presented below in Table 2 (Task 1) and Tables 5-6 (Task 2). The certificates of analysis are provided in Section 6 of the study report

Animals

Receipt and Acclimation. A total of 300 COBS Crl:CD-1, (ICR)BR mice, 150/sex, were supplied by Charles River Breeding Laboratories, Inc., Kingston, NY on August 31, 1984. The choice of test animal species and strain was justified in the study protocol. The animals were 6 weeks of age at the time of receipt. Animals that were to be used for Task 1 were quarantined for two weeks and animals that were to be used for Task 2 were isolated for five weeks. After two weeks of quarantine, two males and two females were sacrificed Serum samples were obtained and shipped to Microbiological Associates, Inc., Bethesda, MD for determination of antibodies (indicative of prior exposure/infection) to 11 mouse viruses The study report states that all sera were negative, but the report from Microbiological Associates was not included.

Housing, Diet and Environment During the quarantine period, 10 animals per sex were caged together. During Task 1, four animals per sex were housed together. During the premating period of Task 2, 5 animals per sex were caged together During the breeding phases of Tasks 2 and 3, breeding pairs were housed together, and during the subsequent 21-day segregation phase, the animals were caged individually In Task 4, the pups that were chosen for the mating phase were housed in groups of 2-3 mice per sex. During the breeding phases of Task 4, breeding pairs were housed together, and during the subsequent 21-day segregation phase, the animals were caged individually The rats were housed in solid bottom polypropylene or polycarbonate cages with stainless steel wire lids All cages were supplied with Ab-Sorb-Dri bedding which was supplied by Laboratory Products, Inc., Garfield, NJ, bedding was

changed weekly The animals were supplied with 14 hours of light and 10 hours of dark; the photoperiod was automatically controlled The room temperature and humidity were monitored with an electronic hygrothermograph supplied by Omnidata International, Inc., Logan, UT. The study protocol stated that room temperatures would be maintained at 70 ± 2°F, but the study report did not specify the actual range or the range of humidity during the study. The air was exchanged 12-14 times per hour

The animals were provided food and water *ad libitum.*

Experimental Design

Assignment to Study Group and Dose Selection. The mice were weight-stratified and randomly assigned to control and treatment groups. The dose levels for Task 1 were based on information available in the literature, and the dose levels for Task 2 were based on the results of Task 1. For Task 1, 8 mice/sex/group were administered dietary concentrations of 0, 0.31, 0 62, 1.25, 2.5 and 5% bisphenol A For Task 2, there were 40 animals/sex in the control group and 20 animals/sex in each treatment group, and the dietary concentrations were 0, 0.25, 0 5 and 1% bisphenol A. The animals in the control and 1% groups were retained for Task 3. For Task 4, the F1 pups from the last litter produced in Task 2 were retained. For the control and treated groups in Task 4, 20 male pups and 20 female pups were randomly selected to include 2 pups per sex from each of 10 randomly selected litters if possible. Our reviewers note that the process of dose selection for each task was clearly outlined in the study protocol and the report narrative.

Dosing Period. The Task 1 animals were 8 weeks of age at the beginning of treatment and were treated for 14 days The Task 2 animals were 11 weeks of age at the beginning of treatment. The animals were treated for 7 days before mating, throughout the 98 day breeding phase and during the subsequent 21 day segregation phase. For Task 3, the control and high-dose animals from Task 2 were retained Following the 21 day segregation phase of Task 2, treatment of Task 3 animals was discontinued for the one week cohabitation phase. The Task 3 animals were then placed back on treatment for 21 days For Task 4, the F1 pups from the last litter of the animals in Task 2 were retained. These animals were placed on the appropriate diets at weaning and these diets were administered for the duration of Task 4.

Clinical Observations

During the study all animals were examined twice daily for mortality, morbidity, and signs of toxicity. A complete physical examination was conducted once weekly.

Body Weight Measurements

The Task 1 animals were weighed at the beginning of the study and weekly thereafter. Body weights of Task 2 animals were recorded at weeks 0, 1, 2, 5, 9, 13 and 18. The Task 2 females were also weighed on the day of delivery. Body weights of the Task 3 animals were recorded weekly for the 4-week duration of Task 3. The Task 4 animals were weighed at birth, weaning, at commencement of the 7-day cohabitation phase at day 74 ± 10, and weekly thereafter.

Food Consumption
For Task 1, food consumption was recorded weekly For Task 2, food consumption was recorded for weeks 1, 2, 5, 9, 13 and 18. For Task 3 and Task 4, food consumption was recorded weekly during the segregation phase

Breeding Procedures

After the 7-day premating phase of Task 2, each female was paired with a male from the same treatment group for 98 consecutive days. The females were allowed to deliver each litter. The pups were sacrificed within 16 hours of birth so that the breeding pairs could mate again at postpartum estrus. The number and proportion of fertile pairs and the number of litters produced per breeding pair were recorded. The last litters produced during Task 2 were retained for Task 4.

The animals in the control and 1% groups of Task 2 were retained for Task 3 Immediately following the 21 day segregation phase of Task 2, the males in the 1% group were randomly paired with half of the control females and the females in the 1% group were randomly paired with half of the control males Each remaining control female was randomly paired with the remaining control males. The animals were cohabited for one week. The females were examined each morning for evidence of mating (presence of a vaginal copulatory plug). The breeding pairs were separated after one week. Vaginal cytology was performed for 5 days on any females that did not have a copulatory plug and were not pregnant by day 144

The F1 animals from the last litter of Task 2 were retained for Task 4. When the F1 animals were 74 $\pm$ 10 days of age, each female was paired with a male from the same treatment group for one week. The females were examined each morning for evidence of mating (presence of a vaginal copulatory plug). The breeding pairs were separated after one week. Vaginal cytology was performed for 5 days on any females that did not have a copulatory plug and did not appear pregnant 16 days after separation of the breeding pairs.

Litters

For each litter produced in Tasks 2, 3 and 4, the live offspring were sexed, counted, pooled and weighed by sex The dead pups were counted, but their sex and weights were not recorded. The animals in the last litter produced during Task 2 were retained for Task 4; their body weights were also recorded at weaning on day 21. The other pups produced in Task 2, as well as those produced in Tasks 3 and 4 were sacrificed and discarded without necropsy.

Scheduled Necropsy Examinations

After 14 days of treatment, the Task 1 animals were sacrificed by asphyxiation with CO_2 and discarded without necropsy. At the end of Task 2, the animals in the 0 25 and 0.5% groups were sacrificed by asphyxiation with CO_2 and discarded without necropsy.

At the end of Tasks 3 and 4, the animals were sacrificed by asphyxiation with CO_2 and terminal body weights were recorded. For males, the weights of the liver, kidneys (weighed together with the adrenals), left testis with attached epididymis, right testis, right epididymis, seminal vesicles (with coagulating glands) and prostate gland were recorded. For females, the weights of the liver, kidneys (weighed

together with the adrenals) were recorded. The uterus with the upper half of the vagina, liver, kidneys/adrenals, seminal vesicles, prostate, brain and pituitary,[2] and partly eviscerated carcass were fixed in 10% neutral-buffered formalin The ovaries and oviducts, the left testis with attached epididymis and right testis were fixed in Bouin's solution. The right cauda epididymis was excised and retained for sperm analyses, the remaining right caput and corpus epididymis were fixed in Bouin's solution. The weight of the cauda was recorded.

For analysis of sperm motility, a drop of modified Tyrode's buffer containing egg yolk (supplied by Environmental Health research and Testing, Lexington, KY) was placed on a prewarmed slide. A small aliquot of seminal fluid was then pressed out onto the slide and a coverslip was placed over the slide. A stage warmer was attached to a microscope and the sample was evaluated for sperm motility using 400X magnification. The percent motile sperm per sample was recorded For determination of sperm count, the cauda was placed in a petri dish containing 2 ml prewarmed (37°C) sterile phosphate-buffered saline solution (pH 7 2). The cauda was gently chopped and the contents were gently "expressed". The sperm were dispersed into the saline solution by swirling the petri dish, the samples were then incubated for 15 minutes at 37°C. The petri dish was then swirled and the sperm suspension was gently flushed through a prewarmed (37°C) Pasteur pipet A 0.5 ml aliquot was then placed in a test tube (15 X 100 mm) and diluted with 1 ml phosphate-buffered saline (pH 7.2). The test tube was agitated by hand and then placed in boiling water for 30 seconds to kill the sperm. The test tube was then agitated and two aliquots of the suspension were counted in a hemocytometer The data were recorded as number of cauda epididymal sperm per mg tissue The remaining original sperm suspension was transferred to a test tube (10 X 75 mm) and one drop of 1% Eosin Y stain in water was added After 45 minutes, the sperm were resuspended Four aliquots were placed on slides, allowed to air dry overnight and coverslipped using Permount mounting medium diluted with xylene. The slides were examined with a microscope under 400X magnification and at least 500 sperm per slide were evaluated for normal and abnormal morphology using the criteria published by Wyrobeck and Bruce (1975) and Soares et al. (1979).

Histopathology

The preserved tissues from Task 3 and 4 animals were shipped to Experimental Pathology Laboratories, Inc. (EPL) for histological examination. EPL prepared cross sections of the medial lobe of the liver, adrenal glands and right kidney, and longitudinal sections of the left kidney for both sexes For all male mice, EPL prepared longitudinal sections of the left testis with attached epididymis, the right testis and the right epididymis, as well as cross sections of the seminal vesicles and prostate. For females, EPL prepared cross sections of the ovary with attached oviduct, both uterine horns and the upper half of the vagina All sections were stained with hematoxylin and eosin and were examined by a pathologist at EPL. The report and a quality assurance report certification which was signed by the Study Director[3] on May 17, 1985 are provided in Section 8 of the study report.

---

[2] Fixed in situ following removal of the (dorsal) cranial roof

[3] The EPL histopathology study (EPL Study No. 229-062) was conducted as a separate study with its own Study Director (J. Hardisty); a protocol was not included for the EPL study

Statistical Evaluation

The number of litters and the number of live pups per litter were computed for each fertile pair of Task 2 animals and then treatment means were determined. For each fertile pair in Tasks 3 and 4, the number of live pups per litter was calculated. The following formula was used to calculate the fertility index.

$$\frac{\text{\# of breeding pairs producing viable litter}}{\text{\# of breeding pairs}} \times 100$$

The statistics used to analyze the various parameters are presented in Table 1.

Table 1.
Statistical Evaluation

Parameter	Statistic
Task 1 - % body weight gain over 14-day period	Analysis of variance and Dunnett's Multiple Range Test
% mortality, proportion of fertile pairs	Chi Square Test for homogeneity and Fisher's Exact test for pairwise comparisons
Mean number of litters, mean litter size, number of live offspring, proportion of live pups, sex of live pups	Chi Square Approximation to the Kruskal-Wallis test, Jonckheere's Test and Mann-Whitney U Test
Mean pup body weights, organ weights	Analysis of variance using the Kruskal-Wallis Test and pair-wise comparisons using the Mann-Whitney U Test and Jonckheere's Test. Analysis of covariance and t-test to judge significance of differences
Terminal body weights	Analysis of variance and t-test to judge significance of differences

Appraisal of Protocol/Experimental Design

The study protocol was provided in Section 5 of the study report The study was well designed and followed NTP's reproductive toxicology testing scheme designated "Fertility Assessment by Continuous Breeding" At the time that the study was conducted, the protocol was new to the scientific and regulatory community. Although FDA and EPA have never formally converted this protocol into a test guideline, it is generally accepted in lieu of a two-generation reproductive toxicity study.

STUDY RESULTS

TASK 1

Analysis of Levels of Bisphenol A in the Feed

The levels of bisphenol A in the feed are presented in Table 2. The actual levels of bisphenol A ranged from 93-102% of the target levels.

Table 2.
Levels of Bisphenol A in the Feed

Sample Identification	Bisphenol A Found (mg/g)[a]	Bisphenol A Found Per Label (%)
Control	< edl[b] < edl[b] < edl[b]	-- -- --
0.312% (3.12 mg/g)	2 93 2.93 2 89	94 94 92 93 ± 1[c]
0.625% (6 25 mg/g)	6.21 5.96 6.08	99 95 97 97 ± 2[c]
1.25% (12.5 mg/g)	12.6 12.7 12 7	101 102 102 102 ± 1[c]
2.5% (25 mg/g)	25.2 24.8 25.2	101 99 101 100 ± 1[c]
5% (50 mg/g)	50 4 50 4 50 6	101 101 101 101 ± 0[c]

a- Bisphenol A measured by gas chromatography
b- Estimated detectable level was 0.2-0 5 mg/g
c- Mean ± SD

Mortality

Six of eight males in the 5% group died during week 2 One female in the 5% group died during week 1 and five others died during week 2 All other animals survived until scheduled termination.

Clinical Observations

The study report states that there were no clinical signs of toxicity noted in the control, 0.31, 0.62 or 1.25% groups. Clinical signs of toxicity were noted in males and females in the 2 5% group and included dehydration, dyspnea, lethargy, tremors and ptosis. Clinical signs of toxicity were noted in males and females in the 5% group and included dehydration, dyspnea, lethargy, piloerection, diarrhea, ptosis and moribundity.

Body Weight

The mean body weights at the beginning and end of Task 1 are presented in Table 3 The percent weight gain for the sexes combined was significantly reduced in the 1 25, 2 5 and 5% groups compared to the control group.

Table 3.
Mean Body Weights Of Task 1 Animals (Mean ± SE)

Group	Initial Body Weight (g)	Final Body Weight (g)	% Change
Control - Male	32.1 ± 0.6	33.3 ± 0 4	4.1 ± 1.3
- Female	24.8 ± 0.2	27.0 ± 0.3	9 3 ± 1.0
- Combined	28.4 ± 1.0	30 2 ± 0.9	6.7 ± 1.0
0.31% - Male	31.9 ± 0 8	34 2 ± 0 7	7.6 ± 1.5
- Female	25.0 ± 0.6	27.6 ± 0.3	10.7 ± 1.6
- Combined	28 4 ± 1.0	30 9 ± 0.9	9 2 ± 1.1
0.62% - Male	32 3 ± 0.6	32.9 ± 0 9	1.7 ± 1 8
- Female	24 8 ± 0.4	27.6 ± 0.6	11.3 ± 1.3
- Combined	28 6 ± 1.0	30.2 ± 0.8	6.5 ± 1.6
1 25% - Male	32.2 ± 0 6	30 8 ± 0 8	-4.1 ± 2.9
- Female	24.8 ± 0.4	25.7 ± 0.3	3.7 ± 1.8
- Combined	28.5 ± 1 0	28.2 ± 0.8	-0.2 ± 1 9[b]
2.5% - Male	32.0 ± 0 5	28.5 ± 1.4	-11.3 ± 3.5
- Female	25.0 ± 0.5	24.0 ± 0 3	-3.8 ± 1.8
- Combined	28.5 ± 1.0	26.3 ± 0 9	-7 6 ± 2 1[b]
5% - Male	31.8 ± 0.3	23.5 ± 0.5[a]	-25.9 ± 2.6
- Female	25.0 ± 0.4	17 8 ± 1.5[a]	-30.8 ± 4 0
- Combined	28 4 ± 0.9	20.6 ± 1.7	-28.4 ± 2 4[b]

a- There were only 2 animals alive at scheduled necropsy.
b- $p < 0.01$

Food Consumption

The mean food consumption during weeks 1 and 2 of Task 1 is presented in Table 4. Mean food consumption was comparable among treated and control groups.

Table 4.
Mean Food Consumption Per Mouse (g; Mean ± SE)

Group	Week 1	Week 2
Control - Male	5 7 ± 0.2	5 1 ± 0.1
- Female	5.5 ± 0.3	5 4 ± 0.7
- Combined	5.6 ± 0.1	5 2 ± 0 1
0.31% - Male	4.8 ± 0.0	4.8 ± 0 1
- Female	5.4 ± 0 5	5.6 ± 0.4
- Combined	5.1 ± 0.1	5 2 ± 0.3
0 62% - Male	5.7 ± 0.7	5 9 ± 0 4
- Female	6.1 ± 0 4	6 1 ± 0.2
- Combined	5.9 ± 0.3	6.0 ± 0.2
1 25% - Male	5.9 ± 0.6	6 4 ± 0 5
- Female	6 2 ± 0.0	6.7 ± 0.1
- Combined	6.1 ± 0 3	6.5 ± 0.2
2.5% - Male	5.7 ± 0.8	5 7 ± 0 1
- Female	6 8 ± 0.5	6 2 ± 0.5
- Combined	6.2 ± 0 5	5.9 ± 0.2
5% - Male	5.7 ± 0 1	5.4[a]
- Female	6.1 ± 0.3	5 0[a]
- Combined	5 9 ± 0.1	5 2 ± 0.2

a- There were only 2 animals alive at scheduled necropsy

TASK 2

Analysis of Levels of Bisphenol A in the Feed

The levels of bisphenol A in the feed during weeks 1 and 6, and weeks 12 and 18 are presented in Tables 5 and 6, respectively. During weeks 1 and 6, the actual levels of bisphenol A ranged from 96-97% and 102-113% of the target levels, respectively During weeks 12 and 18, the actual levels of bisphenol A ranged from 93-100% and 98-104% of the target levels, respectively

Table 5.
Levels of Bisphenol A in the Feed During Weeks 1 and 6

Sample Identification	Bisphenol A Found (mg/g)[a] Week 1	Bisphenol A Found Per Label (%) Week 1	Bisphenol A Found (mg/g)[a] Week 6	Bisphenol A Found Per Label (%) Week 6
Control	< edl[b] < edl[b] < edl[b]	-- -- --	< edl[b] < edl[b] < edl[b]	-- -- --
0.25% (2.5 mg/g)	2.43 2.40 2.39	97 96 96 96 ± 1[c]	2.63 2.54 2.63	105 102 105 104 ± 2[c]
0 5% (5 mg/g)	5 00 4.61 5 01	100 92 100 97 ± 5[c]	4.93 5.29 5 10	99 106 102 102 ± 4[c]
1% (10 mg/g)	9 54 10.00 9.23	95 100 96 96 ± 4[c]	11.2 11 4 11 4	112 114 114 113 ± 1[c]

a- Bisphenol A measured by gas chromatography
b- Estimated detectable level was 0 2-0.5 mg/g
c- Mean ± SD

Table 6.
Levels of Bisphenol A in the Feed During Weeks 12 and 18

Sample Identification	Bisphenol A Found (mg/g)[a] Week 12	Bisphenol A Found Per Label (%) Week 12	Bisphenol A Found (mg/g)[a] Week 18	Bisphenol A Found Per Label (%) Week 18
Control	< edl[b] < edl[b]	-- --	< edl[b] < edl[b]	-- --
0.25% (2.5 mg/g)	2.32 2.28 2.34	93 91 94 93 ± 2[c]	2.57 2 65 2.60	103 106 104 104 ± 2[c]
0.5% (5 mg/g)	4.92 4 83 4.88	98 97 98 98 ± 1[c]	4.80 5.04 4.86	96 101 97 98 ± 3[c]
1% (10 mg/g)	10.2 10.0 9 8	102 100 98 100 ± 2[c]	9.9 10.4 10.1	99 104 101 101 ± 2[c]

a- Bisphenol A measured by gas chromatography
b- Estimated detectable level was 0.2-0 5 mg/g
c- Mean ± SD

Mortality

Two control males died during week 1 and one control female died during week 4 of the study. One female in the 0.25% group died during week 9 and one female in the 0.5% group died during week 4. The study authors did not state the cause of death. The fertility and reproductive performance data for the breeding pair of these animals were not included in the analysis.

Clinical Observations

The study report states that there were no clinical signs of toxicity noted in the control or treated groups. No further details were provided

Body Weight

The mean male body weights for selected weeks of the study are presented in Table 7. Mean body weights were slightly reduced in the 1% group and by week 18 of the study the mean body weights were reduced by 8% compared to the control group. The mean female body weights for selected weeks of the study are presented in Table 8. Interpretation of the data is difficult since some fluctuation in female

body weights reflects the pregnancies occurring throughout the study However, compared to the control group, the mean body weights in the 1% group were reduced by 23% during week 9 and 15% during week 18. A more meaningful comparison can be obtained from the body weight data for the females immediately following delivery of their litters. These data are presented in Table 9. Following delivery of each of the five litters, the mean body weight of the females in the 1% group was significantly reduced compared to the control group, indicating generalized maternal toxicity.

Table 7.
Mean Male Body Weights for Selected Weeks of Task 2 (Mean ± SE)

Study Week	Control (N=38)	0.25% Group (N=20)	0.5% Group (N=20)	1% Group (N=20)
1	34.3 ± 0 3	34 6 ± 0.4	34 3 ± 0.4	33.3 ± 0 6
2	34.6 ± 0.2	35 5 ± 0 4	34 5 ± 0 4	34 0 ± 0 5
5	36.0 ± 0.3	35 2 ± 0.3	36.1 ± 0.4	33 9 ± 0 5
9	36 3 ± 0.4	35 8 ± 0.3	35.3 ± 0.5	34.3 ± 0.5
13	36 1 ± 0.5	35.7 ± 0.2	35 4 ± 0.6	34 9 ± 0 5
18	39 0 ± 0.6	--[a]	--[a]	35 8 ± 0 4

a- Males in the 0 25 and 0.5% groups were sacrificed after week 15.

Table 8.
Mean Female Body Weights for Selected Weeks of Task 2 (Mean ± SE)

Study Week	Control (N=40)[a]	0.25% Group (N=20)[b]	0.5% Group (N=20)[c]	1% Group (N=20)
1	27 1 ± 0 3	27.2 ± 0 3	27.0 ± 0.3	26.8 ± 0.3
2	30.1 ± 0.3	30 8 ± 0.4	29 8 ± 0.3	29 3 ± 0.4
5	35 9 ± 0 5	34 9 ± 0 5	35.7 ± 0 6	33.7 ± 0 8
9	49 1 ± 1.3	48 7 ± 1.7	44 1 ± 1.7	37 7 ± 1.0
13	38.6 ± 0.6	39.7 ± 1 5	40 0 ± 2 0	39.7 ± 1.6
18	45.1 ± 1.0	46.7 ± 0.6	41.7 ± 1.6	38.2 ± 1.3

a- One control female died during week 4· n=39 for weeks 5-18
b- One female in the 0 25% group died during week 9: n=19 for weeks 10-18
c- One female in the 0.5% group died during week 4: n=19 for weeks 5-18.

Table 9
Mean Female Body Weights Following the Delivery of Each Litter (Mean ± SE)

Litter Number	Control (N=37)	0.25% Group (N=19)	0.5% Group (N=18)	1% Group (N=20)
1	35.13 ± 0.39	35.37 ± 0.58	34.59 ± 0 68	32 31 ± 0.51[f]
2	38.15 ± 0.44	38 99 ± 0.68	37.00 ± 0.62	35.67 ± 0.52[f]
3	38.86 ± 0.50	38.68 ± 0.64	39.16 ± 0.60	36.68 ± 0.53[f]
4	41.06 ± 0.50	41 09 ± 0.70[b]	40.55 ± 0.74	38 01 ± 0 68[d,f]
5	41.26 ± 0.63[a]	42.13 ± 0 54[b]	40.02 ± 0.76[c]	37 50 ± 0.70[e,f]

a- N=35
b- N=18
c- N=13
d- N=18
e- N=12
f- Significantly different from control, $p < 0.01$

Food Consumption

The mean food consumption for the males during the first week of the study was 4.7 ± 0.1 g, 5.0 ± 0.1 g, 4 4 ± 0.1 g and 4.3 ± 0.2 g for the control, 0.25, 0.5 and 1% groups, respectively. The mean food consumption for the females during the first week of the study was 4.8 ± 0.1 g, 4 5 ± 0.2 g, 4 8 ± 0.1 g and 4.7± 0 1 g for the control, 0 25, 0.5 and 1% groups, respectively During week 18 of the study, the mean food consumption for the males was 5.3 ± 0 1 g and 6.1 ± 0.3 g for the control and 1% groups, respectively, the mean food consumption for the females was 18.3 ± 1.0 g, 20.4 ± 0.4 g, 14.9 ± 1.3 g and 14.0± 0.9 g for the control, 0 25, 0.5 and 1% groups, respectively During the breeding phase of the study, mean food consumption was calculated for each breeding pair and then expressed on a per mouse basis These values are presented in Table 10. The study authors concluded that there were no treatment-related effects on food consumption. Our reviewers concur.

Table 10.
Mean Food Consumption for Task 2 Animals (g/mouse; Mean ± SE)

Study Week	Control	0.25% Group	0.5% Group	1% Group
2	6.0 ± 0.2	5.3 ± 0.1	5 6 ± 0.1	5 7 ± 0.1
5	5 5 ± 0.1	5 4 ± 0.1	5.4 ± 0.1	6.1 ± 0.1
9	5.2 ± 0 1	5.2 ± 0 1	5.5 ± 0.1	5 9 ± 0.2
13	5.0 ± 0.1	5.1 ± 0 1	5.2 ± 0 1	5.6 ± 0 2

Reproductive Performance

The reproductive performance parameters for the Task 2 animals are presented in Table 11. The fertility index was calculated for each breeding pair, and a breeding pair was considered fertile if it produced one or more litters. The index was comparable among treated and control groups, and was calculated as 100, 100, 95 and 100% for the control, 0.25, 0 5 and 1% groups, respectively The mean number of litters produced per fertile pair was significantly reduced in the 0 5 and 1% groups; the reductions were dose-dependent. In addition, the mean number of live pups per litter (males, females and sexes combined) was significantly reduced in the 0 5 and 1% groups, the reductions were dose-dependent The proportion of pups born alive was significantly reduced in the 1% group. The sex ratio was comparable among control and treated groups. There was a significant increase in mean pup body weight for the males, females and sexes combined in the 0.5% group and for the males and sexes combined in the 1% group. However, when the pup body weights were adjusted for the total number of live and dead pups per litter the differences were not statistically significant Thus, the increased pup body weights in the 0.5 and 1% groups probably reflect the smaller litter size in these two groups.

Table 11.
Reproductive Performance of Task 2 Animals (Mean ± SE)

Parameter	Control N=37	0.25% Group N=19	0.5% Group N=18	1% Group N=20
Litters per pair	4.97 ± 0 05	4.89 ± 0 11	4 72 ± 0.11[b]	4 50 ± 0.15[c]
Live pups per litter				
- Males	6.03 ± 0 19	5.43 ± 0 26	4.74 ± 0.20[c]	3.24 ± 0.24[c]
- Females	6 05 ± 0.23	5.78 ± 0.17	4.93 ± 0.25[c]	3.06 ± 0.33[c]
- Combined	12.07 ± 0.36	11.20 ± 0 32	9 67 ± 0 41[c]	6 03 ± 0.51[c]
Proportion of pups born alive	0 99 ± 0 00	0 99 ± 0.00	0.99 ± 0 05	0 95 ± 0.02[c]
Sex Ratio (# Males/total pups)	0 50 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.54 ± 0.03
Live pup weight (g)				
- Males	1 60 ± 0 02	1 60 ± 0.02	1.66 ± 0.02[b]	1.69 ± 0.04[b]
- Females	1.52 ± 0.01	1.55 ± 0 02	1.61 ± 0.03[c]	1.62 ± 0.05
- Combined	1 56 ± 0 01	1.57 ± 0 02	1.64 ± 0.02[c]	1.65 ± 0.04[b]
Adjusted live pup weight (g)[a]				
- Males	1.65 ± 0 02	1.63 ± 0 02	1.64 ± 0 02	1 58 ± 0.03
- Females	1 59 ± 0.02	1 58 ± 0 03	1 59 ± 0 03	1 48 ± 0.03
- Combined	1.62 ± 0.02	1.60 ± 0.02	1.62 ± 0.02	1.53 ± 0 03

a- Mean live pup weight adjusted for total number of live and dead pups per litter by analysis of covariance

b- Significantly different from the control, $p<0.05$

c- Significantly different from the control, $p<0 01$

Scheduled Necropsy

Since significant effects on reproductive performance were observed, the animals in the control and 1% groups were retained for a crossover mating to determine the affected sex (Task 3). The animals in the 0.25 and 0.5% groups were sacrificed and discarded without necropsy. The pups from the first four litters were sacrificed and discarded without necropsy. The F1 pups from the fifth litter were retained for Task 4.

TASK 3

For Task 3, a cross-over mating was conducted in which half of the control females were mated with half the control males (group 1). The remaining control females were mated with the males in the 1% group (group 2), and the remaining control males were mated with the females in the 1% group (group 3). The animals were cohabited during week 1 and then separated for weeks 2-4 during which the pups were delivered. Treatment was discontinued during the co-habitation/mating phase, but re-instituted during the separation /littering phase.

Mortality

One control female died during week 3 and one male in the 1% group died during week 4. The study report did not state the cause of death. These animals were not included in the analysis of the reproductive performance data.

Clinical Observations

The study report states that there were no clinical signs of toxicity noted in the control or treated groups. No further details were provided.

Body Weight

The mean body weights during weeks 1-4 of Task 3 are presented in Table 12. The mean male body weights were comparable among groups The mean female body weight in the 1% group (group 3) was reduced compared to the controls in groups 1 and 2 However, direct comparison of the three groups is difficult due to fluctuations in body weight during pregnancy. A more meaningful comparison can be determined from the mean body weights immediately following delivery of the litters. The mean female body weight following delivery was 40.3 $\pm$ 0.59 g for the control females (groups 1 and 2 combined) and 38.51 $\pm$ 0.48 g for the females in the 1% group; thus, the mean body weight in the 1% group was reduced by 4% of the control value.

Table 12.
Mean Body Weights (g) for Task 3 Animals (Mean ± SE)

Group	Study Week	Mean Male Body Weight	Mean Female Body Weight
Group 1 Control Males and Control Females (N=19 pairs)	1	37.9 ± 0 9	39.3 ± 0 9
	2	37 7 ± 1 0	43.7 ± 1.3
	3	37.4 ± 1 0	49.7 ± 2 5
	4	37.8 ± 0.9	37.9 ± 0.8
Group 2 Control Females and 1% Males (N=20 pairs)	1	36 0 ± 0.4	38 1 ± 0.7
	2	35.0 ± 0.4	41.6 ± 1.0
	3	35.0 ± 0 5	45.2 ± 2 2[a]
	4	36 3 ± 0.4[b]	36 6 ± 0.7[a]
Group 3 Control Males and 1% Females (N=19 pairs)	1	36.4 ± 0 5	35.9 ± 0 5
	2	36 7 ± 0.8	39.0 ± 0.7
	3	35.8 ± 0 5	42 2 ± 2.1
	4	36.8 ± 0 7	35.5 ± 0 6

a- N = 19
b- N = 19

Food Consumption

Food consumption was not recorded during the mating phase of Task 3. Mean food consumption for weeks 2-4 is presented in Table 13 The mean food consumption for the 1% male group (group 2) was increased by 19-42% during the study period compared to the control males (groups 1 and 3). The mean food consumption for the 1% female group (group 3) was increased by 7-22% during the study period compared to the control females (groups 1 and 2)

Table 13.
Mean Food Consumption (g) for Task 3 Animals (Mean ± SE)

Group	Study Week	Males	Females
Group 1 Control Males and Control Females (N=19 pairs)	2 3 4	4.9 ± 0.1 5 0 ± 0.4 5 0 ± 0 1	6.8 ± 0.2 7.5 ± 0.1 6.0 ± 0.2
Group 2 Control Females and 1% Males (N=20 pairs)	2 3 4	6 6 ± 0 2 6 2 ± 0 3 7.1 ± 0.3[b]	6.3 ± 0 2 7 3 ± 0.2[a] 6.1 ± 0.2[a]
Group 3 Control Males and 1% Females (N=19 pairs)	2 3 4	5 2 ± 0.2 5.2 ± 0.1 5.1 ± 0 2	7.6 ± 0.3 8.0 ± 0 3 7.3 ± 0 2

a- N = 19
b- N = 19

Reproductive Performance

The mating index was 89, 95 and 95% and the fertility index was 84, 89 and 84% in groups 1, 2 and 3, respectively The reproductive performance data are presented in Table 14. There was a significant reduction in the mean number of live pups per litter (males, females, and sexes combined) in group 3 when compared to groups 1 and 2. There was also a significant reduction in the mean number of live pups per litter for sexes combined in group 2 when compared to group 1 The proportion of pups born alive and the sex ratio were comparable among groups Mean pup body weights for females and sexes combined was significantly increased in group 3 compared to group 1; mean male pup body weight was significantly increased compared to groups 1 and 2. However, when pup body weight was adjusted for the number of live and dead pups per litter, mean pup body weights were comparable among groups. Therefore, the apparent increase in pup body weights observed in group 3 were due to the decreased litter size

Table 14.
Reproductive Performance of Task 3 Animals (Mean ± SE)

Parameter	Group 1 Control Males and Control Females (N=16)	Group 2 Control Females and 1% Males (N=17)	Group 3 Control Males and 1% Females (N=16)
Live pups per litter			
- Males	6.13 ± 0 57	4.41 ± 0.58	2.69 ± 0.47[e]
- Females	5.19 ± 0 39	4 00 ± 0.52	2 81 ± 0 31[e]
- Combined	11.31 ± 0.64	8 41 ± 0.98[f]	5 50 ± 0 65[e]
Proportion of pups born alive	0.99 ± 0.01	0.92 ± 0 06	0.94 ± 0.04
Sex Ratio (# Males/total pups)	0.53 ± 0.04	0.53 ± 0.05[b]	0 45 ± 0.05
Live pup weight (g)			
- Males	1.66 ± 0.05	1 71 ± 0.04[c]	1.84 ± 0.04[c,e]
- Females	1.58 ± 0.03	1.67 ± 0.05[d]	1.75 ± 0.06[f]
- Combined	1.61 ± 0 03	1 71 ± 0 04	1 81 ± 0 05[g]
Adjusted live pup weight (g)[a]			
- Males	1.77 ± 0.03	1 72 ± 0.03[c]	1 71 ± 0 03[c]
- Females	1.68 ± 0.04	1 69 ± 0 04[d]	1 63 ± 0.05
- Combined	1.73 ± 0.03	1.72 ± 0 03	1.69 ± 0.03

a- Mean live pup weight adjusted for total number of live and dead pups per litter by analysis of covariance
b- One litter had no live pups
c- One litter had no live male pups
d- One litter had no live female pups
e- Significantly different from group 1 ($p<0.01$) and group 2 ($p < 0.05$)
f- Significantly different from group 1, $p<0.05$
g- Significantly different from group 1, $p<0.01$

Scheduled Necropsy

Organ Weights

At the completion of Task 3, the animals were weighed and necropsied. The mean terminal body and organ weights are presented in Table 15. The mean body weight of the females in the 1% group was significantly reduced compared to control females. There was a significant increase in mean absolute liver weights for males and females in the 1% group compared to controls; the differences remained statistically significant when liver weights were adjusted for body weight by analysis of covariance Combined absolute kidney/adrenal weights were significantly increased in the males in the 1% group compared to control, and adjusted kidney/adrenal weights were significantly increased for males and females in the 1% group compared to controls. Absolute and adjusted seminal vesicle weights were

significantly reduced in the males in the 1% group compared to controls. The weights of the other male reproductive organs were comparable among treated and control groups.

Table 15.
Terminal Body and Organ Weights of Task 3 Animals (Mean ± SE)

Parameter	Control Males (N=38)	Control Females (N=38)	1% Group Males (N=19)	1% Group Females (N=19)
Body Weight (g)	36.55 ± 0 51	35.78 ± 0.50	35.37 ± 0.46	34 19 ± 0.50[c]
Liver (g) - Absolute - Adjusted[d]	2 00 ± 0.04 1.97 ± 0.05	2 17 ± 0.05 2.13 ± 0.04	2.49 ± 0.12[b] 2 55 ± 0.08[b]	2.62 ± 0.09[b] 2 71 ± 0.05[b]
Kidneys/Adrenals (g) - Absolute - Adjusted[d]	 0.80 ± 0 02 0 80 ± 0.01	 0.64 ± 0.01 0.63 ± 0 01	 0 92 ± 0 02[b] 0 93 ± 0.02[b]	 0.67 ± 0.02 0.69 ± 0 01[b]
Left testis & epididymis (mg) - Absolute - Adjusted[d]	194 ± 5 193 ± 5	----- -----	188 ± 6 191 ± 7	----- -----
Right testis (mg) - Absolute - Adjusted[d]	 133 ± 3 131 ± 3	 ----- -----	 129 ± 5 131 ± 4	 ----- -----
Right epididymis (mg) - Absolute - Adjusted[d]	 53 ± 1 52 ± 1	 ----- -----	 53 ± 2 53 ± 2	 ----- -----
Prostate (mg) - Absolute - Adjusted[d]	55 ± 5 55 ± 5	----- -----	53 ± 5 53 ± 7	----- -----
Seminal vesicles (mg) - Absolute - Adjusted[d]	 457 ± 16 453 ± 15	 ----- -----	 358 ± 21[a,b] 366 ± 21[a,b]	 ----- -----

a- Seminal vesicles were not weighed for one animal
b- Significantly different from control, $p<0.01$
c-Significantly different from control, $p<0.05$
d- Organ weights were adjusted for body weight by analysis of covariance

Histopathology

The histologic lesions noted in the weighed organs of the males and females are presented in Tables 16 and 17, respectively. No treatment-related lesions were noted in the reproductive organs or adrenal glands of the treated males and females In addition, evaluation of the cell types in the vaginal mucosa indicated that there were no effects on estrus cyclicity of the treated females. Treatment-related lesions were noted in the liver and kidneys of males and females in the 1% group. In general, the lesions in the livers of the males were more severe than in the females, and lesions in the kidneys of the females were more severe than in the males. Lesions in the liver of the males included centrilobular hepatocytomegaly,[4] multifocal necrosis,[5] and multinucleated giant hepatocytes; lesions in the females included multifocal necrosis and multinucleated giant hepatocytes. Lesions in the kidneys of the males included tubular cell nuclear variability[6] and an increase in the incidence of several spontaneous lesions including interstitial lymphoid accumulations and tubular regeneration These same lesions were also noted in the females in the 1% group, but these animals also had other renal lesions including large microcalculi in the cortical tubules, sometimes associated with attenuated tubular epithelium, tubular regeneration and/or dilated tubules containing proteinaceous or pigmented casts.

---

[4] The EPL study report indicated that the incidence of spontaneous centrilobular hepatocytomegaly is high in female mice of the examined strain, and no treatment-related increases over background were evident.

[5] Multifocal necrosis was accompanied by increases in perisinusoidal lining cells and Kupffer cell pigmentation

[6] The diagnosis "tubular cell nuclear variability" sometimes included karyomegaly and often was accompanied by increased tubular eosinophilia and/or anisocytosis

Table 16.
Histopathology Findings for Task 3 Males

Lesion	Control Group (N=19)	Control Males from Group 3 (N=19)	1% Group (N=19)
Right testes - focal atrophy	0	1	1
Left testes - focal atrophy	0 [a]	0	1
Right epididymis			
- focal chronic inflammation	1	0	1
- focal cystic ducts	0	1	0
- hypospermia	0	1	0
Right Kidney			
- Malignant lymphoma, lymphocytic type	0	1	0
- Tubular cell nuclear variability	0	0	10
- Interstitial lymphoid accumulations	12	11	16
- Tubular regeneration	9	7	15
- Multifocal cortical tubular dilatation	5	1	3
- Cortical tubular casts	2	1	4
- Healed infarct	0	0	2
- Pelvic dilatation	1	1	0
- Pyelonephritis	0	0	1
Left Kidney			
- Malignant lymphoma, lymphocytic type	0	1	0
- Tubular cell nuclear variability	0	0	9
- Interstitial lymphoid accumulations	14	15	14
- Tubular regeneration	7	12	15
- Multifocal cortical tubular dilatation	4	1	3
- Cortical tubular casts	1	2	5
- Healed infarct	1	0	1
- Pelvic dilatation	1	0	0
- Pyelonephritis	0	0	1
- Focal thrombosis	0	1	0
Adrenal Gland			
- Focal capsular cell hyperplasia	5	5 [a]	4
- Acute adrenalitis, zona reticularis	1	2 [a]	0
- Focal lymphoid accumulations, medulla	0	1 [a]	0
Liver			
- Malignant lymphoma, lymphocytic type	0	1	0
- Centrilobular hepatocytomegaly	0	2	16
- Multifocal necrosis	3	1	15
- Multinucleated giant hepatocytes	0	0	15
- Focal hepatocellular hyperplasia	0	0	1
- Multifocal microgranulomas	0	1	0

a- N = 18

Table 17.
Histopathology Findings for Task 3 Females

Lesion	Control Group (N=19)	Control Females from Group 3 (N=19)	1% Group (N=19)
Uterus			
- Focal glandular mineralization	0	0	1
- Stromal hemosiderosis	16	17	16
- Amyloidosis	1	2	4
- Perivascular lymphoid accumulations	3	0	0
- Focal perivascular fibrosis	0	1	1
Vagina			
- Proestrus	2	3	2
- Estrus	5	4	10
- Metestrus-1	3	1	1
- Metestrus-2	6	5	1
- Diestrus	3	6	5
- Focal chronic vaginitis	0	1	1
- Acute vaginitis	1	0	1
Ovary - Cyst	1 [a]	4	2
Right Kidney			
- Tubular cell nuclear variability	0	0	12
- Cortical tubular microcalculi	0	0	4
- Multifocal cortical tubular dilatation	4	2	13
- Cortical tubular casts	4	1	10
- Tubular regeneration	11	8	17
- Interstitial lymphoid accumulations	11	6	11
- Healed infarct	1	0	1
- Focal osseous metaplasia	0	1	0
- Glomerular amyloidosis	2	3	2
- Cysts	1	0	0
Left Kidney			
- Tubular cell nuclear variability	0	0	12
- Cortical tubular microcalculi	0	1	7
- Multifocal cortical tubular dilatation	6	4	16
- Cortical tubular casts	4	2	12
- Tubular regeneration	12	8	18
- Interstitial lymphoid accumulations	10	5	12
- Focal osseous metaplasia	0	1	0
- Glomerular amyloidosis	2	3	2
Adrenal Gland			
- Focal capsular cell hyperplasia	8	8	10
Liver			
- Multifocal necrosis	6	6	11
- Multinucleated giant hepatocytes	0	0	4
- Multifocal microgranulomas	2	3	0
- Multifocal lymphoid accumulations	2	0	0

a - N = 18

Sperm Analyses

Sperm analyses were conducted for the control and treated males in Task 3 and are presented in Table 18. There were no differences among treated and control males in sperm concentration or the % abnormal sperm. Males in the 1% group had a significant reduction in the % motile sperm compared to controls

Table 18.
Sperm Analysis for Task 3 Males (Mean ± SE)

Parameter	Control (N=38)	1% Group (N=19)
% Motile sperm	64.30 ± 4.82[b]	39.00 ± 7.04[c]
Sperm concentration[a]	258 27 ± 28.99	182 80 ± 33 61
% Abnormal sperm	10.85 ± 1.01[b]	9.29 ± 1.26

a- Calculated as no. sperm X 10^3/mg caudal (epididymal) tissue
b- One male had no sperm, N=37
c- Significantly different from the control group, p<0 01

TASK 4

The pups from the fifth litter (F1 generation) of Task 2 were retained for Task 4. Following weaning, the pups were placed on the same diet as the F0 dams

Mortality

There was increased mortality of the pups in the 1% group. One litter in the 1% group died prior to weaning. All male pups in one litter in the 1% group died between days 21-74 and all female pups in two litters in the 1% group died between days 21-74. The study authors noted that of the pups selected at weaning for the mating phase of Task 4, 6.3% (8/126), 3.8% (4/105), 13.9% (11/79) and 37.5% (18/48) of the pups in the control, 0 25, 0 5 and 1% groups, respectively, died between birth and the beginning of the mating phase at day 74 ± 10.

Clinical Observations

The study report states that there were no clinical signs of toxicity noted in the control or treated groups. No further details were provided Our reviewers find it troubling that nearly 40% of the 1% pups died, yet no clinical signs were recorded

Body Weight

The mean body weights of the F1 pups on postnatal days 0, 21 and 74 are presented in Table 19. At birth, there was a significant increase in mean pup body weight in the 0 5 and 1% groups compared to controls. The study authors stated that this may have been due to the reduced litter size in these two

groups Mean body weights of surviving pups were comparable among treated and control groups at weaning on day 21 and at the commencement of the mating phase of the study at day 74 ± 10.

The mean body weights of the animals recorded during the mating phase and 21-day segregation phase of Task 4 are presented in Table 20. Mean body weights were comparable among treated and control groups

Table 19.
Mean Body Weights of F1 Pups Retained for Task 4 (g; Mean ± SE)

Age	Control	0.25% Group	0 5% Group	1% Group
0 Days (Birth)[a]				
- Females	1.57 ± 0.03 (36)	1 56 ± 0.02 (19)	1.72 ± 0.05 (15)[d,i]	1 70 ± 0.05 (13)[e,i]
-Males	1 64 ± 0 04 (35)[c]	1 61 ± 0.03 (19)	1.82 ± 0 07 (16)[i]	1.78 ± 0.05 (15)[i]
21 Days [a,b]				
- Females	9.98 ± 0.53 (10)	9.85 ± 0.61 (10)	12.26 ± 0 72 (10)	11.02 ± 0.63 (8)[f]
-Males	11.02 ± 0.63 (10)	10 40 ± 0 69 (10)	12.90 ± 0 87 (10)	10.97 ± 1.07 (8)[f]
74 ± 10 Days [a]				
- Females	27.22 ± 0.65 (10)	27 04 ± 0 48 (10)	28.61 ± 0.60 (10)	26 87 ± 0.65 (6)[g]
-Males	33.93 ± 0.84 (10)	33.75 ± 0 71 (10)	34 13 ± 1 14 (9)[h]	32.62 ± 0.93 (7)[h]

a- Number of litters evaluated in parentheses
b- At day 21, 8-10 litters were weaned and reared
c- One litter had no male pups
d- One litter had no female pups
e- Two litters had no female pups
f- One litter died before day 21 and another had no male pups, so only 8 litters were retained
g- All female pups in two litters died between day 21-74
h- All male pups in one litter died between day 21-74
i- Significantly different from the control group, $p < 0\ 05$

Table 20.
Mean Body Weights During Task 4 (g; Mean ± SE)

Study Week	Control (N=20)	0 25% Group (N=20)[a]	0 5% Group (N=20)	1% Group (N=11)
1 - Males - Females	32.8 ± 0.6 29.3 ± 0.6	33.0 ± 0.5 29 0 ± 0.5	33.1 ± 0.7 30.3 ± 0.7	32.1 ± 0.7 28.8 ± 0.7
2 - Males - Females	32 7 ± 0.6 36.0 ± 0 8	33.2 ± 0.5 36.6 ± 0.7	32.8 ± 0 6 37 3 ± 1.0	31.7 ± 0 7 34.4 ± 1.1
3 - Males - Females	32.5 ± 0.7 40.2 ± 2.2	33 1 ± 0.5 45.1 ± 2 4[a]	32.8 ± 0.6 41.4 ± 2 4	31.8 ± 0.8 37.9 ± 2.9
4 - Males - Females	32.9 ± 0.7 30.4 ± 0.5	32.7 ± 0 5 30.0 ± 0.3	33.4 ± 0 6 31.1 ± 0 5	32 5 ± 0.7 29.8 ± 0.4
Mean female weight after delivery	34.33 ± 0.77[b]	33.94 ± 0 58[b]	34.84 ± 0 64	32.68 ± 0 68[c]

a- One female died during week 3, N=19
b- N=19
c- N=10

Food Consumption

The mean food consumption was not recorded during the mating phase of Task 4 The mean food consumption for weeks 2-4 is presented in Table 21. Mean food consumption was comparable among treated and control groups.

Table 21.
Mean Food Consumption (g) for Task 4 Animals (Mean ± SE)

Group	Study Week	Males	Females
Control (N=20)	2 3 4	5.9 ± 0.2 5 3 ± 0.1 5 2 ± 0.1	7.1 ± 0 3 7.1 ± 0 4 5 7 ± 0 2
0.25% Group (N=20)	2 3 4	5 4 ± 0.1 5.4 ± 0.1 5.3 ± 0.1	6.2 ± 0.2 6 9 ± 0 1[a] 5 9 ± 0.2[a]
0.5% Group (N=20)	2 3 4	5 8 ± 0.2 5.4 ± 0.1 5.6 ± 0.1	6 7 ± 0 2 6 6 ± 0 1 5.9 ± 0 1
1% Group (N=11)	2 3 4	6 4 ± 0.2 5.8 ± 0 2 6 4 ± 0.3	7.5 ± 0.2 6.4 ± 0 1 6.9 ± 0.2

a- N = 19

Reproductive Performance

The mating index was 95, 100, 100 and 91% and the fertility index was 95, 100, 100 and 91% in the control, 0.25, 0.5 and 1% groups, respectively. The reproductive performance data are presented in Table 22. There were no differences among treated and control groups in the number of liver pups per litter, proportion of pups born alive, sex ratio, mean body weight or adjusted mean body weight.

Table 22.
Reproductive Performance of Task 4 Animals (Mean ± SE)

Parameter	Control (N=19)	0 25% Group (N=19)	0 5% Group (N=20)	1% Group (N=10)
Live pups per litter				
- Males	5.53 ± 0 59	5.37 ± 0.47	6.05 ± 0.35	5.20 ± 0.74
- Females	5.47 ± 0.52	5 37 ± 0 42	6.30 ± 0.42	5.70 ± 0 56
- Combined	11 00 ± 0.61	10.74 ± 0.63	12.35 ± 0.35	10.90 ± 0 67
Proportion of pups born alive	0 95 ± 0.05	0.95 ± 0.03	1.00 ± 0.00	0.99 ± 0 01
Sex Ratio (# Males/total pups)	0.48 ± 0 05	0 50 ± 0.03	0.49 ± 0 03	0 46 ± 0.05
Live pup weight (g)				
- Males	1.53 ± 0.03[b]	1.51 ± 0.05	1.49 ± 0 02	1 46 ± 0.06
- Females	1 42 ± 0.05	1.47 ± 0 04	1 45 ± 0.02	1.40 ± 0.04
- Combined	1.46 ± 0 05	1.49 ± 0.04	1.47 ± 0 02	1.43 ± 0 05
Adjusted live pup weight (g)[a]				
- Males	1 53 ± 0.03[b]	1.49 ± 0.03	1.52 ± 0.03	1.43 ± 0.04
- Females	1.42 ± 0 03	1 45 ± 0 03	1.48 ± 0.03	1 37 ± 0 05
- Combined	1 46 ± 0.03	1.47 ± 0 03	1 50 ± 0.03	1.40 ± 0 04

a- Mean live pup weight adjusted for total number of live and dead pups per litter by analysis of covariance
b- One litter had no live male pups, N = 18

Scheduled Necropsy

Organ Weights

At the completion of Task 4, the animals were weighed and necropsied. The mean terminal body and organ weights for males and females are presented in Tables 23 and 24, respectively. Mean male and female terminal body weights were comparable among treated and control groups. In males, there was a significant increase in absolute liver weight in the 1% group compared to control; when adjusted for body weight by analysis of covariance, liver weights were significantly increased in the 0 25, 0.5 and 1% groups compared to control. Absolute and adjusted kidney weights were significantly increased in all male treated groups compared to control. Absolute and adjusted weights of the left testis with attached epididymis were significantly decreased in the 0.5 and 1% groups. Absolute and adjusted weights of the right testis were significantly reduced in the 0 5% group, and absolute and adjusted weights of the right epididymis were significantly reduced in all treated groups compared to control. Absolute and adjusted seminal vesicle weights were reduced in all treated groups; the differences were statistically significant for absolute and adjusted weights in the 1% group, and adjusted seminal vesicle weights in the 0.25% group. Absolute prostate weights were comparable among treated and control groups In females, there was a significant increase in adjusted liver and kidney/adrenal weights in all treated groups compared to control.

Table 23.
Terminal Body and Organ Weights of Male Task 4 Animals (Mean ± SE)

Parameter	Control (N=20)	0.25% Group (N=20)	0.5% Group (N=20)	1% Group (N=11)
Body Weight (g)	32 24 ± 0.77	32.43 ± 0 48	32.71 ± 0.63[b]	32.67 ± 0.61
Liver (g) - Absolute	1.92 ± 0 08	2 07 ± 0 05	2.10 ± 0.07	2.52 ± 0 07[c]
- Adjusted[a]	1.94 ± 0 04	2.07 ± 0 04[d]	2.07 ± 0.04[b,d]	2.51 ± 0.05[c]
Kidneys/Adrenals (g)				
- Absolute	0.70 ± 0.01	0 81 ± 0.02[c]	0 85 ± 0.02[c]	0 84 ± 0.02[c]
- Adjusted[a]	0.70 ± 0.01	0 81 ± 0 01[c]	0 84 ± 0.02[b,c]	0.84 ± 0 02[c]
Left testis & epididymis (mg) - Absolute	196 ± 4	194 ± 4	178 ± 5[c]	179 ± 5[d]
- Adjusted[a]	197 ± 4	195 ± 4	177 ± 4[b,c]	179 ± 6[c]
Right testis (mg)				
- Absolute	141 ± 3	136 ± 3	126 ± 4[c]	133 ± 4
- Adjusted[a]	142 ± 3	136 ± 3	124 ± 3[b,c]	133 ± 4
Right epididymis (mg)				
- Absolute	55 ± 3	49 ± 1[d]	46 ± 1[c]	45 ± 1[c]
- Adjusted[a]	55 ± 2	49 ± 2[d]	46 ± 2[b,c]	45 ± 3[c]
Prostate (mg) - Absolute	42 ± 5	45 ± 5	46 ± 5	48 ± 6
- Adjusted[a]	42 ± 5	45 ± 5	47 ± 5[b]	48 ± 7
Seminal vesicles (mg)				
- Absolute	341 ± 15	304 ± 11	314 ± 16	248 ± 9[c]
- Adjusted[a]	343 ± 13	305 ± 13[d]	311 ± 13[b]	247 ± 17[c]

a- Organ weights were adjusted for body weight by analysis of covariance
b- Body weight for one male was not recorded
c- Significantly different from control, $p<0\ 01$
d- Significantly different from control, $p<0.05$

Table 24.
Terminal Body and Organ Weights of Female Task 4 Animals (Mean ± SE)

Parameter	Control (N=20)	0 25% Group (N=19)	0.5% Group (N=20)	1% Group (N=11)
Body Weight (g)	29.77 ± 0.59	29 28 ± 0.36	30.72 ± 0 53	29.47 ± 0 43
Liver (g) - Absolute	1.87 ± 0.06	1 95 ± 0.05	2.18 ± 0.06[c]	2.22 ± 0.05[c]
- Adjusted[a]	1.88 ± 0 04	1.99 ± 0.04[b]	2.12 ± 0.04[c]	2.25 ± 0.05[c]
Kidneys/Adrenals (g)				
- Absolute	0.48 ± 0.01	0.53 ± 0 01[c]	0.56 ± 0.02[c]	0.54 ± 0.01[c]
- Adjusted[a]	0.48 ± 0.01	0.54 ± 0 01[c]	0.55 ± 0.01[c]	0.54 ± 0.02[c]

a- Organ weights were adjusted for body weight by analysis of covariance
b- Significantly different from control, $p<0.05$
c- Significantly different from control, $p<0.01$

Histopathology

The histologic lesions noted in the weighed organs of the males and females are presented in Tables 25 and 26, respectively. No treatment-related lesions were noted in the reproductive organs or adrenal glands of the treated males and females. In addition, evaluation of the cell types in the vaginal mucosa indicated that there were no effects on estrus cyclicity of the treated females. Dose-related, treatment-related lesions were noted in the kidneys of males and females in all treated groups and included interstitial lymphoid accumulations, tubular regeneration, multifocal cortical tubular dilation, cortical tubular casts, microcalculi and mineralization of renal cells. The renal lesions were more severe in the females than in the males. Dose-related liver lesions were noted in males at all doses and included centrilobular hepatocytomegaly, multifocal necrosis and multinucleated giant hepatocytes. An increased incidence of multifocal necrosis and multinucleated giant hepatocytes was noted in the females in the 0.5 and 1% groups. The hepatic lesions were more severe in the males than in the females.

Table 25.
Histopathology Findings for Task 4 Males

Lesion	Control (N=20)	0.25% Group (N=20)	0 5% Group (N=20)	1% Group (N=11)
Right testes - focal atrophy	0	0	1	0
Right epididymis				
- focal chronic inflammation	1	0	0	0
Seminal vesicles				
- Focal capsular lymphoid accumulation	0	0	0	1
Right kidney				
- Tubular cell nuclear variability	0	0	0	2
- Interstitial lymphoid accumulations	3	2	6	9
- Tubular regeneration	2	4	6	10
- Multifocal cortical tubular dilatation	2	1	2	5
- Cortical tubular casts	1	1	0	3
- Healed infarct	0	0	0	1
- Cortical tubular microcalculi	0	0	0	3
- Papillary mineralization	0	0	0	1
- Pelvic dilatation	1	0	3	2
- Multifocal interstitial mineralization	0	0	0	3
Left Kidney				
- Tubular cell nuclear variability	0	0	0	2
- Interstitial lymphoid accumulations	3	2	6	8
- Tubular regeneration	2	4	9	8
- Multifocal cortical tubular dilatation	1	4	5	6
- Cortical tubular casts	0	4	3	6
- Healed infarct	0	0	0	3
- Cortical tubular microcalculi	0	0	0	3
- Papillary mineralization	0	0	0	2
- Pelvic dilatation	0	0	2	0
-Total kidney infarcation	0	0	0	1
-Multifocal interstitial mineralization	0	0	0	3
Adrenal Gland				
- Focal capsular cell hyperplasia	1[a]	2	5	2
- Acute adrenalitis, zona reticularis	0[a]	0	0	1
Liver				
- Centrilobular hepatocytomegaly	0	14	18	10
- Multifocal necrosis	0	14	19	11
- Multinucleated giant hepatocytes	0	13	19	10

a- N = 19

Table 26.
Histopathology Findings for Task 4 Females

Lesion	Control (N=20)	0.25% Group (N=19)	0.5% Group (N=20)	1% Group (N=11)
Uterus				
- Focal stromal mineralization	0	0	1	1
- Focal multinucleated giant cells	0	0	0	1
- Stromal hemosiderosis	8	11	9	4
Vagina				
- Proestrus	4[a]	4	3[a]	1
- Estrus	8[a]	3	7[a]	4
- Metestrus-1	0[a]	0	0[a]	0
- Metestrus-2	3[a]	8	3[a]	0
- Diestrus	4[a]	4	6[a]	6
- Acute vaginitis	0[a]	1	0[a]	0
Ovary - Cyst	1	2	0	0
Right Kidney				
- Tubular cell nuclear variability	0	0	0	7
- Cortical tubular microcalculi	0	0	0	9
- Multifocal cortical tubular dilatation	0	1	3	6
- Cortical tubular casts	0	0	3	3
- Tubular regeneration	4	7	6	9
- Interstitial lymphoid accumulations	3	4	4	4
- Multifocal interstitial mineralization	0	0	0	1
Left Kidney				
- Tubular cell nuclear variability	0	0	0	7
- Cortical tubular microcalculi	0	0	0	9
- Multifocal cortical tubular dilatation	0	3	2	6
- Cortical tubular casts	0	2	1	4
- Tubular regeneration	5	10	8	10
- Interstitial lymphoid accumulations	2	4	4	5
- Multifocal interstitial mineralization	0	0	0	1
- Cysts	0	0	1	0
Adrenal Gland				
- Focal capsular cell hyperplasia	4	1	3	3
Liver				
- Multifocal necrosis	4	5	9	11
- Multinucleated giant hepatocytes	0	0	1	4
- Multifocal microgranulomas	2	3	2	0
- Multifocal mineralization	0	0	0	3

a- N = 19

Sperm Analyses

Sperm analyses were conducted for the control and treated males in Task 4 and are presented in Table 27. There were no differences among treated and control males in sperm concentration or the % abnormal sperm. Males in the 0.5% group had a significant reduction in the % motile sperm compared to controls, but the % motile sperm in the 0 25 and 1% groups were comparable to control.

Table 27.
Sperm Analysis for Task 4 Males (Mean ± SE)

Parameter	Control (N=19)	0.25% Group (N=20)	0 5% Group (N=20)	1% Group (N=11)
% Motile sperm	75 32 ± 0.58	63 85 ± 6.44	51.75 ± 5.81[b]	69 55 ± 8 76
Sperm concentration[a]	651 03 ± 57.79	691 87 ± 51.95	583.04 ± 61.56	535.15 ± 55 48
% Abnormal sperm	7 89 ± 1.21	9.35 ± 1.73	6.84 ± 1.02	7.47 ± 0.74

a- Calculated as No. Sperm X 10^3/mg caudal (epididymal) tissue
b- Significantly different from the control group, $p<0.01$

ASSESSMENT

Our reviewers considered this to be a well-designed and well-executed study. Although this study was primarily designed to examine the effects on the reproductive system, our reviewers noted that a NOAEL could not be determined for the F0 animals since organ weights and histopathology were not examined in the low- and mid-dose groups

CONCLUSIONS

In the range-finding study (Task 1), CD-1 mice were administered dietary levels of 0, 0 31, 0.62, 1.25, 2.5 and 5% bisphenol A for two weeks. The actual levels of bisphenol A ranged from 93-102% of the target levels. Six of eight males and 6/8 females in the 5% group died. Clinical signs of toxicity were noted in the 2.5 and 5% groups, and the percent weight gain for the sexes combined was significantly reduced in the 1.25, 2.5 and 5% groups compared to the control group Based on these results, the study authors concluded that dose levels of 0, 0 25, 0 5 and 1% bisphenol A would be appropriate for the continuous breeding phase of the study (Task 2). Our reviewers concur.

During Task 2, the feed was analyzed at various weeks for the level of bisphenol A. During weeks 1 and 6, the actual levels of bisphenol A ranged from 96-97% and 102-113% of the target levels, respectively During weeks 12 and 18, the actual levels of bisphenol A ranged from 93-100% and 98-104% of the target levels, respectively Two control males died during week 1 and one control female died during week 4 of the study. One female in the 0 25% group died during week 9 and one female in the 0.5% group died during week 4. Administration of bisphenol A for the 18-week period resulted in a slight reduction (8%) in the mean body weights of the males in the 1% group Mean body weight data for the

females during the 18-week period are difficult to interpret due to fluctuations during pregnancy A more meaningful comparison can be obtained from the body weight data for the females immediately following delivery of their litters. Following delivery of each of the five litters, the mean body weight of the females in the 1% group was significantly reduced compared to the control group, indicative of generalized maternal toxicity. Mean food consumption was comparable among treated and control groups.

In Task 2, there were no effects on the fertility of the breeding pairs. The mean number of litters produced per fertile pair and the mean number of live pups per litter (males, females and sexes combined) were significantly reduced in the 0.5 and 1% groups, and the proportion of pups born alive was significantly reduced in the 1% group. The sex ratio was comparable among control and treated groups. There was a significant increase in mean pup body weight for the males, females and sexes combined in the 0.5% group and for the males and sexes combined in the 1% group. However, when the pup body weights were adjusted for the total number of live and dead pups per litter the differences were not statistically significant. Thus, the increased pup body weights in the 0.5 and 1% groups probably reflect the smaller litter size in these two groups

Given the observed effects on reproductive performance, the control and high-dose animals were retained for Task 3. A cross-over mating was conducted in which half of the control females were mated with half the control males (group 1). The remaining control females were mated with the males in the 1% group (group 2), and the remaining control males were mated with the females in the 1% group (group 3). One control female died during week 3 and one male in the 1% group died during week 4. During the 4-week study period, administration of bisphenol A had no effect on mean body weights in the males or females. Compared to controls, the mean food consumption was increased by 19-42% and 7-22% for the males and females in the 1% group, respectively.

In Task 3, there were no effects on the fertility of the breeding pairs There was a significant reduction in the mean number of live pups per litter (males, females, and sexes combined) in group 3 when compared to groups 1 and 2. There was also a significant reduction in the mean number of live pups per litter for sexes combined in group 2 when compared to group 1. The proportion of pups born alive and the sex ratio were comparable among groups. Mean pup body weights for females and sexes combined was significantly increased in group 3 compared to group 1, mean male pup body weight was significantly increased compared to groups 1 and 2 However, when pup body weight was adjusted for the number of live and dead pups per litter, mean pup body weights were comparable among groups The study authors concluded that the apparent increase in pup body weights observed in group 3 were due to the decreased litter size. Our reviewers agree.

At the end of Task 3, the animals were necropsied. The mean body weight of the females in the 1% group was significantly reduced compared to control females. Organ weights were adjusted for body weight by analysis of covariance. There was a significant increase in mean adjusted liver and kidney/adrenal weights for males and females in the 1% group. Adjusted seminal vesicle weights were significantly reduced in the males in the 1% group, but the weights of the other male reproductive organs were comparable among treated and control groups. Treated males had significant reductions in sperm motility, but sperm numbers and morphology were unaffected. Histologic examination revealed no lesions in the male or female reproductive organs Hepatic and renal lesions were noted in both sexes in the 1% group. Hepatic lesions in the males included centrilobular hepatocytomegaly, multifocal necrosis and multinucleated giant hepatocytes; lesions in the females included multifocal necrosis and

multinucleated giant hepatocytes. Renal lesions in the males included tubular cell nuclear variability and an increase in the incidence of several spontaneous lesions including interstitial lymphoid accumulations and tubular regeneration These same lesions were also noted in the females, but these animals also had other renal lesions including large microcalculi in the cortical tubules, sometimes associated with effaced tubular epithelium, tubular regeneration and/or dilated tubules containing proteinaceous or slightly pigmented tubular casts.

For Task 4, selected F1 pups from the last litter of Task 2 were mated on day 74 $\pm$ 10. There was excessive mortality in the 1% group; 6.3% (8/126), 3 8% (4/105), 13.9% (11/79) and 37.5% (18/48) of the pups in the control, 0.25, 0.5 and 1% groups, respectively, died between birth and the beginning of the mating phase at day 74 $\pm$ 10 At birth, there was a significant increase in mean pup body weight in the 0 5 and 1% groups compared to controls. The study authors stated that this may have been due to the reduced litter size in these two groups. Mean body weights were comparable among treated and control groups for the duration of the study. Mean food consumption was comparable among treated and control groups.

Administration of bisphenol A to the F1 animals did not affect fertility and there were no effects on the number of liver pups per litter, proportion of pups born alive, sex ratio, mean body weight or adjusted mean body weight.

At the end of Task 4, the animals were necropsied. Organ weights were adjusted for body weight by analysis of covariance. In males, adjusted weights of the liver and kidney were significantly increased in the 0 25, 0.5, and 1% groups. The adjusted weight of the left testis with attached epididymis was significantly decreased in the 0 5 and 1% groups. The adjusted weight of the right epididymus was significantly decreased in the 0.5 and 1.0% groups and the adjusted weight of the right testis was significantly decreased in the 0.5% group Adjusted seminal vesicle weights were reduced in all treated groups; the differences were statistically significant for the 0.25 and 1% groups. In females, there was a significant increase in adjusted liver and kidney/adrenal weights in all treated groups. Sperm analyses showed decreased sperm motility in 0 5% males, but no clear dose-related responses on sperm motility, concentration or morphology Histologic examination revealed no treatment-related lesions in the reproductive organs or adrenal glands of the treated males and females. In addition, evaluation of the cell types in the vaginal mucosa indicated that there were no effects on estrus cyclicity of the treated females Dose-related lesions were noted in the kidneys of males and females in all treated groups and included interstitial lymphoid accumulations, tubular regeneration, multifocal cortical tubular dilation, cortical tubular casts, microcalculi and mineralization of renal cells. The renal lesions were more severe in the females than in the males. Dose-related liver lesions were noted in males at all doses and included centrilobular hepatocytomegaly, multifocal necrosis and multinucleated giant hepatocytes. An increased incidence of multifocal necrosis and multinucleated giant hepatocytes was noted in the females in the 0 5 and 1% groups. The hepatic lesions were more severe in the males than in the females

EXECUTIVE SUMMARY

This study was conducted according to the NTP's protocol "Fertility Assessment by Continuous Breeding" This study design can include up to four studies which are called tasks. In this particular study, Tasks 1-4 were conducted. Task 1 is a 14-day repeat dose range-finding study Task 2 is the continuous breeding phase to ascertain effects on reproduction in the F0 animals Task 3 is a cross-over

mating of the control and high-dose animals to determine the affected sex Task 4 is designed to assess the reproductive performance of the F1 animals obtained from the final mating during Task 2.

For Task 1, groups of 8 male and 8 female CD-1 mice were administered dietary levels of 0, 0.31, 0.62, 1 25, 2.5 and 5% bisphenol A for two weeks. Body weights and food consumption were recorded weekly. At study termination, the animals were sacrificed and discarded The actual levels of bisphenol A ranged from 93-102% of the target levels. Six of eight males and 6/8 females in the 5% group died. Clinical signs of toxicity were noted in the 2.5 and 5% groups, and the percent weight gain for the sexes combined was significantly reduced in the 1.25, 2.5 and 5% groups compared to the control group.

For Task 2, CD-1 mice were administered dietary levels of 0, 0.25, 0 5 or 1% bisphenol. The control group contained 40 males and 40 females, and each treatment group contained 20 males and 20 females. The animals were treated for 7 days prior to mating, throughout the 98-day breeding phase and for 3 weeks after breeding. For breeding, one male and one female from the same treatment group were cohabited for 98 consecutive days The females were allowed to deliver their litters. The pups were sacrificed within 16 hours of birth so that the breeding pairs could mate again at postpartum estrus. The litters from the final mating were retained for Task 4. The number and proportion of fertile pairs and the number of litters produced per breeding pair was recorded For each litter, the live offspring were sexed, counted, pooled and weighed by sex. The dead pups were counted, but their sex and weights were not recorded At the end of the 18-week study period, the animals in the control and 1% groups were retained for Task 3. The animals in the 0.25 and 0.5% groups were sacrificed and discarded

During weeks 1 and 6, the actual levels of bisphenol A ranged from 96-97% and 102-113% of the target levels, respectively During weeks 12 and 18, the actual levels of bisphenol A ranged from 93-100% and 98-104% of the target levels, respectively. Two control males died during week 1 and one control female died during week 4 of the study. One female in the 0.25% group died during week 9 and one female in the 0 5% group died during week 4. Administration of bisphenol A for the 18-week period resulted in a slight reduction (8%) in the mean body weights of the males in the 1% group. Mean body weight data for the females during the 18-week period are difficult to interpret due to fluctuations during pregnancy. A more meaningful comparison can be obtained from the body weight data for the females immediately following delivery of their litters Following delivery of each of the five litters, the mean body weight of the females in the 1% group was significantly reduced compared to the control group, indicative of generalized maternal toxicity. Mean food consumption was comparable among treated and control groups

In Task 2, there were no effects on the fertility of the breeding pairs. The mean number of litters produced per fertile pair and the mean number of live pups per litter (males, females and sexes combined) were significantly reduced in the 0.5 and 1% groups, and the proportion of pups born alive was significantly reduced in the 1% group. The sex ratio was comparable among control and treated groups. There was a significant increase in mean pup body weight for the males, females and sexes combined in the 0.5% group and for the males and sexes combined in the 1% group However, when the pup body weights were adjusted for the total number of live and dead pups per litter the differences were not statistically significant. Thus, the increased pup body weights in the 0 5 and 1% groups probably reflect the smaller litter size in these two groups

In Task 3, a cross-over mating was conducted in which half of the control females were mated with half the control males (group 1). The remaining control females were mated with the males in the 1% group

(group 2), and the remaining control males were mated with the females in the 1% group (group 3). The animals were cohabited for one week immediately after the completion of Task 2 and then separated for the 21-day segregation phase The dams were allowed to deliver their pups For each litter, the live offspring were sexed, counted, pooled and weighed by sex The dead pups were counted, but their sex and weights were not recorded. At the end of task 3, the F0 animals were sacrificed and necropsied. Selected organ weights were recorded and the tissues were examined histologically In addition, sperm analyses (count, motility and morphology) were conducted. The F1 pups were sacrificed and discarded.

One control female died during week 3 and one male in the 1% group died during week 4. During the 4-week study period, administration of bisphenol A had no effect on mean body weights in the males or females. Compared to controls, the mean food consumption was increased by 19-42% and 7-22% for the males and females in the 1% group, respectively.

In Task 3, there were no effects on the fertility of the breeding pairs. There was a significant reduction in the mean number of live pups per litter (males, females, and sexes combined) in group 3 when compared to groups 1 and 2. There was also a significant reduction in the mean number of live pups per litter for sexes combined in group 2 when compared to group 1. The proportion of pups born alive and the sex ratio were comparable among groups. Mean pup body weights for females and sexes combined was significantly increased in group 3 compared to group 1; mean male pup body weight was significantly increased compared to groups 1 and 2. However, when pup body weight was adjusted for the number of live and dead pups per litter, mean pup body weights were comparable among groups. The study authors concluded that the apparent increase in pup body weights observed in group 3 were due to the decreased litter size. Our reviewers agree

At scheduled necropsy, the mean body weight of the females in the 1% group was significantly reduced compared to control females. Organ weights were adjusted for body weight by analysis of covariance There was a significant increase in mean adjusted liver and kidney/adrenal weights for males and females in the 1% group. Adjusted seminal vesicle weights were significantly reduced in the males in the 1% group, but the weights of the other male reproductive organs were comparable among treated and control groups. Treated males had significant reductions in sperm motility, but sperm numbers and morphology were unaffected. Histologic examination revealed no lesions in the male or female reproductive organs. Hepatic and renal lesions were noted in both sexes in the 1% group. Hepatic lesions in the males included centrilobular hepatocytomegaly, multifocal necrosis and multinucleated giant hepatocytes; lesions in the females included multifocal necrosis and multinucleated giant hepatocytes. Renal lesions in the males included tubular cell nuclear variability and an increase in the incidence of several spontaneous lesions including interstitial lymphoid accumulations and tubular regeneration These same lesions were also noted in the females, but these animals also had other renal lesions including large microcalculi in the cortical tubules, sometimes associated with effaced tubular epithelium, tubular regeneration and/or dilated tubules containing proteinaceous or slightly pigmented tubular casts

For Task 4, the F1 pups from the last litter produced in Task 2 were retained For the control and treated groups in Task 4, 20 male pups and 20 female pups were randomly selected to include 2 pups per sex from each of 10 randomly selected litters if possible The F1 animals were placed on the appropriate diet at weaning and treatment was continued for the duration of the study. When the F1 animals were 74 $\pm$ 10 days of age, each female was mated to a male in the same treatment group fro a period of 7 days. The animals were then separated for the 21-day segregation phase The dams were allowed to deliver their pups. For each litter, the live offspring were sexed, counted, pooled and weighed by sex. The dead pups

were counted, but their sex and weights were not recorded. At the end of Task 4, the F1 animals were sacrificed and necropsied. Selected organ weights were recorded and the tissues were examined histologically. In addition, sperm analyses (count, motility and morphology) were conducted. The F2 pups were sacrificed and discarded

There was excessive mortality in the 1% group; 6.3% (8/126), 3.8% (4/105), 13.9% (11/79) and 37 5% (18/48) of the pups in the control, 0.25, 0.5 and 1% groups, respectively, died between birth and the beginning of the mating phase at day 74 $\pm$ 10 At birth, there was a significant increase in mean pup body weight in the 0.5 and 1% groups compared to controls. The study authors stated that this may have been due to the reduced litter size in these two groups. Mean body weights were comparable among treated and control groups for the duration of the study. Mean food consumption was comparable among treated and control groups.

Administration of bisphenol A to the F1 animals did not affect fertility and there were no effects on the number of liver pups per litter, proportion of pups born alive, sex ratio, mean body weight or adjusted mean body weight.

At scheduled necropsy, mean body weights were comparable among groups. Organ weights were adjusted for body weight by analysis of covariance In males, adjusted weights of the liver and kidney were significantly increased in the 0.25, 0.5, and 1% groups The adjusted weight of the left testis with attached epididymis was significantly decreased in the 0.5 and 1% groups. The adjusted weight of the right epididymus was significantly decreased in the 0 5 and 1.0% groups and the adjusted weight of the right testis was significantly decreased in the 0.5% group. Adjusted seminal vesicle weights were reduced in all treated groups; the differences were statistically significant for the 0.25 and 1% groups. In females, there was a significant increase in adjusted liver and kidney/adrenal weights in all treated groups. Sperm analyses showed decreased sperm motility in 0.5% males, but no clear dose-related responses on sperm motility, concentration or morphology. Histologic examination revealed no treatment-related lesions in the reproductive organs or adrenal glands of the treated males and females In addition, evaluation of the cell types in the vaginal mucosa indicated that there were no effects on estrus cyclicity of the treated females Dose-related lesions were noted in the kidneys of males and females in all treated groups and included interstitial lymphoid accumulations, tubular regeneration, multifocal cortical tubular dilation, cortical tubular casts, microcalculi and mineralization of renal cells. The renal lesions were more severe in the females than in the males. Dose-related liver lesions were noted in males at all doses and included centrilobular hepatocytomegaly, multifocal necrosis and multinucleated giant hepatocytes. An increased incidence of multifocal necrosis and multinucleated giant hepatocytes was noted in the females in the 0 5 and 1% groups. The hepatic lesions were more severe in the males than in the females.

For the F0 generation, the LOAEL for reproductive toxicity was 0 5% based on a reduction in the mean number of live pup per litter, and the NOAEL was 0.25%. For the F1 generation, the LOAEL for reproductive toxicity was 0.25% based on significant changes in reproductive organ weights in males at all dose levels, and a NOAEL was not determined. For the F0 generation, the LOAEL/NOAEL for systemic toxicity could not be determined since only the high-dose animals were necropsied. For the F1 generation, the LOAEL for systemic toxicity in was 0.25% based on renal toxicity, and a NOAEL was not determined.

LITERATURE CITED

Soares ER, Sheridan W, Haseman JK, Segal M. 1979. Increased frequencies of aberrant sperm as indicators of mutagenic damage in mice *Mutat Res* 64:27-35

Wyrobeck AJ, Bruce JR 1975. Chemical induction of sperm abnormalities in mice. *Proc Natl Acad Sci (USA)* 72:4425-4429.

Bisphenol A: Reproduction and Fertility Assessment in CD-1 Mice When Administered in the Feed

SIGNATURE AND DATE

Technical Reviewer:

/s/ /s/ Date 7/27/01

Jennifer Seed, Ph.D.

QA/QC Reviewer:

/s/ /s/ Date 7-30-01

Jennifer L. Rojko, D.V.M., Ph.D., D.A.C.V.P.

Appendix A: Data Validation

Bisphenol A (BPA): Reproduction and Fertility Assessment in CD-1 Mice When Administered in the Feed

I. Animals Followed Throughout the Study

Twenty percent of all animals were selected for review using the random number generator in Microsoft EXCEL. Data for all animals selected for review were checked in all tables containing individual animal data. No abnormalities were noted in examining the individual animal data.

Males:				Females:			
0.0% BPA	0.25	0.5	1.0	0.0% BPA	0.25	0.5	1.0
D045	D013	D004	D155	D311	D261	D318	D331
D131	D103	D030	D111	D270	D200	D333	D221
D156	D154	D072	D037	D222	D263	D240	D310
D113	D092	D133	D125	D217	D257	D206	D176
D107				D327			
D130				D180			
D006				D241			
D090				D300			

II. Critical Effects and Spot Checks

A comparison of the study protocol to the materials and methods was performed. Standard error was calculated because the data were included in the study report. Information included in the objective, abstract, protocol, and tables were examined

Group mean values were verified through calculation using individual animal data in the following tables:

- Table 4: Reproductive Performance of Fertile Pairs During Continuous Breeding (live pups per litter, live pup weight for control and 1% groups);
- Table 5: Postpartum F_0 Dam Weights Following the Delivery of Each Litter (Litter 1,3,5 for control and 1% groups);
- Table 6: Mating and Fertility of Pairs After a Mating Trial to Determine the Affected Sex (Number with copulatory plug/Number cohabited for all groups);
- Table 9: Male Body Weight and Organ Weights at Necropsy. Combined Control Males vs. 1.0% BPA Males (body weight, right testis weight, and prostate weight);
- Table 11: Female Body Weight and Organ Weights at Necropsy: Combined Control Females vs. 1 0% BPA Females (body weight, lever weight, and kidney/adrenal weight);
- Table 13. Sperm Analysis: Combined Control Males vs. 1 0% BPA Males (1% group);
- Table 15. Mating and Fertility of Pairs of Final Task 2 Offspring: Effect of Continuous Exposure to BPA (Number with copulatory plug/Number cohabited for all groups);
- Table 17: Postpartum F_1 Dam Weights Following the Delivery of a Single Litter (control and 1% groups);
- Table 18. Male Body Weight and Organ Weights of Final Task 2 Offspring at Necropsy (body weight, right testis weight, and prostate weight for control and 1% groups);
- Table 20: Female Body Weight and Organ Weights of Final Task 2 Offspring at Necropsy (body weight, liver weight, and kidney/adrenal weight for control and 1% groups); and

- Table 22. Sperm Analysis for Final Task 2 Male Offspring: Effect of Continuous Exposure to Bisphenol A (control and 1% groups).

The following deviations were noted:

- In Table 4, the calculation for mean live female pup weight per litter at 1.0% BPA was given as 1.62 ± 0.05. Our calculations resulted in a mean live weight of 1.61 ± 0.05. This difference may be due to rounding error.

- Several differences were noted in the data reported in Table 6 compared with the individual data given in Table A8 The differences are noted in the table below.

Group	No. with copulatory plugs/No. cohabited	
	Data from Table 6	Individual data from Table A8
control x control	17/19	14/19
1.0% BPA male x control female	18/19	19/20
control male x 1.0% BPA female	18/19	17/19

- Slight differences were found in the calculated means found in Table A17 and the summary table in Table 18. Our calculations corresponded with the means reported in Table A17. The differences are noted below.

Concentration	Mean from Table 18	Mean from Table A17
0.0	32.24 ± 0.77	32.25 ± 0.77
1.0	32.67 ± 0.61	32.67 ± 0.6

- Our reviewers were unsure as to how percent tailless sperm were calculated in Table 13 and 22, therefore, percent tailless sperm data were not validated with the individual data found in Tables A10 and A16.

- The sperm concentration calculated from the control group in the individual data in Table A16, 651.3 ± 57 7, did not correspond with the data reported in the summary data in Table 22 of 651.03 ± 57 79. These differences may be due to rounding and typographical errors.

III. Findings

As noted above, discrepancies or inconsistencies revealed in this data validation included the appearance of some abnormal values. It also included some differences in actual and calculated means and standard errors. These issues, however, do not affect the confidence in this data study report.

/s/

/s/

Lilia Chen, B.S.

7/12/00

Date

MAY 3 0 2001

/s/

/s/

Contract Number

223-96-2302

Work Assignment Number

2000-19
(ICF 419)

Task Number

05

FDA Study Identification Number

NTP 84-105

Signature of Program Manager

/s/

/s/

Date

5-25-01

STUDY TITLE

Bisphenol A: Reproduction and Fertility Assessment in CD-1 Mice
When Administered Via Subcutaneous Silastic Implants

TESTING LABORATORY

Research Triangle Institute
Chemistry and Life Sciences Group
Life Sciences and Toxicology Division
P.O. Box 12194
Research Triangle Park, NC 27709

COMPLETION DATE OF STUDY

January 20, 1984

SPONSOR OF STUDY

National Toxicology Program
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

LABORATORY STUDY NUMBER

RTI #31U-2344

STUDY SUMMARY

Compliance Statement

The study protocol indicated that the study would be conducted according to FDA GLP regulations. The study report was signed by the Study Director on January 11, 1984, but did not contain a specific compliance statement.

Quality Assurance Statement

A quality assurance statement was included with the study, and was signed by the Quality Assurance Unit on January 12, 1984.

Study Objective

The study objective was to evaluate the effects of bisphenol administered via subcutaneous Silastic implants in CD-1 mice on mating performance, fertility, and pregnancy in the F0 generation. The study was conducted according to NTP's "Fertility Assessment by Continuous Breeding" which consists of five tasks. Task 1 is a 14-day repeat dose range-finding study. Task 2 is the continuous breeding phase to ascertain effects on reproduction in the F0 animals. Task 3 is only conducted when positive findings are noted in Task 2, and consists of cross-over breeding of the control and high-dose F0 animals to determine the affected sex. Task 4 is only conducted if no effects are noted in Task 2, and evaluates reproduction in the F1 animals from the control and high-dose groups. Task 5 evaluates reproductive hormone patterns in plasma samples obtained at necropsy of animals in Task 3 or 4. In this study, only Tasks 1 and 2 were conducted.

MATERIALS AND METHODS

Test Article

Description and Receipt. The test article bisphenol A ($C_{15}H_{16}O_2$; CAS No. 80-05-7) was supplied by Alpha Products. The compound was received by Midwest Research Institute (MRI), Kansas City, MO on February 18, 1982 The lot number was 011681 and the batch number was 1. Information on the purity and stability of the compound was supplied by MRI. The compound was subsequently shipped to Research Triangle Institute (RTI)

Analysis of Purity, Homogeneity and Stability. MRI conducted spectrophotometric and chromatographic analyses of the compound, and determined that the test article was approximately 95% pure MRI found that the test article was easily suspended in corn oil by shaking and stirring. Rehomogenization was easy in samples that had settled for five days For the analysis of homogeneity, solutions of bisphenol A in corn oil were prepared for the prechronic and chronic dose levels of 400 mg/ml and 50 mg/ml, respectively Five 200 ml portions of the suspension were poured into 250 ml glass-stoppered flasks The contents of one of the five flasks were stirred using a magnetic stir bar. Using a syringe with a 16-gauge needle, two 1-ml aliquots were removed from the flask and placed in 5ml septum vials for analysis. Each of the remaining four flasks were similarly sampled over the next four days. The analyses showed a maximum of 1.8% variation in dose concentration from the mean values determined for the suspension The suspension prepared at the chronic dose level was analyzed for stability, and found to be stable for at

least 3 weeks in the dark at room temperature. The certificate of analysis is provided in Appendix 8 4 of the study report

Preparation of Implants. The implants were prepared from Dow-Corning Silastic tubing, with an inner diameter of 0.078 inches and an outer diameter of 0.125 inches. The tubing was cut into varying lengths as shown in Table 1 One end of the tubing was sealed with Dow-Corning medical grade elastomer. A disposable plastic syringe fitted with a 1 ½ inch needle was used to fill a 50% (w/v) suspension of bisphenol A in corn oil. The other end of the tubing was then sealed with Dow-Corning medical grade elastomer. The implants were placed in individual glass vials and stored at 4°C.

Implant Analysis At the beginning and the end of Task 1, one implant per dose group was sent to MRI for analysis. At the beginning of Task 2, one implant per dose group was sent to MRI, and at the end of Task 2 the implants from all animals were sent to MRI. The contents from each implant were dissolved in acetone. Subsequently, the samples were diluted with a solution of N-phenylcarbazolein acetone which was the internal standard and quantified by gas chromatography. The certificate of analysis is presented in Appendix 8.1 of the study report

Animals

Receipt and Acclimation. A total of 300 COBS Crl.CD-1, (ICR)BR mice, 150/sex, were supplied by Charles River Breeding Laboratories, Inc., Kingston, NY on October 25, 1982. The animals were 6 weeks of age at the time of receipt. Animals that were to be used for Task 1 were maintained in quarantine for two weeks and animals that were to be used for Task 2 were isolated for five weeks. After two weeks of quarantine, two males and two females were sacrificed. Serum samples were obtained and shipped to Microbiological Associates, Inc , Bethesda, MD for determination of antibodies (indicative of prior exposure/infection) to 11 mouse viruses The study report states that all sera were negative, but the report from Microbiological Associates was not included

Housing, Diet and Environment. During the quarantine period, 10 animals per sex were caged together and during the premating period of Task 2, 5 animals per sex were caged together. Subsequently, the Task 1 and 2 animals were caged individually except during the mating phase of Task 2 The rats were housed in solid bottom polypropylene or polycarbonate cages with stainless steel wire lids. All cages were supplied with Ab-Sorb-Dri bedding which was supplied by Laboratory Products, Inc., Garfield, NJ. The cages were sanitized weekly with Life-Sci-100 detergent and 180°F water; the detergent was supplied by Dubois Chemical Co , Cincinnati, OH The animals were supplied with 14 hours of light and 10 hours of dark; the photoperiod was automatically controlled. The room temperature and humidity were monitored with a DP220 Datapod Electronic Hygrothermograph supplied by Omnidata International, Inc., Logan, UT. Room temperature ranged from 17 6 - 21.5°C and the humidity ranged from 48 - 70% during the study.

The animals were provided food (Purina rodent chow #5002) and water ad libitum.

Placement of Implants. The animals were weighed and anesthetized by an intraperitoneal injection of 60 mg/kg of a 7:1 (w/w) solution of ketamine and xylazine. For animals receiving a single implant, the tubing containing the bisphenol A was implanted subcutaneously in the cervical dorsum For animals receiving two implants, a single dorsomedial incision was made in animals receiving two implants, and each piece of tubing was placed on opposite sides lateral to the spine.

The implants were expelled from several animals in all groups through cutaneous lesions that developed adjacent to the implant or at the original incision. This happened to some animals as many as four times during the study. A new piece of tubing was then implanted in the manner described above. Pregnant animals were allowed to deliver their litters prior to implant replacement

Experimental Design

Assignment to Study Group and Dose Selection. The mice were weight-stratified and randomly assigned to control and treatment groups. The dose levels for Task 1 were based on information available in the literature, and the dose levels for Task 2 were based on the results of Task 1. The dose levels, length of implant, volume of the bisphenol A/corn oil in the implant and the number of implants per animal for Tasks 1 and 2 are presented in Table 1. For Task 1, there were 8 animals/sex/group. For Task 2, there were 40 animals/sex in the control group and 20 animals/sex in each treatment group

TABLE 1

Dose Levels and Implant Lengths for Tasks 1 and 2

Task	Dose Level (mg bisphenol A per implant)	Length of Implant (inches)[a]	Volume in Implant (μl)[b]	No. Implants per Animal
1	0	1.125	100 0	2
	6.25	0.250	12.5	1
	12 5	0.375	25 0	1
	25	0 625	50.0	1
	50	1.125	100.0	1
	50	1 125	100.0	2
2	0	1.125	100.0	2
	25	0.625	50.0	1
	50	1.125	100.0	1
	50	1.125	100 0	2

a- Length of plugs on both ends included (0.125 inches total)
b- The volume of corn oil or a 50% (w/v) suspension of bisphenol A in corn oil per implant

Dosing Period. The Task 1 animals were 8 weeks of age at the beginning of treatment and were treated for 14 days. The Task 2 animals were 11 weeks of age at the beginning of treatment. The animals were treated for 7 days before mating, throughout the 98 day breeding phase and during the subsequent 21 day segregation phase.

Clinical Observations

During the study all animals were examined twice daily for mortality, morbidity, and signs of toxicity. A complete physical examination was conducted once weekly.

Body Weight Measurements

Each Task 1 and Task 2 animal was weighed at the beginning of the study and weekly thereafter. Body weights of Task 2 animals were also recorded at termination

Breeding Procedures for Task 2

Following the 7 day premating period, each female was paired with a male from the same treatment group for 98 consecutive days The females were allowed to deliver each litter. The pups were sacrificed within 16 hours of birth so that the breeding pairs could mate again at postpartum estrus. The number and proportion of fertile pairs and the number of litters produced per breeding pair were recorded.

Litters

For each litter, the live offspring were sexed, counted, pooled and weighed by sex. The dead pups were counted, but their sex and weights were not recorded

Scheduled Necropsy Examinations

After 14 days of treatment, the Task 1 animals were bled by cardiac puncture. The samples for each sex and treatment group were pooled and centrifuged at 2000 rpm for 15 minutes. The plasma was harvested and then frozen and stored at -20°C. The frozen plasma samples were shipped to MRI for analysis of bisphenol A levels by electron capture gas chromatography. The certificate of analysis is included in Appendix 8.1 of the study report. The weight of the female reproductive tract (ovaries, oviduct, uterus and upper half of vagina) was recorded Otherwise, the animals were not necropsied.

At the end of Task 2, the animals in the control and 100 mg groups were asphyxiated with CO_2 and bled by cardiac puncture. The weights of the liver, intact female reproductive tract (ovaries, oviduct, uterus and upper half of vagina), testis with attached epididymis (each side weighed separately), seminal vesicles and prostate gland were recorded The heads were removed and the cranial roofs excised The exposed brain and pituitary were fixed in situ by immersion in 10% neutral-buffered formalin. After fixation, the brain (cerebrum, cerebellum and medulla) and pituitary were dissected and the weights were recorded. The livers were discarded and the testes were preserved in Bouin's solution. The remaining reproductive tissues were preserved in 10% neutral-buffered formalin The animals in the 25 and 50 mg groups were sacrificed and discarded.

Statistical Evaluation

The number of litters and the number of live pups per litter were computed for each fertile pair of Task 2 animals and then treatment means were determined. The following formula was used to calculate the fertility index:

$$\frac{\text{\# of breeding pairs producing viable litter}}{\text{\# of breeding pairs}} \times 100$$

The statistics used to analyze the various parameters are presented in Table 2

Table 2

Statistical Evaluation

Parameter	Statistic
Task 1 - % body weight gain over 14-day period, female reproductive tract weight	Analysis of variance and Dunnett's Multiple Range Test
% mortality, proportion of fertile pairs	Chi Square Test for homogeneity and Fisher's Exact test for pairwise comparisons
Mean number of litters, mean litter size, number of live offspring, proportion of live pups, sex of live pups	Chi Square Approximation to the Kruskal-Wallis test, Jonckheere's Test and Mann-Whitney U Test
Mean pup body weights, organ weights	Analysis of variance using the Kruskal-Wallis Test and pair-wise comparisons using the Mann-Whitney U Test and Jonckheere's Test. Analysis of covariance and t-test to judge significance of differences
Task 2 - terminal body weights	Analysis of variance and t-test to judge significance of differences

Appraisal of Protocol/Experimental Design

The study protocol was provided in Appendix 8.4 of the study report. The study was well designed and followed NTP's reproductive toxicology testing scheme designated "Fertility Assessment by Continuous Breeding". At the time that the study was conducted the protocol was new to the scientific and regulatory community. Although FDA and EPA have never formally converted this protocol into a test guideline, it is generally accepted in lieu of a two-generation reproductive toxicity study.

STUDY RESULTS

TASK 1

Analysis of Levels of Bisphenol A in Implants and Serum

The levels of bisphenol A in the implants on day 0 and day 14 of Task 1 are presented in Table 3 and the plasma levels are presented in Table 4. At the beginning of Task 1, the implants contained 69-88% of the target dose of bisphenol A and at the end of the 14-day treatment period, the implants contained 55-62% of the target dose. There were not substantial differences in the amount of released bisphenol A in the 6.25, 12.5 and 25 mg groups Based on the amount of bisphenol A present in the implants at the beginning and at the end of the study, the total dose per mouse over the 14-day treatment period was

calculated as 1.6, 1.4, 1 6, 5.6 and 10.5 mg bisphenol A for the 6.25, 12.5, 25, 50 and 100 mg groups, respectively. Actual plasma levels were below the limit of detection in the 6 25 mg group. Our reviewers note that these values represent less than 11% of the target values. The mean plasma level was 7.0 and 7.7 ng/ml for the females and males in the 12.5 mg group, respectively and 8.3 and 8.4 ng/ml for the females and males in the 25 mg group, respectively. As shown by the amount of bisphenol A released from the implants, there were not substantial differences in the plasma levels in these two dose groups. In the next two higher dose groups there were differences in the plasma levels for males and females. The mean plasma levels were 13 1 and 18.5 ng/ml for the females and males in the 50mg group, respectively and 56.2 and 31.5 ng/ml for the females and males in the 100 mg group, respectively. The study authors could not provide a reason for this sex difference.

Table 3

Levels of Bisphenol A in Implants (Task 1 Animals)

Study Day	Sample Identification	Bisphenol A Found (mg) [a]	Bisphenol A Found Per Label (%)
0	0 mg/implant	< mdl[b]	--
	6.25 mg/implant	5.5	88
	12.5 mg/implant	8.3	66
	25 mg/implant	16.7	67
	50 mg/implant	34.0	68
	50 mg/implant	34.5	69
14	0 mg/implant	< mdl[b]	--
	6 25 mg/implant	3 9	62
	12.5 mg/implant	6 9	55
	25 mg/implant	15.1	60
	50 mg/implant	28.4	57
	50 mg/implant	29.5	59

a- Bisphenol A measured by gas chromatography
b- Minimum detectable level was 0.3 mg

Table 4

Plasma Levels of Bisphenol A (Task 1 Animals)

Group	Sex	Bisphenol A (ng/ml)[a]	Mean ± SD
Control	M F	< mdl[b] < mdl	< mdl < mdl
6.25	M F	< mdl < mdl	< mdl < mdl
12.5	M F	8.2, 7.2 5.3, 8.6	7.7 ± 0.7 7.0 ± 2.3
25	M F	7.8, 8 9 8.3[c]	8.4 ± 0 8 8.3[c]
50	M F	19.8, 16.5, 19.3 10 2, 13.9, 15.3	18.5 ± 1 8 13 1 ± 2.6
100	M F	32.2, 26.7, 35.7 57.4, 55.0	31.5 ± 4 5 56.2 ± 1.7

a- Plasma was pooled within treatment and sex
b- Minimum detectable limit was 3 ng/ml
c- Duplicate lost due to hemolysis of the sample

Mortality

One female in the 12.5 mg group died during necropsy and one male in the 25 mg group was killed by his cage mates. In addition, one female in the 50 mg group and one male and one female in the 100 mg group were removed from the study because the implants were lost All other animals survived until scheduled termination

Clinical Observations

The study report states that there were no clinical signs of toxicity noted in the control or treated groups. No further details were provided.

Body Weight

The mean body weights at the beginning and end of Task 1 are presented in Table 5. Mean body weights and the percent change in body weight over the two week treatment period were comparable among treated and control groups.

Table 5

Mean Body Weights Of Task 1 Animals (Mean ± SE)

Group	Initial Body Weight (g)	Final Body Weight (g)	% Change
Control - Male (N=8) - Female (N=8)	33.6 ± 0 6 26.3 ± 0.5	37.9 ± 0 8 27 5 ± 0.5	12.9 ± 2 2 4 6 ± 1 0
6.25 - Male (N=8) - Female (N=8)	34.0 ± 0.7 26.4 ± 0.5	36 0 ± 0.8 28 2 ± 0.6	6.0 ± 2.2 7 0 ± 2.3
12.5 - Male (N=8) - Female (N=7)	33.9 ± 0 7 26.1 ± 0 5	35.5 ± 0 8 27.4 ± 0 8	4.9 ± 1 9 4.9 ± 1 5
25 - Male (N=7) - Female (N=8)	33 9 ± 1.1 26.5 ± 0.5	37.1 ± 1.2 27.9 ± 0.4	9.2 ± 1.1 5.4 ± 1.5
50 - Male (N=8) - Female (N=7)	33.7 ± 0.5 26 6 ± 0.7	36.7 ± 0.6 28 1 ± 0.5	9.2 ± 1.9 5.8 ± 1.7
100 - Male (N=7) - Female (N=7)	33.9 ± 0.9 26 2 ± 0.4	37.5 ± 0.9 28.2 ± 0.8	10.7 ± 2.4 7.6 ± 2.0

Scheduled Necropsy

No gross necropsies were conducted on the Task 1 animals In females, the reproductive tract (ovaries, oviduct, uterus and upper half of vagina) was removed intact and the weights were recorded. The mean weights were 159.0 ± 9.7, 175 6 ± 22.9, 179 0 ± 10 8, 186 4 ± 25.3, 146.6 ± 9.3 and 241.3 ± 36.9 for the control, 6 25, 12.5, 25, 50 and 100 mg groups, respectively. The mean weight of the reproductive tract was higher in the 100 mg group than in the control group, but due to the high variability statistical significance was not achieved. The study authors stated that this may be indicative of a potential effect that would have to be confirmed in Task 2.

TASK 2

Analysis of Levels of Bisphenol A in Implants

The levels of bisphenol A in the implants on day 0 and at the end of the 18-week study period of Task 2 are presented in Table 6. No bisphenol A was detected in the control implants At the onset of the study, the implants contained 65-71% of the target amount of bisphenol A At the end of the study, the implants contained 18-30% of the initial target level of bisphenol A Based on the amount of bisphenol A present in the implants at the beginning and at the end of the study, the total dose per mouse over the 18-week treatment period was calculated as 11-12.3 mg for the 25 mg group, 17.7-19.3 mg for the 50 mg group and 37 2-40 mg for the 100 mg group. Our reviewers note that these values represent less than half the target values.

Table 6

Levels of Bisphenol A in Implants (Task 2 Animals)

Study Week	Sample Identification	Bisphenol A Found (mg) [a]	Bisphenol A Found Per Label (%)
Week 0	0 mg/implant	< mdl[b]	--
	25 mg/implant	16.9	68
	50 mg/implant	32.3	65
	50 mg/implant	35.4	71
Week 18[c] (Mean ± SD)	0 mg/implant	< mdl	--
	0 mg/implant	< mdl	--
	25 mg/implant	4.6 ± 1 8	18 0 ± 7 0
	25 mg/implant	5.9 ± 1.2	24 0 ± 5 0
	50 mg/implant	13.0 ± 1.9	26 0 ± 4 0
	50 mg/implant	14.6 ± 3.2	30 0 ± 6 0
	50 mg/implant X 2	27.7 ± 4.7	27.0 ± 4.0
	50 mg/implant X 2	30.5 ± 5.5	30.0 ± 6.0

a- Bisphenol A measured by gas chromatography
b- Minimum detectable level was 0.3 mg
c- Mean ± SD, the first value is for males and the second value is for females

Mortality

One control female and one female in the 50 mg group died during the study. The study authors did not state the cause of death. The fertility and reproductive performance data for the breeding pair of these two females was not included in the analysis.

Clinical Observations

The study report states that there were no clinical signs of toxicity noted in the control or treated groups No further details were provided.

Several animals developed irritation and cutaneous lesions at the site of the implants. Often the implants were expelled at these sites (presumably through ulcerated lesions) or through the site of the initial incision (presumably through incompletely healed skin) Our reviewers note that the study authors did not indicate whether or not the dorsomedial skin incisions were closed (via clip, suture, or staple) or left open to heal by granulation.

Body Weight

The mean body weight for selected weeks of the study are presented in Tables 7 and 8 for males and females, respectively. Mean body weights were comparable among treated and control groups. The fluctuation in female body weights reflects the pregnancies occurring throughout the study

Table 7

Mean Male Body Weights for Selected Weeks of Task 2 (Mean ± SE)

Study Week	Control (N=40)	25 mg Group (N=20)	50 mg Group (N=20)	100 mg Group (N=20)
1	37.7 ± 0 4	37.1 ± 0 6	37.6 ± 0 5	37.8 ± 0.6
3	38.0 ± 0.4	37.1 ± 0.5	40.1 ± 1 6	37 7 ± 0 6
5	38.4 ± 0.4	36.9 ± 0.5	37.7 ± 0.4	37 0 ± 0 6
7	36.5 ± 0.4	36.5 ± 0.5	37.7 ± 0 5	34 8 ± 1 1
9	39.7 ± 0.5	38.7 ± 0.6	39.6 ± 0 5	38.5 ± 0.6
11	38.9 ± 0 4	38 0 ± 0 6	38 9 ± 0.5	38.4 ± 0.8
13	39.1 ± 0.6	38.1 ± 0.6	38.6 ± 0.7	36.3 ± 1.5
15	40 1 ± 0.4	37.3 ± 1 8	39.3 ± 0 6	38 6 ± 0.8
18	40.2 ± 0.4	39.8 ± 0.7	39.8 ± 0 6	39.1 ± 0.6

Table 8

Mean Female Body Weights for Selected Weeks of Task 2 (Mean ± SE)

Study Week	Control (N=40)	25 mg Group (N=20)	50 mg Group (N=20)	100 mg Group (N=20)
1	29.0 ± 0.3	28.4 ± 0.4	29.1 ± 0 4	28 8 ± 0.4
3	47 9 ± 7 2	37 9 ± 1 0	39.6 ± 1 3	40.3 ± 1.1
5	37 7 ± 0.6	36 9 ± 0.9	39.8 ± 1 7	38.0 ± 0 9
7	41.2 ± 1.2	40.2 ± 1.5	43.5 ± 2.0	39 1 ± 1.5
9	48 2 ± 1.1	45.3 ± 1.6	47.4 ± 1.9	47.1 ± 1.8
11	44.3 ± 1.4	44.3 ± 1 8	45 2 ± 1.8	45.4 ± 1.9
13	43 2 ± 1.2[a]	40.9 ± 0.9	47.1 ± 1.7	43.3 ± 1.7
15	48.6 ± 1.2	52.0 ± 2.1	46.4 ± 1.6	49 5 ± 1 6
18	37.2 ± 0.5	36.9 ± 0.7	38.9 ± 1.0	37.7 ± 0.7

a- One control animal died during week 12: n=39 for weeks 12-18.

Reproductive Performance

The reproductive performance parameters for the Task 2 animals are presented in Table 9. One breeding pair in the 50 mg group had only one litter consisting of all dead pups. The sex and weight of the pups was not determined so the data are not included in Table 9. The fertility index was comparable among treated and control groups The index was 97, 95, 100 and 100% for the control, 25, 50 and 100 mg groups, respectively. The mean number of litters produced per fertile pair and the proportion of pups born alive were comparable among treated and control groups. There was a significant increase in the mean number of male pups per litter in the 50 mg group compared to all other groups (treated and control) and a significant increase in the mean number of live pups per litter in the 50 mg group compared to the control and 100 mg group. The biological significance of this is unclear since a similar increase was not observed in the 100 mg group The mean male pup body weight was significantly increased in the 100 mg group relative to the control and 25 mg groups, and the mean female body weight in the 100 mg group was significantly increased relative to the 50 mg group However, the mean combined pup body weights were comparable among treated and control groups. The body weights were adjusted for the total number of live and dead pups per litter by analysis of covariance. The adjusted male body weight in the 100 mg group was significantly increased compared to the control and 25 mg groups and in the 50 mg group compared to the control group The adjusted body weight of males and females combined was significantly increased in the 100 mg group compared to the 25 mg group The adjusted female body weight was significantly increased in the 100 mg group compared to the control and 25 mg groups.

Table 9

Reproductive Performance of Task 2 Animals (Mean ± SE)

Parameter	Control N=38	25 mg Group N=19	50 mg Group N=19	100 mg Group N=20
Litters per pair	4 39 ± 0 12	4.26 ± 0.20	4.53 ± 0.23	4 45 ± 0.17
Live pups per litter				
- Males	4.88 ± 0 18	4 59 ± 0.31	5.56 ± 0 39[c]	4.77 ± 0.27
- Females	5.06 ± 0.19	4.95 ± 0.39	5.10 ± 0.38	4.72 ± 0.28
- Combined	9.93 ± 0.29	9 54 ± 0.58	10.66 ± 0.71[d]	9.49 ± 0 48
Proportion of pups born alive	1.00 ± 0 00	0 99 ± 0.00	0.95 ± 0.05	0.99 ± 0.00
Sex Ratio (# Males/total pups)	0.49 ± 0.01	0.48 ± 0 02	0 52 ± 0.01[a]	0.50 ± 0 02
Live pup weight (g)				
- Males	1.69 ± 0.02	1.70 ± 0.03	1 73 ± 0 04[a]	1.78 ± 0.03[e]
- Females	1.64 ± 0 02	1 64 ± 0.03	1.63 ± 0.03[a]	1.74 ± 0.04[f]
- Combined	1.68 ± 0.02	1.67 ± 0.03	1 68 ± 0 03[a]	1.75 ± 0.03
Adjusted live pup weight (g)[b]				
- Males	1.69 ± 0 02	1.69 ± 0.03	1.76 ± 0.03[a,g]	1.77 ± 0 03[e]
- Females	1.64 ± 0.02	1 63 ± 0.03	1.67 ± 0.03[a]	1 72 ± 0 03[h]
- Combined	1.67 ± 0.02	1.66 ± 0.03	1.72 ± 0 03[a]	1.73 ± 0.02[i]

a- Data for 18 fertile pairs
b- Mean adjusted for total number of live and dead pups per litter by analysis of covariance
c- Significantly different from the control, 25 and 100 mg groups, $p<0.05$
d- Significantly different from the control and 100 mg groups, $p<0.05$
e- Significantly different from the control and 25 mg groups, $p<0.05$
f- Significantly different from the 50 mg group, $p<0.05$
g- Significantly different from the control group, $p<0.05$
h- Significantly different from the control and 25 mg groups, $p<0.05$
i- Significantly different from the 25 mg group, $p<0.05$

Scheduled Necropsy

The terminal body and organ weights of the control and 100 mg groups are presented in Table 10. The mean body weights and organ weights were comparable among treated and control groups.

Table 10

Terminal Body and Organ Weights of Task 2 Animals (Mean ± SE)

Parameter	Control Males (N=40)	Control Females (N=39)	100 mg Group Males (N=20)	100 mg Group Females (N=20)
Body Weight (g)	39.87 ± 0.43[a]	37 22 ± 0.48	39.17 ± 0.55[a]	37.90 ± 0 67
Liver (g)	2 168 ± 0.033	2.286 ± 0 057	2 127 ± 0.028	2 286 ± 0.064
Brain (g)	0.453 ± 0.005	0.471 ± 0 006	0.459 ± 0.008	0.483 ± 0.007
Pituitary (mg)	2.3 ± 0.1[b]	4 2 ± 0.2[c]	2.1 ± 0 1[b]	4.1 ± 0.2[b]
Female reproductive tract (g)	-----	0 393 ± 0.178	-----	0.383 ± 0.025
Left testis and epididymis (mg)	193 ± 5.0	-----	194.0 ± 5.0	-----
Right testis (mg)	137 0 ± 4.0	-----	137.0 ± 6.0	-----
Right epididymis (mg)	58.0 ± 1.0	-----	59.0 ± 2 0	-----
Prostate (mg)	57 0 ± 3.0	-----	58.0 ± 4.0	-----
Seminal vesicles (mg)	434.0 ± 20 0	-----	432 0 ± 30.0[d]	-----

a- One animal was not weighed
b- One pituitary was lost due to improper dissection
c- Two pituitaries were lost due to improper dissection
d- Seminal vesicles were not weighed for one male by accident

ASSESSMENT

Although this was a well-designed and well-executed study, it was flawed by two factors: (1) the release of the test article from the implants was too low, and (2) the rate of implant expulsion/loss was excessive.

CONCLUSIONS

From the results of the range-finding study (Task 1), the study authors concluded that it is possible to administer bisphenol A via the Silastic implants The bisphenol A was released from the implants and was detectable in the plasma of the animals. Based on the amount of bisphenol A present in the implants at the beginning and at the end of the study, the total dose per mouse over the 14-day treatment period was calculated as 1.6, 1 4, 1.6, 5 6 and 10 5 mg bisphenol A for the 6.25, 12.5, 25, 50 and 100 mg groups, respectively. Our reviewers note that these values represent less than 11% of target values for Task 1 animals. Actual plasma levels were below the limit of detection in the 6.25 mg group. The mean plasma level was 7.0 and 7.7 ng/ml for the females and males in the 12 5 mg group, respectively and 8.3 and 8.4 ng/ml for the females and males in the 25 mg group, respectively. The mean plasma levels were 13.1 and

18 5 ng/ml for the females and males in the 50 mg group, respectively and 56 2 and 31 5 ng/ml for the females and males in the 100 mg group, respectively Administration of bisphenol A did not affect mean body weights of the animals over the two week period. The mean weight of the female reproductive tract was higher in the 100 mg group than in the control group, but due to the high variability statistical significance was not achieved. The study authors stated that this may be indicative of a potential effect that would have to be confirmed in Task 2.

For the continuous breeding phase of the study (Task 2), dose levels of 0, 25, 50 and 100 mg bisphenol A were used. The study authors concluded that bisphenol A was released from the implants. Based on the amount of bisphenol A present in the implants at the beginning and at the end of the study, the total dose per mouse over the 18-week treatment period was calculated as 11-12.3 mg for the 25 mg group, 17.7-19.3 mg for the 50 mg group and 37.2-40 mg for the 100 mg group. Our reviewers note that these values represent less than 50% of target values for Task 2 animals. Plasma levels were not measured

Administration of bisphenol A for the 18-week period did not affect the mean body weights of the animals. There were no effects on the fertility of the breeding pairs or on the mean number of litters produced. There were some statistically significant increases in mean pup body weights. However, the study authors concluded that there was no dose-response relationship and therefore were not treatment related. At scheduled sacrifice, mean absolute and relative organ weights were comparable among the control and high dose groups. Our reviewers concur with the study authors' conclusions.

There were a number of problems associated with the implants. A number of animals expelled their implants during the 18-week study period In addition, a number of animals developed cutaneous lesions at the implant sites indicating that bisphenol A was locally toxic. For these reasons, the study authors concluded that the implants were not suitable for long-term delivery of bisphenol A and the potential reproductive toxicity of bisphenol A should be evaluated by another route of exposure.

EXECUTIVE SUMMARY

This study was conducted according to the NTP's protocol "Fertility Assessment by Continuous Breeding". This study design can include up to five studies which are called tasks. In this particular study, tasks 1 and 2 were conducted. Task 1 is a 14-day repeat dose range-finding study. Task 2 is the continuous breeding phase to ascertain effects on reproduction in the F0 animals. For Task 1, groups of 8 male and 8 female CD-1 mice were administered bisphenol A via subcutaneous Silastic implants at dose levels of 0, 6.25, 12.5, 25, 50 or 100 mg for two weeks. The amount of bisphenol A in the implants was analyzed at the beginning and at the end of the study period. The plasma levels of bisphenol A were analyzed at the end of the study period. Body weights were recorded weekly. At study termination, the female reproductive tract was weighed.

The bisphenol A was released from the implants and was detectable in the plasma of the animals. Based on the amount of bisphenol A present in the implants at the beginning and at the end of the study, the total dose per mouse over the 14-day treatment period was calculated as 1.6, 1 4, 1.6, 5.6 and 10.5 mg bisphenol A for the 6.25, 12.5, 25, 50 and 100 mg groups, respectively. Our reviewers note that these values represent less than 11% of target values for Task 1 animals Actual plasma levels were below the limit of detection in the 6.25 mg group. The mean plasma level was 7 0 and 7 7 ng/ml for the females and males in the 12.5 mg group, respectively and 8.3 and 8.4 ng/ml for the females and males in the 25 mg group, respectively. The mean plasma levels were 13 1 and 18 5 ng/ml for the females and males in the 50 mg group, respectively and 56.2 and 31 5 ng/ml for the females and males in the 100 mg group,

respectively. Administration of bisphenol A did not affect mean body weights of the animals over the two week period. The mean weight of the female reproductive tract was higher in the 100 mg group than in the control group, but due to the high variability statistical significance was not achieved. The study authors stated that this may be indicative of a potential effect that would have to be confirmed in Task 2.

For Task 2, CD-1 mice were administered bisphenol A via subcutaneous Silastic implants at dose levels of 0, 25, 50 or100 mg. The control group contained 40 males and 40 females, and each treatment group contained 20 males and 20 females The animals were treated for 7 days prior to mating, throughout the 98-day breeding phase and for 3 weeks after breeding. For breeding, one male and one female from the same treatment group were cohabited for 98 consecutive days. The females were allowed to deliver their litters The pups were sacrificed within 16 hours of birth so that the breeding pairs could mate again at postpartum estrus. The number and proportion of fertile pairs and the number of litters produced per breeding pair was recorded. For each litter, the live offspring were sexed, counted, pooled and weighed by sex. The dead pups were counted, but their sex and weights were not recorded. At the end of the 18-week study period, the animals in the control and 100 mg groups were subjected to a gross necropsy The weights of the liver, intact female reproductive tract (ovaries, oviduct, uterus and upper half of vagina), testis with attached epididymis (each side weighed separately), seminal vesicles, prostate gland, brain (cerebrum, cerebellum and medulla) and pituitary were recorded, and the tissues were preserved. The animals in the 25 and 50 mg groups were sacrificed and discarded.

Based on the amount of bisphenol A present in the implants at the beginning and at the end of the study, the total dose per mouse over the 18-week treatment period was calculated as 11-12.3 mg for the 25 mg group, 17.7-19.3 mg for the 50 mg group and 37.2-40 mg for the 100 mg group. Our reviewers note that these values represent less than 50% of target values for Task 2 animals. Plasma levels were not measured.

Administration of bisphenol A for the 18-week period did not affect the mean body weights of the animals. There were no effects on the fertility of the breeding pairs or on the mean number of litters produced. There were some statistically significant increases in mean pup body weights. However, the study authors concluded that there was no dose-response relationship and therefore were not treatment related At scheduled sacrifice, mean absolute and relative organ weights were comparable among the control and high dose groups.

There were a number of problems associated with the implants A number of animals expelled their implants during the 18-week study period. In addition, a number of animals developed cutaneous lesions at the implant sites indicating that bisphenol A was locally toxic For these reasons, the study authors concluded that the implants were not suitable for long-term delivery of bisphenol A and the potential reproductive toxicity of bisphenol A should be evaluated by another route of exposure. Given these problems, it was not appropriate to establish a LOAEL/NOAEL for this study. Our reviewers agree.

Bisphenol A. Reproduction and Fertility Assessment in CD-1 Mice
When Administered Via Subcutaneous Silastic Implants

SIGNATURE AND DATE

Technical Reviewer:

/s/ /s/ Date 5/18/01

Jennifer Seed, Ph.D.

QA/QC Reviewer:

/s/ /s/ Date 5-24-01 5/24/01

Jennifer L. Rojko, D.V.M., Ph.D., D.A.C.V.P.

Appendix A: Data Validation

Bisphenol A: Reproduction and Fertility Assessment in CD-1 Mice When Administered via Subcutaneous Silastic Implants

I. Animals Followed Throughout the Study

Twenty percent of all animals were selected for review using the random number generator in Microsoft EXCEL. Data for all animals selected for review were checked in all tables containing individual animal data. No abnormalities were noted in examining the individual animal data.

Males:				Females:			
0.0 mg	**25.0 mg**	**50.0 mg**	**100.0 mg**	**0.0 mg**	**25.0 mg**	**50.0 mg**	**100.0 mg**
G95	G109	G79	G61	G199	G291	G206	G263
G115	G150	G41	G53	G197	G296	G189	G201
G48	G45	G17	G90	G211	G181	G164	G207
G8	G67	G6	G58	G229	G223	G273	G262

II. Critical Effects and Spot Checks

A comparison of the study protocol to the materials and methods was performed. Standard error was calculated because the data was included in the study report. Information included in the summary, methods, results, discussion, and tables were examined. All findings are presented below.

- The protocol outlined five tasks that were to be performed in this study. However, only task 1 and 2 were actually performed in the study because Bisphenol A (BPA) did not exert adverse effects on fertility and reproduction in male and female CD-1 mice. The study authors noted that further studies are required to determine a more optimal route of exposure for reproductive toxicity of BPA in CD-1 mice.

- The methodology (pg. 30) indicated that fertility and reproductive performance data for breeding pairs in Task 2 were not included in the statistical analysis of the data if either partner died during the task. However, the data for animal G231 were included in Table A3 in the statistical analysis even though this animal died during the task. Results are shown below.

Pair G231 + G141	Mean No. Live Pups per Litter	Mean Total No. Pups per Litter
Data included in the mean and standard error (from Table A3)	10.7 ± 0.7	11.0 ± 0.7
Data not included in the mean and standard error	10.6 ± 0.7	11.1 ± 0.5

- In Table A6 (organ weights), male individual G94 in the 100 mg exposure group had a very high prostate weight of .119 grams. The mean prostate weight in the exposure group was .058 grams. No outliers were mentioned in the study report.

- Table 6 from the index of tables (pgs. 99-115) was found missing from the study report.

Group mean values were verified through calculation using individual animal data in the following tables:

- Table A1 Male body weight (control and 100 mg/kg/day exposure groups, week 1,5,10,15,18),
- Table A2: Female body weight (control and 100 mg/kg/day exposure group, week 1,5,10,15,18);
- Table 6: Group Means of Live Pup Weight (litter per pair, live pups per litter, and live pup weight for control and 50 mg/kg/day exposure groups);
- Table 7 Female body weight and organ weights at necropsy (control and 100 mg/kg/day exposure groups); and
- Table 8. Group Means of Mouse Organ Weights (control and 100 mg/kg/day exposure groups).

The following deviations were noted:

- For Tables A1 and A2, no calculations were performed for weeks 5, 10, or 15 because individual body weights were only included for weeks 1 and 18. The mean for male body weight at week 18 in the 25 mg treatment group was given at 39.8 grams, but was calculated at 39.7 grams. The mean for male body weight at week 18 in the 50 mg treatment group was given at 39.8 grams, but was calculated at 39.9 grams. These discrepancies may be due to rounding errors.

- All the results in Table 6 for reproductive performance at the 0 treatment group did not correspond with the calculations in both Table A3 (mean and standard error data), and Table 6 (summary table), as presented below.

At 0.0 mg treatment group

Reproductive Parameter	Mean and Standard Error (from Table 6)	Mean and Standard Error (from Table A3)	Calculated Mean and Standard Error (Calculated from Table A3)
Litters per Pair	4.39 ± 0.12	4.3 ± 0.2	4.23 ± 0.17
Live pups per litter (combined)	9.93 ± 0.29	10.0 ± 0.3	9.99 ± 0.29
Live pup weight (male)	1.69 ± 0.02	1.7 ± 0	1.7 ± 0
Live pup weight (female)	1.64 ± 0.02	1.6 ± 0	1.7 ± 0
Live pup weight (combined)	1.68 ± 0.02	NC	1.7 ± 0

NC Not calculated

- The means and standard error data in Table A3 corresponded with calculated data, however, that data did not correspond to the summary data in Table 6, as illustrated below.

At 50.0 mg treatment group

Reproductive Parameter	Mean and Standard Error (from Table 6)	Calculated Mean and Standard Error (Calculated from Table A3)
Litters per Pair	4 53 ± 0.23	4.45 ± 0.24
Live pups per litter (combined)	10.66 ± 0.71	10.65 ± 0.68
Live pup weight (male)	1.73 ± 0.04	1.72 ± 0 04
Live pup weight (female)	1.63 ± 0.03	1.64 ± 0.03
Live pup weight (combined)	1.68 ± 0.03	1.68 ± 0.04

- The study authors noted that the total number of fertile pairs for the 0 treatment group was 38 but data from table A3 for reproductive performance for individual breeding pairs show 39 fertile pairs.

III. Findings

As noted above, discrepancies or inconsistencies revealed in this data validation included missing data and the appearance of some abnormal values. It also included some differences in actual and calculated means and standard errors. These issues, however, do not affect the confidence in this data study report.

/S/ /s/

Lilia Chen, B S.

7/11/00

Date

MAY 17 2001

/s/

/s/

Contract Number

223-96-2302

Work Assignment Number

2000-19
(ICF 419)

Task Number

06

FDA Study Identification Number

NTP-85-088

Signature of Program Manager

/s/

/s/

Date

5-10-01

STUDY TITLE

Teratologic Evaluation of Bisphenol A (CAS No. 80-05-7) Administered to CD-1 Mice on Gestational Days 6 through 15 (NTP-85-088)

TESTING LABORATORY

Research Triangle Institute (RTI)
Post Office Box 12194
Research Triangle Park, NC 27709

COMPLETION DATE OF STUDY

Report Date. February 15, 1985

SPONSORS OF STUDY

The National Toxicology Program
Research Triangle Park, NC 27709

and

National Center for Toxicological Research
Division of Teratogenesis
Jefferson, AR 72079

LABORATORY STUDY NUMBER

RTI Study Code No M182-BPA
RTI Project No 31U-2077

STUDY SUMMARY

GLP Compliance/Quality Assurance

The protocol (Appendix I, page 29) indicated that the testing facility was in compliance with Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies (1978) and that the laboratory was accredited by the American Association for Accreditation of Laboratory Animal Care The protocol further stated that the study would be conducted in compliance with GLP guidelines However, a statement confirming that the study complied with GLP guidelines was not included in the study report

The study report included a signed and dated (October 15, 1984) Quality Assurance Statement (pages 4-5), which reported a list of five Quality Assurance Unit (QAU) inspections conducted during the study as well as a Final Report Audit and Data Record Audit. A footnote to the list of inspections, however, indicated that several of the inspections were performed on a comparable study rather than on

the present study The study report was considered by the QAU to be an accurate account of the study procedures and findings.

Objective

The objective of this study was to evaluate the toxic and teratogenic effects of the test material (bisphenol A) administered orally to pregnant mice during the period of organogenesis (gestation days 6-15)

Test Material

Test Article

The test article was identified as Bisphenol A (CAS No. 80-05-7, molecular formula $C_{15}H_{16}O_2$, molecular weight 228.28) and was obtained from Midwest Research Institute (MRI), Kansas City, MO MRI obtained the test article from Alfa Products (Lot No 011681, Batch No 01) (Appendix II, page 10) MRI described the test article as a white crystalline powder (Appendix II, page 15) Although the product label indicated 98% purity (Appendix II, page 10), MRI estimated a purity of approximately 95% by high performance liquid chromatography (HPLC), which identified two impurities comprising approximately 3 9% and 1 0% of the sample (Appendix II, page 11) Nonaqueous titration of the phenol groups indicated a purity of approximately 99%, however, this method could not detect any phenolic or acidic impurities in the sample (Appendix II, page 11). The major impurity was later identified as the 2,4' isomer of Bisphenol A by capillary gas chromatography/mass spectrometry, which estimated the impurity to be about 3.4% of the sample (Appendix II, page 48). According to MRI, the test article was stable when stored for two weeks protected from light at temperatures up to 60°C (Appendix II, page 20) At MRI, the test article was stored sealed in plastic bags inside a 10-gallon metal can at 5°C (Appendix II, page 14), the storage conditions at RTI were not provided in the study report. The expiration date of the test article also was not provided in the study report There were no significant differences, however, in Bisphenol A samples returned to MRI from RTI before the present study (9/14/82) and after the present study (1/27/83) compared to frozen reference samples maintained by MRI (Appendix II, pages 41, 59, and 65)

Vehicle

The vehicle was Mazola® Corn Oil (commercial food grade) The corn oil was stored at 4°C The lot/batch number, purity, stability, and expiration date of the vehicle were not provided in the study report Prior to use, however, each bottle of corn oil was tested for the presence of peroxides as an indication of rancidity (a sample with peroxide content >10 mEq/kg would have been considered rancid) Analysis of samples of the corn oil used to formulate each dosing suspension did not reveal any peroxides

Dose Preparation

Prior to the study, dosing formulations were prepared by combining appropriate amounts of the test substance with corn oil in flasks and shaking (chemical/vehicle mixing protocol described in detail in Appendix II, page 31). A sufficient quantity of each formulation was prepared to be used throughout each replicate of the study All dosing formulations were stored at room temperature in screw cap vials To ensure that the study was conducted blind for dose, random 5-digit number codes and color codes

were used to identify the concentration in each vial The suspensions were thoroughly agitated prior to dosing and were stirred continuously on a magnetic stir plate during dosing to maintain homogeneity.

Formulation Analysis

Both pre-dosing and post-dosing analyses of the concentrations of the dosing formulations were conducted. All pre-dosing analyses for replicate I and replicate II of the study yielded values within 90-110% of the nominal concentration for each formulation. In both replicates of the study, post-dosing analyses yielded values within 90-110% of the nominal concentration for each formulation [1]

Homogeneity and stability of bisphenol A in corn oil were evaluated by MRI (Appendix II, pages 20-26) The results of the homogeneity analyses indicated that by using appropriate mixing procedures, bisphenol A could be suspended in corn oil formulations suitable for gavage administration. Furthermore, suspensions stored for five days could be rehomogenized by stirring. The stability analyses revealed that bisphenol A suspended in corn oil was stable for three weeks when at room temperature sealed in the dark. Bisphenol A suspended in corn oil was stable for three hours when open to air in the light. Homogeneity and stability analyses of the actual dosing solutions used by RTI do not appear to have been conducted

Test Animals

Receipt

A total of 210 female and 70 male caesarean-originated, barrier-sustained (COBS) CD-1® (ICR)BR outbred albino mice were received from the Charles River Breeding Laboratory, Inc. (Kingston, NY). Upon arrival, females and males were 8-10 weeks and 10-12 weeks old, respectively

Species selection was justified in the study report (page 22)

Housing

Upon receipt, animals were randomly assigned to cages for a seven day quarantine period Individually coded identification tags were attached to the ear of each mouse during this time. Males were housed individually, and females were group-housed (ten per cage) The cages were solid bottomed polypropylene or polycarbonate with stainless steel wire lids and molded filter tops. Ad-Sorb-Dri® cage bedding was used Cages were organized on galvanized-steel racks with adjustable shelves Following mating, females were again group-housed (four per cage) with other females assigned to the same treatment group.

Environment

The animals were housed in rooms that received 12 hours of continuous light and 12 hours of continuous dark each day. The rooms were air-conditioned, and at least 12-14 air changes were provided per hour The rooms were monitored continuously for temperature and humidity The temperature range was

---

[1]Formulations used in the preliminary study phase ranged from 86-123% of nominal values

68 0-70 0 °F, which was within the range specified in the protocol (70 ± 2 °F) The relative humidity range was 38-61%; a target range was not specified in the protocol

Diet

Purina Certified Rodent Chow® (5002) and deionized/filtered water were available, *ad libitum* Analyses of the feed and water were not included in the study report

Mating

No male breeder was used more than twice in any given dose group Prior to breeding, females were "primed" in order to increase the likelihood of successful mating A single male was placed in a wire mesh cage inside the home cage of 10 females Forty-eight hours later, each female was caged overnight with an individually caged male (i e , only one male and one female per cage). The females were considered to be fertilized if a copulation plug was present on the following morning The day on which a plug was found was designated gestational day zero (gd 0). In addition to the individually coded ear tags previously attached to each mouse, sperm-positive females were assigned a dam number on gd 0 According to the study report, all 154 sperm-positive females were within the weight range 20-35 g on gd 0, however, individual animal data were not provided Actual weight ranges on gd 0 were provided only for the 114 females confirmed pregnant at sacrifice (26-34 g).

Study Design

Dose Selection

Dose levels were selected based on two preliminary studies In the first study, bisphenol A suspended in corn oil was administered by gavage in a volume of 10.0 mL/kg to 56 nonpregnant CD-1 mice (8/dose) at dose levels of 0, 120, 250, 500, 1000, 1500, or 2000 mg/kg/day for 10 days (days 6-15 of the study) Animals were sacrificed two days after the last dose (day 17) The animals were observed for mortality or clinical signs at least once daily during the treatment period, and body weights were determined on day 0, days 6-15, and day 17 (immediately after sacrifice) Liver weights were determined on day 17 after sacrifice. Animals that died on study were subjected to gross necropsy to determine the cause of death.

One 1000-mg/kg/day mouse was removed from the study because it had not been dosed on day 7 The mortality rate was 0% (0/8), 0% (0/8), 0% (0/8), 0% (0/8), 0% (0/7), 75% (6/8), and 87.5% (7/8) at 0, 120, 250, 500, 1000, 1500, or 2000 mg/kg/day, respectively The most common clinical signs observed at daily observation were rough coat, clinical weight loss, piloerection, and lethargy Other signs observed sporadically included ataxia, comatose behavior, dyspnea, chirping, bloating, hunched back, hyperactivity (circling) after dosing, and death There were no significant effects on body weight, body weight gain, or liver weight The 2000-mg/kg/day group was not analyzed statistically because only one animal survived. Probit analysis indicated that the oral LD_{10} for nonpregnant mice in this study was 1031 mg/kg/day. Doses were selected for the second preliminary toxicity on the basis of these results.

In the second study, bisphenol A suspended in corn oil was administered by gavage in a volume of 10 0 mL/kg to 86 time-mated female CD-1 mice (14-15/dose) at dose levels of 0, 250, 500, 750, 1000, and 1250 mg/kg/day on gestation days 6-15 The dams were observed for mortality or clinical signs at

least once daily during the treatment period, and body weights were determined on gd 0, gd 6-15, and gd 17. Animals that died on study were necropsied to determine the cause of death At or following sacrifice on gd 17, dams were evaluated for clinical signs of toxicity, body weight, liver weight, gravid uterine weight, and uterine contents (i e., implantation sites, resorptions, dead fetuses, and live fetuses) Live fetuses were weighed and examined for gross malformations Based on maternal mortality and other clinical signs of toxicity through gd 17, dose selection for this preliminary teratology study was considered adequate to justify using the study as replicate I of the definitive teratology study. Therefore, fetuses also were examined for visceral and skeletal malformations

Four dams were removed from the study: one 250-mg/kg/day dam and one 750-mg/kg/day dam whose deaths were attributed to dosing error (punctured esophagus) and two 1000-mg/kg/day dams who delivered litters prior to schedules sacrifice on gd 17 The rates of maternal mortality were 0% (0/14), 0% (0/13), 14 3% (2/14), 7.7% (1/13), 7 7% (1/13), and 13 3% (2/15) at 0, 250, 500, 750, 1000, and 1250 mg/kg/day, respectively The most common clinical signs observed at daily observation were rough coat and weight loss Other signs observed sporadically included lethargy, piloerection, vaginal bleeding, arched back, body sores, dyspnea, wheezing, and death Maternal body weight on gd 15 and 17 exhibited a significant downward trend with increasing dose and a significant overall effect for dose Maternal body weight on gd 15 and 17 was significantly lower than controls in high-dose dams Maternal weight gain during gestation and treatment, absolute maternal weight gain (i e , weight gain during gestation minus gravid uterine weight), and gravid uterine weight exhibited significant downward trends with increasing dose (despite the fact that values for these parameters actually increased at lower doses) and significant overall effects for dose The high-dose group (1250 mg/kg/day) was significantly lower than controls for maternal weight gain during gestation and treatment, absolute maternal weight gain, and gravid uterine weight Maternal liver weight exhibited an overall effect for dose with the 250, 500, 750, and 1000 mg/kg/day groups significantly higher than the control group

There were no effects on. the number of implantation sites per litter, the percent of resorbed, dead, nonlive, and affected fetuses per litter, the number of litters with resorbed, dead, nonlive, and affected fetuses, or the number of live fetuses per litter Average fetal body weight per litter exhibited a significant downward trend with increasing dose and a significant overall effect for dose The high-dose group (1250 mg/kg/day) exhibited significantly lower average fetal body weight per litter compared to the control group There was no significant difference in the incidence of gross malformations in offspring of treated mothers. Gross malformations were observed in 0/131, 0/129, 0/144, 1/135, 5/119, and 1/65 fetuses from the 0, 250, 500, 750, 1000, and 1250 mg/kg/day groups, respectively

According to the study author, these results justified the use of the second preliminary study as the first replicate of the definitive teratology study, with the exclusion of the 250 mg/kg/day group This decision was based on (1) apparent dose-related increase in clinical signs with minimal evidence of toxicity at the low dose (i.e , 500 mg/kg/day), (2) significant dose-related trends for maternal and fetal body weight parameters with significant pairwise differences from control in the high dose group but no significant pairwise differences from the control in the low dose group; (3) no reduction in live litter size, even at maternal and fetotoxic doses, thus allowing the evaluation of an equivalent number of live fetuses across dose groups; and (4) low enough maternal mortality (13.3%) in the high dose group to permit evaluation of maternal and fetal toxicity at this dose Therefore, the doses for the definitive teratology study were 0, 500, 750, 1000, and 1250 mg/kg/day

Our reviewers noted that the use of the second preliminary study as the first replicate of the definitive teratology study had been proposed in the original study protocol and was appropriate

Animal Assignment

Plug-positive females were randomly assigned to the dose groups indicated in Table 1 using a procedure based on stratified body weight to ensure comparable group mean body weights for each treatment group. The experimental design involved two replicates

Table 1. Animal Assignment

Dose (mg/kg/day)	Number of Sperm-Positive Females		
	Replicate I [a]	Replicate II [b]	Total
0	14	15	29
500	14	15	29
750	14	15	29
1000	15	19	34
1250	15	18	33

[a]Gestational day 0 for Replicate I 8/13-8/16, 1982
[b]Gestational day 0 for Replicate II: 9/10-9/15, 1982

Administration

All doses were administered once daily by gavage, on gestation days 6 through 15, at a constant dose volume of 10 mL/kg of body weight/day. Although the dams were weighed daily prior to dosing on gd 6-15, the study report does not specify that dosing was based on the most recent body weight determination

Maternal Observations

Mortality/Clinical Observations

The animals were checked for mortality or clinical signs at least once daily during the treatment period (at the time of daily dosing) The study report indicates that animals were also observed for mortality or clinical signs one hour and four hours post-dosing, when staff scheduling allowed. Clinical signs were also recorded prior to sacrifice on gd 17 (p VII-2)

Body Weight/Food Consumption

Body weight data were recorded on gestation days 0, 6 through 15, and 17 (immediately following sacrifice) Non-pregnant animals were excluded from the analyses. Individual food consumption data were not determined

Gross Pathology/Cesarean Examinations/Organ Weights

Animals that died between gd 0 and gd 17 were subjected to a gross necropsy to determine the cause of death According to the study report (p. 31), surviving dams were sacrificed on gd 17 (cervical dislocation) and evaluated (ante-mortem) for clinical signs of toxicity. body weight, liver weight, gravid uterine weight, total number of corpora lutea, and status of uterine implantations (i e , implantation site, resorptions, dead fetuses, and live fetuses) Appendix VII further indicated that any internal abnormalities observed during dissection were noted, and affected tissues, if any, were removed, labeled, preserved in 10% neutral-buffered formalin, and placed in storage (p VII-2) Maternal kidneys and livers were weighed and similarly fixed/stored in formalin The uteri of dams with no apparent implants were treated with a solution of 10% ammonium sulfide in order to visualize possible implantation sites The viable fetuses were dissected from the uterus and immediately placed on a moist paper towel on a tray of ice According to the study report, this procedure induces anesthesia by lowering core body temperature below 25 °C Individual live fetuses were weighed and sexed

Histopathology

Histopathology examinations were not conducted in this study

Fetal Observations

External Examinations

All viable fetuses were examined macroscopically for any external abnormalities as detailed in Appendix VII

Visceral Examinations

Using the Staples' fresh tissue dissection method, all live fetuses were examined for visceral abnormalities as detailed in Appendix VII. Prior to dissection, half of the fetuses were decapitated, the heads were fixed in Bouin's solution for free hand sectioning and examination.

Skeletal Examinations

All fetal carcasses (50% without heads) were examined for skeletal abnormalities following fixation with Alizarin Red S stain as detailed in Appendix VII Rare or difficult to describe malformations were photographed or sketched

Data Analysis

Indices

No indices were calculated

Statistical Evaluation

Data for all measures were pooled across replicates and analyzed parametrically or non-parametrically, as appropriate Maternal weight data (body weight, body weight gain, gravid uterine weight, and absolute and relative liver weight) and per litter data (implantation sites per litter, % resorptions per litter, % dead fetuses per litter, % nonlive implants per litter, % affected fetuses per litter, live fetuses per litter, average fetal weight per litter, average male fetal weight per litter, average female fetal weight per litter, % males per litter, % male fetuses malformed per litter, % female fetuses malformed per litter, and % fetuses malformed per litter) were evaluated using parametric techniques Litter derived percentage data (% resorptions per litter, % dead fetuses per litter, % nonlive implants per litter, % affected fetuses per litter, % males per litter, % male fetuses malformed per litter, % female fetuses malformed per litter, and % fetuses malformed per litter) were first subjected to arcsine-square root transformation Bartlett's test for homogeneity of variance was conducted on all data to be analyzed using parametric techniques. The study report indicates, however, that Bartlett's test was not used to decide whether analysis of variance (ANOVA) procedures should be used on selected data (page 45) A two-way ANOVA (dose x replicate) was used to determine whether significant dose effects, replicate effects, or dose x replicate interactions had occurred in any of the parameters listed above. For maternal body weights or weight gain measures, a three-way ANOVA (dose x replicate x day) was used if no significant dose effect was observed in the two-way ANOVA. In addition, average male and female fetal body weight per litter and percent males and females malformed per litter were evaluated using a three-way ANOVA (dose x replicate x sex) to test for significant effects of sex The Linear Trends Test also was conducted on all data analyzed by ANOVA to determine the significance of the dose-response relationship When ANOVA indicated significant ($p<0.05$) effects for dose, Williams' and/or Dunnett's Multiple Comparison Test was used to compare individual treatment groups to the control group A one-tailed test was used for all parameters except measures of maternal body and organ weight, fetal body weight, and percent males per litter for which a two-tailed test was used When significant ($p<0.05$) replicate effects or dose x replicate effects were noted, data were further analyzed for dose effects separately for each replicate of the study using a one-way ANOVA, Linear Trend Test, and Williams' and/or Dunnett's Multiple Comparison Test Nominal scale measures (number of litters with resorptions, number of litters with dead fetuses, number of litters with nonlive implants, number of litters with affected fetuses, number of litters with gross malformations, number of litters with visceral malformations, number of litters with skeletal malformations, and number of litters with malformations) were analyzed using non-parametric techniques The Chi-Square Test for Independence was used to assess differences among treatment groups. When Chi-Square indicated significant ($p<0.05$) differences among treatment groups, a one-tailed Fisher's Exact Test was used to compare individual treatment groups to the control group When significant ($p<0.05$) replicate effects or dose x replicate interactions were noted for parameters analyzed by ANOVA, associated nominal scale data were further analyzed for dose effects separately for each replicate of the study

Archive

Copies of the final study report, the original study data sheets, work sheets, and computer printouts generated in the statistical analysis of data, and all biological samples were stored at RTI Copies of the final report also were provided to the National Center for Toxicological Research The study report indicates that records were to be maintained in accordance with the FDA GLP regulations for Nonclinical Laboratory Studies (1978) The data and reports were to be retained in storage for a minimum of two years The chemical and biological samples were to be retained in storage for a minimum of two years or "for as long as the quality of the preparation affords evaluation," (narrative, p. 37) whichever was less

Critical Appraisal of the Protocol and Study Design

A protocol was included in the study report Overall, the study was adequately designed, and the study personnel were clearly indicated in the protocol (page 28) and study report (pages 35-36)

Our reviewers noted several deficiencies in the study design with respect to the test article and vehicle Specifically, the storage conditions at RTI and the expiration date of the test article were not provided. In addition, the lot/batch number, purity, stability, and expiration date of the vehicle were not provided Although the homogeneity and stability of sample dosing solutions were evaluated by the supplier (MRI), the actual dosing solutions were not analyzed for homogeneity or stability under the storage conditions used at RTI Degradation of the test article or the vehicle prior to preparation of the dosing solutions or degradation of the dosing solutions prior to administration could have affected the study results However, test articles samples from RTI were comparable to frozen reference samples maintained by the supplier (MRI) In addition, analysis of samples of the corn oil used to formulate each dosing suspension did not reveal any peroxides (an indicator of rancidity). Finally, pre-dosing and post-dosing analyses of the concentrations of the dosing formulations yielded values that were within the range of 90-110% of nominal Therefore, our reviewers concluded that the integrity of the study was not affected by these deficiencies

Our reviewers also noted several deficiencies in the study design with respect to the test animals For example, the age of the animals at mating was not specified in the study report The use of animals that are too young or too old could alter reproductive parameters. The study report indicated that males were 10-12 weeks of age and females were 8-10 weeks of age upon arrival at the testing facility, however, the protocol indicated that both males and females were to be 8-10 weeks old upon arrival The age at mating could not be determined despite the fact that gd 0 dates were provided because the arrival date of the animals was not provided. The lack of information on the exact age of the animals at mating is particularly problematic due to the use of two replicates for the definitive teratology study Unless two separate shipments of animals were received, the animals in replicate II would have been about one month older than the animals in replicate I Although the study report did not specify that two shipments were received, this possibility seems likely due to the number of animals in the initial shipment compared to the number of animals required The study report indicated that 210 female CD-1 mice were received from the breeder and used for the preliminary range-finding studies and the definitive teratology study, however, according to the data tables, 56 nonpregnant females were assigned to the first preliminary study, 86 plug-positive females were assigned to the second preliminary study (which became replicate I of the definitive teratology study), and 82 plug-positive females were assigned to replicate II of the definitive teratology study (i e , a total of 224 females). A second group of animals could have been used for replicate II of the study These deficiencies and discrepancies may be an

indication of incomplete data audit, incomplete quality control, or incomplete quality assurance review Nevertheless, they are considered by our reviewers to not have affected the interpretation of the study results

Our reviewers also noted several deficiencies in the study design with respect to animal husbandry In particular, the females were group-housed rather than housed individually Standard toxicity testing guidelines suggest that females should be single-caged except during mating In addition, the study report did not include analyses of the animal diet and water for levels of contaminants. Although control and treatment animals presumably would have been exposed equally to any contaminants present in the diet and water, there is a potential for synergistic effects between potential contaminants and the test article.

One deficiency was noted by our reviewers regarding administration of the dosing solutions The body weight determination on which dosing was based on was not specified Dosing is assumed to have been based on the most recent available body weight, and the animals were weighed daily during the treatment period.

Our reviewers also noted several deficiencies in the study design regarding maternal toxicity parameters Specifically, dams were checked for mortality and clinical signs of toxicity at least once daily prior to dosing The study report indicates that animals were also observed for mortality or clinical signs one hour and four hours post-dosing, when staff scheduling allowed The study results did not indicate when, or if, these additional daily checks were made Nevertheless, standard toxicity testing guidelines suggest that dams should be observed a minimum of twice daily for mortality and clinical signs. In addition, food consumption data were not recorded in this study Potential effects on maternal body weight could result from either a direct toxic effect of bisphenol A or from a decrease in food consumption due to the irritant properties of bisphenol A. According to the study report, weight depression in previously conducted chronic studies was attributed to significantly reduced food consumption (page 17)

Finally, our reviewers noted deficiencies in data reporting Specifically, individual animal data were not included in the study report for the following parameters daily observations of dams for mortality and clinical signs, maternal body weight measurements, maternal organ weight determinations (gravid uterine weight and liver weight), gross necropsy findings, and all reproductive parameters (total number of corpora lutea, implantation sites, resorptions, dead fetuses, and live fetuses as well as fetal sex and fetal body weight) This lack of individual animal data for these parameters precludes verification/validation of the corresponding results reported.

STUDY RESULTS

Maternal Observations

Mortality and Clinical Observations

Two dams were removed from the study following accidental puncturing of the esophagus during dosing (one 500-mg/kg/day dam and one 750-mg/kg/day dam), and two dams were removed because they delivered their pups prior to scheduled sacrifice (both in the 1000 mg/kg/day group) All the removals occurred in Replicate I. The maternal mortality rate was 0% (0/29), 7 1% (2/28), 3.6% (1/28), 6 3% (2/32), and 18 2% (6/33) for the 0, 500, 750, 1000, and 1250 mg/kg/day groups, respectively

Clinical signs observed in the 500, 750, 1000, and 1250 mg/kg/day groups during the treatment period were lethargy (500, 1000, and 1250 mg/kg/day only), piloerection, rough coat, vaginal bleeding (500, 1000, and 1250 mg/kg/day only), clinical weight loss, and death Other signs observed sporadically in the 500, 750, 1000, or 1250 mg/kg/day groups included: arched back, vocalization, alopecia, wheezing, bloating, coughing, and dyspnea Rough coat and weight loss also occurred sporadically in the vehicle control group

Body Weight

Maternal body weight data are provided in Table 2 On gd 0, gd 6, and gd 11, there were no significant dose effects on maternal body weight Maternal body weight on gd 15 and on gd 20 exhibited a significant downward trend with respect to increasing dose and a significant overall effect for dose Significant differences from the control group were noted in the 1250-mg/kg/day group at both observations In addition, a significant main effect for replicate was observed maternal body weight on gd 0 ($p \leq 0\ 0001$) and maternal body weight on gd 6 ($p = 0.0178$) There were no dose x replicate interactions

Maternal body weight gain data are provided in Table 3 Maternal weight gain during gestation and treatment exhibited a significant downward trend with respect to increasing dose and a significant overall effect for dose ($P<0\ 001$) Significant differences from the control group were noted in the 1250-mg/kg/day group for both parameters There was a significant dose effect on absolute weight gain ($p = 0\ 0187$), however, no significant pairwise differences between treatment groups and the control were found There were no significant replicate effects or dose x replicate interactions for any maternal body weight gain parameters

Table 2 Mean Maternal Body Weight During Gestation (g)[a]

		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
GD 0	MEAN	29 36	29.21	29 29	29 57	29 82
	S E M.	0 34	0 36	0 32	0.34	0 36
	N	26	23	21	23	21
GD 6	MEAN	31 95	32.30	32 49	32.59	32 72
	S.E M	0 32	0.40	0 30	0 41	0 43
	N	26	23	21	23	21
GD 11	MEAN	35 63	36.60	36.88	36 19	35 16
	S E M.	0.36	0 55	0 50	0 58	0 72
	N	26	23	21	23	21
GD 15	MEAN	43.58††	44.54	44 95	41 96	39.29*
	S E M	0 53	0 98	0 77	1 12	1 29
	N	26	23	21	23	21
GD 20	MEAN	49.15††	51 02	50 02	47 65	43.29*
	S E M	0 86	1.33	1 04	1 54	2 15
	N	26	23	21	23	21

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included in body weight calculations

††Significant dose-related downward trend ($p<0.01$)

*Significantly different from control, Dunnett's Test ($P<0.05$)

Table 3 Mean Maternal Body Weight Gain During Gestation (g)[a]

		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
Gestation (gd 0-20)	MEA N S E.M. N	19.78†† 0 88 26	21.80 1 18 23	20 73 1 01 21	18.08 1.59 23	13 47* 2 07 21
Treatment (gd 6-15)	MEA N S.E M N	11 63††† 0 45 26	12 24 0 84 23	12 46 0.72 21	9 37 1 10 23	6 57* 1 15 21
Absolute[b]	MEA N S.E M N	4 92 0 38 26	6 19 0.47 23	5 97 0 77 21	4.65 0.75 23	3 51 0 69 21

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included in body weight calculations

[b]Weight gain during gestation minus gravid uterine weight

††Significant dose-related downward trend (p<0.01)

†††Significant dose-related downward trend (p<0 001)

*Significantly different from control, Dunnett's Test (P<0 05)

Food Consumption

Food consumption data were not recorded in this study.

Maternal Necropsy Findings

According to Table 14 in the study report (p 87), the following clinical signs were among those observed on gd 17: cysts around ovary (or ovaries), pale kidney (s), larger than normal gall bladder, fluid-filled cyst(s) on the kidney(s), pale liver, kidney capsule filled with yellow fluid, and enlarged kidney/hydroureter. There was no apparent relationship to treatment. The nature of these reported clinical signs, however, suggests that they were observed during the dissection following sacrifice. Piloerection, rough coat, and vaginal bleeding, which also were noted as clinical signs on gd 17 in the study report, may have been observed prior to sacrifice and necropsy Animals that died on study were to have been necropsied, however, these findings were not included in the study report

Reproductive Parameters/Organ Weights

Data on reproductive parameters and organ weights are provided in Table 4. The number of corpora lutea per dam exhibited a significant dose-related downward trend, however, there were no significant differences among treatment groups in the ANOVA and no significant pairwise differences between

treatment groups and the control group There was a significant dose-related upward trend and significant overall effect for dose in the percent of resorptions per litter and the percent of nonlive fetuses per litter, with the high-dose group significantly greater than control values in pairwise comparisons for both parameters There were no significant effects on the number of implantation sites per litter, the preimplantation loss percentage, or the percent of dead fetuses per litter.

A dose-related downward trend and an overall significant effect for dose was observed for fetal body weight, and average fetal body weight per litter in the high-dose group was significantly lower than the control group These effects were present when male and female fetuses were considered separately. No significant effects were observed with respect to the number of live fetuses per litter and the percent of male fetuses per litter

A significant dose-related decrease in gravid uterine weight was observed This parameter also exhibited a significant overall effect for dose with the high-dose group significantly lower than control values Maternal liver weight was significantly greater in the 500, 750, and 1000-mg/kg/day groups (but not the 1250-mg/kg/day group) compared to the control group. The effect of bisphenol A treatment on liver weight did not exhibit a dose-related trend An overall effect for dose and a significant dose-related upward trend were noted for relative liver weight All treatment groups were significantly greater than the control group in pairwise comparisons

Our reviewers consider the lack of increase in absolute liver weight in the high-dose dams to be consistent with the decreases in maternal body weight and body weight gain seen in this group We note that liver weight depends on food intake and body weight and that the increases in relative liver weight in all the bisphenol A-treated females were consistent with a treatment-related effect on the maternal liver at all doses examined

Table 4. Reproductive Parameters and Organ Weights[a]

		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
Subjects						
Number Treated		29	29	29	34	33
Number Removed		0	1[b]	1[b]	2[c]	0
Number Dead		0	2	1	2	6
Number (%) Pregnant at Sacrifice		26 (89.7)	23 (88.5)	21 (77 8)	23 (76.7)	21 (77 8)
All Litters						
Number of Litters		26	23	21	23	21
Number of Corpora Lutea per Dam	Mean S E M	11 88[d]†† 0.40	11 77[e] 0 74	11 84[d] 0 51	10.05[d] 0 86	9 16[d] 1 15

		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
Number of Implantation Sites per Litter	Mean S E M	11.88 0.43	12.30 0 52	11 71 0 73	12 09 0 45	11 81 0 49
Preimplantation Loss Percentage	Mean S E.M	2 65 1.00	2 81 1 42	10 25 4 32	1 49 1 12	1 23 0 67
Percent Resorptions per Litter	Mean S E M	14 13† 4.21	12 86 4 51	7 50 1.59	18.42 6 92	40 14* 9.56
Percent Dead per Litter	Mean S.E M.	0 60 0 41	0 00 0 00	2 03 1 01	2 69 1 33	0.87 0 60
Percent Nonlive per Litter[f]	Mean S.E M	14 73† 4.18	12 86 4 51	9 53 1.70	21 12 6.76	41 02* 9.62
Live Litters						
Number of Litters with Live Fetuses		25	22	21	20	14
Number of Live Fetuses per Litter[g]	Mean S E M	10 76 0 47	11 55 0.52	10.67 0.71	11 20 0 46	10.93 0 77
Percent Male Fetuses per Litter	Mean S E M.	51 43 3 32	51.40 3 38	47 88 3 02	52 54 3.67	50 02 3 48
Average Fetal Weight (g) per Litter[g]	Mean S.E M	0 955†† 0 016	0 943 0.022	0 943 0 035	0 869 0 036	0 820* 0 029
Average Male Fetal Weight (g) per Litter[g]	Mean S E.M.	0 964†† 0.021	0.964 0 023	0 959 0.037	0.880 0 038	0 824* 0 031
Average Female Fetal Weight (g) per Litter[g]	Mean S E M	0 939†† 0 019	0.922 0 021	0 919 0 033	0 859 0.035	0 818* 0 026

		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
Organ Weights						
Gravid Uterus Weight (g)	Mean S E M N	14 73[h]†† 0 81 25	15 61 0 93 23	14 82[h] 0.89 20	13 43 1.21 23	9 96* 1 54 21
Maternal Liver Weight (g)	Mean S E M N	2 65 0 05 26	3 02[i]* 0 08 22	3 08* 0 11 21	3 01* 0 13 23	2 83[i] 0 12 20
Relative Maternal Liver Weight (% body weight)	Mean S E M N	5 42††† 0.10 26	5 92*# 0 10 23	6.14*## 0 14 21	6.33*## 0 18 23	6 77*## 0 24 21

[a]Dams that were not pregnant at sacrifice, that died, or that were otherwise removed from the study were not included
[b]One dam was removed following death due to dosing error
[c]Two dams were removed because they delivered pups prior to sacrifice on gd 17
[d]Number of corpora lutea for two dams was inadvertently not recorded.
[e]Number of corpora lutea for one dam was inadvertently not recorded
[f]Nonlive includes both dead and resorbed fetuses.
[g]Includes only dams with live fetuses.
[h]Gravid uterine weight for one dam was inadvertently not recorded
[i]Liver weight for one dam was incorrectly recorded and therefore not included.
†Significant dose-related trend (p<0 05)
††Significant dose-related trend (p<0.01)
†††Significant dose-related trend (p<0.001)
*Significantly different from control, Dunnett's Test (p<0 05)
#Significantly different from control, Williams' Test (p<0.05)
##Significantly different from control, Williams' Test (p<0 01)

Significant replicate effects occurred for the number of implantation sites per litter (p = 0 0050), the number of live fetuses per litter (p = 0 0223), and average fetal body weight per litter (p = 0 0048) Average fetal body weight per litter also exhibited a significant main effect for sex (p = 0 0005); males fetuses were heavier than female fetuses

The preimplantation loss percentage and maternal liver weight exhibited significant dose x replicate interactions Therefore, these parameters were analyzed for dose effects by replicate These results are presented in Table 5 In replicate II, preimplantation loss percentage exhibited a significant effect for dose, but only the 750-mg/kg/day group was significantly different from control values in pairwise comparisons. The study author noted that two animals in the 750-mg/kg/day group "exhibited a wide disparity between the total number of corpora lutea and the total number of implantation sites" (narrative, p 44) and further suggested that these two animals accounted for the significantly increased

preimplantation loss percentage in that group There were no significant effects on preimplantation loss percentage in replicate I

Significant differences among treatment groups were noted for maternal liver weight in replicate I, however, these differences did not exhibit a dose-related trend Pairwise comparisons of the replicate I groups to the control group revealed that the 500-, 750- and 1000-mg/kg/day groups were significantly increased, whereas the 1250-mg/kg/day group was significantly decreased There were no significant differences among treatment groups in replicate II although a significant dose-related upward trend was observed. As discussed previously, our reviewers consider the lack of increase in absolute liver weight in the high-dose group to be consistent with decreases in maternal body weight and body weight gain seen in this group

Table 5 Analysis by Replicate of Reproductive and Organ Weight Parameters that Exhibited Dose by Replicate Interactions

Replicate		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
Preimplantation Loss Percentage						
I	MEA N S E M N	3 42 1 81 11	1.82 1.30 11	0 79 0.79 9	0 00 0.00 8	1.87 1 24 8
II	MEA N S E M N	2 00‡ 1 08 13	3 80 2 57 11	18 76* 7 30 10	2.41 1 79 13	0 76 0.76 11
Maternal Liver Weight (g)						
I	MEA N S E M N	2 57‡‡‡ 0 04 12	3 23*# 0 07 11	2.94# 0 14 11	3 01*# 0 15 10	2.51# 0 13 9
II	MEA N S E M N	2.72† 0 08 14	2.82 0 13 11	3 24 0.16 10	3 01 0.20 13	3.10 0.15 11

†Significant dose-related upward trend (p<0 05)
‡Significant difference among dose groups (p<0 05)
‡‡‡Significant difference among dose groups (p<0 001)
*Significantly different from control, Dunnett's Test (P<0.05)
#Significantly different from control, Williams' Test (p<0 05)

Histopathology

Tissues were not examined histologically

Fetal Observations

The results of analyses of selected fetal parameters are shown in Table 6 The percent of fetuses malformed per litter, percent of male fetuses malformed per litter, and percent of female fetuses malformed per litter were comparable among treatment groups. There were no significant replicate effects for these parameters

Table 6 Summary of Fetal Observations

		0 mg/kg/day	500 mg/kg/day	750 mg/kg/day	1000 mg/kg/day	1250 mg/kg/day
Number of Fetuses Examined[a]		269	254	224	224	153
Number of Litters Examined[b]		25	22	21	20	14
Percent Fetuses Malformed per Litter	MEAN S E M.	2 70 1 02	2 44 0.96	1 28 0 73	5 69 4 30	5 79 2 25
Percent Male Fetuses Malformed per Litter	MEAN S E.M.	3 13 1 78	5.76 3.19	2.55 1 46	5 29 3.68	6 09 3.05
Percent Female Fetuses Malformed per Litter	MEAN S E M	2 25 1 07	0.51 0 51	0 00 0 00	6 25 5.09	6 58 2 76

[a]Only live fetuses were examined for malformations.

[b]Only litters with live fetuses were included

Fetal External Examination

The occurrence of external malformations was not evaluated statistically In total, external malformations were observed in two 500-mg/kg/day fetuses (two litters), two 750-mg/kg/day fetuses (two litters), eleven 1000-mg/kg/day fetuses (one litter), and four 1250-mg/kg/day fetuses (three litters); there were no external malformations in control fetuses. Grossly observed changes occurred sporadically and included cleft palate, exencephaly, gastroschisis, and ectocardia (heart outside body cavity). The total fetal and litter incidences of external variations were not provided, nor were the variations reported divided into external, visceral, and skeletal categories External variations included hematomas (tail, head, face, lower limb, neck, back, and thorax), open eye(s), and blood in the amniotic sac.

Fetal Visceral Examination

The occurrence of visceral malformations was not evaluated statistically In total, visceral malformations were observed in five control fetuses (four litters). three 500-mg/kg/day fetuses (two litters), one 750-mg/kg/day fetus, four 1000-mg/kg/day fetuses (two litters), and one 12500-mg/kg/day fetus. Enlarged epididymis, enlarged uterine horn, enlarged auricular flaps, extra vessel, kidneys half of normal size, and hydrocephaly were the reported findings The total fetal and litter incidences of visceral variations were not provided, nor were the variations reported divided into external, visceral, and skeletal categories Visceral variations included very soft kidney tissue, cream colored liver lobe(s), distended ureter(s), and white spots in the left ventricle

Fetal Skeletal Examination

The occurrence of skeletal malformations was not evaluated statistically In total, skeletal malformations were observed in two control fetuses (two litters), two 500-mg/kg/day fetuses (two litters), one 750-mg/kg/day fetus, three 1000-mg/kg/day fetuses (two litters), and seven 1250-mg/kg/day fetuses (three litters) Missing rib, ribs fused to each other, thoracic centra off center, fused sternebrae, misshapen skull. missing thoracic arch, and short rib were the most common findings. The total fetal and litter incidences of skeletal variations were not provided, nor were the variations reported divided into external, visceral, and skeletal categories. Skeletal variations included misaligned sternebrae, bipartite centra, extra ossification site(s) on the sternum, incomplete ossification of the pelvic girdle, wavy rib, and frontal and parietal bones fused on lateral edges

ASSESSMENT

The purpose of this study was to evaluate the toxic and teratogenic effects of the test material (bisphenol A) administered orally to pregnant mice during the period of organogenesis (gestation days 6-15) The deficiencies and deviations in experimental design were discussed above. The lack of food consumption data is the major deficiency noted by our reviewers The effects on body weight observed in the dams may have been due to a treatment-related decrease in food consumption However, our reviewers comment that the study data clearly indicate that high doses of bisphenol A are toxic and are associated with an approximately 18% increase in mortality as well as effects on liver weight In contrast, at lower doses of the test article, decreases in food consumption might explain decreases in body weight Our reviewers noted that other study design deficiencies were not likely to have affected the study results

CONCLUSIONS

The administration of the test article, bisphenol A, resulted in 7.1, 3 6, 6.3, and 18 2% mortality for the 500, 750, 1000, and 1250 mg/kg/day groups, respectively The high-dose group exhibited significant decreases in body weight on gd 15 and 17, body weight gain during the treatment period (gd 6-15) as well as the entire gestation period (gd 0-17), and gravid uterus weight Significantly increases in maternal liver weight occurred in the 500, 750, and 1000 mg/kg/day groups, but not in the 1250 mg/kg/day group However, relative maternal liver weight was significantly increased in all treatment groups compared to the control The study author indicated that the liver weight effects may be indicative of hepatotoxicity and further noted the hepatotoxic findings of a previous NTP carcinogenicity assay The high-dose group exhibited a significantly increased percent of resorptions and nonlive

implants per litter as well as statistically insignificant decreases in corpora lutea The average body weight of fetuses from the high-dose group also was significantly reduced. There were no effects on any other reproductive parameters or on the percent of fetuses malformed per litter Our reviewers note, however, that the maternal body weight effects can not be clearly attributed to the direct toxic effect of bisphenol A in the absence of food consumption data Nevertheless, the LOAEL for maternal toxicity is 500 mg/kg/day based on liver weight effects, no maternal NOAEL can be established Our reviewers also note that the embryotoxic and fetal toxic effects observed in the high-dose group (i e., increased resorptions and nonlive implants and decreased fetal body weight) could have been secondary effects of the maternal toxicity that occurred in that dose group (i.e., decreased body weight and weight gain). According to standard toxicity testing guidelines, however, it should be assumed that developmental effects that occur in the presence of minimal maternal toxicity are evidence of developmental toxicity Therefore, the LOAEL for developmental toxicity is 1250 mg/kg/day based on increased resorptions and nonlive implants as well as decreased fetal body weight, the NOAEL for developmental toxicity is 1000 mg/kg/day

EXECUTIVE SUMMARY

In a developmental toxicity study, bisphenol A in corn oil was administered to mated female (COBS) CD-1® (ICR)BR outbred albino mice (29-34/dose) by gavage at dose levels of 0, 500, 750, 1000, or 1250 mg/kg/day from days 6 through 15 of gestation Mortality occurred in 7.1, 3.6, 6 3, and 18 2% of dams in the 500, 750, 1000, and 1250 mg/kg/day groups, respectively The high-dose group exhibited significant dose-related effects on maternal body weight (gd 15 and gd 17), body weight gain (gestation and treatment), and gravid uterine weight. Because food consumption data were not recorded, it is unclear whether these body weight effects are the result of direct bisphenol A toxicity or the result of treatment-related decreases in food consumption A significantly increased maternal liver weight occurred in the 500, 750, and 1000 mg/kg/day groups, but not in the 1250 mg/kg/day However, relative maternal liver weight was significantly increased in all treatment groups compared to the control The high-dose group exhibited a significantly increased percent of resorptions and nonlive implants per litter The average body weight of fetuses from the high-dose group also was significantly reduced There were no effects on any other reproductive parameters or on the percent of fetuses malformed per litter Therefore, the LOAEL for maternal toxicity is 500 mg/kg/day based on liver weight effects, no maternal NOAEL can be established The LOAEL for developmental toxicity is 1250 mg/kg/day based on increased resorptions and nonlive implants as well as decreased fetal body weight, the NOAEL for developmental toxicity is 1000 mg/kg/day

Teratologic Evaluation of Bisphenol A (CAS No 80-05-7) Administered to CD-1 Mice on Gestational Days 6 through 15 (NTP-85-088)

Technical Reviewer.

/s/

/s/

Jennifer Wetham, M S

Date: 4-26-01

QA/QC Reviewer:

/s/

/s/

Jennifer Rojko, D.V.M , Ph/D , D A C.V.P.

Date 4-30-01

Appendix A. Data Validation

Teratologic Evaluation of Bisphenol A (CAS No. 80-05-7) Administered to CD-1 Mice on Gestational Days 6 through 15 (NTP-85-088)

I. ANIMALS FOLLOWED THROUGHOUT THE STUDY

The limited nature of the data provided for individual animals in the study precluded the traditional random review throughout the study. Individual data were provided in Table 19 (Malformations and Variations Exhibited) and Appendix IV (maternal weight gain during treatment and gestation), but there were no means by which to validate these data.

II. CRITICAL EFFECTS AND SPOTCHECKS

A comparison of the study Materials and Methods section to the study Protocol was done. The initial Protocol was amended as noted in Appendix I, Amendment 1 of the study. The Protocol called for males and females, approximately 6-10 weeks of age upon arrival. The Materials and Methods section noted males to arrive between 10-12 weeks of age and females arrived at 8-10 weeks of age. In addition, the Protocol (Section 9.2) called for a probit analysis of maternal mortality through gestational day 17 for the Preliminary Toxicity Study II which was not discussed in the materials and methods section. It should also be noted that the data collected in the Preliminary Toxicity Study II were used as Replicate I for the Teratology Study.

The limited nature of the data provided for individual animals in the study restricted the validation of mean values, incidence rates, and statistical analyses. The summary data (Table 12) for Maternal Weight Gain during gestation and treatment (data found in Appendix IV) were checked for all five dosing groups (0, 500, 750, 1000, and 1250 mg/kg/day) and no inconsistencies were found. Using the fetal malformation and variation data provided (Table 19), a spotcheck of the summary incidence rates (Table 18) showed no inconsistencies.

III. FINDINGS

The stated findings of the study appear to be accurately reflected in the data provided. The deviations from the protocol do not appear to effect the validity of the study results. The limited amount of individual animal data actually presented did not allow for a complete and random validation effort. Even so, there appears to be no reason to doubt the validity of the study results.

/s/

/s/

Siddha Parker, B A.

8/7/00

Date