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Ms. Christine Todd Whitman
Administrator
U. S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

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Dear Ms. Whitman:

The American Chemistry Council (Council) makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following six final reports that the Council's Brominated Flame Retardant Industry Panel (BFRIP) recently conducted are enclosed:

- Tetrabromobisphenol A (TBBPA):
 - An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A (TBBPA) in Rats;
 - *In Vitro* Mammalian Chromosome Aberration Test;
 - Analytical Method Verification for the Determination of Tetrabromobisphenol A (TBBPA) in Freshwater;
 - Analytical Method Verification for the Determination of Tetrabromobisphenol A (TBBPA) in Soil;
 - Determination of the n-Octanol/Water Partition Coefficient of Tetrabromobisphenol A (TBBPA); and,
 - Determination of the Vapor Pressure of Tetrabromobisphenol A (TBBPA) using the Spinning Rotor Gauge Method.

These reports do not include confidential information.

If you have any questions, please contact Wendy K. Sherman, the BFRIP Manager, at 703/741-5639 or via email [wendy.sherman@americanchemistry.com].

Sincerely yours,

Elizabeth Festa Watson
Managing Director, CHEMSTAR

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Enclosures (6)



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**AN ORAL PRENATAL DEVELOPMENTAL TOXICITY STUDY WITH
TETRABROMOBISPHENOL A IN RATS**

TEST ARTICLE: Tetrabromobisphenol A

PERFORMING LABORATORY: MPI Research
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Mattawan, MI 49071-9399

**LABORATORY STUDY
IDENTIFICATION:** 474-005

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DATE OF STUDY COMPLETION: September 20, 2001

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1. QUALITY ASSURANCE STATEMENT

Below are the inspections conducted by the Quality Assurance Department and the dates the inspections were reported to the Study Director and Management:

Date(s) of Inspection	Study Phase Inspected	Date(s) Reported to Study Director/Management
1/29/01, 2/22/01	Protocol Review	2/22/01, 3/8/01
2/27/01	Test Material Administration	3/8/01
3/6/01	Test Material Preparation	4/10/01
3/13/01	Protocol Amendment No. 1	4/10/01
4/11/01	Protocol Amendment No. 2	5/4/01
7/11/01 - 7/25/01	Data Review	7/25/01
7/11/01 - 7/25/01	Report Review	7/25/01
7/26/01 - 8/2/01	Data Review	8/2/01
7/26/01 - 8/2/01	Report Review	8/2/01
8/6/01	Report Review	8/6/01
9/16/01	Report Review	9/17/01

Karyn M. Webber-Deines
 Karyn M. Webber-Deines, B.A.
 Auditor, Quality Assurance

9/20/01
 Date

2. SUMMARY

The objective of this study was to provide general information concerning the effects of oral treatment of the pregnant rat with Tetrabromobisphenol A (TBBPA) on the developing organism. This included death, structural abnormalities or altered growth, and assessment of maternal effects. This study consisted of 3 treatment groups and 1 vehicle (corn oil) control group (25 mated female rats/group). Female CD[®] rats were mated in-house and received the TBBPA at dose levels of 0, 100, 300, and 1000 mg/kg/day at a constant volume of 5 mL/kg. The control animals received the vehicle at the same volume and dosing regimen as the treated groups. The test article was administered orally by gavage as a single daily dose. Dosing initiated on Day 0 of gestation and continued through to include Day 19 of gestation. The day on which evidence of mating was observed was considered Day 0 of gestation. Observations of the dams included clinical signs, gestational body weights, and food consumption. Females were euthanized on Day 20 of gestation and given a postmortem macroscopic examination. Gross lesions were saved in 10% neutral buffered formalin for possible future examination. Gravid uterine weights and liver weights were recorded. Litters were delivered by cesarean section. The total number of corpora lutea, uterine implantations, early and late resorptions, viable and nonviable fetuses, and the sex and individual weights of fetuses were recorded. All fetuses were given a gross external examination for malformations and variations. Approximately one-half of the fetuses in each litter were fixed in Bouin's solution, and the remaining fetuses were skinned and preserved in alcohol. Bouin's-fixed fetuses from control and all treated groups were examined for visceral abnormalities (freehand razor blade sectioning procedure), and the remaining fetuses from all groups were stained with Alizarin Red S and Alcian Blue and evaluated for skeletal/cartilaginous malformations and ossification variations. The maternal Day 20 gestation examinations and cesarean sections and subsequent fetal evaluations were performed blind to treatment.

Pretest analyses confirmed that the suspensions as prepared were homogeneous and stable for at least 14 days when stored refrigerated. Periodic analysis of dosing suspensions used on study ranged from 88 to 113% of nominal and confirmed that animals were receiving the appropriate dose levels.

No treatment-related mortality was seen. The death of 1 animal in the 300 mg/kg/day group on Gestation Day 5 was attributed to an intubation injury. All other animals survived to scheduled euthanasia.

Salivation was seen among the TBBPA-treated animals, occurring most frequently at the 300 and 1000 mg/kg/day dose levels. Because of its sporadic occurrence, this was not considered to represent a direct effect of treatment with TBBPA, but more likely was in response to the taste of residual amounts of test article on the dosing catheter. No other effects of treatment were seen from the clinical examinations, and no effect of treatment was evident from gestational parameters (body weight, body weight gain, or food consumption), uterine implantation data, liver weights, or necropsy findings. Likewise, no effect of treatment was evident from fetal body weights, fetal sex distribution, or from fetal external, visceral, or skeletal examinations.

Thus, in this oral developmental toxicity study in rats with TBBPA, the NOAEL (No Observable Adverse Effect Level) for maternal and developmental toxicity was 1000 mg/kg/day, the highest dose level evaluated.

3. INTRODUCTION

The study described in this report was conducted in accordance with MPI Research Standard Operating Procedures and the protocol as approved by the Sponsor. Procedures pertinent to this study are described in this report.

The protocol met or exceeded the United States EPA (Environmental Protection Agency), OPPTS (Office of Prevention, Pesticides, and Toxic Substances) Guideline No. 870.3700, Prenatal Developmental Toxicity Study, August, 1998 and OECD (Organization for Economic Co-operation and Development) Guideline Study No. 414 (Draft Document, August 1996).

3.1. Objective

The objective of this study was to provide general information concerning the effects of oral treatment of the pregnant rat with Tetrabromobisphenol A on the developing organism. This included death, structural abnormalities or altered growth, and assessment of maternal effects.

3.2. Species Selection

The current state of scientific knowledge does not provide any acceptable alternatives, *in vitro* or otherwise, to the use of live animals to accomplish the purpose of this study. The rat is a universally used model for evaluating developmental toxicity of various classes of chemicals. This laboratory has historical control data on the incidence of fetal malformations and variations and other fetal endpoints in the strain of rat used on study.

3.3. Study Schedule

Protocol Approved by the Sponsor	December 27, 2000
Study Initiation Date (Protocol Signed by the Study Director)	January 26, 2001
Arrival of Males	January 17, 2001
Arrival of Females	February 12, 2001
Randomization (Commencement of Treatment)	February 27-28, March 1-3, and March 6-7, 2001
Uterine Examinations	March 19-23 and March 26-27, 2001
Draft Report	August 8, 2001
Final Report	September 20, 2001

4. MATERIALS AND METHODS

4.1. Test Article and Vehicle Control Information

4.1.1. Test Article and Vehicle Control Receipt

The test article, Tetrabromobisphenol A (TBBPA), used on study was a composite of 3 lots of commercial TBBPA produced by Albemarle Corporation, Great Lakes Chemical Corporation, and the Dead Sea Bromine Group. The sample used on this study was received from Wildlife International Limited, Easton, Maryland, on January 10, 2001. A description, lot number, storage conditions, safe handling procedures, as well as other relevant information for the test article, have been documented in the study data. One week prior to initiation of dosing and approximately 1 month following completion of the in-life portion of the study, a sample (approximately 5 grams) of the test article was collected and shipped to the Sponsor for analysis. The Sponsor has provided documentation on the strength, purity, composition, and stability on the test article. Pertinent information on the test article is presented in Appendix A.

The vehicle, corn oil, was received in 2 lots from Spectrum Chemical Manufacturing Corporation, Gardena, California, on January 25, 2001. Descriptions, lot numbers, storage conditions, expiration dates, safe handling procedures, as well as other relevant information for the vehicle, have been documented in the study data. Documentation on the strength, purity, composition, stability, physical properties, and method of synthesis, fabrication, and/or derivation of the batches of corn oil used on study are limited to that information listed on the label of this commercially available vehicle. Pertinent information on the vehicle is presented in Appendix A.

4.1.2. Test Article and Vehicle Control Preparation

On a weekly basis during the study, approximately 350 mL of vehicle was dispensed into amber glass containers. One container was dispensed each day for dosing the control animals. This was done before handling the test article to prevent any contamination of vehicle used to dose control animals. The individual containers with vehicle were stored refrigerated until used.

The test article was used as received, and no adjustment was made for purity. The test article was mixed with the vehicle to achieve the desired concentrations. The method of preparation of the dosing suspensions was determined based upon physical characteristics of the test article and size of batches required. Suspensions for each dose level were prepared separately. Fresh suspensions were prepared for each dose level weekly. In preparing dosing suspensions, test article inventory and usage records were maintained in accordance with MPI Research Standard Operating Procedures # TMC 3-1 "Preparation of Test Material Suspensions" and # TMC 8-1 "Test Article Inventory." These data are retained with the study data file.

To prepare the dosing suspensions, the required amount of test article was weighed directly into a calibrated beaker. Vehicle was added to the beaker to yield a final volume of 350 mL. The contents of the beaker were suspended using a Polytron[®] tissue homogenizer and mixed using a magnetic stir bar and stir plate until dispensed using a syringe into individual amber glass containers, 1 container/day. The containers were stored refrigerated until dispensed for dosing.

4.1.3. Test Article Analysis

All analytical work was conducted by KAR Laboratories, Inc., Kalamazoo, Michigan, in compliance with Good Laboratory Practices and subjected to review by the Quality Assurance Unit of KAR Laboratories, Inc.

KAR Laboratories developed and validated a method for the determination of TBBPA in corn oil. A copy of the complete validation report is presented in Appendix B.

4.1.3.1. Homogeneity

Prior to initiation of test article administration, test batches of the test article suspensions at the low and high concentrations used in the study (20 and 200 mg/mL, respectively) were prepared to assess the homogeneity of the test preparations employing the same method and batch size to be used during the study.

While the suspensions were stirring, 6 samples (2 top, 2 middle, and 2 bottom) were collected from each mix for analysis to assess homogeneity. Following acceptance of the analytical results by the Study Director, any remaining samples were properly disposed.

4.1.3.2. Stability

Appropriately sized portions of the prepared suspensions mixed for the homogeneity analysis were stored refrigerated for up to 14 days. Samples of these suspensions were collected following re-suspending after 7 and 14 days and analyzed to determine the stability of the test article in the vehicle. Following acceptance of the analytical results by the Study Director, any remaining samples were properly disposed.

4.1.3.3. Concentration

Samples of test article suspensions at each concentration level and control vehicle were collected from the first and last mixes used on study and analyzed to verify test article concentrations.

4.1.4. Reserve Sample

A reserve sample from each batch of test article used in this study was taken and archived at MPI Research.

4.2. Experimental Design

4.2.1. Animal Acquisition and Maintenance

On February 12, 2001, a total of 140 female CD[®] [CrI: CD[®] (SD)IGS BR] rats were received from Charles River Laboratories, Portage, Michigan. Upon arrival at the laboratory, all rats were individually housed and permitted an acclimation period of at least 2 weeks prior to initiation of mating. During this acclimation period, female rats were observed daily for any clinical signs of disease and given a detailed clinical examination prior to pairing with males. The females were 8 weeks old at arrival and 10 weeks at the initiation of pairing with males. Mated females weighed between 203 to 274 grams on Day 0 of gestation.

On January 17, 2001, 135 male CD[®] [CrI: CD[®] (SD)IGS BR] rats were received from Charles River Laboratories, Portage, Michigan. These males came from a shipment received in error, and 60 were used for breeding. Upon arrival, all rats were individually housed. The male rats were 6 weeks of age at receipt and 12 weeks of age at the time of breeding. Males used for breeding were euthanized and discarded after all females used on study were mated. Males not used for breeding were euthanized and discarded.

4.2.2. Randomization and Assignment to Study

Mated female rats considered suitable for study were weighed on Day 0 of gestation prior to treatment. Using these body weights, the animals were sorted into groups using a simple randomization where group homogeneity of mean weights was used as a criterion of acceptance. The body weight range of individual animals in each group did not exceed $\pm 20\%$ of the mean for the group. One hundred, mated, female rats were assigned to the control and treatment groups identified in the following table. Extra female rats obtained for the study, but not placed on study, were euthanized and discarded.

Group Assignment						
Group Number	Dose Level (mg/kg/day)	Dose Volume mL/kg	Dose Concentration mg/mL	Number of Mated Females		
				Initial	Laparohysterectomy/ Necropsy	
1	0 (Vehicle Control)	5	-	25	25	
2	100	5	20	25	25	
3	300	5	60	25	25	
4	1000	5	200	25	25	

Each animal was individually identified by a metal ear tag bearing a unique number. Each animal's cage was identified by the animal number, group, and sex. The individual animal number plus the MPI Research study number comprised a unique identification for each animal.

Throughout the study, all rats were kept in an environmentally controlled room. Temperature and relative humidity in the animal quarters were monitored and recorded daily and maintained between 65 and 79° F and 38 and 63%, respectively. Fluorescent lighting

provided illumination approximately 12 hours per day. Diet (Certified Lab Diet® meal rodent Chow #5002, PMI Nutrition International, Inc., St. Louis, Missouri) and tap water were available *ad libitum*. Documentation of each lot number of diet used during the study is retained in the study records. Water was supplied using an automatic watering system. From acclimation until euthanasia, the rats were housed individually in suspended, stainless steel, wire-mesh type cages, except during pairing when animals were co-housed 1:1 (male:female) in the male's cage. Nesting material was not provided because the dams were euthanized prior to delivery.

Certification analysis of each diet lot was performed by the manufacturer, and these data are retained in the archives of MPI Research. No additional analysis of the diet was conducted. The drinking water, provided to the laboratory and available to the test animals, is monitored for specified contaminants at periodic intervals according to Standard Operating Procedures of MPI Research, and therefore, no further analysis of the water was conducted. These water monitoring data are maintained in the Archives of MPI Research. The Study Director is not aware of any contaminants present in the water or feed that could have interfered with the results of the study.

4.2.3. Test Article and Vehicle Control Administration

4.2.3.1. Justification for Route of Administration

The oral route by gavage is an acceptable and standard method for administering test article per OECD Guideline No. 414 and OPPTS Guideline No. 870.3700.

4.2.3.2. Justification for Dose Levels and Duration of Treatment

The dose levels of TBBPA used in this study (100, 300, and 1000 mg/kg/day) were selected by the Sponsor on the basis of available data from previous studies. Animals were treated from fertilization (Day 0 of gestation) to Day 19, which was 1 day prior to scheduled euthanasia and laparohysterectomy (Day 20 of gestation). This dosing regimen is consistent with that proposed in the guideline referenced in the Justification for Route of Administration section (Section 4.2.3.1.). It assumes from preliminary studies that there is not a high potential for preimplantation loss at these dose levels.

4.2.3.3. Administration

The test and vehicle control articles were administered by oral gavage once each day. The dose was delivered using an appropriately sized, plastic, disposable syringe attached to a cut 15-gauge, stainless steel hypodermic needle with a polyurethane umbilical catheter fitted over the needle for administration. Dosing formulations were stirred during dosing. Test article administration began on Day 0 of gestation and continued through to include Day 19 of gestation. Animals were treated at a constant volume of 5 mL/kg/day. The control animals received the vehicle at the same dose volume. Individual doses were based on the most recent body weights.

4.3. In-Life Examinations

4.3.1. Mortality and Cageside Observations

All study animals were observed at least twice a day, 7 days a week, for morbidity, mortality, signs of injury, and availability of food and water. Any findings were recorded. Mortality or other signs of toxicity were recorded on the day they were observed.

4.3.2. Detailed Clinical Observations

Daily from Gestation Days 0 to 20, each animal was removed from the cage and given a detailed clinical examination. These examinations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration. During the treatment period, these examinations were conducted approximately 1 hour after dosing.

4.3.3. Body Weights and Body Weight Changes

Individual body weights were recorded on Days 0, 3, 6, 9, 12, 15, 18, and 20 of gestation. Body weight changes were calculated and reported for the following gestational day intervals: 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20, and 0-20. Adjusted Day 20 gestation body weights (actual Day 20 gestation body weight minus gravid uterine weight) and adjusted body weight gain (Days 0-20 of gestation) are also reported.

4.3.4. Food Consumption

Food consumption was recorded for study animals on the corresponding body weight days and reported for the following intervals: Gestation Days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20, and 0-20.

4.4. Ovarian and Uterine Examinations

On Day 20 of gestation, each female was euthanized with an overdose of carbon dioxide and immediately subjected to a laparohysterectomy. The uterus was excised, and the gravid uterine weight was recorded. Beginning at the distal end of the left uterine horn, the location of viable and nonviable fetuses and early and late resorptions along each uterine horn, position of the cervix, and the number of total implantations were recorded. The uterus was opened, the embryonic membrane of each fetus was gently removed, and each fetus was pulled away from the placenta, fully extending the umbilical cord. The placentae were grossly examined *in situ*. The number of corpora lutea on each ovary was also recorded. A necropsy was conducted on each dam as described in the Postmortem Study Evaluations section (Section 4.6.). The livers of all maternal animals were weighed and discarded. Maternal gross lesions were saved in 10% neutral buffered formalin for possible microscopic examination, and the carcasses were discarded.

The maternal Gestation Day 20 evaluations and subsequent fetal examinations were conducted blind to treatment levels. Each day, all maternal laparohysterectomies and fetal body weight measurements were completed within 2 hours from start to finish. Fetal external examinations and processing of the fetuses for subsequent evaluations were performed outside this time period, generally after all uterine examinations had been completed. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites (Kopf, *et al.*, 1964). If stained foci were identified, the female was considered pregnant, and the foci considered representative of early resorptions.

4.5. Teratologic Examinations

Fetuses were individually weighed, sexed, tagged with an identification number, and examined for external malformations and variations. Approximately one-half of the fetuses in each litter were placed in Bouin's solution for subsequent soft tissue examination using the Wilson (1965) razor-blade sectioning technique. The remaining fetuses in each litter were skinned, fixed in alcohol, and processed for staining of the skeletal and cartilaginous tissues using Alizarin Red S and Alcian Blue by a method similar to that described by Kimmel and Trammell (1981). These specimens were cleared with glycerin for subsequent examination of bone and cartilage. Fetal findings were classified as malformations or developmental variations under the supervision of a developmental toxicologist. In the distribution of fetuses for visceral and skeletal examinations, every attempt was made to equalize the ratio of male and female fetuses/litter processed for each evaluation.

4.6. Postmortem Study Evaluations

A complete necropsy was performed on the female rats under the supervision of a veterinary pathologist. Special emphasis was placed on structural abnormalities or pathologic changes that may have influenced the pregnancy. Gross lesions from the dams were saved in 10% neutral buffered formalin for possible future microscopic examination, and the carcasses were discarded. The livers of all maternal animals were weighed. A glossary of macroscopic terms used in this study is included in this report.

4.7. Statistics

The table below defines the sets of comparisons used in the statistical analyses described in this section.

Statistical Comparisons	
Control Group	Treatment Group
1	2, 3, 4

The endpoints that were analyzed and the methods of analysis that were employed are presented in the table below.

Statistical Analysis Methods	
Endpoint	Analysis
Parental In-life Data	
Gestation Body Weights	Group Pair-wise Comparisons
Gestation Body Weight Changes	Group Pair-wise Comparisons
Gestation Food Consumption	Group Pair-wise Comparisons
Adjusted Day 20 Gestation Body Weights and Body Weight Gains over Days 0-20 of gestation using the Adjusted Day 20 Body Weights	Group Pair-wise Comparisons
Liver Weights, absolute and relative to the Adjusted Day 20 Gestation Body Weight	Group Pair-wise Comparisons
Fertility Indices	
Pregnancy Index	Fisher's Exact Test
Uterine and Ovarian Exam	
Gravid Uterine Weights (mean/dam)	Group Pair-wise Comparisons
Corpora Lutea (mean/dam)	Group Pair-wise Comparisons
Total Implantations (mean/dam)	Group Pair-wise Comparisons
Fetal Sex Ratio (mean % viable male fetuses/total no. viable fetuses)	Arcsin-Square-Root Transformation
Viable Fetuses (mean/dam)	Group Pair-wise Comparisons
Mean Percent Viable Fetuses/Implants	Arcsin-Square-Root Transformation
Nonviable Fetuses (mean/dam)	Descriptive Statistics
Early Resorptions (mean/dam)	Group Pair-wise Comparisons
Mean Percent Early Resorption/Implants	Arcsin-Square-Root Transformation
Late Resorptions (mean/dam)	Descriptive Statistics
Preimplantation Loss (mean%/dam)	Arcsin-Square-Root Transformation
Postimplantation Loss (mean%/dam)	Arcsin-Square-Root Transformation
Mean Fetal Body Weights (mean/dam)	Covariate Analysis
Malformation by type and total (external, visceral, and skeletal) – litter is the experimental unit ^a	Fisher's Exact Test
Developmental Variations by type and total (external, visceral, and skeletal) – litter is the experimental unit ^a	Fisher's Exact Test

^a Both fetal and litter incidences are reported, but only the litter incidences were analyzed statistically.

4.7.1. Group Pair-wise Comparisons

For each specified endpoint and for all collection intervals, Levene's test (Milliken and Johnson, 1992) was used to assess homogeneity of group variances. If the test was not significant ($p > 0.01$), Dunnett's test (Dunnett, 1964) was used to compare each group receiving test article with the control group. If Levene's test was significant ($p < 0.01$), comparisons with the control group were made using Welch's t-test (Welch, 1937) with a Bonferroni correction. Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests unless indicated otherwise.

4.7.2. Arcsine-Square-Root Transformation

Data comprised of percent values were transformed using the arcsin of the square root (Steele and Torrie, 1980). The analysis described in Section 4.7.1. was then used to analyze the transformed percentage values.

4.7.3. Fisher's Exact Test

For binomial endpoints (excluding sex ratios), each treatment group was compared to the control group using a Fisher's exact test with a Bonferroni correction. Results were reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests unless indicated otherwise.

4.7.4. Covariate Analysis

For fetal body weights, litter size was included as a covariate in the model used to conduct the Dunnett's test (Dunnett, 1964). Each treatment group was compared with the control group, and results were reported at the 0.05 and 0.01 significance levels. These data were analyzed using two-tailed tests unless indicated otherwise.

4.7.5. Descriptive Statistics

Descriptive statistics consisted of means, standard deviations, and number of animals for each group and time period.

4.8. Computer Systems

The following computer systems were used during the conduct of this study.

Computer Systems	
In-life Systems:	ARTEMiS II
Randomization:	ARTEMiS II
Developmental and Reproduction System:	ARTEMiS II
Statistical Analysis:	SAS
Reporting:	Microsoft Office Professional

4.9. Data and Specimen Retention

All raw data, documentation, MPI-generated records, protocols, reserve samples, specimens (wet tissues), and the final report generated as a result of this study will be retained at MPI Research for a period of 10 years following completion of the study (final report issue date). Retention of materials after the times stated above will be subject to future contractual agreements between the Sponsor and MPI Research.

5. RESULTS AND DISCUSSION

5.1. Test Article Analysis

Individual analytical values are presented in Appendix B.

5.1.1. Homogeneity

Homogeneity of the dosing suspensions at the low and high concentration levels was assessed pretest on mock batches prepared at the same size as intended for use on study. These data are summarized below and demonstrate that the mixing procedure developed for this study produced homogeneous suspensions.

Nominal Concentration (mg TBBPA /mL)	Analytical Concentration [mg TBBPA/mL] (% of nominal)			
	Top Level	Middle Level	Bottom Level	Mean of all sampling levels ±%RSD
20	20.7/20.9 Mean 20.8 (104)	20.5/20.9 Mean 20.7 (104)	20.9/20.8 Mean 20.9 (104)	20.8 ± 0.8 (104)
200	227/228 Mean 228 (114)	218/228 Mean 223 (112)	221/228 Mean 225 (113)	225 ± 1.9 (112)

Key: RSD – Relative Standard Deviation.

5.1.2. Stability

Suspensions prepared to determine homogeneity were stored refrigerated and found to be stable for at least 14 days following preparation. Results of the stability analyses at 7 and 14 days after preparation are summarized below.

Nominal Concentration (mg TBBPA/mL)	Analytical Concentration [mg TBBPA/mL] (% of Day 0 homogeneity analytical results)		
	Preparation Day 0	Days after preparation (% of Day 0)	
		7	14
20	20.8	21.6/21.2 Mean 21.4 (103)	21.9/20.4 Mean 21.2 (102)
200	225	218/220 Mean 219 (97)	214/243 Mean 228 (101)

5.1.3. Concentration

Results of analyses of dosing suspensions used for dosing for the first and last week of study are summarized below.

Group (mg/kg/day)	Nominal Concentration (mg TBBPA /mL)	Analytical Concentration [mg TBBPA/mL] (% of Nominal)	
		First Week	Last Week
1 (0)	0	0	0
2 (100)	20	19.1/19.0 Mean 19.0 (95)	19.5/20.1 Mean 19.8 (99)
3 (300)	60	52.0/53.7 Mean 52.8 (88)	59.9/59.9 Mean 59.9 (100)
4 (1000)	200	197/211 Mean 204 (102)	232/221 Mean 226 (113)

Analysis of dosing suspensions from the first and last week of study ranged from 88 to 113% of nominal and confirmed that animals were receiving the appropriate doses.

5.2. Mortality and Clinical Findings

Clinical findings are summarized in Table 1 and presented individually in Appendix C. An animal termination history (i.e., Individual Record of Animal Fate and Disposition) is presented in Appendix L.

No treatment-related mortality was seen in this study. One animal (Number 962) in the 300 mg/kg/day group died on Day 5 of gestation after 4 days of treatment. At necropsy, this animal was noted with a perforation of the pharynx, oily fluid in the thoracic cavity, and hemorrhage in the pericardial sac. Death was considered related to a dosing injury. All other animals in the control and treated groups survived to scheduled euthanasia.

Salivation was seen among the TBBPA-treated animals and occurred most frequently at the 300 and 1000 mg/kg/day dose levels. This finding was seen in 1 animal at 100 mg/kg/day, 11 animals at 300 mg/kg/day, and 13 animals at 1000 mg/kg/day. It was not seen in the controls. The salivation seen in the treated groups occurred sporadically with no trend evident. Therefore, its occurrence was not considered to represent a direct effect of treatment with TBBPA. It is more likely that this salivation was in response to the taste of residual amounts of test article on the dosing catheter. Other clinical findings seen in the treated groups occurred at low incidence affecting 1 to 2 animals, and their occurrence in this study was considered incidental and unrelated to treatment. These findings, though not seen in the concurrent controls, were considered typical for this laboratory with this strain and age of animal.

5.3. Body Weights and Body Weight Changes

Maternal body weights and body weight changes during gestation are summarized in Tables 2 and 3, respectively, and individual animal values are presented in Appendix D.

No adverse effect of treatment with TBBPA was evident from gestation body weights or body weight gain. Gestation body weights for the treated groups did not differ statistically from controls and were considered comparable between the groups. Likewise, no effect of treatment was evident from body weight gain during gestation. In the 100 mg/kg/day group, body weight gain for Days 0-3 of gestation was statistically lower than controls. However, in the absence of a similar response at the higher dose levels, this was considered incidental and unrelated to treatment. For all other intervals during gestation and over the entire Day 0-20 gestation period, body weight gain for the treated groups was comparable to controls.

5.4. Food Consumption

Maternal food consumption data during gestation are summarized in Table 4, and individual animal data are presented in Appendix E.

No effect of treatment with TBBPA was evident from gestation food consumption. There were a few intervals during gestation when food consumption for the 100 and 300 mg/kg/day groups was statistically lower than controls but in the absence of a similar response in the 1000 mg/kg/day group, these were considered incidental and unrelated to treatment.

5.5. Macroscopic Observations

Maternal macroscopic postmortem observations are summarized in Table 5 and presented individually in Appendix F.

No effect of treatment with TBBPA was evident from the maternal macroscopic observations. The few macroscopic findings seen among the treated animals occurred in low incidence and were considered unrelated to treatment.

5.6. Maternal Liver Weights

Mean maternal liver weights, absolute and relative to the adjusted Day 20 gestation body weights, are summarized in Table 8. Individual animal values are presented in Appendix I.

No effect of treatment with TBBPA was evident from maternal liver weights. Liver weights, absolute and relative to the adjusted Day 20 gestation body weights, in the 100 mg/kg/day group were statistically lower than controls but in the absence of a similar response at the higher dose levels, this was considered incidental and unrelated to treatment. Liver weights, absolute and relative to body weight, in the 300 and 1000 mg/kg/day groups did not differ statistically from controls and were considered comparable between the groups.

5.7. Ovarian and Uterine Examinations

Corpora lutea and uterine examination data are summarized in Table 6 and presented individually in Appendices G and K. Gravid uterine weights, adjusted Day 20 gestation body weights, and body weight gains over Days 0-20 of gestation using the adjusted body weights are summarized in Table 7, and individual animal data are presented in Appendix H.

Pregnancy rates were 100% in the control and 300 mg/kg/day groups and 96% in the 100 and 1000 mg/kg/day groups. No animals delivered prematurely. One female (Number 999) in the 1000 mg/kg/day group was confirmed pregnant on the basis of uterine foci poststaining. The number of Day 20 gestation litters with viable fetuses for evaluation ranged from 23 to 24 in the treated groups. In the control group there were 25 litters for evaluation.

No adverse effect of treatment with TBBPA was evident from uterine implantation data. The mean number of corpora lutea, uterine implantations, viable fetuses, and resorptions per dam in the treated groups was comparable to controls. Likewise, mean pre- and postimplantation loss indices for the treated groups did not differ statistically from controls and were considered comparable between the groups. A 9.75% postimplantation loss in the 1000 mg/kg/day group, while about 2-fold higher than controls (4.95%), was largely attributable to the 100% postimplantation loss seen in the 1 female whose pregnancy was confirmed on the basis of stained uterine foci. Excluding data for this female, the postimplantation loss in the 1000 mg/kg/day group was 5.8% and similar to controls.

Gravid uterine weights, adjusted Day 20 gestation body weights, and adjusted body weight gain over Days 0-20 of gestation for the treated groups were comparable to controls, and no effect of treatment was evident from these data.

5.8. Fetal Evaluations

5.8.1. Fetal Body Weights

Mean fetal body weights are summarized in Table 9. Individual fetal body weights and mean fetal body weights by litter are presented in Appendices K and G, respectively.

No effect of treatment with TBBPA was evident from fetal body weights. Fetal body weights, distinguished by sex and for both sexes combined, for the treated groups did not differ statistically from controls and were considered comparable between the groups.

5.8.2. Fetal Sex Ratios

Fetal sex distributions presented as the mean percentage of male fetuses per litter are summarized in Table 6. The number of fetuses of each sex by litter and the sex of individual fetuses are presented in Appendices G and K, respectively.

No effect of treatment with TBBPA was evident from fetal sex ratios. These ratios for the treated groups did not differ statistically from controls and were considered comparable between the groups.

5.8.3. Fetal External Observations

Fetal external observations are summarized in Tables 10 and 13, and individual fetal findings are presented in Appendix J.

No effect of treatment was evident from the fetal external examinations. The few external findings (malformations and variations) seen in the treated groups occurred with low incidence on both a per fetus and per litter basis and were considered unrelated to treatment. The litter incidences for these findings for the treated groups did not differ statistically from controls.

5.8.4. Fetal Visceral Observations

Fetal visceral observations are summarized in Tables 11 and 14, and individual fetal findings are presented in Appendix J.

No effect of treatment was evident from the fetal visceral examinations. The few visceral malformations seen in the 100 and 300 mg/kg/day groups occurred at low incidence, and in the absence of similar findings among fetuses at the 1000 mg/kg/day dose group, their occurrence was considered incidental and unrelated to treatment. A low incidence of visceral malformations was also seen in the control group. The litter incidences of these visceral malformations in the 100 and 300 mg/kg/day groups did not differ statistically from controls. No visceral malformations were seen in fetuses from the 1000 mg/kg/day group.

No visceral variations were seen among fetuses from the control or treated groups.

5.8.5. Fetal Skeletal Observations

Fetal skeletal observations are summarized in Tables 12 and 15, and individual fetal findings are presented in Appendix J.

No effect of treatment was evident from the fetal skeletal examinations. No skeletal malformations were seen in fetuses from the 100 and 300 mg/kg/day groups or in control fetuses. The only skeletal malformation seen in the study was bent scapula, and this was seen in a single fetus from the 1000 mg/kg/day group. The low incidence in occurrence of this malformation was considered incidental and unrelated to treatment.

No effect of treatment was evident from ossification variation data. A slight increase in the litter incidence of rudimentary rib(s) was seen in the treated groups. However, since the differences from controls were not statistically significant or dose related, this was considered to represent an incidental occurrence unrelated to treatment. Rudimentary rib is a common ossification variation seen in this laboratory in the Day 20 gestation fetal rat (see Appendix M), and historically, litter incidences in controls have reached as high as 72%. Other ossification variations seen among the treated fetuses occurred at low incidence or with similar frequency as controls, and no effect of treatment was evident from these data. The overall incidence of litters containing fetuses with ossification variations for the treated groups (Table 15) was comparable to controls.

5.8.6. Overall Incidence of Malformations

The incidence of litters containing fetuses with malformations for the treated groups was comparable to controls (Table 16). These litter incidences for the control, 100, 300 and 1000 mg/kg/day groups were 12.0%, 8.3%, 4.2%, and 8.7%, respectively.

6. CONCLUSION

In this oral developmental toxicity study in rats with Tetrabromobisphenol A, the NOAEL (No Observable Adverse Effect Level) for maternal and developmental toxicity was 1000 mg/kg/day, the highest dose level evaluated.

7. REFERENCES

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REPRODUCTION GLOSSARY

Abnormality - A morphologic or functional deviation from normal limits (anomaly).

Activity - Action or process of exerting energy.

Cesarean Section - Laparotomy to deliver the fetus(es) is performed on gestation day 20 (rat).

Conception - The onset of pregnancy; formation of a visible zygote.

Corpora Lutea - Ovarian follicle cells which have discharged their ova and become hypertrophied, assuming a yellow color. Regressing c. - corpora lutea in process of degeneration due to death of embryos *in utero*.

Development - Gradual growth or expansion, especially from a lower to a higher stage of complexity.

- Arrested Development - Cessation of the developmental process at some stage prior to its normal completion.
- Prenatal Development - That which occurs before birth.
- Postnatal Development - That which occurs after birth.

Developmental Toxicity - Toxicity incurred by the conceptus during development. There are four manifestations: (1) growth retardation, (2) death, (3) terata or malformation, and (4) functional deficit.

The term supercedes older terms "embryotoxicity" (death, malformation) and "fetotoxicity" (death, growth retardation), thereby eliminating temporal considerations.

Developmental Variations - [Also formerly referred to as variations (skeletal, anatomic, homoeotic), common variants, aberrations, retardations, anomalies, deviations].

Defined recently by a regulatory agency as a "divergence beyond the usual range of structural constitution, and which may not be as severe an effect as a malformation" [EPA, *Fed. Reg.*, 51 (185): 34028, 1986].

For purposes of this laboratory, variations are defined as those alterations in anatomic structure that are considered to have no significant adverse biological effect on animal health or body conformity, representing slight deviations from normal. Most examples placed in this category are minor variations in size and form of normally present ossification centers. While these are evaluated on a precise day of development, some variation is expected related to when conception and implantation actually occurred. Thus, differences in the pattern of ossification, manifested either as retardation or as acceleration of apparent osteogenesis, are common findings. Also included in this category are slight misshapening or misalignment of structures, and processes involving continued development (bilateral skeletal centers not yet fused, incomplete maturation of renal papillae, presence of vestigial structures, etc.), and development of extra ossification sites. Slight malpositioning and hypoplasia are also considered variations in development.

Developmental variations correlate in some instances with maternal and/or developmental toxicity.

Distal - Distant from the trunk.

REPRODUCTION GLOSSARY

Embryo - The early or developing stage of an organism, especially the developing product of fertilization of an egg. Its development is termed embryogenesis. The embryonic period is as follows:

Rat 9-14 days postconception

Fecundity Index or Pregnancy Index -

$\frac{\text{No. females pregnant}}{\text{No. females with evidence of mating}} \times 100$

Fetus - The unborn offspring; the developing young following embryogenesis. The fetal period is as follows:

Rat 15-22 days postconception

Gestation - The period of intrauterine development; conception to birth.

Gestation Day 0 - Day in which positive evidence of mating has been ascertained.

Gestation Duration - The length of gestation; term of pregnancy. The duration is as follows for the common species:

Rat 21 days

GLPs - Good Laboratory Practices.

Legislation first enacted in June, 1979 to assure the quality and integrity of safety data filed pursuant to products regulated by the U.S. FDA. The Final Rule became effective October 5, 1987 [*Fed. Reg.*, 52 (172: 33768, 1987)]. Similar GLPs have since been adopted by the U.S. EPA (effective October 16, 1989 for pesticides, and September 18, 1989 for TSCA-regulated chemicals), Japan Koseisho (April 1, 1983) and Japan MAFF (October 1, 1984).

Gravid - An animal with uterine evidence of implantation which delivers a conceptus or contains developing young (pregnant).

Implantation - Attachment of the blastocyst to the epithelial lining of the uterus, its penetration through the epithelium, and its embedment in the compact layer of the endometrium (nidation).

It occurs after fertilization as follows:

Rat 5.5 - 6 days

Implantation sites not discernible by placental remains may be visualized by staining of the uterus with ammonium sulfide (Kopf method, *Naunyn Schmiedebergs Arch. Pharmacol.*, 247: 121-135, 1964).

The types of implants as defined by SOP are as follows: a) viable fetus - responds to touch, b) nonviable fetus - does not respond to touch, no signs of autolysis, c) late resorption - recognizable fetal form, but undergoing autolysis, d) early resorption - implantation site, tissue has no recognizable fetal characteristics.

Lesion - Any pathological or traumatic discontinuity of tissue or loss of function of a part; a circumscribed area of pathologically altered tissue.

Litter Size - In polytocous animals, pregnancy consists of multiple offspring in the litter; this averages for the common species in our laboratory of (live + dead):

Rat 13.4

Malformation - Defective or abnormal function; anatomic or morphologic abnormality; deformity (dysmorphism, cacomorphosis). Defined recently by a regulatory agency as "a permanent deviation which generally is incompatible with or severely detrimental to normal postnatal survival or development" [*EPA, Fed. Reg.*, 51 (185): 34028, 1986].

REPRODUCTION GLOSSARY

For practical purposes we use the following definition in this laboratory: Malformations are those structural anomalies that alter general body conformity, disrupt or interfere with body function, or are generally thought to be incompatible with life. Specific examples of processes that result in maldevelopment include marked or severe misshapening, asymmetry or irregularity of structure brought about by fusion, splitting, disarticulation, malalignment, hiatus, enlargement, lengthening, thickening, thinning, or branching. Absence (agenesia) of parts or whole structures is also considered a malformative process.

Malformations occur when normal organogenesis is interrupted.

Mating Index –

$\frac{\text{No. females with evidence of mating}}{\text{No. females paired}} \times 100$

$\frac{\text{No. males with evidence of mating}}{\text{No. males paired}} \times 100$

Moribund - In a state of dying.

NOAEL - No Observable Adverse Effect Level.

Nonviable - A nonliving conceptus; includes early and late resorptions and dead fetuses.

Postimplantation Loss -

$\frac{\text{No. implantations} - \text{No. viable fetuses}}{\text{No. implantations}} \times 100$

Preimplantation Loss -

$\frac{\text{No. corpora lutea} - \text{No. implantations}}{\text{No. corpora lutea}} \times 100$

Regressing (Corpora Lutea) - Involution of corpora lutea in response to cessation of pregnancy maintenance.

Reproductive Effects - Those findings associated with reproductive parameters in generation studies. They are usually classed as primary (effects related to sex organs or reproductive capacity of adults), or secondary (changes that may be dependent upon or related to other signs of toxicity).

A tabulation of primary reproductive effects might include (Christian, *J. Am. Coll. Toxicol.*, 5: 161, 1986):

- estrous
- mating performance
- fertility
- fecundity
- duration of gestation
- delivery complications
- pathology of reproductive organs
- litter size
- litter viability at birth
- litter survival
- sex ratios of litters
- growth & functional
- development of litter, birth to weaning

Resorption - A conceptus which, having implanted in the uterus, subsequently died and is being, or has been, resorbed.

SOPs - Standard Operating Procedures.

Spontaneous Malformations - The normal background incidence of maldevelopment unrelated to known causes. The rate of spontaneous malformation for common species is as follows (Schardein, *Chemically Induced Birth Defects*, Dekker 1993).

Rat 0.02 - 2%

REPRODUCTION GLOSSARY

Teratogen - An agent or factor that causes the production of physical defects in the developing embryo. The production of defects is termed **teratogenesis**.

Uterine Examination - The excision of the uterus to determine pregnancy status. The location of viable and nonviable fetuses (embryos), early and late resorptions and the number of total implantations is determined through the unopened uterine wall.

Viable - Capable of living.

ANATOMIC PATHOLOGY GLOSSARY

Alopecia - A natural or abnormal deficiency of hair.

Discoloration, tan - A change in color from normal or from surrounding tissue to a yellowish-brown hue.

Discoloration, white - A change in color from normal or from surrounding tissue to a whitish or pale hue.

Fluid, white - The presence of liquid of a whitish color.

Irregular surface (Pitting) - Having an external aspect or surface that is non-conforming or irregular.

Hemorrhage - The presence of blood outside the vascular system.

Pale - Whitish or pallid; lighter in color than normal.

Perforation - A hole made through a part or substance.

Thickened - Having relatively great depth; of considerable extent from one surface or side to the opposite. Not as thin as normally expected.

Within normal limits - Tissue considered to be normal, under the conditions of the study and considering the age, sex and strain of the animal concerned. Alterations may be present which, under other circumstances, would be considered deviations from normal.

MPI Research Study Number 474-005
 An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A in Rats

Summary of Gestation Clinical Findings*

Observation	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Number of Animals Alive at Start of Interval	25	25	25	25
Animals with No Abnormalities Detected	25	21	12	10
Behavior/Activity				
Activity decreased	0/0	0/0	0/0	2/1
Salivation	0/0	1/1	23/11	55/13
External Appearance				
Discharge, Red, Anogenital region	0/0	0/0	0/0	1/1
Emaciated	0/0	0/0	0/0	8/1
Material around mouth, Red	0/0	0/0	0/0	2/1
Material around nose, Red	0/0	0/0	0/0	3/2
Eye/Ocular				
Eye discolored, Cloudy, Eye/right	0/0	10/1	0/0	0/0
Eye discolored, White, Eye/right	0/0	4/1	0/0	0/0
Pelage/Skin				
Hair absent, Abdominal region	0/0	0/0	9/1	0/0
Hair absent, Anogenital region	0/0	1/1	0/0	0/0
Hair absent, Forefoot/left	0/0	0/0	0/0	1/1
Hair absent, Forefoot/right	0/0	0/0	0/0	1/1
Hair absent, Forelimb/left	0/0	0/0	9/1	5/2
Hair absent, Forelimb/right	0/0	0/0	11/2	5/2
Hair discolored, Brown, Anogenital region	0/0	1/1	0/0	0/0
Hair discolored, Yellow, Anogenital region	0/0	0/0	0/0	8/1
Hair sparse, Forelimb/left	0/0	0/0	2/1	0/0
Hair sparse, Forelimb/right	0/0	0/0	8/1	0/0
Skin cold to touch	0/0	0/0	0/0	4/1
Skin discolored, Pale, Entire body	0/0	0/0	0/0	4/1

+ - Number of times observed/Total number of animals affected No statistical analysis performed

MPI Research Study Number 474-005
 An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A in Rats

Summary of Gestation Clinical Findings⁺

Observation	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Pelage/Skin				
Unkempt appearance	0/0	0/0	0/0	2/2
Respiration				
Breathing rapid	0/0	0/0	0/0	4/1

+ - Number of times observed/Total number of animals affected No statistical analysis performed

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Table 2 Summary of Gestation Body Weight Values*

Endpoint Body Weight	Day of Gestation	0 mg/kg/day (Vehicle Control)			100mg/kg/day			300 mg/kg/day			1000 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
	0	229.7	16.89	25	231.6	11.68	24	236.2	13.70	25	237.2	13.55	23
	3	245.9	16.48	25	243.3	11.79	24	251.6	12.65	25	253.4	13.27	23
	6	261.5	18.24	25	257.8	12.26	24	266.5	14.06	24	268.1	14.97	23
	9	275.3	17.32	25	271.1	11.70	24	279.9	13.94	24	282.7	16.11	23
	12	291.4	18.55	25	286.8	12.90	24	295.1	17.14	24	300.0	17.45	23
	15	311.8	19.39	25	306.1	15.38	24	312.5	17.99	24	319.1	16.88	23
	18	355.0	21.42	25	346.8	19.89	24	351.1	24.82	24	358.7	25.24	23
	20	390.7	22.90	25	382.0	23.59	24	386.4	30.83	24	397.3	29.98	23

N - Number of measures used to calculate mean
 SD - Standard Deviation

*No statistical significance observed

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 An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A in Rats

Table 3 Summary of Gestation Body Weight Change Values

Endpoint	Day of Gestation	0 mg/kg/day (Vehicle Control)				100 mg/kg/day				300 mg/kg/day				1000 mg/kg/day			
		Mean	SD	N		Mean	SD	N		Mean	SD	N		Mean	SD	N	
Body Weight Change g	0-3	16.2	5.11	25		11.7*	6.54	24		15.4	5.69	25		16.2	5.16	23	
	3-6	15.6	5.19	25		14.5	6.65	24		14.9	6.60	24		14.7	5.20	23	
	6-9	13.8	4.63	25		13.4	3.37	24		13.5	4.38	24		14.6	4.91	23	
	9-12	16.1	5.06	25		15.7	5.30	24		15.2	5.96	24		17.3	4.56	23	
	12-15	20.4	4.67	25		19.3	7.70	24		17.5	6.67	24		19.1	5.88	23	
	15-18	43.2	7.93	25		40.7	8.34	24		38.6	11.62	24		39.6	14.94	23	
	18-20	35.6	6.56	25		35.2	6.91	24		35.3	9.19	24		38.6	7.81	23	
	0-20	161.0	15.01	25		150.5	19.29	24		150.3	26.72	24		160.1	24.56	23	

N - Number of measures used to calculate mean
 SD - Standard Deviation

*Significantly different from control; (p<0.05)

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 An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A in Rats

Table 4

Summary of Gestation Food Consumption Values

Endpoint	Day of Gestation	0 mg/kg/day (Vehicle Control)			100 mg/kg/day			300 mg/kg/day			1000 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Food Consumption g/animal/day	0-3	18.9	2.28	25	16.9 ^b	1.99	24	16.6 ^b	2.36	25	17.9	1.82	23
	3-6	21.0	2.29	24	19.7	2.04	24	19.5	2.63	24	22.2	7.66	23
	6-9	21.7	2.01	25	20.3	2.05	24	20.5	2.57	23	21.6	2.14	23
	9-12	22.3	2.26	25	21.5	2.39	24	20.9	4.32	24	23.1	1.96	23
	12-15	23.2	2.17	25	21.8	2.07	24	21.2 ^b	2.69	24	22.3	1.84	23
	15-18	25.2	3.98	25	24.9	5.25	24	23.7	3.09	24	23.9	4.44	23
	18-20	24.2	2.59	25	23.5	2.41	24	23.2	2.87	24	24.0	2.66	23
	0-20	22.2	1.80	24	21.2	1.40	24	20.8 ^a	2.25	23	22.1	1.54	23

MPI Research Study Number 474-005
 An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A in Rats

Summary of Macroscopic Observations
 Terminal Sacrifice: Rat

TISSUE OBSERVATION	Severity	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
NUMBER OF ANIMALS EXAMINED					
NUMBER WITHIN NORMAL LIMITS					
All Tissues		25	25	25	25
Within normal limits		25	22	21	22
<u>Eye</u>		(0)	(1)	(0)	(0)
Discolored, white	-mild	0	1	0	0
<u>Heart</u>		(0)	(0)	(1)	(0)
Pericardial Sac, hemorrhage	-moderate	0	0	1	0
<u>Lungs</u>		(0)	(0)	(0)	(1)
Pale		0	0	0	1
<u>Kidney</u>		(0)	(0)	(1)	(0)
Pitting, right	-mild	0	0	1	0
<u>Pharynx</u>		(0)	(0)	(1)	(0)
Perforation	-mild	0	0	1	0
<u>Skin</u>		(0)	(1)	(1)	(2)
Hair absent		0	1	1	2

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Summary of Macroscopic Observations
 Terminal Sacrifice: Rat

Table 5 Cont.

TISSUE OBSERVATION	Severity	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Thoracic Cavity Contains oily material Discoloration, white		(0) 0 0 0	(1) 0 1 1	(2) 1 1 0	(1) 0 0 0
	-moderate -severe	0 0	0 0	1 0	0 1
Fluid filled, white		0	0	0	1
Thymus Discoloration, tan Thickened,		(0) 0 0	(0) 0 0	(0) 0 0	(1) 1 1
	-moderate	0	0	0	1

CODE: () = NUMBER OF ANIMALS WITH MACROSCOPIC OBSERVATIONS

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Table 6 Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Females on Study	25	25	25	25
No. Not Pregnant	0	1	0	1
No. Pregnant	25	24	25	24
Pregnancy Index Percent*	100.0	96.0	100.0	96.0
No. Died Pregnant	0	0	1	0
No. Abortions	0	0	0	0
No. Early Deliveries	0	0	0	0
No. Females Pregnant by Stain	0	0	0	1
No. Females with Viable Fetuses Day 20 Gestation	25	24	24	23

*No statistical significance observed

No. - Number

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Table 6 Cont. Summary of Maternal and Developmental Observations at Uterine Examination*

Endpoint	0 mg/kg/day (Vehicle Control)			100 mg/kg/day			300 mg/kg/day			1000 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Corpora Lutea No. per Animal	16.8	2.72	25	16.6	2.21	24	16.8	3.60	24	18.0	2.57	23
Implantation Sites No. per Animal	15.4	1.76	25	15.3	2.48	24	15.4	3.52	24	15.6	2.36	24
Preimplantation Loss % per Animal	7.25	7.540	25	7.77	9.699	24	10.18	15.696	24	10.65	8.059	23
Viable Fetuses No. per Animal	14.6	1.68	25	14.5	2.64	24	14.1	3.71	24	14.3	3.43	24
Viable Fetuses/Implant % per Implant	95.05	6.636	25	94.63	7.523	24	92.34	12.560	24	90.25	20.023	24

No. - Number
 SD - Standard Deviation
 N - Number of measures used to calculate mean
 *No statistical significance observed

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Table 6 Cont. Summary of Maternal and Developmental Observations at Uterine Examination*

Endpoint	0 mg/kg/day (Vehicle Control)			100 mg/kg/day			300 mg/kg/day			1000 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Fetal Sex Ratio % Males per Animal	52.9	12.20	25	50.7	15.95	24	47.5	16.58	24	52.5	13.64	23
Postimplantation Loss % Implants per Animal	4.95	6.636	25	5.37	7.523	24	7.66	12.560	24	9.75	20.023	24
Nonviable Fetuses No. per Animal	0.0	0.00	25	0.0	0.00	24	0.0	0.00	24	0.0	0.00	24
Resorptions: Early No. per Animal	0.8	1.12	25	0.8	1.13	24	1.3	2.01	24	1.3	1.73	24
Early Resorptions/Implant % per Implant	4.95	6.636	25	5.37	7.523	24	7.66	12.560	24	9.75	20.023	24
Resorptions: Late No. per Animal	0.0	0.00	25	0.0	0.00	24	0.0	0.00	24	0.0	0.00	24

*No statistical significance observed

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mean

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Table 8 **Summary of Organ Weight Values**

Endpoint	0 mg/kg/day (Vehicle Control)			100 mg/kg/day			300 mg/kg/day			1000 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Adjusted Final Body Weight												
g	307.720	19.830	25	300.750	16.752	24	308.917	17.315	24	313.565	23.308	23
Liver												
g	16.702	1.378	25	15.453*	1.563	25	16.048	1.706	25	16.761	2.050	24
Liver/Adjusted Final Body Weight %												
%	5.429	0.322	25	5.197*	0.332	24	5.243	0.364	24	5.443	0.336	23

N - Number of measures used to calculate mean
 SD - Standard Deviation
 *Significantly different from control; (p<0.05)

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Table 7 Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values*

Endpoint	0 mg/kg/day (Vehicle Control)			100 mg/kg/day			300 mg/kg/day			1000 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, g	83.0	8.21	25	81.3	14.17	24	77.5	19.54	24	83.7	9.71	23
Day 20 Body Weight, g	390.7	22.90	25	382.0	23.59	24	386.4	30.83	24	397.3	29.98	23
Adjusted Day 20 Body Weight, g	307.7	19.83	25	300.8	16.75	24	308.9	17.32	24	313.6	23.31	23
Weight Change from Day 0, g	161.0	15.01	25	150.5	19.29	24	150.3	26.72	24	160.1	24.56	23
Adjusted Weight Change From Day 0, g	78.0	12.16	25	69.2	11.47	24	72.8	12.44	24	76.4	18.18	23

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Table 9

		Summary of Fetal Body Weights, g*			
		0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Fetal Weight	Males	Mean 3.81 (3.81)	3.81 (3.81)	3.67 (3.66)	3.75 (3.75)
		SD 0.258	0.319	0.240	0.357
		N 25	24	23	23
Females		Mean 3.62 (3.62)	3.63 (3.63)	3.53 (3.53)	3.56 (3.56)
		SD 0.262	0.276	0.208	0.293
		N 25	24	24	23
Males + Females		Mean 3.72 (3.72)	3.72 (3.72)	3.59 (3.58)	3.66 (3.67)
		SD 0.254	0.296	0.221	0.322
		N 25	24	24	23

SD - Standard Deviation

N - Number of measures used to calculate mean

() - Least square mean

* No statistical significance observed

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Table 10 Summary of Individual Fetal External Observations*

Observation	Classification	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated		25	24	24	23
No. Fetuses Evaluated		366	348	339	344
Forelimb(s)					
Digits, ectrodactyly	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Entire, abnormal flexure	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)
Head					
Entire, edema	V				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		2 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Hand limb(s)					
Entire, abnormal flexure	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)

No. - Number

M - Malformation

V - Variation

¹ Not statistically analyzed

* No statistical significance observed

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Table 11 Summary of Individual Fetal Visceral Observations*

Observation	Classification	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated		25	24	23	23
No. Fetuses Evaluated		177	179	166	175
Brain					
Lateral ventricle, hydrocephaly	M				
No. Litters (%)		1 (4.0)	2 (8.3)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		1 (0.6)	2 (1.1)	0 (0.0)	0 (0.0)
Head					
Eye lens, smaller than normal	M				
No. Litters (%)		0 (0.0)	1 (4.2)	1 (4.3)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	1 (0.6)	2 (1.2)	0 (0.0)
Retina, folded retina	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
Thoracic cavity					
Aortic arch, missshapen	M				
No. Litters (%)		0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Diaphragm, diaphragmatic hernia	M				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)

No.-Number

M- Malformation

¹Not statistically analyzed

*No statistical significance observed

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Table 11 Cont. Summary of Individual Fetal Visceral Observations*

Observation	Classification	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated		25	24	23	23
No. Fetuses Evaluated		177	179	166	175
Thoracic cavity Cont.					
Pulmonary artery, misshapen	M				
No. Litters (%)		0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Pulmonary trunk, misshapen					
No. Litters (%)	M	1 (4.0)	1 (4.2)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		1 (0.6)	1 (0.6)	0 (0.0)	0 (0.0)

No. - Number

M - Malformation

¹Not statistically analyzed

*No statistical significance observed

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Table 12 Summary of Individual Fetal Skeletal Observations*

Observation	Classification	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated		25	24	24	23
No. Fetuses Evaluated		189	169	173	169
Cervical vertebra(e)					
Neural arch(es), additional ossification center	V	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)
No. Litters (%)		0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
Neural arch(es), incompletely ossified	V	1 (4.0)	0 (0.0)	1 (4.2)	1 (4.3)
No. Litters (%)		1 (0.5)	0 (0.0)	1 (0.6)	1 (0.6)
No. Fetuses (%) ¹		1 (0.5)	0 (0.0)	1 (0.6)	1 (0.6)
Pectoral girdle					
Scapula, bent	M	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Pelvic girdle					
Ischium, incompletely ossified	V	1 (4.0)	0 (0.0)	1 (4.2)	1 (4.3)
No. Litters (%)		1 (0.5)	0 (0.0)	2 (1.2)	1 (0.6)
No. Fetuses (%) ¹		1 (0.5)	0 (0.0)	2 (1.2)	1 (0.6)

*No statistical significance observed
¹Not statistically analyzed
 No.-Number
 M- Malformation
 V- Variation

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Table 12 Cont. Summary of Individual Fetal Skeletal Observations*

Observation	Classification	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated		25	24	24	23
No. Fetuses Evaluated		189	169	173	169
Rib(s)					
Rib(s), bent	V				
No. Litters (%)		1 (4.0)	0 (0.0)	2 (8.3)	1 (4.3)
No. Fetuses (%) ¹		1 (0.5)	0 (0.0)	2 (1.2)	1 (0.6)
Rib(s), rudimentary	V				
No. Litters (%)		7 (28.0)	14 (58.3)	10 (41.7)	13 (56.5)
No. Fetuses (%) ¹		16 (8.5)	27 (16.0)	22 (12.7)	29 (17.2)
Skull					
Frontal bone, incompletely ossified	V				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Hyoid, not ossified	V				
No. Litters (%)		0 (0.0)	1 (4.2)	0 (0.0)	1 (4.3)
No. Fetuses (%) ¹		0 (0.0)	1 (0.6)	0 (0.0)	1 (0.6)
Interparietal bone, incompletely ossified	V				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)

¹Not statistically analyzed

*No statistical significance observed

No.-Number

V- Variation

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Table 12 Cont. Summary of Individual Fetal Skeletal Observations*

Observation	Classification	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated		25	24	24	23
No. Fetuses Evaluated		189	169	173	169
Skull Cont.					
Jugal, incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Nasal bone, incompletely ossified	V				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Parietal bone, incompletely ossified	V				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	2 (8.7)
No. Fetuses (%) ¹		2 (1.1)	0 (0.0)	0 (0.0)	2 (1.2)
Squamosal, incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Supra occipital bone, incompletely ossified	V				
No. Litters (%)		1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹		2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Sternum					
Sternebra(e), bipartite	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%) ¹		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)

¹Not statistically analyzed

*No statistical significance observed

No. -Number

V - Variation

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Table 12 Cont. Summary of Individual Fetal Skeletal Observations*

Observation	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated	25	24	24	23
No. Fetuses Evaluated	189	169	173	169
Sternum Cont.				
Sternebra(e), not ossified				
No. Litters (%)	18 (72.0)	14 (58.3)	16 (66.7)	13 (56.5)
No. Fetuses (%) ¹	35 (18.5)	17 (10.1)	29 (16.8)	25 (14.8)
	V			

No.-Number

V - Variation

¹ Not statistically analyzed

*No statistical significance observed

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Table 13 Summary of External Malformations and Developmental Variations*

	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated	25	24	24	23
No. Fetuses Evaluated	366	348	339	344
Total Malformations				
No. Litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%) ¹	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Total Variations				
No. Litters (%)	1 (4.0)	0 (0.0)	1 (4.2)	1 (4.3)
No. Fetuses (%) ¹	2 (0.5)	0 (0.0)	1 (0.3)	2 (0.6)

No. - Number Not statistically analyzed; *No statistical significance observed

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Table 14 Summary of Visceral Malformations and Developmental Variations*

	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated	25	24	23	23
No. Fetuses Evaluated	177	179	166	175
Total Malformations				
No. Litters (%)	3 (12.0)	2 (8.3)	1 (4.3)	0 (0.0)
No. Fetuses (%) ¹	3 (1.7)	3 (1.7)	3 (1.8)	0 (0.0)
Total Variations				
No. Litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%) ¹	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

No. - Number

¹Not statistically analyzed

^{*}No statistical significance observed

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Table 15 Summary of Skeletal Malformations and Developmental Variations*

	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated	25	24	24	23
No. Fetuses Evaluated	189	169	173	169
Total Malformations				
No. Litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%) ¹	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Total Variations				
No. Litters (%)	22 (88.0)	20 (83.3)	19 (79.2)	18 (78.3)
No. Fetuses (%) ¹	52 (27.5)	44 (26.0)	52 (30.1)	52 (30.8)

No. - Number

Not statistically analyzed

*No statistical significance observed

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Table 16 Summary of External, Visceral, and Skeletal Malformations*

	0 mg/kg/day (Vehicle Control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
No. Litters Evaluated	25	24	24	23
No. Fetuses Evaluated	366	348	339	344
Total Malformations				
No. Litters (%)	3 (12.0)	2 (8.3)	1 (4.2)	2 (8.7)
No. Fetuses (%) ¹	3 (0.8)	3 (0.9)	3 (0.9)	2 (0.6)

No. - Number

¹Not statistically analyzed

* No statistical significance observed