NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology Studies of Tetrabromobisphenol A (CASRN 79-94-7) in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies)

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NTP Technical Report on the Toxicology Studies of Tetrabromobisphenol A (CASRN 79-94-7) in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies)

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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Explanation of Levels of Evidence of Carcinogenic Activity

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

Peer Review

The members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology Studies of Tetrabromobisphenol A (CASRN 79-94-7) in F344/Ntac Rats and B6c3f1/N Mice and Toxicology and Carcinogenesis Studies of Tetrabromobisphenol A in Wistar Han [Crl:Wi(Han)] Rats and B6c3f1/N Mice (Gavage Studies)* on October 29, 2013, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers had five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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Abstract

Tetrabromobisphenol A is a flame retardant used in epoxy resin circuit boards, in electronic enclosures (of polycarbonate-acrylonitrile-butadiene-styrene plastics), in paper, and in textiles. It may also be used as a chemical intermediate for the synthesis of other flame retardants. Tetrabromobisphenol A was nominated by the NIEHS for toxicity and carcinogenicity studies based on its high production volume, the potential for widespread human exposures, and the absence of standard toxicity and carcinogenicity studies reported in the scientific literature. Male and female F344/NTac rats and B6C3F1/N mice were administered tetrabromobisphenol A (purity of greater than 99%) in corn oil by gavage for 3 months, and male and female Wistar Han [Crl:WI(Han)] rats (referred to as Wistar Han rats) and B6C3F1/N mice were administered tetrabromobisphenol A (purity of approximately 99%) in corn oil by gavage for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

Three-month Study in F344/NTac Rats

Groups of 10 male and 10 female core study rats were administered 0, 10, 50, 100, 500, or 1,000 mg tetra-bromobisphenol A/kg body weight in corn oil by gavage, 5 days per week for up to 14 weeks. Additional special study groups of 10 male and 10 female rats were administered the same doses for 23 days. All core study rats survived to the end of the study. Mean body weights of dosed groups of male and female rats were similar to those of the vehicle controls.

Dose-related decreases in total thyroxine concentrations occurred on day 4 and at week 14 in 500 and 1,000 mg/kg males and females; this effect was observed with less consistency in the 100 mg/kg groups. Hematology findings on day 23 suggested small decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in 500 and 1,000 mg/kg males and females. By week 14, there was some amelioration in the severity of the erythron decreases in males and females. By week 14, there was some amelioration in the severity of the erythron decreases in these groups. At week 14, serum activities of alanine aminotransferase and sorbitol dehydrogenase generally demonstrated decreases in males and females administered 100 mg/kg or greater.

Significant increases occurred in liver weights of 500 and 1,000 mg/kg rats and significant decreases occurred in spleen weights of 500 and 1,000 mg/kg males. No treatment-related histopathologic lesions were observed in rats in the 3-month study.

Three-month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 10, 50, 100, 500, or 1,000 mg tetra-bromobisphenol A/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. All mice survived to the end of the study. Mean body weights of dosed groups of male and female mice were similar to those of the vehicle controls.

Liver weights of 500 mg/kg males and 1,000 mg/kg males and females were significantly greater than those of the vehicle controls. Kidney weights were significantly decreased and spleen weights were significantly increased in 1,000 mg/kg males.

In the kidney, incidences of renal tubule cytoplasmic alteration were significantly increased in 500 and 1,000 mg/kg male mice, and the severity of the lesion in the 1,000 mg/kg group was greater than that in the 500 mg/kg group.

Two-year Study in Wistar Han Rats

Groups of 60 male and 60 female rats were administered 0 or 1,000 mg tetrabromobisphenol A/kg body weight and 50 male and 50 female rats were administered 250 or 500 mg/kg in corn oil by gavage, 5 days per week for up to 104 (males) or 105 (females) weeks. Survival of dosed groups was similar to that of the vehicle control groups. Mean body weights of 500 and 1,000 mg/kg males were at least 10% less than those of the vehicle control group after week 25. Ten vehicle control and ten 1,000 mg/kg rats of each sex were evaluated at 3 months to allow comparison to 3-month endpoints in the F344/NTac rats. At the 3-month interim evaluation, there were no treatment-related lesions in males or females, but thymus weights of 1,000 mg/kg rats were significantly less than those of the vehicle control groups, and there were increased liver weights in the 1,000 mg/kg groups similar to those seen in the 3-month F344/NTac rats.

In the original transverse review of the uterus, there were significant positive trends in the incidences of adenoma and adenocarcinoma, and the incidences of adenocarcinoma in the 500 and 1,000 mg/kg groups were greater than that in the vehicle control group. Malignant mixed Müllerian tumors were also found in treated rats. When combined, the incidences of adenoma, adenocarcinoma, or malignant mixed Müllerian tumor were significantly increased in the 500 and 1,000 mg/kg groups. Additional longitudinal reviews of residual uterine tissue were conducted and more neoplasms were identified. When the two reviews were combined, there were significant positive trends in the incidences of adenocarcinoma and of adenoma, adenocarcinoma, or malignant mixed Müllerian tumor (combined), and the incidences were significantly increased in the 500 and 1,000 mg/kg groups. In the residual longitudinal review, a new and potentially preneoplastic lesion of endometrial atypical hyperplasia was identified as statistically significant in all dosed groups.

Mutation analyses were performed comparing the mutation spectra of uterine adenocarcinomas from tetrabromobisphenol A-dosed Wistar Han rats and spontaneous uterine adenocarcinomas from control Wistar Han rats from a variety of NTP studies. Results of these analyses indicated that the rate of *Tp53* mutations was significantly increased in uterine adenocarcinomas from rats dosed with tetrabromobisphenol A compared to spontaneous uterine adenocarcinomas.

In the testis, incidences of interstitial cell adenoma were slightly increased in 500 and 1,000 mg/kg males.

In the ovary, the incidences of rete ovarii cyst in 500 and 1,000 mg/kg females were significantly greater than that in the vehicle controls.

Two-year Study in Mice

Groups of 50 male and 50 female mice were administered 0, 250, 500, or 1,000 mg tetrabromobisphenol A/kg body weight in corn oil by gavage, 5 days per week for 105 weeks. Survival of 1,000 mg/kg males and females was significantly less than that of the vehicle control groups. Mean body weights of 1,000 mg/kg females were at least 10% less than those of the vehicle controls after week 25.

In the liver, the incidence of multiple hepatocellular adenoma was significantly increased in 500 mg/kg males. In addition, the incidences of hepatoblastoma and of hepatocellular carcinoma or hepatoblastoma (combined) in 250 mg/kg males were significantly greater than those in the vehicle controls. The incidences of clear cell focus in 500 mg/kg males and eosinophilic focus in 250 and 500 mg/kg males were significantly increased.

The incidences of adenoma or carcinoma (combined) of the cecum or colon and the incidences of hemangiosarcoma (all organs) occurred with significant positive trends in males.

In the kidney, incidences of renal tubule cytoplasmic alteration were significantly increased in 250 and 500 mg/kg males.

In the forestomach, the incidences of ulcer, mononuclear cell cellular infiltration, inflammation, and epithelium hyperplasia were significantly increased in 500 mg/kg males and 250 and 500 mg/kg females.

Genetic Toxicology

Tetrabromobisphenol A was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, or *E. coli* strain WP2 *uvr*A/pKM101, with or without exogenous metabolic activation. In vivo, no increases in micronucleated normochromatic erythrocytes were observed in male or female B6C3F1/N mice following 3 months of administration of tetrabromobisphenol A by gavage; no significant changes in the percentage of circulating polychromatic erythrocyteswere observed in dosed mice, suggesting that tetra-bromobisphenol A did not induce bone marrow toxicity over the dose range tested.

Conclusions

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* (see Explanation of Levels of Evidence of Carcinogenic Activity; see summary of the peer review panel comments and the public discussion on this Technical Report in Appendix N) of tetrabromobisphenol A in male Wistar Han rats based on the occurrence of testicular adenoma. There was *clear evidence of carcinogenic activity* of tetrabromobisphenol A in female Wistar Han rats based on increased incidences of uterine epithelial tumors (predominantly uterine adenocarcinoma). There was *some evidence of carcinogenic activity* of tetrabromobisphenol A in male B6C3F1/N mice based on increased incidences of hepatoblastoma. The increased incidences of large intestine neoplasms and hemangiosarcoma (all organs) may have been related to chemical administration. There was *no evidence of carcinogenic activity* of tetrabromobisphenol A in female B6C3F1/N mice administered 250 or 500 mg/kg.

Administration of tetrabromobisphenol A resulted in increased incidences of non-neoplastic lesions of the uterus and ovary in female rats, the liver and kidney in male mice, and the forestomach in male and female mice.

Synonyms: 2,2-Bis(3,5-dibromo-4-hydroxyphenyl)propane; 2,2-bis(4-hydroxy-3,5-dibromophenyl)propane; 4,4′ isopropylidenebis(2,6 dibromophenol); 4,4′-(1 methylethylidene)bis(2,6-dibromophenol); 2,2′,6,6′-tetrabromobisphenol A; 3,3′,5,5′ tetrabromobisphenol A; 2,2′,6,6′-tetrabromo-4,4′-isopropylidenediphenol; tetrabromodian; tetrabromodiphenylpropane

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of Tetrabromobisphenol A

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice[a]	Female B6C3F1/N Mice[a]
Doses in corn oil by gavage	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg
Body weights	500 and 1,000 mg/kg groups at least 10% less than the vehicle control group after week 25	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	1,000 mg/kg group at least 10% less than the vehicle control group after week 25
Survival rates	33/50, 28/50, 38/50, 39/50	35/50, 34/50, 29/50, 33/50	33/50, 26/50, 39/50, 12/50	40/50, 31/50, 36/50, 4/50
Non-neoplastic effects	None	Uterus: endometrium, hyperplasia, atypical (residual longitudinal review-2/50, 13/50, 11/50, 13/50) Ovary: rete ovarii cyst (1/50, 0/49, 6/50, 6/49)	Liver: clear cell focus (11/50, 10/50, 25/50); eosinophilic focus (20/50, 33/50, 40/50) Kidney: renal tubule, cytoplasmic alteration (0/50, 20/50, 47/50) Forestomach: ulcer (9/50, 9/49, 19/50); infiltration cellular, mononuclear cell (5/50, 8/49, 21/50); inflammation (9/50, 10/49, 20/50); epithelium, hyperplasia (10/50, 13/49, 27/50)	Forestomach: ulcer (2/50, 15/50, 40/50); infiltration cellular, mononuclear cell (2/50, 13/50, 33/50); inflammation (2/50, 14/50, 41/50); epithelium, hyperplasia (4/50, 16/50, 39/50)

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice[a]	Female B6C3F1/N Mice[a]
Neoplastic effects	None	Uterus: adenoma (original transverse review-0/50, 0/50, 3/50, 4/50); adenocarcinoma (original transverse review-3/50, 3/50, 8/50, 9/50; original transverse and residual longitudinal reviews, combined-4/50, 10/50, 15/50, 16/50); malignant mixed Müllerian tumor (original transverse review-0/50, 4/50, 0/50, 2/50); adenoma, adenocarcinoma, or malignant mixed Müllerian tumor (original transverse review-3/50, 7/50, 11/50, 13/50; original transverse and residual longitudinal reviews, combined-6/50, 11/50, 16/50, 19/50)	Liver: hepatoblastoma (2/50, 11/50, 8/50)	None
Equivocal findings	Testis: interstitial cell, adenoma (0/50, 0/50, 1/50, 3/50)	None	Large intestine (cecum or colon): adenoma or carcinoma (0/50, 0/50, 3/50) Hemangiosarcoma (all organs): (1/50, 5/50, 8/50)	None
Level of evidence of carcinogenic activity	Equivocal evidence	Clear evidence	Some evidence	No evidence
Genetic toxicology				
Bacterial gene mutations:			Negative in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, with or without hamster or rat liver S9. Negative in *S. typhimurium* strains TA98 and TA100 and in *E. coli* strain WP2 *uvrA*/pKM101 with and without rat liver S9.	
Micronucleated erythrocytes				
Mouse peripheral blood in vivo:			Negative in males and females	

[a]Due to early mortality, lesion incidences for the 1,000 mg/kg group are not presented.

Introduction

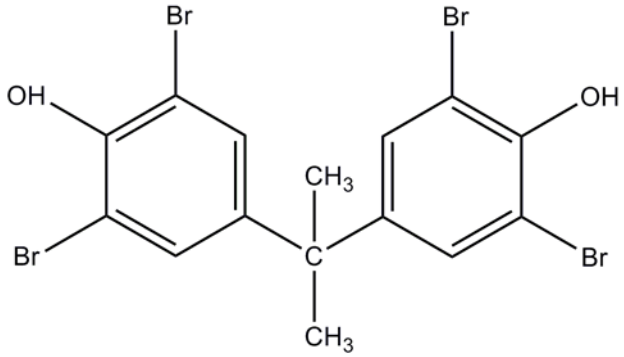

Figure 1. Tetrabromobisphenol A (CASRN 79-94-7; Chemical Formula: $C_{15}H_{12}Br_4O_2$; Molecular Weight: 543.88)

Synonyms: 2,2-Bis(3,5-dibromo-4-hydroxyphenyl)propane; 2,2-bis(4-hydroxy-3,5-dibromophenyl)propane; 4,4′-isopropylidenebis(2,6-dibromophenol); 4,4′-(1-methylethylidene)bis(2,6-dibromophenol); 2,2′,6,6′-tetrabromobisphenol A; 3,3′,5,5′-tetrabromobisphenol A; 2,2′,6,6′-tetrabromo-4,4′-isopropylidenediphenol; tetrabromodian; tetrabromodiphenylpropane.
Trade names: Bromdian, Fire Guard 2000, Firemaster BP 4A, Saytex RB 100PC.

Chemical and Physical Properties

Tetrabromobisphenol A is an off-white powder with a melting point in the range of 179° to 181°C and a density of 2.2 kg/L at 4°C; it is insoluble in water but is soluble in oxygenated solvents[1]. (Tetrabromobisphenol A contains 58.4% bromine, and under basic conditions, both hydroxyl groups of tetrabromobisphenol A react with epichlorohydrin to give the diglycidyl ether, which is widely used in epoxy resin formulations[2].

Production, Use, and Human Exposure

Tetrabromobisphenol A is produced by the bromination of bisphenol A in the presence of solvents such as a halocarbon, water, 50% hydrobromic acid, aqueous alkyl monoethers, or aqueous acetic acid. When methanol is used, methyl bromide is formed as a coproduct[3]. The United States annual tetrabromobisphenol A production is between 100 and 500 million pounds[4]. Other reports list global tetra-bromobisphenol A annual production at 145,000 tonnes (320,000,000 lbs)[5; 6]. It is estimated that tetrabromobisphenol A accounts for 59% of all brominated flame retardants used worldwide[7].

Tetrabromobisphenol A is a flame retardant used in epoxy resin circuit boards, in electronic enclosures (of polycarbonate-acrylonitrile-butadiene-styrene plastics), in paper, and in textiles. Tetrabromobisphenol A is used as a chemical intermediate for the synthesis of other flame retardants [e.g., tetrabromobisphenol A allyl ether, tetrabromobisphenol A bis(2-hydroxyethyl ether), tetrabromobisphenol A carbonate oligomers, and tetrabromobisphenol A diglycidyl ether][2; 3; 8].

Products containing tetrabromobisphenol A have been shown to release tetrabromobisphenol A into the environment[9]. Tetrabromobisphenol A has been found in sewage sludge, soil, sediments, birds, fish, and air, and it has been detected in cow and human milk, human serum, human adipose tissue, umbilical cord serum, and in household dust[10]. A study in Boston found that 35%

of human milk samples contained tetrabromobisphenol A[11]. Tetrabromobisphenol A is present in arctic wildlife indicating the ability for long-range transport from point sources[12]. Its half-life was approximately 50 days in a 64-day aerobic and anaerobic soil study and 48 to 84 days in a sediment/water degradation study[13]. Tetrabromobisphenol A and derivatives have been found at increased levels in soil and sediment downstream from a brominated flame retardant factory[14].

Bacteria can debrominate tetrabromobisphenol A to tri-, di-, and monobromobisphenol A[15]. Photodegradation of tetrabromobisphenol A in water by UV radiation has the following half-lives: 10.2 days in spring, 6.6 days in summer, 25.9 days in autumn, and 80.7 days in winter; cloud cover increases the half-life[3]. The main breakdown product of tetrabromobisphenol A by photodegradation is 2,4,6-tribromophenol. Other decomposition products identified include di- and tribromobisphenol A; dibromophenol; 2,6-dibromo-4-(bromoisopropylene) phenol; 2,6-dibromo-4-(dibromoisopropylene) phenol; and 2,6-dibromo-1,4-hydroxybenzene[16].

Exposure to tetrabromobisphenol A may be from inhalation of ambient air, dermal contact, or ingestion[3]. A recent survey of various fish species in China indicates that tetrabromobisphenol A may be present in fish at concentrations up to 39 ng/g[17]. The United States Environmental Protection Agency[4] reports that up to 1,000 people may be exposed during manufacturing and processing of tetrabromobisphenol A in the workplace.

Exposure to tetrabromobisphenol A may also occur through the disposal, recycling, incineration, and landfilling of electronic waste (e-waste)[18]. Tetrabromobisphenol A is typically detected at parts per million (ppm) concentrations in sediments and sewage sludge near brominated flame retardant production facilities[19; 20]. The environmental persistence of tetrabromobisphenol A is due to its high lipophilicity (log K_{ow} = 5.9), low volatility (7.0×10^{-11} atm m^3/mol), and low water solubility (4.16 mg/L at 25°C)[19; 20]

In a recent review in Europe, tetrabromobisphenol A was found in food samples, mothers' milk, outdoor air samples, indoor dust, soil samples, and wildlife[21]. The estimated daily intake of tetrabromobisphenol A is up to 2.6 ng/kg body weight per day in adults and up to 257 ng/kg per day in infants[21].

Regulatory Status

The threshold for reporting releases of tetrabromobisphenol A is 100 lbs[22]. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain identified a lower confidence limit for a benchmark response of a 10% relative decrease in serum thyroxine (T_4) levels of 16 mg/kg body weight[21].

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

Experimental Animals

[^{14}C]-labeled tetrabromobisphenol A was rapidly absorbed, metabolized, and excreted following oral administration to rats[23-25]. Results of these studies indicated minimal sex and strain differences in the absorption and excretion of tetrabromobisphenol A in this rodent model. Over 90% of the [^{14}C] in single oral doses ranging from 2 to 1,000 mg/kg was recovered within 72 hours in feces of male Sprague Dawley rats Hakk et al.[23] male F344 rats Kuester et al.[24], and

female Wistar Han rats[25]. Comparative intravenous dosing and bile duct-cannulated rat experiments demonstrated that most of the [^{14}C] in feces was due to biliary excretion following absorption of tetrabromobisphenol A from the gut[23; 24]. The balance of the administered doses recovered within 72 hours in these studies was excreted in urine (up to 3%) or remained in tissues at negligible amounts (less than 1%). No disposition data for tetrabromobisphenol A in mice were found in the literature.

The kinetics studies conducted by Kuester et al.[24] and Knudsen et al.[25] demonstrated rapid clearance of [^{14}C]-labeled tetrabromobisphenol A from the blood of either male (F344) or female (Wistar Han) rats following single oral or intravenous administration. The T_{max} of [^{14}C] in blood after oral administration was observed at 32 ± 19 minutes in male rats (200 mg/kg; fasted) and at 114 ± 42 minutes in female rats (250 mg/kg; nonfasted). Tetrabromobisphenol A had terminal half-lives of less than 5 hours and systemic bioavailability was less than 5% in these animals. Tissues contained little or no detectable [^{14}C] 24 hours following 1, 5, or 10 consecutive daily oral doses of 20 mg/kg in male F344 rats in the study conducted by[24]. Further, Kang et al.[26] observed no accumulation in tissues of male Sprague Dawley rats receiving 14 consecutive daily doses of 1,000 mg/kg tetrabromobisphenol A. The authors reported no saturation of single doses in the range of 200 to 1,000 mg/kg. However, Kuester et al.[24] reported some initial delay of excretion of a single oral dose of 200 mg/kg over that of lower doses (2 and 20 mg/kg) in F344 male rats (indicative of saturation of transport/metabolism in the liver). An initial delay in fecal excretion of a single oral dose of 1,000 mg/kg over that of 25 and 250 mg/kg was also observed in Wistar Han female rats[25]. In both instances, the effect was transitory and the amount excreted in feces was similar across the dosing range within 72 hours of administration.

Tetrabromobisphenol A was rapidly conjugated with glucuronic acid or sulfate in disposition and metabolism studies conducted in rats (Figure 2). Further, evidence from these studies indicated that tetrabromobisphenol A underwent enterohepatic circulation through a cycle of deconjugation by gut microflora and reabsorption in the gut. A monoglucuronide, a diglucuronide, and a mixed glucuronide-sulfate metabolite were identified in bile of male F344 and Sprague Dawley rats[23; 24]. Glucuronide and sulfate conjugates of tetrabromobisphenol A were detected in bile of female Wistar Han rats[25], in serum of male Sprague Dawley rats dosed orally with 300 mg/kg[27], and in *Xenopus laevis* tadpoles following exposure to tetrabromobisphenol A in water[28]. In a study conducted by Zalko et al.[29], tetrabromobisphenol A was primarily oxidized in rat subcellular liver fractions, resulting in products derived from cleavage of the molecule. Glucuronide and glutathione conjugates were also detected. Oxidative cleavage of tetrabromobisphenol A may occur in the rat as evidenced by detection of a 2,6-dibromobenzosemiquinone radical in the bile of tetrabromobisphenol A-treated male Sprague Dawley rats[30] (Figure 2). Tribromobisphenol A (Figure 2) has been detected in feces of female Wistar rats receiving a single intraperitoneal dose of 250 mg/kg[31] and in plasma and feces of male Sprague Dawley rats receiving a single oral dose of 300 mg/kg tetrabromobisphenol A[27]. It is speculated that microflora may reduce tetrabromobisphenol A in the gut[29; 31]. Reductive dehalogenation of tetrabromobisphenol A occurs in the environment as the result of bacterial activity[32].

NTP has found that a structurally similar flame retardant, tetrabromobisphenol A bis (2,3-dibromo-propyl ether), was poorly absorbed from the gastrointestinal tract and eliminated primarily in the feces of male F344 rats[33]. Because of the low absorption and little to no metabolism, it is predicted that this chemical would have a low order of toxicity.

Humans

Tetrabromobisphenol A was absorbed and metabolized rapidly in healthy human volunteers receiving a single oral dose of 0.1 mg/kg[27]. It was below the limit of detection in all blood samples, including the initial timepoints of 1, 2, and 4 hours. However, tetrabromobisphenol A glucuronide was present at all timepoints up to 72 hours, with peak concentrations detected between 2 and 6 hours. Traces of tetrabromobisphenol A glucuronide were also detected in urine samples. Tetrabromobisphenol A metabolism in human liver subcellular fractions was qualitatively similar to that described above in rat liver subcellular fractions[29].

Occupational exposure has resulted in detection of tetrabromobisphenol A in humans. Up to 4 ng/g lipid was present in serum of workers (n = 4) in an electronics dismantling plant or computer technicians (n = 19) working in a hospital environment[34; 35]. Hagmar et al.[34] calculated a half-life of 2.2 days for tetrabromobisphenol A in the factory workers. Other studies have measured environmental exposures in the general population. Serum lipid of Norwegian subjects (n= 29) contained a mean concentration of 0.65 ng/g (cohort from 1999), serum of Japanese subjects (n = 5) contained 7 ± 1 pg/g, adipose tissue of subjects in New York City (n = 20) contained 0.05 ± 0.1 ng/g lipid, and up to 0.55 ng/g lipid was detected in human milk from a Boston cohort (n = 43)[11; 36-38].

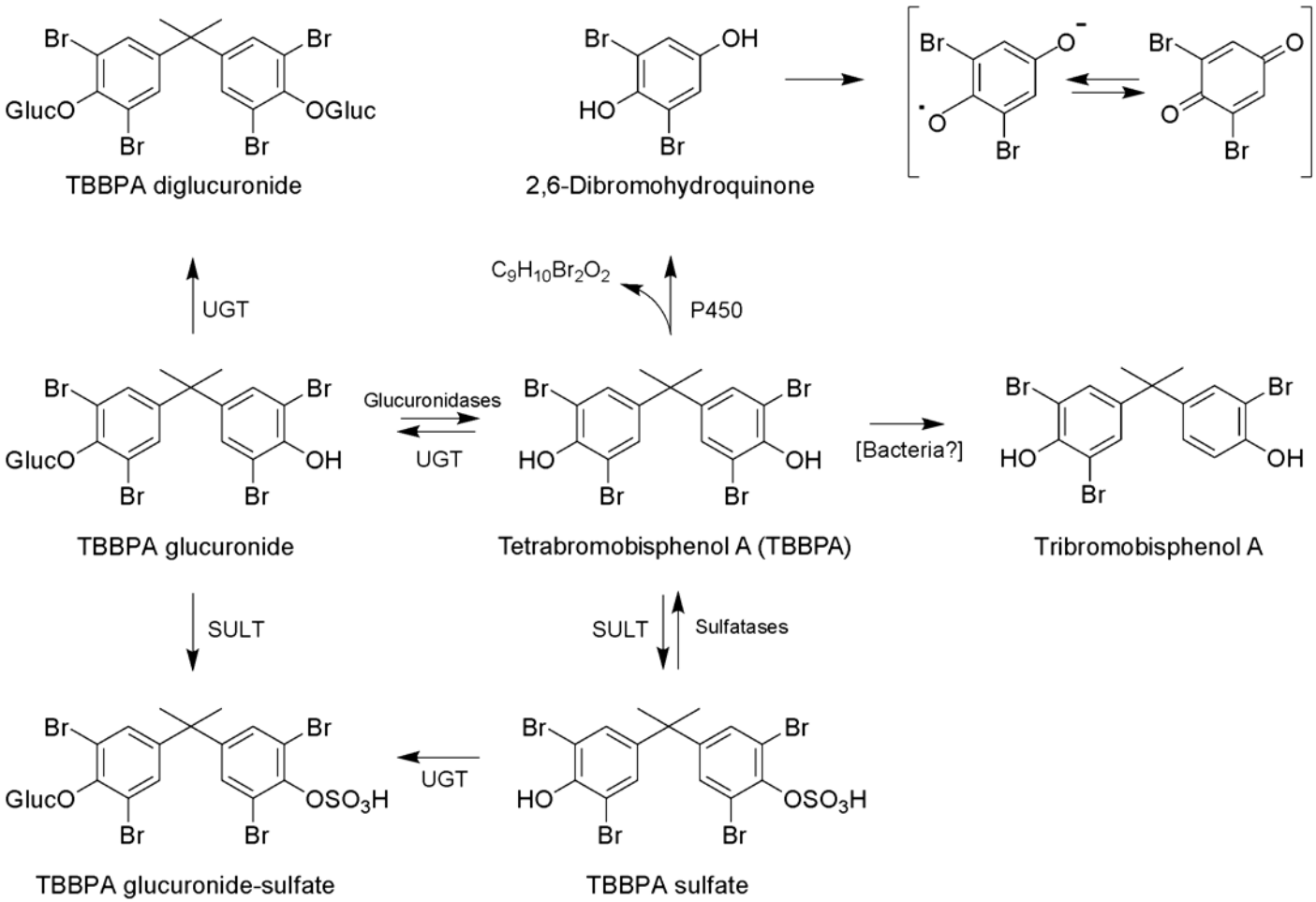

Figure 2. Metabolic Scheme of Tetrabromobisphenol A in Rats Constructed from Studies Conducted by Hakk et al., 2000; Schauer et al., 2006; Kuester et al., 2007; and Chignell et al., 2008

Gluc = C6H9O6; UGT = UDP-glucuronosyltransferases; SULT = sulfotransferases.

Toxicity

Experimental Animals

General Toxicology and Neurotoxicology

The high tetrabromobisphenol A LC_{50} and LD_{50} values for mice, rats, guinea pigs, and rabbits indicate that the acute toxicity of tetrabromobisphenol A is low[39]. For the mouse and guinea pig, LC_{50} values were greater than 500 mg/m^3 (22.5 ppm), while for the rat, the LC_{50} was greater than 10,920 mg/m^3 (490.88 ppm). Oral LD_{50} values for mice and rats were greater than 2,000 mg/kg, while an intubation LD_{50} value greater than 50,000 mg/kg (92 mmol/kg) was calculated for the rat. Dermal LD_{50} values greater than 1,000 mg/kg (2 mmol/kg) were reported for rabbits and guinea pigs. The intraperitoneal LD_{50} values were greater than or equal to 3,200 mg/kg (5.883 mmol/kg) for the mouse and rat.

No standard tetrabromobisphenol A 3-month subchronic rodent toxicity studies have been reported in the peer-reviewed scientific literature. However, the USEPA[13; 40], the World Health Organization (WHO)[3], the European Union[41], and the EFSA[21] report that they have reviewed several unpublished subchronic toxicity studies. The tetra-bromobisphenol A studies reviewed generally report a low level of acute toxicity in rodents. These unpublished toxicity studies, as reviewed by the USEPA, WHO, or the EU, are summarized below.

When groups of 25 male and 25 female Charles River CD rats were fed tetrabromobisphenol A (estimated to deliver 0, 0.05, 0.5, 5, or 50 mg tetrabromobisphenol A per kg body weight per day) for 28 days, no gross or microscopic lesions were noted[3].

In a 90-day CD rat study, there were no treatment-related deaths, clinical signs, neurobehavioral effects, or histopathologic changes after tetrabromobisphenol A was administered by oral gavage in corn oil at doses of 0, 100, 300, or 1,000 mg/kg body weight per day[13]. Total bilirubin levels were higher in 1,000 mg/kg males and in 300 and 1,000 mg/kg females than in the vehicle controls. Mean serum alkaline phosphatase activity was elevated in females at 1,000 mg/kg. Serum thyroid stimulating hormone (TSH) and triiodothyronine (T_3) levels were statistically comparable between vehicle controls and treated rats. Reduced T_4 levels were seen at all doses on days 33 and 90, but these hormone levels returned to baseline after the recovery period.

Tetrabromobisphenol A given to Sprague Dawley rat dams at concentrations of 100, 1,000 or 10,000 ppm in a soy-free diet from gestation day (GD) 10 until postnatal day (PND) 20 resulted in a slight decrease in serum T_3 concentration in pups at PND 20, but there was no evidence for developmental brain effects[42].

When tetrabromobisphenol A was administered in the diet to B6C3F1 mice [0, 500, 4,900, 15,600 or 50,000 ppm (corresponding to 0, 71, 700, 2,200, or 7,100 mg/kg body weight)] for 3 months, all animals at 50,000 ppm died, but no deaths were observed at the lower exposures[3]. Body weight gains were decreased at 15,600 and 50,000 ppm, though food intake did not change. Red blood cells, hemoglobin, hematocrit, serum triglycerides, and total serum proteins decreased at 15,600 ppm. Treatment-related organ weight changes and pathologic changes were not detected, except in the spleen, where organ weight increased and some blood was observed outside the red pulp. The no-observed-adverse-effect level was 4,900 ppm.

In a 28-day study in Wistar Han rats (tetrabromobisphenol A in feed at doses to deliver 0, 30, 100, or 300 mg/kg per day), there were dose-related decreases in plasma T_4 levels and increases in plasma T_3 levels[43]. In a related article by the same group, Germer et al.[44] reported no evidence for alterations of liver cytochrome levels in treated rats.

In a 28-day IMP:Wistar female rat tetrabromobisphenol A study (10, 50, or 250 mg tetrabromobisphenol A/kg intragastrically), there were reported increases in liver glutathione and malondialdehyde levels at 50 mg/kg, while 5-aminolevulinate synthase activity was decreased at 250 mg/kg[45]. Changes in heme synthesis were noted as measured by increases in porphyrin levels in urine after 2 weeks of dosing. Histopathologic examination of the liver showed no treatment-related changes in any of the treated groups.

Nephrotoxicity was reported to occur in newborn rats given tetrabromobisphenol A orally at 0, 40, 200, or 600 mg/kg for 18 days from PND 4 until weaning at PND 21[46]. The nephrotoxicity was seen at PND 22 in the 200 and 600 mg/kg groups and was characterized by polycystic kidney lesions. At 85 days of age, nephrotoxic lesions were still present in the 200 and 600 mg/kg groups. However, when tetra-bromobisphenol A dosing started in female rats at 5 weeks of age and continued for 18 days (0, 2,000, or 6,000 mg/kg), there was no evidence for kidney toxicity[46].

In a tetrabromobisphenol A inhalation study, five male and five female Charles River CD rats were exposed to 0, 2,000, 6,000, or 18,000 mg/m^3 for 4 hours daily, 5 days/week for 2 weeks[3]. Clinical signs included salivation, red or clear nasal discharge, and lacrimation at 6,000 or 18,000 mg/m^3. There were no treatment-related effects on mortality, body weight, feed consumption, or hematologic or clinical chemistry endpoints, and no treatment-related gross or microscopic lesions were observed.

No neurotoxicity was reported when NMRI mice were given one dose of tetrabromobisphenol A (0, 0.75, or 11.5 mg/kg body weight) on PND 10 and spontaneous motor behavior was measured 2 or 4 months after administration[47]. However, cholinergic effects were observed when tetrabromobisphenol A was administered to neonatal NMRI mice. [^{14}C]-labeled tetrabromobisphenol A was reported to accumulate in the hippocampus of NMRI mice given one oral dose at PND 10[48]. Three hours after 3-week-old male ddY mice received an oral tetrabromobisphenol A dose of 5 mg/kg, neurotoxicity responses were observed using a variety of open field test responses, and tetrabromobisphenol A was found to accumulate in the brain (striatum)[49]. Tetrabromobisphenol A exposure caused alterations in pup brain development on PND 20 (as measured by an increase in interneurons in the dentate hilus expressing reelin, suggestive of aberration of neuronal migration) when 10,000 ppm was given to the Sprague Dawley rat dam on GD 10 to 20, but there was no evidence for altered thyroid hormone levels[50].

Motor activity was measured in Sprague Dawley rat pups, and there was no effect on motor activity on PNDs 1, 21, or 60 after oral gavage administration of tetrabromobisphenol A at 0, 10, 100, or 1,000 mg/kg to dams from 10 weeks premating through gestation, lactation, and weaning of F_2 litters[51].

In vitro Studies

In vitro studies show that tetrabromobisphenol A has weak estrogenic activity and causes a modest decrease in T_4 levels.

Tetrabromobisphenol A (1 to 10 µM) caused cell proliferation of the human breast cancer estrogen-sensitive cell line, MCF-7[52; 53]. Tetrabromobisphenol A binds to the estrogen receptor (ER) but to a lower degree than bisphenol A[52]. Two metabolites of tetrabromobisphenol A [2,6-dibromo-4-(2-hydroxy-propane-2-yl) phenol and 2,6-dibromo-4-(2-methoxy-propane-2-yl) phenol], produced in fungal cultures, have been shown to also have estrogenic activity in the MCF-7 cell line[54].

Tetrabromobisphenol A (administered by intraperitoneal injection) increased uterine weight in the uterotrophic assay with ovariectomized mice[53]. Uterine weight increased by 24% after exposure to 20 mg/kg tetrabromobisphenol A; uterine weight was increased 147% by 20 mg/kg bisphenol A.

Tetrabromobisphenol A (0.016 µM) was a potent inhibitor of estradiol sulfotransferase (inhibition of sulfation may increase the bioavailability of endogenous estrogen) in Chemical Activated Luciferase gene eXpression® assays, which use reporter cell lines carrying a luciferase gene under the transcriptional control of response elements for activated receptors[55].

Tetrabromobisphenol A was an estrogen receptor (ERα) agonist and progesterone receptor (PR) antagonist in yeast strains respectively transformed with the ERα gene or the PR gene[56]. A series of phenol compounds were tested for estrogen activity in yeast strains transformed with the human ERα gene, androgen receptor (AR) gene, or the PR gene, and tetrabromobisphenol A was an ERα agonist and PR antagonist in this system. Tetrabromobisphenol A (10 µM) did not show any agonist or antagonist activity for the AR gene.

Tetrabromobisphenol A disrupted thyroid hormone activity in the rat pituitary cell line GH3[53]. Tetrabromobisphenol A (0.1 µM), was a T_4 competitor in the transthyretin-binding assay, but did not show any antiandrogenic activity[55]. Tetrabromobisphenol A was reported to bind to transthyretin[57].

Tetrabromobisphenol A did not show androgenic activity in the mouse fibroblast cell line NIH3T3[53]. Growth of the rat pituitary gland tumor cell line MtT/E-2 is estrogen dependent; tetrabromobisphenol A enhanced proliferation of cells in this cell line but to a lower extent than bisphenol A[58]. Using a digest of these cells, tetrabromobisphenol A was reported to bind to the thyroid hormone receptor, while bisphenol A did not (10 to 100 µM).

Tetrabromobisphenol A was reported to be a γ-amino-butyric acid receptor agonist and an antagonist on human excitatory α4β2 nicotinic acetylcholine (nACh) receptors expressed in *Xenopus* oocytes[59]. Tetrabromobisphenol A inhibited calcium permeable nACh receptors in neuronal B35 cells[59].

Tetrabromobisphenol A affected neurotransmitter transport in synaptosomes and calcium mobility in both granulocytes and cerebellar granule cells in vitro (rat cerebellar granule cells, rat brain synaptosomes, human neutrophil granulocytes)[60-62]. Effects in these studies were seen in doses ranging from 1 to 20 µM.

Immunotoxicity

The potential for tetrabromobisphenol A to be an immunotoxin was noted in several studies. Irregular changes in cytokine production and immune cell populations due to tetrabromobisphenol A treatment (1% in the diet for 28 days) were suggested to cause

exacerbation of pneumonia in respiratory syncytial virus-infected mice[63]. In an in vitro study in natural killer (NK) cells, tetrabromobisphenol A (5 μM) decreased the level of cell surface proteins thereby possibly interfering with NK cell function[64].

Humans

In several patch tests with human subjects, tetrabromobisphenol A was nonirritating and nonsensitizing[41]. In one in vitro study, human lymphocytes showed that tetrabromobisphenol A decreased lytic function of human NK cells (lymphocytes)[65]. However, systematic studies to identify tetrabromobisphenol A toxicity in humans have not been reported in the literature.

Reproductive and Developmental Toxicity

Experimental Animals

In a one-generation (F_1) reproduction study in Wistar rats (10 parental rats per group), tetrabromobisphenol A was administered in the diet (daily exposures estimated at 0, 3, 10, 30, 100, 300, 1,000, or 3,000 mg/kg per day)[43]. Exposure of parental rats started 10 days or 2 weeks before mating for males and females, respectively, and was continued throughout mating, gestation, and lactation. After weaning, offspring received continued exposure throughout their lives. The authors reported there were no treatment-related effects on fertility or fecundity or changes in sex ratios in F_1 litters. Individual female pups exposed to tetrabromobisphenol A showed a slight decrease in anogenital distance measured on PND 7 that was not observed on PNDs 4 or 21, and there was a delay in time to vaginal opening. There was no effect on time to balanopreputial separation. Total plasma T_4 was decreased in male and female pups and T_3 was increased in plasma (measured only in female pups). The most sensitive endpoint was in the F_1 generation as reflected by increased testicular and pituitary gland weights in males. This group also reported that hypothyroxinemia correlated to a cluster of developmental parameters in the Wistar rat including delayed sexual development in females, decreased pup mortality, and effects on brainstem auditory evoked potentials[66].

The USEPA[40] reported results of a two-generation Sprague Dawley rat study in which tetrabromobisphenol A was administered daily by oral gavage. Further details on this study were found in the European Union tetrabromobisphenol A summaries[21; 41]. Sprague Dawley rats were exposed to 10, 100, or 1,000 mg tetrabromobisphenol A/kg body weight per day by gavage in the F_0 generation during 10 weeks premating and during a 2-week mating period. Females were treated also during gestation and lactation. The same treatment regime as in F_0 animals was also applied in F_1 animals. The F_0 generation was sacrificed after the pups were weaned, and decreases in T_4 levels were found at the high dose in males and females. In the F_1 generation, lower serum T_4 concentrations were observed in both sexes at 100 and 1,000 mg/kg. T_3 serum levels were significantly lower only in F_0 males of the 1,000 mg/kg group. No changes in serum TSH levels, compared to vehicle control animals, were observed in any of the treated groups. No treatment-related histopathologic changes were observed. Fertility and fecundity were not affected.

In a study in ICR mice where tetrabromobisphenol A was administered in the diet (0%, 0.01%, 0.1%, or 1%) to dams from GD 0 to weaning at PND 27, there were no exposure-related effects on litters[67]. Total serum cholesterol levels and liver weights of treated dams and offspring were

higher than those of the control mice. Histologic findings in treated dams or off-spring showed increases in focal necrosis of hepatocytes and inflammatory cell infiltration in the liver, and increases in dilation or atrophy of renal tubules and cysts in the kidney.

In a study in CD1 outbred mice, tetrabromobisphenol A was administered in drinking water to deliver an estimated dose of 1 µg tetrabromobisphenol A per day (35 µg/kg per day)[68]. Various exposure groups were included in the study including one in which dams received tetrabromobisphenol A during gestation and lactation and pups were exposed during the prepubertal and pubertal periods and up to adulthood. An increased incidence of apoptosis in the testes and decreased thickness of the seminiferous tubule epithelium were noted.

No studies were found in the literature that evaluated the prenatal toxicity potential of tetrabromobisphenol A in rodents, lagomorphs, or nonhuman primates.

In studies in fish (flounder), tetrabromobisphenol A (greater than or equal to 0.047 M) exposure caused reductions in egg production, survival, and overall reproductive success[69]. The estrogenic effects in adult fish affect pathways critical for coordinated signaling in gonadal development and normal reproduction[70]. Disruption of the hypothalamic-pituitary-thyroid axis has also been demonstrated in fish[71]. Specific molecular targets such as hormone receptors and markers for oxidative stress have been found in fish after tetrabromobisphenol A exposure[72]. Embryonic exposure to tetrabromobisphenol A resulted in truncated bodies and tails in developing zebrafish suggesting an impairment in the remodeling of tissues in the caudal region of the embryo[20].

Exposure of *Xenopus tropicalis* embryos (NF10) to 0.01, 0.1, or 1 mg/L tetrabromobisphenol A with or without 70 µg/L T_3 affected development[73]. Compared to the controls, 1 mg/L tetrabromobisphenol A significantly reduced the body length of embryos after 24, 36, and 48 hours of exposure. Treated embryos showed multiple malformations, including abnormal eyes, skin hypopigmentation, enlarged proctodaeum, narrow fins, and pericardial edemas.

Humans

No studies were found in the literature that evaluated the reproductive or developmental toxicity potential of tetrabromobisphenol A in humans. The detection of tetrabromobisphenol A in cord serum collected from women during caesarian deliveries confirms that transplacental transfer occurs in humans[74-76].

Carcinogenicity

No studies that evaluated the carcinogenic potential of tetrabromobisphenol A in rodent models or epidemiology studies examining potential carcinogenic effects of tetrabromobisphenol A in humans were found in the literature.

Genetic Toxicity

Tetrabromobisphenol A (up to 10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in tests conducted with and without exogenous metabolic activation[77]. In a report commissioned by the European Union[41] to review the available genetic toxicity data for tetrabromobisphenol A, negative results were reported in several well-conducted bacterial and yeast mutagenicity tests, and in an in vitro chromosomal

aberration assay in human lymphocytes. All of these assays were conducted with and without metabolic activation. There are no in vivo genotoxicity data available for tetrabromobisphenol A.

Study Rationale

Tetrabromobisphenol A was nominated by the NIEHS for toxicity and carcinogenicity studies based on its high production volume, the potential for widespread human exposures, and the absence of standard toxicity and carcinogenicity studies reported in the scientific literature.

Materials and Methods

Procurement and Characterization

Tetrabromobisphenol A

Tetrabromobisphenol A was obtained from Albemarle Corporation (Baton Rouge, LA) in three lots (25317K-1, C16263X, and 25337XX-8). Lot 25317K-1 was used during the 3-month studies; lots 25317K-1 and C16263X were combined into one lot and renamed lot M032607KA, which was used in the 2-year studies; lot 25337XX-8 was used for dose formulation studies performed at the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO) and was not used in any of the animal studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, and identity was confirmed by the study laboratory at Battelle Columbus Operations (Battelle) (Columbus, OH) (Appendix J). Reports on analyses performed in support of the tetrabromo-bisphenol A studies are on file at the National Institute of Environmental Health Sciences.

Lots 25317K-1 and M032607KA of the test chemical, a white, crystalline powder, were identified as tetra-bromobisphenol A by infrared and proton nuclear magnetic resonance spectroscopy and melting point. Purity of each lot was determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The purity profile for lot 25317K-1 had one major peak and one impurity at two detection wavelengths with areas of 0.7% and 0.8% relative to the total peak area. The overall purity of lot 25317K-1 was determined to be greater than 99%. For lot M032607KA, the analysis indicated one major peak and one impurity at two detection wavelengths with areas of 0.8% and 1.1% relative to the total peak area. The impurity was determined to be tribromobisphenol A by liquid chromatography/mass spectrometry, based on the isotopic pattern in the mass spectrum indicating the presence of three bromine atoms and the m/z of 460.9 ($[M-H]^-$), consistent with a mass of 461.8. The overall purity of lot M032607KA was determined to be approximately 99%.

To ensure stability, the bulk chemical was stored in sealed glass bottles protected from light at room temperature. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month and 2-year studies using HPLC/UV. No degradation of the test chemical was detected.

Corn Oil

National Formulary-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and from Sigma-Aldrich (St. Louis, MO) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than the rejection level of 3 mEq/kg.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared monthly for the 3-month studies and every 6 weeks for the 2-year studies by mixing tetrabromobisphenol A with corn oil. Homogeneity studies of 0.5 and 600 mg/mL formulations and stability studies of a 0.5 mg/mL formulation were performed by

the analytical chemistry laboratory using HPLC/UV. Homogeneity was confirmed; stability was confirmed for at least 42 days for dose formulations stored in sealed glass vials, protected from light, at temperatures up to 25°C, and for at least 3 hours under simulated animal room conditions. The dose formulations were stored in sealed glass bottles protected from light for up to 42 days at room temperature. The study laboratory conducted homogeneity studies of 1, 2, 10, 25, 50, 100, 200, and 400 mg/mL formulations using HPLC/UV; gavageability studies of 100, 200, and 400 mg/mL formulations were also performed. Homogeneity was confirmed, and gavageability was confirmed for the 100 and 200 mg/mL formulations.

Periodic analyses of the dose formulations of tetra-bromobisphenol A were conducted by the study laboratory using HPLC/UV. During the 3-month studies, the dose formulations were analyzed monthly; all 15 of the dose formulations for rats and all 15 for mice were within 10% of the target concentrations (Table J-2). Animal room samples of these dose formulations were also analyzed; all 15 for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months (Table J-3); of the dose formulations analyzed and used during the studies, all 72 for rats and all 45 for mice were within 10% of the target concentrations. Animal room samples were also analyzed; seven of nine animal room samples for rats and eight of nine for mice were within 10% of the target concentrations.

Animal Source

Male and female F344/NTac rats were obtained from the commercial colony at Taconic Farms, Inc. (Germantown, NY). B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc., for the 3-month studies. Male and female Wistar Han [Crl:WI(Han)] rats were obtained from Charles River Laboratories (Raleigh, NC), and male and female B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc., for use in the 2-year studies. The rationale for change of rat strain from F344/N to F344/NTac was a programmatic decision. For many years NTP used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over a period of time, the F344/N strain exhibited sporadic seizures and idiopathic chylothorax and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative purposes, a change in the rat model was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies to allow NTP time to evaluate different rat models between 2005 and 2006[78]. The Wistar Han rat, an outbred rat stock, was then selected because it was projected to have a long lifespan, resistance to disease, large litter size, and low neonatal mortality.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetrabromobisphenol A and to determine the appropriate doses to be used in the 2-year studies. Tetrabromobisphenol A was given orally to mimic an oral exposure. The doses selected for the tetrabromobisphenol A 3-month studies were based on findings reported by the World Health Organization (WHO)[3] and NTP chemistry findings. In the WHO report, in a Charles River CD rat subchronic study, tetrabromobisphenol A was administered by oral gavage in corn oil to deliver doses of 0, 100, 300, or 1,000 mg/kg body weight per day. There were no treatment-related deaths, clinical findings, neurobehavioral effects, or histopathologic changes. In another study in the WHO report, tetrabromobisphenol A was given in the diet to B6C3F1 mice at 0, 500, 4,900, 15,600, or 50,000 ppm (corresponding to 0, 71, 700, 2,200, or 7,100 mg/kg body weight for 3 months). All animals fed 50,000 ppm died during the study, probably because of malnutrition and anemia. NTP found that the maximum amount of tetrabromobisphenol A that could be constituted for oral gavage was 1,000 mg/kg, and thus, this was the high dose selected for the 3-month rat and mouse studies.

On receipt, the rats were 3 to 4 weeks old, and mice were 4 to 5 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and mice for 13 (females) or 14 (males) days; rats were 5 to 6 weeks old and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female rats and mice were administered tetrabromobisphenol A in corn oil by gavage at doses of 0, 10, 50, 100, 500, or 1,000 mg/kg body weight, 5 days per week for 14 weeks. Additional special study groups of 10 male and 10 female rats were administered the same doses for 23 days. Vehicle control animals received the corn oil vehicle alone. Dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded and animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Hematology, clinical chemistry, and thyroid hormone analyses were performed on special study rats on days 4 (except hematology) and 23 and on core study rats at study termination. Hematology analyses were performed on mice at study termination. Blood was collected from the retroorbital plexus of rats and mice for hematology analyses and from the retroorbital plexus of special study rats and from the heart of core study rats for clinical chemistry and thyroid hormone analyses. Samples were collected into tubes containing EDTA for hematology or serum separator tubes for clinical chemistry and thyroid hormone determinations. Hematology parameters were determined using an Advia 120 analyzer (Bayer Diagnostic Division, Tarrytown, NY). Clinical chemistry parameters and total thyroxine were determined using a Hitachi 911 analyzer (Roche Diagnostics Corporation, Indianapolis, IN). Total triiodothyronine and thyroid stimulating hormone were determined by radioimmunoassay using a commercial kit. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice administered 0, 100, 500, or 1,000 mg/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Liver samples were collected from special study rats on day 23 and from core study rats and mice at the end of the studies for cytochrome P450 and uridine diphosphate-glucuronosyl transferase (UDP-GT) activity determinations. Microsomal suspensions were prepared as described by Battelle[79; 80]. The concentration of protein in each suspension was determined using a BCA Protein Assay Kit (Pierce Chemical Co., Rockford, IL). 7-Ethoxyresorufin-*O*-deethylase (CYP1A1) and 7-pentoxyresorufin-*O*-dealkylase (CYP2B) activities were determined spectrofluorimetrically[81], acetanilide-4-hydroxylase (CYP1A2) activity was determined by HPLC with ultraviolet detection[82-84], and UDP-GT activity toward T_4 was determined by quantifying the amount of ^{125}I-T_4-glucuronide produced[85].

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on all vehicle control and 1,000 mg/kg rats and mice. The liver was examined in all groups of rats and mice, and the kidney was examined in all groups of mice. In the original review of the uterus, a transverse section through each uterine horn, approximately 0.5 cm cranial to the cervix, was collected for histopathology review. For the residual tissue review, all remaining cervical, vaginal, and uterine tissue remnants were stored in 10% neutral buffered formalin, processed, and sectioned longitudinally. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved

by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[86] and Boorman et al.[87].

Two-year Studies

Study Design

Groups of 60 male and 60 female Wistar Han rats were administered 0 or 1,000 mg tetrabromobisphenol A/kg body weight, 50 male and 50 female rats were administered 250 or 500 mg/kg, and 50 male and 50 female mice were administered 0, 250, 500, or 1,000 mg/kg in corn oil by gavage, 5 days per week for up to 104 (male rats) or 105 weeks. Ten vehicle control and ten 1,000 mg/kg rats of each sex were evaluated at 3 months to allow comparison to 3-month endpoints in the F344/NTac rats. Vehicle control animals received corn oil only. Dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice.

Rats were quarantined for 8 or 9 days and mice were quarantined for 11 or 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 6 to 7 weeks old and mice were 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Rats were housed three (males) or five (females) per cage and mice were housed one (males) or five (females) per cage. Feed and water were available ad libitum. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks beginning week 5 and at the end of the studies. Body weights were recorded on day 1, weekly for 13 weeks, monthly thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At the 3-month interim evaluation in rats, the heart, right kidney, liver, lung, right testis, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes were initially placed in Davidson’s solution and testes were initially placed in modified Davidson’s solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Original transverse and residual longitudinal reviews of uterine tissue from female Wistar Han rats, including the 3-month interim evaluation animals, were conducted as described for the 3-month study in F344/NTac rats. In addition, cytokeratin and vimentin immunohistochemical stains were used to better characterize specific lesions that occurred in the uterus. Tissues examined microscopically are listed in Table 1. For the 2-year studies, samples of grossly observed tumors (uterine adenocarcinomas) were collected at the time of necropsy, flash frozen in liquid nitrogen, and stored at −80°C for molecular analysis (Appendix M).

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver and uterus of rats and mice; the nose of rats; and the forestomach, large intestine, and kidney of mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[86] and Boorman et al.[87]. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al.[88].

Table 1. Experimental Design and Materials and Methods in the Gavage Studies of Tetrabromobisphenol A

Three-month Studies	Two-year Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/NTac rats B6C3F1/N mice	Wistar Han rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 8 (males) or 9 (females) days Mice: 11 (females) or 12 (males) days
Average Age When Studies Began	
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 6 to 7 weeks Mice: 5 to 6 weeks
Date of First Dose	
Rats: December 12 (males) or 13 (females), 2005 Mice: December 14 (females) or 15 (males), 2005	Rats: July 25 (males) or 26 (females), 2007 Mice: August 6 (females) or 7 (males), 2007
Duration of Dosing	
Core studies: 14 weeks Special study rats: 23 days	3 months (interim evaluation rats), 104 weeks (male rats), or 105 weeks
Date of Last Dose	
Rats: March 13 (males) or 14 (females), 2006 Mice: March 15 (females) or 16 (males), 2006	Rats: July 21 (males) or 23 (females), 2009 Mice: August 4 (females) or 6 (males), 2009
Necropsy Dates	
Rats: March 14 (males) or 15 (females), 2006 Mice: March 16 (females) or 17 (males), 2006	Rats: October 24 (males) or 25 (females), 2007 (interim evaluation), or July 20-22 (males) or 22-24 (females), 2009 (2-year study) Mice: August 3-5 (females) or 5-7 (males), 2009
Average Age at Necropsy	
Rats: 18 to 19 weeks Mice: 19 to 20 weeks	109 or 111 weeks
Size of Study Groups	
10 males and 10 females	Rats: 0 and 1,000 mg/kg, 60 males and 60 females; 250 and 500 mg/kg, 50 males and 50 females Mice: 50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies

Three-month Studies	Two-year Studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed at least weekly	Same as 3-month studies
Water	
Tap water (Columbus municipal supply) via automatic rack watering system (Edstrom Industries, Waterford, WI), available ad libitum	Same as 3-month studies
Cages	
Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly (male mice) or twice weekly (rats and female mice) and rotated every 2 weeks	Same as 3-month studies
Bedding	
Irradiated Sani-Chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (male mice) or twice weekly (rats and female mice)	Same as 3-month studies
Rack Filters	
Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment	
Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses	
0, 10, 50, 100, 500, or 1,000 mg/kg in corn oil; dosing volumes 5 mL/kg (rats) or 10 mL/kg (mice)	0, 250, 500, or 1,000 mg/kg in corn oil; dosing volumes 5 mL/kg (rats) or 10 mL/kg (mice)
Type and Frequency of Observation	
Observed twice daily; clinical findings were recorded and core study animals were weighed initially, weekly, and at the end of the studies.	Observed twice daily; clinical findings were recorded every 4 weeks beginning at week 5 and at the end of the studies; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies.

Three-month Studies	Two-year Studies
Method of Kill	
Rats: Exsanguination while under CO2/O2 anesthesia (core study) or carbon dioxide asphyxiation (special study group) Mice: Carbon dioxide asphyxiation	Carbon dioxide asphyxiation
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed at the 3-month interim evaluation in rats were the heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology	
Blood was collected from the retroorbital plexus of special study rats on days 4 and 23 and of core study rats and mice at the end of the studies; blood was also collected from the heart of core study rats at the end of the study. Hematology parameters were measured on day 23 (rats) and at the end of the studies (rats and mice). Clinical chemistry and thyroid hormones were measured in rats on days 4 and 23 and at the end of the study. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids. ***Thyroid hormones:*** total triiodothyronine, thyroid stimulating hormone, and total thyroxine.	None
Liver Toxicity	
Liver samples were collected from special study rats on day 23 and from core study rats and mice at study termination for determination of acetanilide-4-hydroxylase, 7-ethoxyresorufin-O-deethylase, 7-pentoxyresorufin-O-dealkylase, and uridine diphosphate-glucuronosyl transferase activities.	None
Histopathology	

Three-month Studies	Two-year Studies
Complete histopathology was performed on vehicle control and 1,000 mg/kg core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The liver of rats and mice and the kidney of mice were also examined in the remaining core study groups.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, cervix, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.
Sperm Motility and Vaginal Cytology	
At the end of the studies, spermatid and sperm samples were collected from male rats and mice in the vehicle control, 100, 500, and 1,000 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats and mice in the vehicle control, 100, 500, and 1,000 mg/kg groups.	None

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[89] and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's[90] method for testing two groups for equality and Tarone's[91] life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or non-neoplastic lesions are presented in Table A-1, Table A-4, Table B-1, Table B-4, Table C-1, Table C-4, Table D-1, and Table D-3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Table A-2, Table B-2, Table C-2, and Table D-2) and all non-neoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Table A-2, Table B-2, Table C-2, and Table D-2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Non-neoplastic Lesion Incidences

The Poly-k test[92-94] was used to assess neoplasm and non-neoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time[92]. Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier[92] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344/N rats and B6C3F1/N mice[95]. Bailer and Portier[92] showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does

not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams[96].

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N). For neoplasms and non-neoplastic lesions detected at the 3-month interim evaluation, the Fisher exact test[97], a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[98] and Williams[99; 100]. Hematology, clinical chemistry, thyroid hormone, cytochrome P450, UDP-GT, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[101] (as modified by Williams[102]) and Dunn[103]. Jonckheere's test[104] was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey[105] were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test[97]. Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager[106]. For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[107-109]. In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current mouse study. The

current 2-year rat study is the only one in Wistar Han rats using corn oil as a gavage vehicle in the historical control database; therefore, only historical control incidences for all routes and all vehicles are used for Wistar Han rats in this Technical Report. The historical control database does not contain data for residual tissue evaluations or step sections of tissues.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[110]. In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of tetrabromobisphenol A was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division[111; 112]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity[113] and the somatic mutation theory of cancer[114; 115]. However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites[116]. A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens)[117; 118]. Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test[119; 120]. However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative

results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies[121]. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR-587.

Three-month Study in F344/NTac Rats

All core study rats survived to the end of the study (Table 2). The final mean body weights and mean body weight gains of dosed groups of males and females were similar to those of the vehicle control groups (Table 2 and Figure 3). No clinical findings related to tetrabromobisphenol A administration were observed.

Table 2. Survival and Body Weights of F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	86 ± 3	345 ± 5	259 ± 5	
10	10/10	85 ± 3	354 ± 6	269 ± 5	103
50	10/10	85 ± 3	350 ± 7	265 ± 8	101
100	10/10	85 ± 3	352 ± 7	267 ± 5	102
500	10/10	85 ± 3	339 ± 5	254 ± 3	98
1,000	10/10	84 ± 2	337 ± 6	253 ± 6	98
Female					
0	10/10	81 ± 2	185 ± 2	104 ± 2	
10	10/10	81 ± 2	189 ± 4	108 ± 3	102
50	10/10	81 ± 2	191 ± 2	110 ± 3	103
100	10/10	82 ± 3	186 ± 5	104 ± 4	100
500	10/10	82 ± 2	189 ± 4	107 ± 4	102
1,000	10/10	82 ± 2	187 ± 3	106 ± 2	101

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at 14 weeks/number initially in group.

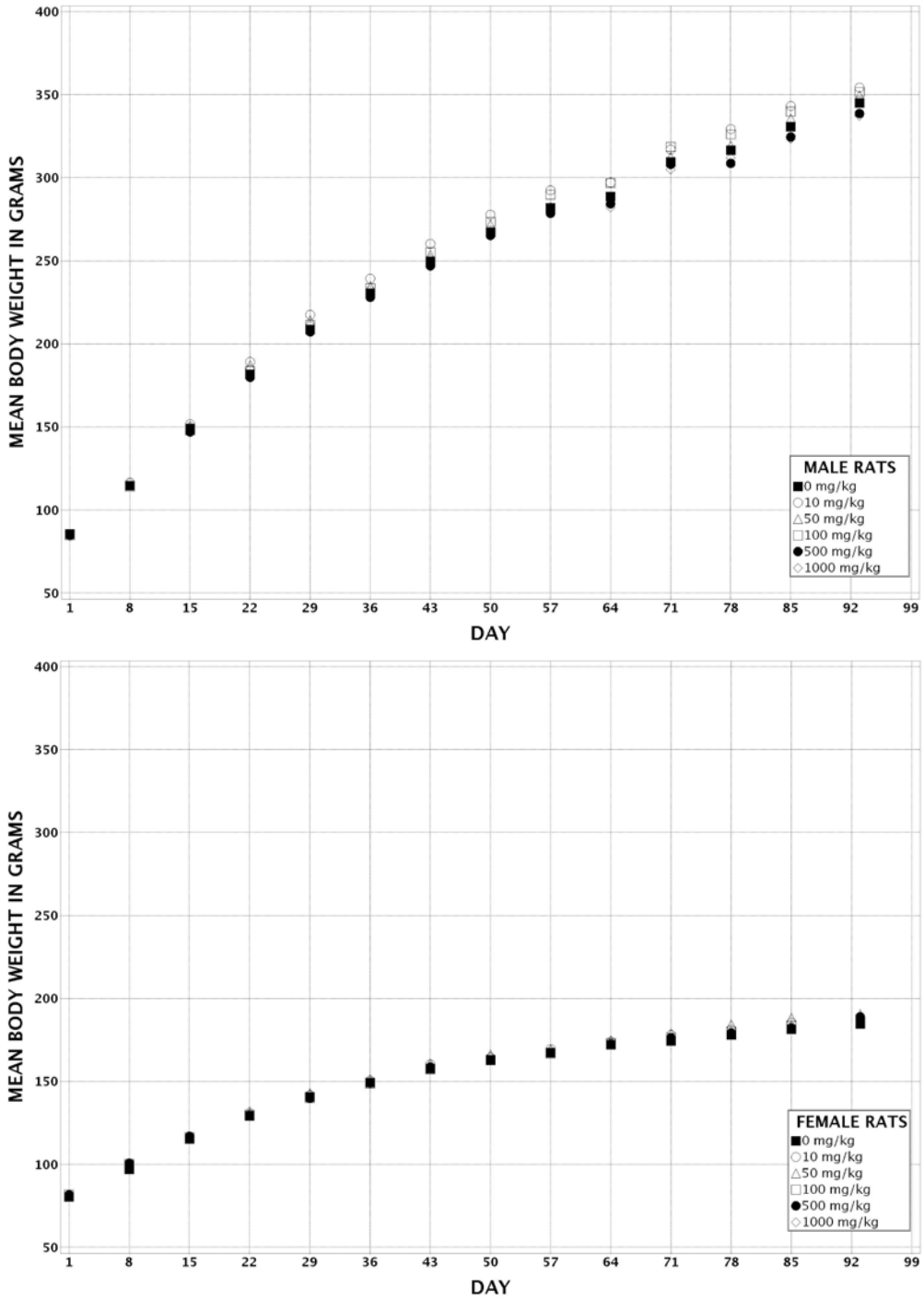

Figure 3. Growth Curves for F344/NTac Rats Administered Tetrabromobisphenol A by Gavage for Three Months

Assays for total thyroxine (T_4) and thyroid stimulating hormone (TSH) were conducted on days 4 and 23 and at week 14; for total triiodothyronine (T_3), assays were conducted on day 23 and at week 14 (Table F-1). Consistent, progressive, and dose-related decreases in total T_4 concentrations occurred in 500 and 1,000 mg/kg males and females; this effect was observed with less consistency in the 100 mg/kg groups. On day 4, T_4 was decreased by approximately 30% in the 1,000 mg/kg animals; by week 14, it was decreased by approximately 45%. The decreases in T_4 were not accompanied by decreases in T_3 concentrations or increases in TSH concentrations.

On day 23 and at week 14, the hematology findings suggested small (≤10%) decreases in the estimators of the circulating red cell mass in 500 and 1,000 mg/kg males and females (Table F-1). The erythron decrease was evidenced by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. The greatest magnitude of the decreases, approximately 10%, occurred in 1,000 mg/kg males on day 23. By week 14, there was some amelioration in the severity of the erythron decrease (≤5%) in the 500 and 1,000 mg/kg groups. The erythrocytes were of normal size and hemoglobin content and no changes in reticulocyte counts were observed.

Serum concentrations of total bile acids, a marker of hepatic function/injury and cholestasis, demonstrated transient increases (twofold or greater) in 500 and 1,000 mg/kg males and females on day 4; the effect had essentially resolved by day 23 (Table F-1). However, another marker of cholestasis, alkaline phosphatase, demonstrated little to no change on day 4. Thus, it would appear the transient increases in bile acid concentrations were probably not related to a cholestatic event, but rather a transient effect on hepatic function involving bile acid metabolism. At week 14, markers of hepatocellular injury, serum activities of alanine aminotransferase and sorbitol dehydrogenase, generally demonstrated decreases in males and females administered 100 mg/kg or greater.

Decreases in cytochrome P450 enzyme and UDP-glucuronosyl transferase activities were seen on day 23 and at week 14 in dosed groups of males and females (Table G-1); however no liver enzyme changes were considered to be biologically significant with the exception of 4- to 23-fold increases over the vehicle control value in 7-pentoxyresorufin-*O*-dealkylase (PROD) activities in 500 and 1,000 mg/kg males and females at week 14. The increased levels indicated some disturbance of liver function, but this was not accompanied by treatment-related liver lesions.

There were significant increases in the absolute and relative liver weights of 500 and 1,000 mg/kg males and females (Table H-1). Significant decreases occurred in the absolute and relative spleen weights of 500 and 1,000 mg/kg males and the absolute thymus weight of 1,000 mg/kg males.

There were no significant differences between the reproductive organ weights or sperm parameters of dosed and vehicle control groups of male rats (Table I-1). Dosed females exhibited a slight but significant increase in time in extended estrus compared to females in the vehicle control group (Table I-2 and Table I-3). This effect was minimal and manifested as a slight increase in the frequency of rats exhibiting 2 sequential days of estrus (compared to proestrus followed by estrus) (Figure I-1). Nevertheless, the rats were exhibiting normal duration cycles.

Therefore, tetrabromobisphenol A was not considered to exhibit the potential to be a reproductive toxicant in male or female F344/NTac rats under the conditions of these studies.

No treatment-related histopathologic lesions were observed in F344/NTac rats in the 3-month study.

Dose Selection Rationale: The 3-month F344/NTac rat results were used to set doses for the 2-year study. There were no chemical-related effects on mortality, body weights, or lesion incidences in the 3-month study in F344/NTac rats. Chemical-related effects on organ weights, thyroid hormones, hematology parameters, and liver enzymes were not considered severe enough to compromise survival in the 2-year study. Therefore, doses selected for the 2-year gavage study in Wistar Han rats were 250, 500, and 1,000 mg/kg. The highest dose that could be administered by gavage was 1,000 mg/kg. Because NTP switched the laboratory rat strain from the F344/NTac rat to the Wistar Han rat after the tetrabromobisphenol A 3-month studies were conducted, a 3-month interim evaluation was added to the 2-year Wistar Han rat study.

Two-year Study in Wistar Han Rats

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 4). Survival of dosed groups was similar to that of the vehicle control groups.

Organ Weights

At the 3-month interim evaluation, the absolute and relative thymus weights of 1,000 mg/kg rats were significantly less than those of the vehicle control groups and the relative liver weights of these dosed groups were significantly greater than those of the vehicle controls (Table H-2).

Body Weights and Clinical Findings

The mean body weights of 500 and 1,000 mg/kg males were generally at least 10% less than those of the vehicle control group after week 25; body weights of dosed groups of female rats were similar to those of the vehicle controls throughout the study (Table 4 and Table 5; Figure 5). There were no clinical findings related to tetrabromobisphenol A administration.

Table 3. Survival of Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Male				
Animals initially in study	60	50	50	60
Three-month interim evaluation[a]	10	0	0	10
Accidental deaths[a]	1	0	0	3
Moribund	14	18	8	6
Natural deaths	2	4	4	2
Animals surviving to study termination	33[e]	28	38	39
Percent probability of survival at end of study[b]	67	56	76	83
Mean survival (days)[c]	642	669	697	688
Survival analysis[d]	P = 0.021N	P = 0.496	P = 0.357N	P = 0.096N
Female				
Animals initially in study	60	50	50	60
Three-month interim evaluation[a]	10	0	0	10
Accidental deaths[a]	3	0	0	4
Moribund	8	14	15	10
Natural deaths	4	2	6	3
Animals surviving to study termination	35[e]	34	29	33[e]
Percent probability of survival at end of study	72	68	58	72
Mean survival (days)	678	685	645	672
Survival analysis	P = 0.943	P = 0.732	P = 0.111	P = 1.000

[a]Censored from survival analyses.
[b]Kaplan-Meier determinations.
[c]Mean of all deaths (uncensored, censored, and terminal kill); does not include interim evaluation animals.
[d]The result of the life table trend test[91] is in the vehicle control column, and the results of the life table pairwise comparisons[90] with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.
[e]Includes one animal that died during the last week of the study.

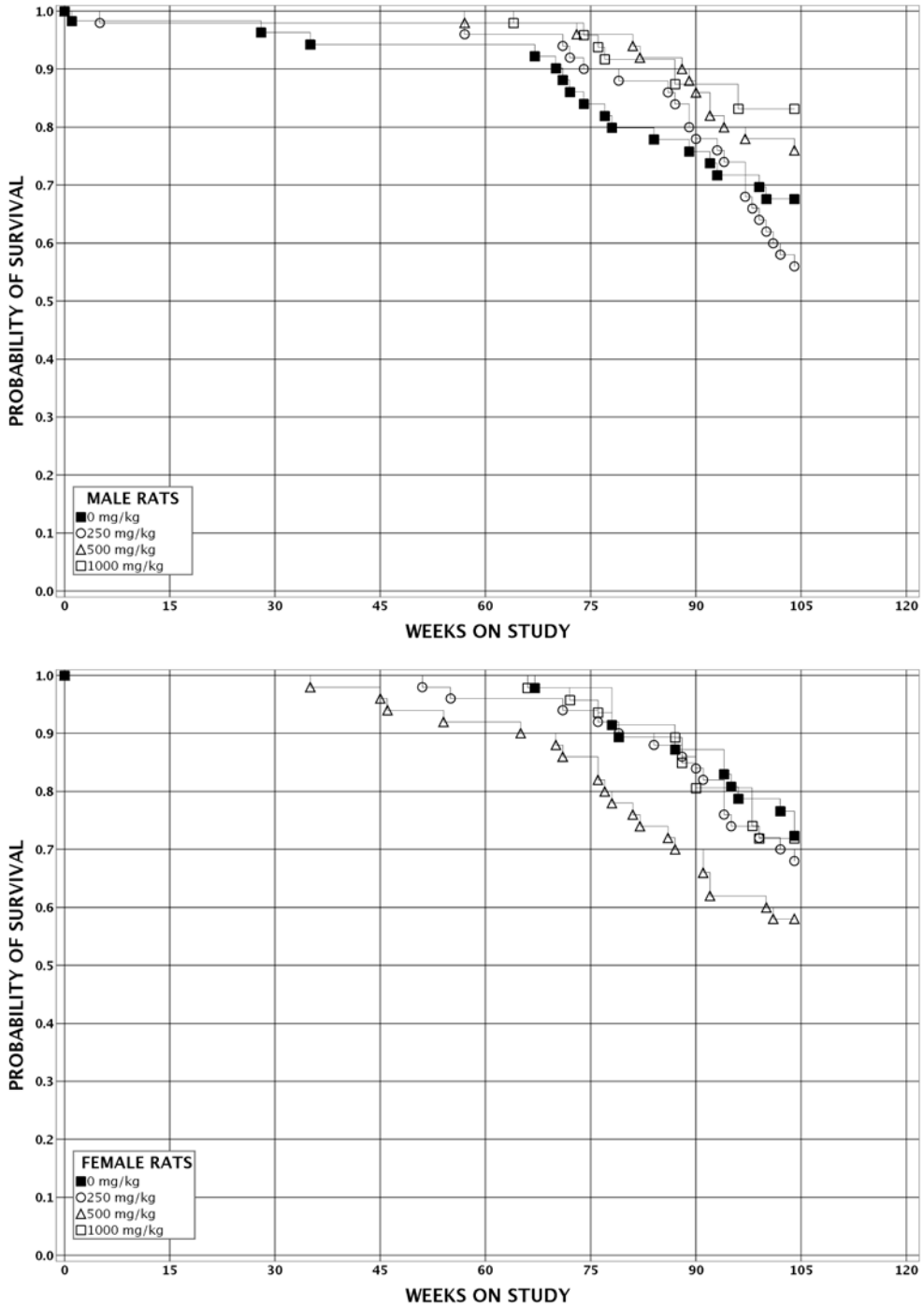

Figure 4. Kaplan-Meier Survival Curves for Wistar Han Rats Administered Tetrabromobisphenol A by Gavage for Two Years

Table 4. Mean Body Weights and Survival of Male Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A

Day	Vehicle Control		250 mg/kg			500 mg/kg			1,000 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	162	60	161	99	50	161	99	50	161	99	60
8	207	59	207	100	50	205	99	50	206	99	60
15	246	59	248	101	50	242	99	50	245	100	60
22	275	59	273	99	50	265	97	50	268	97	60
29	299	59	296	99	50	290	97	50	291	98	60
36	321	59	317	99	49	309	96	50	312	97	60
43	337	59	333	99	49	327	97	50	330	98	60
50	350	59	349	100	49	341	98	50	345	99	60
57	361	59	365	101	49	357	99	50	359	99	60
64	374	59	374	100	49	364	97	50	366	98	60
71	384	59	382	100	49	374	98	50	376	98	60
78	393	59	394	100	49	384	98	50	386	98	60
85	404	59	409	101	49	397	98	50	397	98	60
113	436	49[a]	421	97	49	404	93	50	401	92	50[a]
141	461	49	440	96	49	420	91	50	421	91	50
169	474	49	455	96	49	432	91	50	431	91	50
197	491	48	470	96	49	444	90	50	444	90	50
225	504	48	482	96	49	455	90	50	455	90	50
253	519	46	493	95	49	465	90	50	461	89	50
281	532	46	504	95	49	476	89	50	468	88	50
309	547	46	518	95	49	487	89	50	481	88	50
337	560	46	528	94	49	497	89	50	489	87	50
365	566	46	534	94	49	504	89	50	493	87	50
393	579	46	549	95	49	511	88	49	498	86	49
421	593	46	564	95	48	526	89	49	517	87	49
449	605	46	579	96	48	545	90	49	533	88	48
477	612	45	590	97	48	556	91	49	540	88	48
505	620	42	599	97	46	562	91	49	546	88	47
533	630	40	604	96	45	569	90	48	552	88	45
561	642	39	615	96	44	579	90	48	566	88	44
589	642	38	615	96	44	577	90	46	566	88	44
617	644	38	609	94	42	586	91	45	576	89	41
645	640	36	613	96	38	590	92	41	574	90	41
673	656	35	606	92	36	597	91	39	588	90	39
701	663	33	615	93	30	599	90	39	584	88	39
Mean for Weeks											
1–13	316	–	316	100	–	309	98	–	311	98	–
14–52	503	–	479	95	–	453	90	–	450	89	–
53–101	623	–	592	95	–	562	90	–	549	88	–

[a]Interim evaluation occurred during week 13.

Table 5. Mean Body Weights and Survival of Female Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A

Day	Vehicle Control		250 mg/kg			500 mg/kg			1,000 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	136	60	137	101	50	136	100	50	135	100	60
8	157	60	157	100	50	157	100	50	156	99	60
15	172	60	173	101	50	176	102	50	175	102	60
22	185	60	184	100	50	184	100	50	183	99	60
29	195	60	196	101	50	194	100	50	194	99	60
36	204	60	203	100	50	202	99	50	200	99	60
43	209	60	209	100	50	209	100	50	207	99	60
50	215	60	216	100	50	218	101	50	216	100	60
57	218	60	221	101	50	224	102	50	223	102	60
64	224	60	225	100	50	228	102	50	226	101	60
71	227	60	226	100	50	231	102	50	229	101	60
78	229	60	232	101	50	232	101	50	230	101	60
85	233	60	239	102	50	240	103	50	239	103	60
113	240	50[a]	245	102	50	245	102	50	243	101	50[a]
141	249	50	254	102	50	251	101	50	247	99	50
169	254	50	258	102	50	256	101	50	252	99	50
197	258	50	264	102	50	260	101	50	256	99	49
225	263	50	269	102	50	263	100	50	259	98	49
253	268	50	274	102	50	268	100	49	262	98	49
281	276	50	280	102	50	271	99	49	267	97	49
309	283	50	287	102	50	279	99	49	272	96	49
337	292	50	294	101	50	283	97	47	277	95	49
365	297	47	299	101	49	288	97	47	284	96	49
393	301	47	308	102	48	294	98	46	289	96	49
421	309	47	315	102	48	301	97	46	297	96	49
449	317	47	327	103	48	311	98	46	306	97	47
477	326	46	335	103	48	315	97	45	312	96	46
505	334	46	344	103	47	326	97	43	320	96	45
533	339	46	353	104	46	329	97	40	329	97	44
561	347	42	365	105	45	339	98	38	344	99	43
589	352	42	372	106	44	344	98	37	351	100	43
617	361	41	378	105	43	352	98	35	357	99	39
645	361	41	382	106	41	355	98	31	364	101	37
673	370	37	392	106	37	363	98	31	374	101	37
701	375	37	403	107	36	368	98	30	375	100	33
Mean for Weeks											
1–13	200	–	201	101	–	202	101	–	201	101	–
14–52	265	–	269	102	–	264	100	–	259	98	–
53–101	338	–	352	104	–	330	98	–	331	98	–

[a]Interim evaluation occurred during week 13.

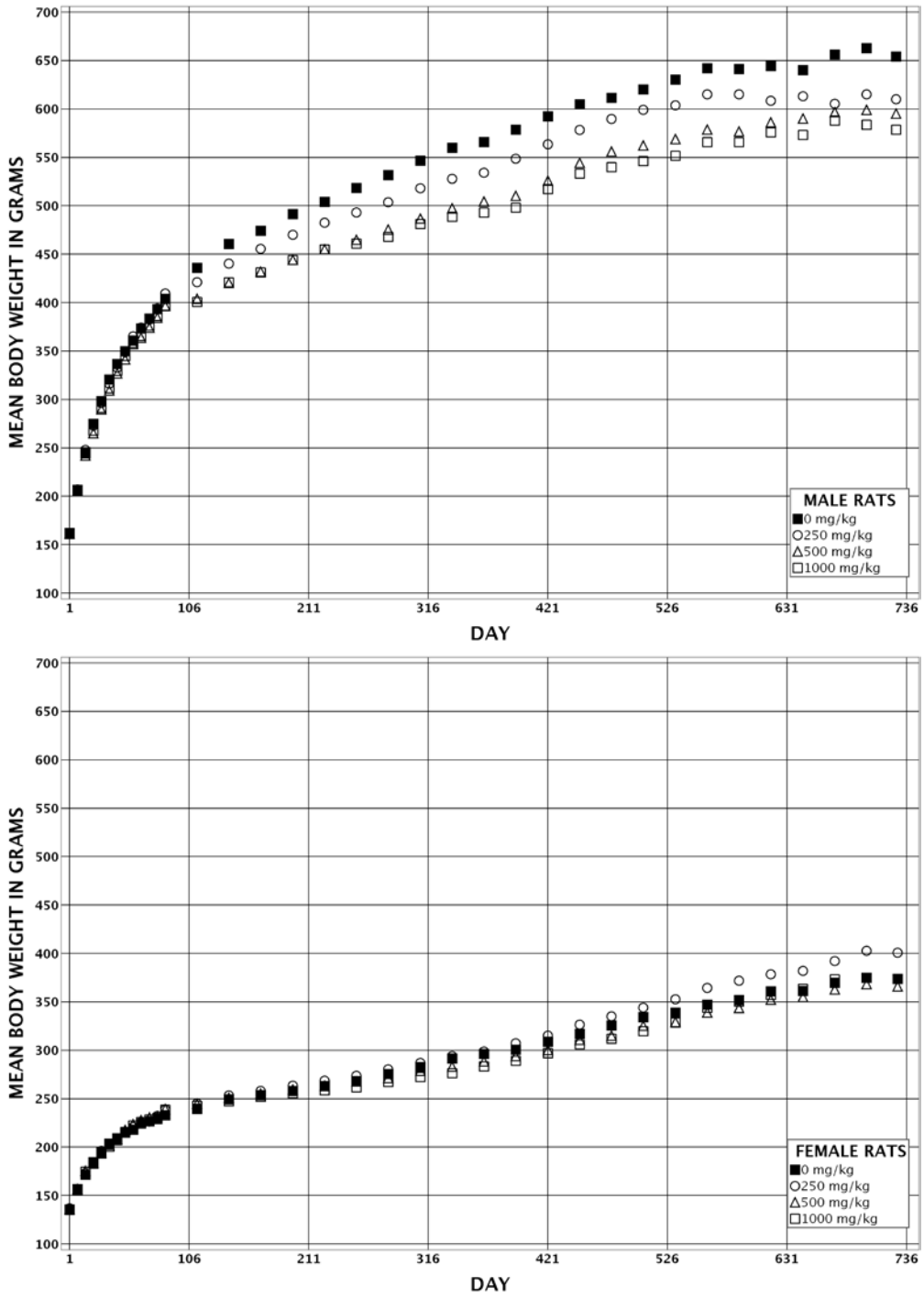

Figure 5. Growth Curves for Wistar Han Rats Administered Tetrabromobisphenol A by Gavage for Two Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or non-neoplastic lesions of the uterus, testis, and ovary. Summaries of the incidences of neoplasms and non-neoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group,

and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

No treatment-related lesions occurred in 1,000 mg/kg rats at the 3-month interim evaluation.

Uterus: Neoplasms occurred in all dosed groups of females in the original transverse review of the uterus; some vehicle control females also had uterine neoplasms (Table 6, Table B-1, and Table B-2). Statistical evaluations were performed for primary tumors identified in the original transverse review, the residual longitudinal review, and the combined original transverse and residual longitudinal reviews. Tumor types were evaluated for statistical significance either individually or combined according to epithelial origin (adenoma, adenocarcinoma, or malignant mixed Müllerian tumor) or mesenchymal origin (stromal polyp, stromal sarcoma, or leiomyosarcoma). For adenoma, there was a positive trend in the original transverse review. For adenocarcinoma, there was a positive trend in the original transverse review and positive trends and significantly increased incidences in the 500 and 1,000 mg/kg groups in the residual longitudinal review and the combined reviews. For the combination of adenoma, adenocarcinoma, or malignant mixed Müllerian tumor there were positive trends and significantly increased incidences in the 500 and 1,000 mg/kg groups in both reviews and when the reviews were combined.

Adenomas were generally solitary, well delineated lesions composed of a collection of endometrial glands that were typical in appearance, with little to no compression of surrounding tissue and no invasion of the adjacent endometrium or myometrium. The glands were lined by a single layer of well-differentiated cuboidal to columnar epithelium without stratification and surrounded by a delicate fibrous stroma. Occasionally adenomas were present on a broad stalk and projected in the uterine lumen.

Adenocarcinomas were often quite large, completely obliterating the normal uterine architecture. Some also invaded distant organs, including the intestines, liver, mesentery, pancreas, adrenal gland, ovary, lymph node, spleen, thymus, subcutaneous tissue, skeletal muscle, lung, and kidney. Histologically, masses were characterized by enlarged pleomorphic epithelial cells arranged as solid nests, cords, papillary, or acinar structures, within or supported by a fibrovascular stroma (Figure 9 and Figure 10). There was moderate to marked cellular pleomorphism and atypia. The epithelium was anaplastic in some cases with stratification of multiple cell layers forming solid sheets of epithelial cells that extended through the uterine wall. Large areas of necrosis and suppurative inflammation were also associated with larger tumors. Proliferation of fibroblasts and formation of thick stroma were present in many cases. If there was more than one adenocarcinoma and the tumors were clearly independent, distinct, and not connected, then a diagnosis of multiple adenocarcinoma was made.

An increased incidence of point mutations in the rat *Tp53* gene was observed in uterine adenocarcinomas from tetrabromobisphenol A-exposed animals (10/16; 63%) compared to spontaneous uterine adenocarcinomas in control animals (1/9; 11%). Additionally, uterine adenocarcinomas from two rats exposed to tetrabromobisphenol A harbored multiple mutations. The results are presented in Appendix M.

Malignant mixed Müllerian tumors were composed of a mixture of neoplastic epithelial and neoplastic mesenchymal cells (Figure 11). Cytokeratin and vimentin immunohistochemical stains were used to better characterize these lesions (Figure 12 and Figure 13). Cytokeratin

staining revealed neoplastic epithelial elements with granular cytoplasmic staining; however, these positive cells were admixed with neoplastic mesenchymal cells that showed positive cytoplasmic staining with vimentin. Stained serial sections showed that some individual neoplastic cells were biphasic and stained with both cytokeratin and vimentin. All tumors were very large and infiltrative, composed of areas with glandular formation and also areas with a more solid growth pattern. In the areas of glandular formation, these tumors were similar to adenocarcinomas in morphology. In the areas with a more solid growth pattern, the neoplastic cells were arranged in sheets, streams, and/or interweaving bundles. In these areas, individual neoplastic cells were large and pleomorphic with large round to elongate nuclei with an open chromatin pattern and a single prominent magenta nucleolus. Bizarre mitotic figures were frequent. One malignant mixed Müllerian tumor had areas of neoplastic bone formation (heterologous type). Tumors in four animals in the 250 mg/kg group had extensive metastases to the liver, mesentery, pancreas, stomach, ovary, spleen, subcutaneous tissue, lung, and kidney.

Table 6. Incidences of Neoplasms and Non-neoplastic Lesions of the Uterus in Female Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Original Transverse Review				
Number Necropsied	50	50	50	50
Endometrium, Hyperplasia, Cystic[a]	8 (2.1)[b]	13 (1.5)	11 (2.0)	18* (1.9)
Adenoma[c]				
Overall rate[d]	0/50 (0%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate[e]	0.0%	0.0%	7.8%	9.4%
Terminal rate[f]	0/34 (0%)	0/34 (0%)	3/29 (10%)	2/33 (6%)
First incidence (days)	–[h]	–	728 (T)	625
Poly-3 test[g]	P = 0.010	–[i]	P = 0.100	P = 0.059
Adenocarcinoma, Multiple	1	0	1	0
Adenocarcinoma (includes multiple)[j]				
Overall rate	3/50 (6%)	3/50 (6%)	8/50 (6%)	9/50 (18%)
Adjusted rate	7.0%	6.7%	19.8%	20.9%
Terminal rate	2/34 (6%)	0/34 (0%)	4/29 (14%)	5/33 (15%)
First incidence (days)	713	548	321	607
Poly-3 test	P = 0.016	P = 0.644N	P = 0.078	P = 0.058
Malignant Mixed Müllerian Tumor[c]	0	4	0	2
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor[j]				
Overall rate	3/50 (6%)	7/50 (14%)	11/50 (22%)	13/50 (26%)
Adjusted rate	7.0%	15.4%	27.3%	29.9%
Terminal rate	2/34 (6%)	1/34 (3%)	7/29 (24%)	7/33 (21%)
First incidence (days)	713	548	321	607
Poly-3 test	P = 0.003	P = 0.181	P = 0.013	P = 0.005
Residual Longitudinal Review				
Number Necropsied	50	50	50	50
Endometrium, Hyperplasia, Cystic	23	30	28	31
Endometrium, Hyperplasia, Atypical	2	13**	11**	13**
Adenoma	3	2	1	3
Adenocarcinoma (includes multiple)				
Overall rate	4/50 (8%)	9/50 (18%)	15/50 (30%)	15/50 (30%)
Adjusted rate	9.3%	19.9%	36.4%	33.8%
Terminal rate	3/34 (9%)	4/34 (12%)	9/29 (31%)	10/33 (30%)
First incidence (days)	713	548	321	442
Poly-3 test	P = 0.003	P = 0.137	P = 0.002	P = 0.005

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Malignant Mixed Müllerian Tumor	0	0	0	1
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor				
Overall rate	6/50 (12%)	10/50 (20%)	16/50 (32%)	16/50 (32%)
Adjusted rate	13.9%	22.1%	38.8%	35.8%
Terminal rate	3/34 (9%)	5/34 (15%)	10/29 (35%)	10/33 (30%)
First incidence (days)	668	548	321	442
Poly-3 test	P = 0.008	P = 0.237	P = 0.007	P = 0.015
Original Transverse and Residual Longitudinal Reviews (Combined)				
Number Necropsied	50	50	50	50
Endometrium, Hyperplasia, Cystic	24	31	30	32
Endometrium, Hyperplasia, Atypical	2	13**	11**	13**
Adenoma	3	2	4	6
Adenocarcinoma (includes multiple)				
Overall rate	4/50 (8%)	10/50 (20%)	15/50 (30%)	16/50 (32%)
Adjusted rate	9.3%	22.0%	36.4%	35.9%
Terminal rate	3/34 (9%)	4/34 (12%)	9/29 (31%)	10/33 (30%)
First incidence (days)	713	548	321	442
Poly-3 test	P = 0.002	P = 0.089	P = 0.002	P = 0.002
Malignant Mixed Müllerian Tumor	0	4	0	2
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor				
Overall rate	6/50 (12%)	11/50 (22%)	16/50 (32%)	19/50 (38%)
Adjusted rate	13.9%	24.2%	38.8%	42.2%
Terminal rate	3/34 (9%)	5/34 (15%)	10/29 (35%)	11/33 (33%)
First incidence (days)	668	548	321	442
Poly-3 test	P < 0.001	P = 0.168	P = 0.007	P = 0.002

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
(T) Terminal kill
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical control incidence for 2-year studies (all routes): 0/150.
[d]Number of animals with neoplasm per number of animals necropsied.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.
[h]Not applicable; no neoplasms in animal group.
[i]Value of statistic cannot be computed.
[j]Historical control incidence for 2-year studies (all routes): 7/150 (includes one endometrium carcinoma).

In the original transverse review, the incidences of cystic endometrial hyperplasia were increased in all dosed groups of females, and the increase in the 1,000 mg/kg group was significant (Table 6 and Table B-4). In the residual longitudinal review, additional incidences of cystic endometrial hyperplasia were found and the treatment-related effect was not supported when the reviews were combined. A new and potentially preneoplastic lesion of endometrial atypical hyperplasia was identified in all dose groups during the residual longitudinal review of the uterus (Figure 14 to Figure 18).

Cystic endometrial hyperplasia was diagnosed when there were three or more dilated glands. Microscopically, a single layer of normal appearing endometrial epithelium, either cuboidal or columnar, lined affected glands. In earlier stages, the glands were lined by more crowded epithelial cells and associated with a neutrophilic infiltrate. This lesion was diagnosed as minimal severity when approximately three to five dilated glands were present with little to no distortion or extension into the uterine lumen. Mild cystic endometrial hyperplasia was diagnosed when greater than five dilated glands were present and bulged into, but did not fill, the uterine lumen. In mild hyperplasia, dilated glands were larger than those noted in cases with minimal severity and some glandular crowding and reduction in surrounding stroma was noted. Diagnoses of moderate severity involved increases in glandular density with extension and filling of the entire uterine lumen in most cases. The diameter of the dilated glands often varied in size from small to greater than half the size of the uterine lumen.

Uterus endometrium atypical hyperplasia was not present in the cross sections of originally examined tissues but was only diagnosed in the longitudinal tissues. The lesion affected either glandular epithelium or uterine surface epithelium, and occasionally both types occurred together. Clusters of enlarged glands separated by little to no stroma characterized this lesion. Affected glands were lined by very tall, stratified, disorganized epithelium that piled up to six cell layers thick in some cases. Epithelial cells lining affected glands often displayed loss of nuclear polarization, karyomegaly, mitoses, and cellular pleomorphism. The thickened epithelium frequently projected into glandular lumens forming multiple thickened infoldings and projections. Despite the atypical features, these proliferative lesions were not considered adenomas as they did not form a distinct mass or compress the surrounding uterine architecture. Morphologic features were different in areas of atypia affecting the surface epithelium. The papillary type consisted of numerous small branching projections of epithelium that extended into the uterine lumen, occasionally on small fibrovascular stalks. Epithelial blebbing and loss of nuclear polarization were noted.

Testis: The incidences of interstitial cell adenoma were slightly increased in 500 and 1,000 mg/kg males, and the incidence in the 1,000 mg/kg group exceeded the historical control incidence for all routes of administration (Table 7, Table A-1, Table A-2, and Table A-3). Atrophy of the testicular germinal epithelium was identified in seven treated males, and the severity of the lesion increased with increasing dose (Table 7 and Table A-4). Affected testes were shrunken with a convoluted tunica albuginea. Approximately 50% to 90% of seminiferous tubules were affected in most cases. Seminiferous tubules were small, thin, and widely separated by pale eosinophilic fluid (edema). Interstitial cells appeared prominent. Seminiferous tubules were lined by low flattened epithelium with lumens devoid of spermatozoa.

The testicular interstitial cell adenomas were characterized as a mass of proliferating interstitial cells with prominent cystic spaces that caused compression of adjacent seminiferous tubules. The

four animals with adenomas (three unilateral and one bilateral) had tumors that ranged from small (an area of about one sixth of the testis) to large (effacing about 70% of the testis). The neoplastic cells had distinct cell borders, were larger and paler than normal interstitial cells, and contained eosinophilic, finely vacuolated cytoplasm, round nuclei with stippled chromatin, and a single prominent magenta nucleolus. The cystic spaces were filled with pale eosinophilic material and clear vacuoles were present around the periphery. There was a scant and sometimes inapparent fibrovascular stroma. Invasion of the capsule was not a feature.

Ovary: The incidences of rete ovarii cyst were significantly increased in 500 and 1,000 mg/kg females (Table 7 and Table B-4).

Table 7. Incidences of Neoplasms and Non-neoplastic Lesions of the Testis and Ovary in Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Male				
Testis[a]	50	50	50	50
Germinal Epithelium, Atrophy[b]	0	4 (2.8)[c]	1 (3.0)	2 (3.5)
Interstitial Cell, Adenoma, Bilateral	0	0	1	0
Interstitial Cell, Adenoma (includes bilateral)[d]				
Overall rate[e]	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate[f]	0.0%	0.0%	2.2%	6.8%
Terminal rate[g]	0/33 (0%)	0/28 (0%)	1/38 (3%)	3/39 (8%)
First incidence (days)	–[i]	–	727 (T)	727 (T)
Poly-3 test[h]	P = 0.023	–[j]	P = 0.526	P = 0.138
Female				
Ovary	50	49	50	49
Rete Ovarii, Cyst	1	0	6*	6*

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
(T) Terminal kill.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for 2-year studies (all routes): 4/150.
[e]Number of animals with neoplasm per number of animals with testis examined microscopically.
[f]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[g]Observed incidence at terminal kill.
[h]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[i]Not applicable; no neoplasms in animal group.
[j]Value of statistic cannot be computed.

Mice

Three-month Study

All mice survived to the end of the study (Table 8). The final mean body weights and mean body weight gains of dosed groups of males and females were similar to those of the vehicle control groups (Table 8 and Figure 6). No clinical findings related to tetrabromobisphenol A administration were observed.

No changes in hematology parameters were attributable to the administration of tetrabromobisphenol A (Table F-2).

Acetanilide-4-hydroxylase, 7-ethoxyresorufin-*O*-deeth-ylase, and PROD activities in the liver of 500 and 1,000 mg/kg males were significantly less (30% to 40%) than those of the vehicle controls at the end of the study; in 1,000 mg/kg females, PROD activity was significantly decreased (30%) at week 14 (Table G-2). These effects were less pronounced in mice than in rats in the 3-month study.

Compared to those of the vehicle controls, absolute and relative liver weights were significantly increased in 500 mg/kg males and 1,000 mg/kg males and females; absolute and relative spleen weights in 1,000 mg/kg males were also significantly increased (Table H-3). Absolute and relative kidney weights were significantly decreased in 1,000 mg/kg male mice.

Tetrabromobisphenol A did not exhibit the potential to be a reproductive toxicant in B6C3F1/N mice under the conditions of these studies (Table I-4, Table I-5, and Table I-6; Figure I-2).

Table 8. Survival and Body Weights of Mice in the Three-month Gavage Study of Tetrabromobisphenol A[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	22.6 ± 0.4	37.4 ± 0.9	14.8 ± 0.9	–
10	10/10	22.3 ± 0.4	34.7 ± 0.7	12.4 ± 0.6	93
50	10/10	22.6 ± 0.3	38.4 ± 0.6	15.8 ± 0.6	103
100	10/10	22.8 ± 0.4	36.2 ± 1.0	13.4 ± 0.9	97
500	10/10	22.8 ± 0.5	37.1 ± 0.9	14.2 ± 0.8	99
1,000	10/10	22.8 ± 0.4	35.2 ± 1.2	12.4 ± 1.2	94
Female					
0	10/10	18.4 ± 0.3	27.5 ± 0.6	9.1 ± 0.6	–
10	10/10	18.4 ± 0.3	29.3 ± 1.0	10.9 ± 0.8	106
50	10/10	18.5 ± 0.3	28.6 ± 0.7	10.1 ± 0.5	104
100	10/10	18.3 ± 0.3	26.2 ± 0.7	7.8 ± 0.5	95
500	10/10	18.5 ± 0.3	29.2 ± 0.7	10.7 ± 0.6	106
1,000	10/10	18.4 ± 0.3	27.7 ± 0.6	9.4 ± 0.6	101

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at 14 weeks/number initially in group.

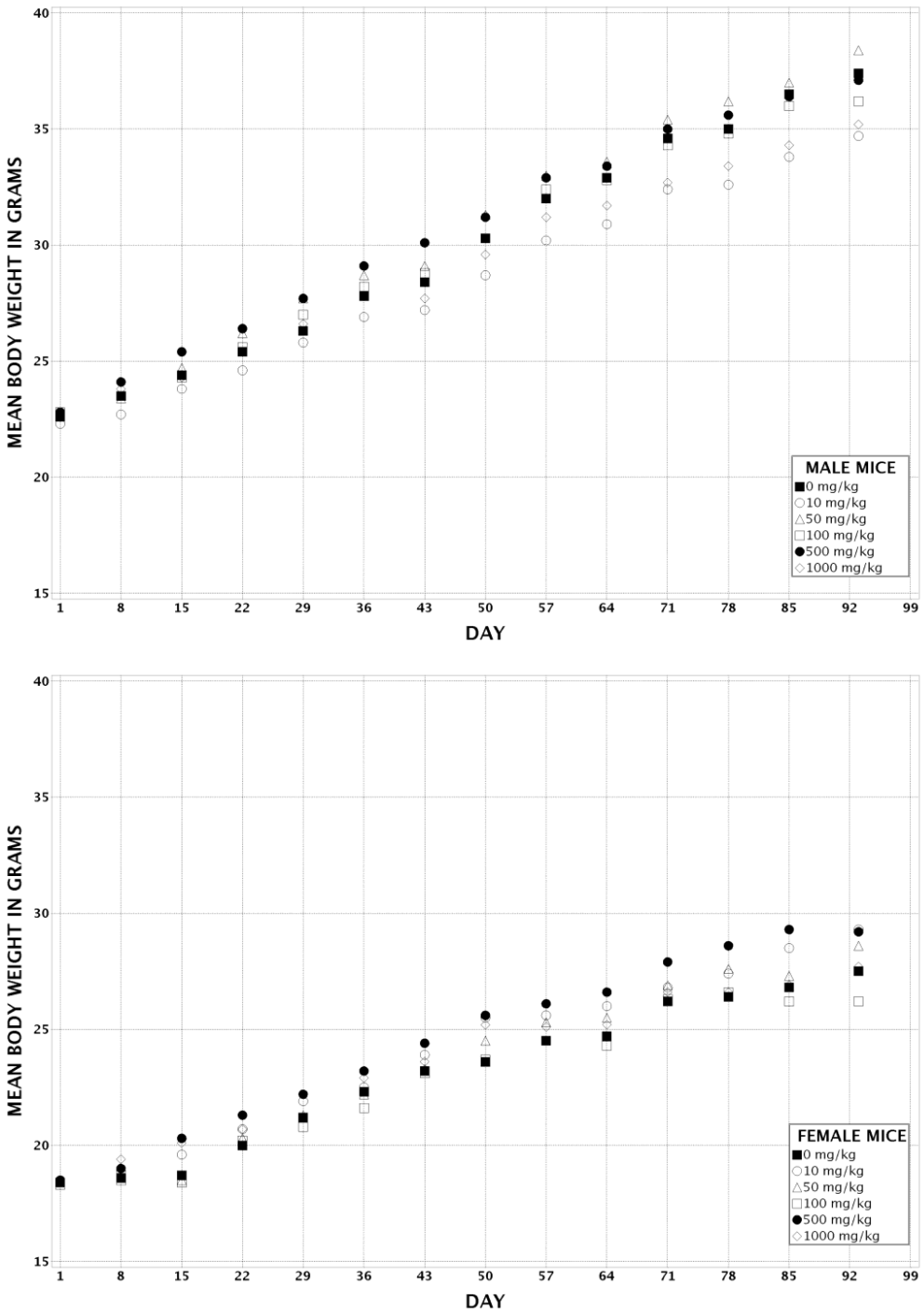

Figure 6. Growth Curves for Mice Administered Tetrabromobisphenol A by Gavage for Three Months

Significantly increased incidences of renal tubule cytoplasmic alteration occurred in 500 and 1,000 mg/kg male mice, and the severity of the lesion in the 1,000 mg/kg group was greater than that in the 500 mg/kg group (Table 9). Renal tubule cytoplasmic alteration was characterized by a decrease or absence of the normal vacuoles present in the cortical proximal tubules.

Dose Selection Rationale: No effects on mortality, body weights, or hematology parameters were observed in the 3-month study. Chemical-related effects on liver enzyme activities, organ weights, and kidney lesion incidences were not expected to cause increased mortality in a 2-year study. Therefore, doses selected for the 2-year gavage study in mice were 250, 500, and 1,000 mg/kg.

Table 9. Incidences of Cytoplasmic Alteration of the Kidney in Male Mice in the Three-month Gavage Study of Tetrabromobisphenol A

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Number Examined Microscopically	10	10	10	10	10	10
Renal Tubule, Cytoplasmic Alteration[a]	0	0	0	0	10** (1.0)[b]	10** (2.0)

**Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 7). Survival of 1,000 mg/kg males and females was significantly less than that of the vehicle control groups. Increased mortality was seen as early as 6 months into the study and coincided with the initial divergence of body weight gain in the 1,000 mg/kg females. Analysis of the pathology findings suggests that decreased survival may have been due in part to gastrointestinal toxicity, although the severities of the various gastrointestinal lesions in the high dose groups were not always increased over those in the other dosed groups.

Body Weights and Clinical Findings

The mean body weights of 1,000 mg/kg females were 10% to 25% less than those of the vehicle controls after week 25 (Figure 8; Table 11 and Table 12). Body weights of all dosed groups of males and of 250 and 500 mg/kg females were generally similar to those of the vehicle control groups throughout the study. No clinical findings related to chemical exposure were observed.

Table 10. Survival of Mice in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	9	10	6	12
Natural deaths	8	14	5	25
Animals surviving to study termination	33[e]	26[e]	39	12
Percent probability of survival at end of study[b]	66	50	78	25
Mean survival (days)[c]	687	678	702	577
Survival analysis[d]	P < 0.001	P = 0.200	P = 0.260N	P < 0.001
Female				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	6	8	3	7
Natural deaths	4	11	11	38
Animals surviving to study termination	40	31	36	4
Percent probability of survival at end of study	80	62	72	8
Mean survival (days)	711	703	695	413
Survival analysis	P < 0.001	P = 0.081	P = 0.421	P < 0.001

[a]Censored from survival analyses.
[b]Kaplan-Meier determinations.

[c]Mean of all deaths (uncensored, censored, and terminal kill).
[d]The result of the life table trend test[91] is in the vehicle control column, and the results of the life table pairwise comparisons[90] with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.
[e]Includes one animal that died during the last week of the study.

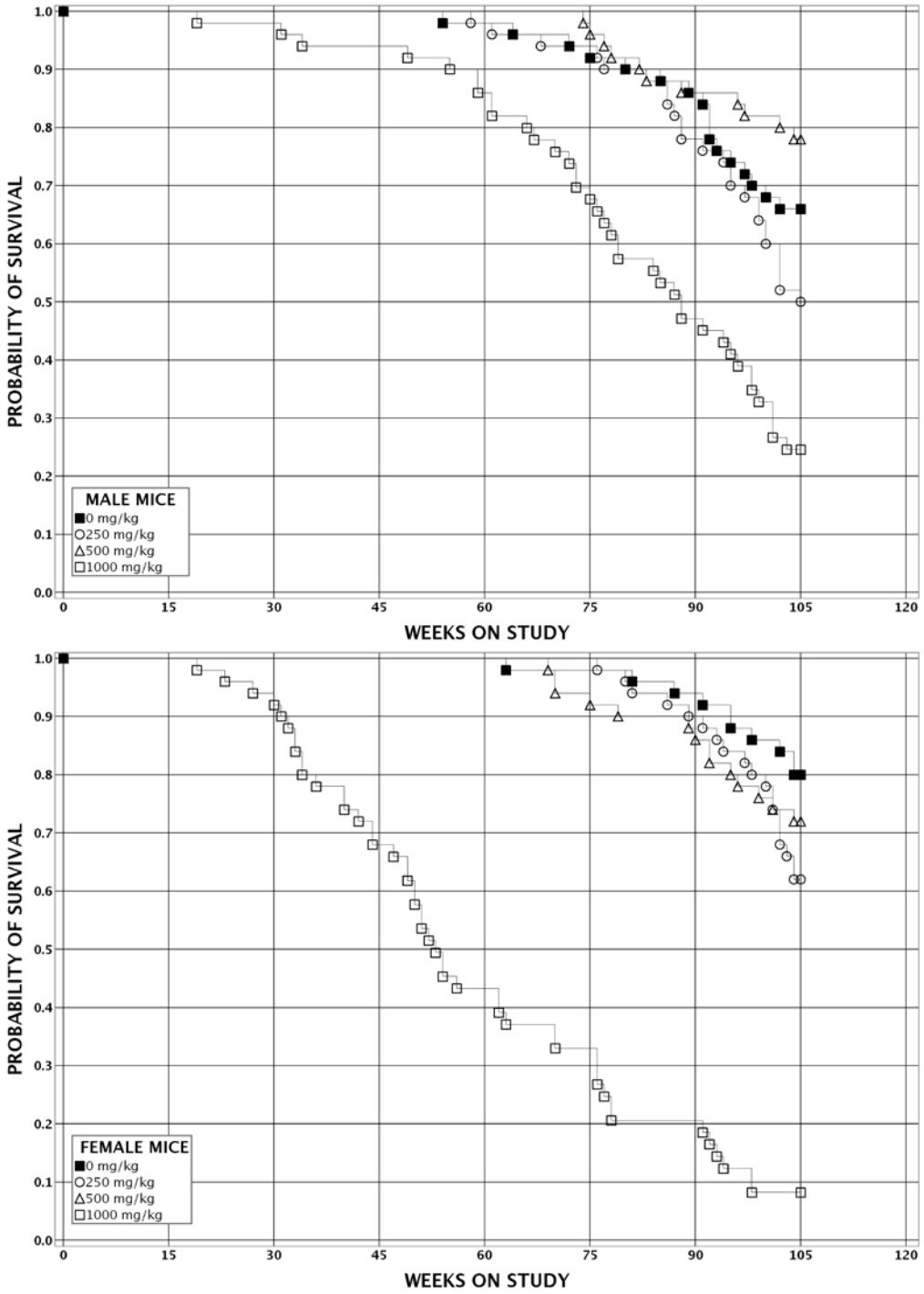

Figure 7. Kaplan-Meier Survival Curves for Mice Administered Tetrabromobisphenol A by Gavage for Two Years

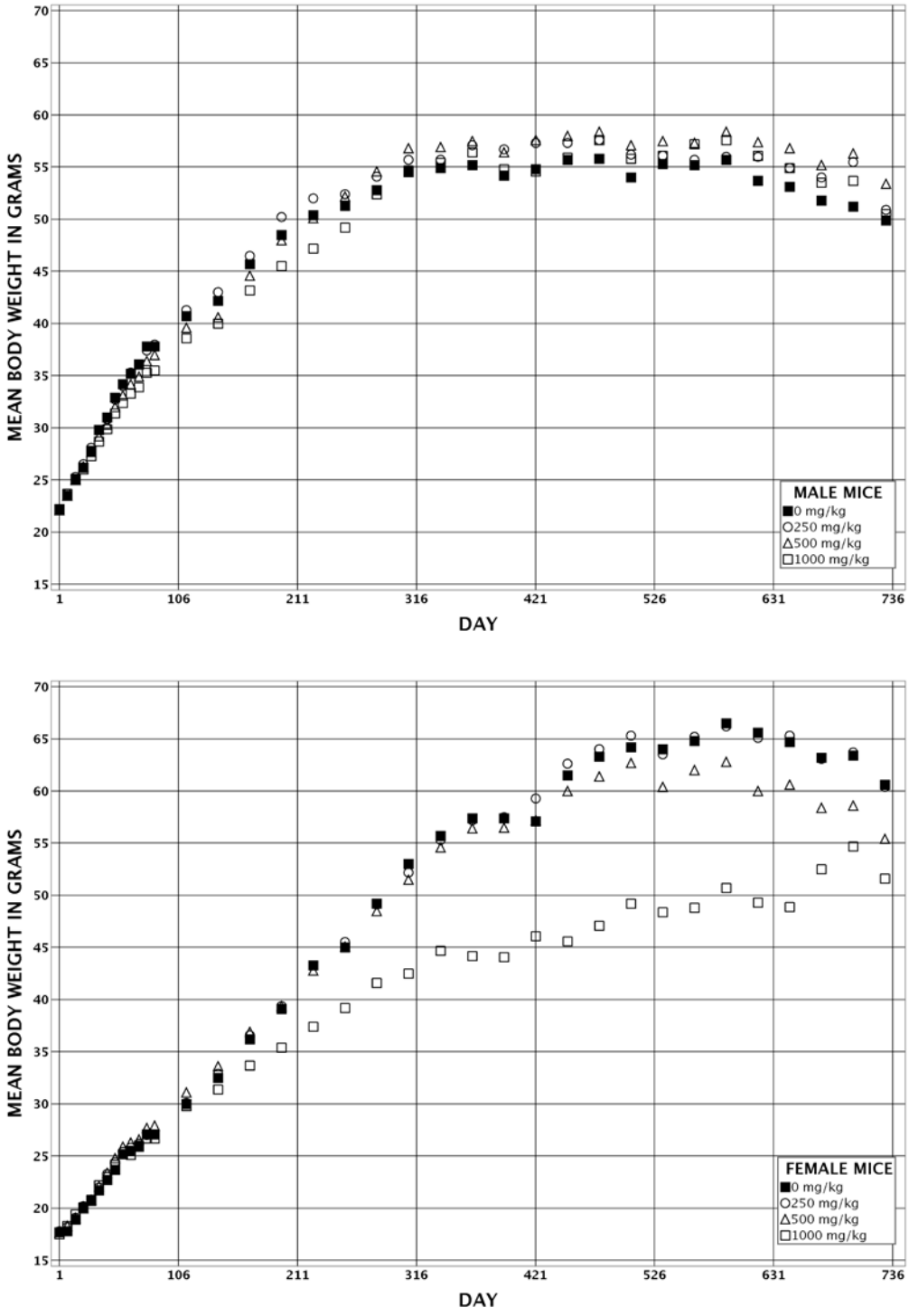

Figure 8. Growth Curves for Mice Administered Tetrabromobisphenol A by Gavage for Two Years

Table 11. Mean Body Weights and Survival of Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A

Day	Vehicle Control		250 mg/kg			500 mg/kg			1,000 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	22.2	50	22.2	100	50	22.1	100	50	22.2	100	50
8	23.5	50	23.7	101	50	23.5	100	50	23.7	101	50
15	25.1	50	25.3	101	50	25.0	100	50	25.1	100	50
22	26.2	50	26.5	101	50	26.3	101	50	26.0	99	50
29	27.8	50	28.1	101	50	27.7	100	50	27.3	98	50
36	29.8	50	29.8	100	50	29.2	98	50	28.7	96	50
43	31.0	50	30.8	99	50	30.3	98	50	29.9	96	50
50	32.9	50	32.7	99	50	32.0	97	50	31.4	96	50
57	34.2	50	34.0	99	50	33.1	97	50	32.4	95	50
64	35.2	50	35.3	101	50	34.2	97	50	33.3	95	50
71	36.1	50	36.1	100	50	34.9	97	50	33.9	94	50
78	37.8	50	37.4	99	50	36.4	96	50	35.3	93	50
85	37.8	50	38.0	100	50	37.0	98	50	35.5	94	50
113	40.7	50	41.3	102	50	39.6	97	50	38.6	95	50
141	42.2	50	43.0	102	50	40.6	96	50	40.0	95	49
169	45.7	50	46.5	102	50	44.6	98	50	43.2	95	49
197	48.5	50	50.2	104	50	48.0	99	50	45.5	94	49
225	50.4	50	52.0	103	50	50.1	99	50	47.2	94	48
253	51.3	50	52.4	102	50	52.2	102	50	49.2	96	47
281	52.8	50	54.1	102	50	54.6	103	50	52.4	99	47
309	54.5	50	55.7	102	50	56.8	104	50	54.7	100	47
337	54.9	50	55.7	102	50	56.9	104	50	55.4	101	47
365	55.2	50	57.1	103	50	57.5	104	50	56.4	102	46
393	54.2	49	56.7	105	50	56.4	104	50	54.8	101	45
421	54.8	49	57.3	105	49	57.6	105	50	54.6	100	42
449	55.7	48	57.3	103	48	58.0	104	50	55.9	100	41
477	55.8	48	57.6	103	47	58.4	105	50	57.6	103	38
505	54.0	47	56.2	104	47	57.1	106	50	55.8	104	36
533	55.3	46	56.1	101	46	57.5	104	48	56.1	101	32
561	55.2	45	55.7	101	45	57.3	104	46	57.2	104	28
589	55.7	45	56.0	101	44	58.4	105	44	57.6	104	27
617	53.7	44	56.0	104	39	57.4	107	43	56.1	105	23
645	53.1	38	54.9	103	38	56.8	107	43	54.9	103	22
673	51.8	36	54.0	104	34	55.2	107	42	53.5	103	19
701	51.2	34	55.5	108	30	56.3	110	41	53.7	105	16
Mean for Weeks											
1–13	30.7	–	30.8	100	–	30.1	98	–	29.6	96	–
14–52	49.0	–	50.1	102	–	49.3	101	–	47.4	97	–
53–101	54.3	–	56.2	103	–	57.2	105	–	55.7	103	–

Table 12. Mean Body Weights and Survival of Female Mice in the Two-year Gavage Study of Tetrabromobisphenol A

Day	Vehicle Control		250 mg/kg			500 mg/kg			1,000 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	17.7	50	17.8	101	50	17.7	100	50	17.5	99	50
8	17.8	50	18.2	102	50	18.3	103	50	18.3	103	50
15	18.9	50	19.1	101	50	19.1	101	50	19.4	102	50
22	20.0	50	20.2	101	50	20.0	100	50	20.1	101	50
29	20.7	50	20.8	100	50	20.8	101	50	20.8	101	50
36	21.7	50	22.0	102	50	22.1	102	50	22.2	102	50
43	22.7	50	23.3	103	50	23.4	103	50	23.0	102	50
50	23.7	50	24.4	103	50	24.8	105	50	24.2	102	50
57	25.2	50	25.4	101	50	25.9	103	50	25.2	100	50
64	25.5	50	25.8	101	50	26.3	103	50	25.1	98	50
71	26.0	50	26.2	101	50	26.6	102	50	25.9	100	50
78	27.1	50	27.0	100	50	27.7	102	50	26.7	98	50
85	27.1	50	27.1	100	50	27.9	103	50	26.7	98	50
113	30.0	50	30.1	101	50	31.1	104	50	29.8	100	50
141	32.5	50	32.9	101	50	33.6	104	50	31.4	97	49
169	36.2	50	36.6	101	50	36.9	102	50	33.7	93	48
197	39.1	50	39.4	101	50	39.4	101	50	35.4	90	47
225	43.3	50	43.3	100	50	42.8	99	50	37.4	86	44
253	45.0	50	45.5	101	50	45.1	100	50	39.2	87	39
281	49.2	50	49.1	100	50	48.5	99	50	41.6	85	37
309	53.0	50	52.2	99	50	51.5	97	50	42.5	80	34
337	55.7	50	55.3	99	50	54.6	98	50	44.7	80	32
365	57.4	50	57.2	100	50	56.4	98	50	44.2	77	25
393	57.4	50	57.5	100	50	56.5	98	50	44.1	77	21
421	57.1	50	59.3	104	50	57.2	100	50	46.1	81	21
449	61.5	49	62.6	102	50	60.0	98	50	45.6	74	18
477	63.3	49	64.0	101	50	61.4	97	50	47.1	74	18
505	64.2	49	65.3	102	50	62.7	98	47	49.2	77	16
533	64.0	49	63.5	99	49	60.4	95	46	48.4	76	13
561	64.8	49	65.2	101	47	62.0	96	45	48.8	75	10
589	66.5	48	66.2	100	47	62.8	95	45	50.7	76	10
617	65.6	47	65.1	99	46	60.0	92	45	49.3	75	10
645	64.7	46	65.3	101	43	60.6	94	41	48.9	76	8
673	63.2	44	63.1	100	41	58.4	92	39	52.5	83	6
701	63.4	43	63.7	101	38	58.6	92	37	54.7	86	4
Mean for Weeks											
1–13	22.6	–	22.9	101	–	23.1	102	–	22.7	100	–
14–52	42.7	–	42.7	100	–	42.6	100	–	37.3	87	–
53–101	62.6	–	62.9	100	–	59.8	96	–	48.4	77	–

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of hemangioma and hemangiosarcoma and of neoplasms and/or non-neoplastic lesions of the liver, large intestine, kidney, forestomach, bone, and ovary. Due to early mortality, data for neoplasms are not presented for the 1,000 mg/kg groups in this section. Summaries of the incidences of neoplasms and non-neoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: The incidence of multiple hepatocellular adenoma was significantly increased in 500 mg/kg males (Table I-3 and Table C-1). The incidences of hepatoblastoma and of hepatocellular carcinoma or hepatoblastoma (combined) were significantly increased in 250 mg/kg males; the incidences of hemangiosarcoma were slightly increased in 250 and 500 mg/kg males (Table 13, Table C-1, and Table C-2). The incidences of hepatoblastoma in the 250 and 500 mg/kg groups exceeded the historical control ranges for corn oil gavage studies and all routes of administration in male B6C3F1/N mice (Table 13 and Table C-3). The incidences of clear cell focus in 500 mg/kg males and eosinophilic focus in 250 and 500 mg/kg males were significantly increased; the incidence of mixed cell focus was increased in 500 mg/kg males, though not significantly (Table 13 and Table C-4).

Hepatocellular adenomas were generally solitary, well-circumscribed lesions occupying an area greater than one liver lobule and causing distinct compression of adjacent parenchyma. They were either solid masses or composed of irregular hepatic plates, one to three cell layers thick. The hepatic plates at the margins impinged at sharp angles to the surrounding normal hepatic plates. An absence of normal lobular architecture was common. Central veins and portal tracts were rare and sometimes trapped within the expanding mass near the periphery. The sinusoids were either compressed or dilated and angiectasis was occasionally present; they were composed of well-differentiated hepatocytes that were variable in size. The tinctorial characteristics of the cytoplasm were variable, and could be eosinophilic, basophilic, clear, vacuolated, or a combination thereof. Cellular atypia was rare and mitoses were variable.

Hepatocellular carcinomas were generally spherical masses with irregular borders, showing local invasion and compression. They were characterized by an abnormal growth pattern, such as trabecular, glandular, and/or solid. The trabecular pattern was composed of cords that were three or more cell layers thick. Cytologic atypia and mitotic figures were common. Nuclei were variable in size, usually enlarged and hyperchromatic. Nucleoli were large, distinct, and generally centrally located.

Hepatoblastomas were irregular-shaped proliferative masses that were often found adjacent to, or arising from, hepatocellular adenomas or carcinomas. If the hepatoblastoma was in close proximity, and appeared to be arising within an adenoma or carcinoma, then only the hepatoblastoma was diagnosed. Hepatoblastomas were composed of small to medium sized neoplastic cells with scant basophilic cytoplasm arranged in sheets and palisading cords separated by thin connective tissue stroma. Neoplastic cells had a stippled chromatin pattern and contained distinct nucleoli. Mitotic figures were often numerous.

Table 13. Incidences of Neoplasms and Non-neoplastic Lesions of the Liver in Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg
Number Examined Microscopically	50	50	50
Clear Cell Focus[b]	11	10	25**
Eosinophilic Focus	20	33**	40**
Mixed Cell Focus	7	8	12
Hemangiosarcoma	0	4	3
Hepatocellular Adenoma, Multiple	12	20	28*
Hepatocellular Adenoma (includes multiple)[c]	32	33	38
Hepatocellular Carcinoma, Multiple	2	4	5
Hepatocellular Carcinoma (includes multiple)[d]	11	15	17
Hepatocellular Adenoma or Hepatocellular Carcinoma	39	39	43
Hepatoblastoma[e]			
Overall rate[f]	2/50 (4%)	11/50 (22%)	8/50 (16%)
Adjusted rate[g]	4.6%	25.6%	17.6%
Terminal rate[h]	1/33 (3%)	7/25 (28%)	7/39 (18%)
First incidence (days)	619	535	722
Poly-3 test[i]	P = 0.065	P = 0.006	P = 0.052
Hepatocellular Carcinoma or Hepatoblastoma[j]			
Overall rate	12/50 (24%)	24/50 (48%)	20/50 (40%)
Adjusted rate	26.8%	52.8%	41.5%
Terminal rate	7/33 (21%)	12/25 (48%)	12/39 (31%)
First incidence (days)	521	535	513
Poly-3 test	P = 0.099	P = 0.008	P = 0.099
Hepatocellular Adenoma, Hepatocellular			
Carcinoma, or Hepatoblastoma	39	42	43

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Due to early mortality, data for the 1,000 mg/kg group are not presented.
[b]Number of animals with lesion.
[c]Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 145/250 (58.0% ± 5.1%), range 52%-64%; all routes: 594/949 (62.6% ± 9.1%), range 48%-78%.
[d]Historical incidence for corn oil gavage studies: 87/250 (34.8% ± 10.9%), range 22%-44%; all routes: 348/949 (36.7% ± 11.4%), range 22%-56%.
[e]Historical incidence for corn oil gavage studies: 9/250 (3.6% ± 2.6%), range 0%-6%; all routes: 40/949 (4.2% ± 3.5%), range 0%-12%.
[f]Number of animals with neoplasm per number of animals with liver examined microscopically.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Observed incidence at terminal kill.
[i]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[j]Historical incidence for corn oil gavage studies: 93/250 (37.2% ± 10.0%), range 24%-48%; all routes: 371/949 (39.1% ± 11.6%), range 22%-54%.

Large Intestine: The incidences of adenoma or carcinoma (combined) of the cecum or colon occurred with a significant positive trend in males and the incidence in the 500 mg/kg group exceeded the historical control ranges for corn oil gavage studies and for all routes of administration (Table 14, Table C-1, Table C-2, and Table C-4). One 500 mg/kg female had an adenoma of the rectum (Table 14 and Table D-1). The adenomas consisted of a collection of irregular-sized glands lined by a hyperchromatic atypical columnar epithelium with closely packed nuclei. The glandular lumens were variably filled with inflammatory cells, mucus, and cell debris. The carcinomas were composed of anaplastic, invasive cells that formed pleomorphic glandular structures with inflammation. The cells were hyperchromatic and columnar to cuboidal, with closely packed nuclei and numerous mitotic figures.

Hemangioma and Hemangiosarcoma: In males, the incidences of hemangiosarcoma (all organs) occurred with a significant positive trend and the incidence in the 500 mg/kg group was significantly increased; the incidences of hemangioma or hemangiosarcoma (combined) occurred with a significant positive trend (Table 15, Table C-1 and Table C-2). The incidences of these neoplasms in both dosed groups were within the historical control ranges for corn oil gavage studies and all routes of administration (Table 15 and Table C-5). These lesions occurred in a variety of organs such as the bone marrow, liver, lung serosa, lymph nodes, skin, spleen, and vertebra.

Table 14. Incidences of Neoplasms of the Large Intestine in Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg
Male			
Cecum or Colon: Adenoma or Carcinoma[b]			
Overall rate[c]	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[d]	0.0%	0.0%	6.5%
Terminal rate[e]	0/33 (0%)	0/25 (0%)	3/39 (5%)
First incidence (days)	–[g]	–	513
Poly-3 test[f]	P = 0.039	–[h]	P = 0.131
Female			
Rectum[i]	50	50	50
Adenoma[j]	0	0	1

[a]Due to early mortality, data for the 1,000 mg/kg groups are not presented.
[b]Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 0/250; all routes: 4/950 (0.4% ± 0.8%), range 0%-2%.
[c]Number of animals with neoplasm per number of animals necropsied.
[d]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[e]Observed incidence at terminal kill.
[f]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[g]Not applicable; no neoplasms in animal group.
[h]Value of statistic cannot be computed.
[i]Number necropsied.
[j]Number of animals with neoplasm.

Table 15. Incidences of Hemangioma or Hemangiosarcoma (All Organs) in Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg
Number Necropsied	50	50	50
All Organs: Hemangioma[b]	2	0	1
All Organs: Hemangiosarcoma[c]			
Overall rate[d]	1/50 (2%)	5/50 (10%)	8/50 (16%)
Adjusted rate[e]	2.3%	11.9%	17.6%
Terminal rate[f]	0/33 (0%)	3/25 (12%)	8/39 (21%)
First incidence (days)	645	602	730 (T)
Poly-3 test[g]	P = 0.014	P = 0.093	P = 0.019
All Organs: Hemangioma or Hemangiosarcoma[h]			
Overall rate	3/50 (6%)	5/50 (10%)	9/50 (18%)
Adjusted rate	6.9%	11.9%	19.8%
Terminal rate	2/33 (6%)	3/25 (12%)	9/39 (23%)
First incidence (days)	645	602	730 (T)
Poly-3 test	P = 0.047	P = 0.338	P = 0.069

(T) Terminal kill.
[a]Due to early mortality, data for the 1,000 mg/kg group are not presented.
[b]Number of animals with neoplasm.
[c]Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 28/250 (11.2% ± 6.4%), range 2%-18%; all routes: 92/950 (9.7% ± 4.5%), range 2%-18%.
[d]Number of animals with neoplasm per number of animals necropsied.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h]Historical incidence for corn oil gavage studies: 32/250 (12.8% ± 5.4%), range 6%-18%; all routes: 105/950 (11.1% ± 4.2%), range 4%-18%.

Kidney: Incidences of renal tubule cytoplasmic alteration were significantly increased in all dosed groups of males and the severities increased with increasing dose; incidences of nephropathy in the 250 and 500 mg/kg groups were significantly decreased (Table 16 and Table C-4). Renal tubule cytoplasmic alteration was characterized by a decrease or absence of the normal vacuoles present in the cortical proximal tubules (Figure 19 and Figure 20).

Forestomach: The incidences of ulcer, mononuclear cell cellular infiltration, inflammation, and epithelium hyperplasia were significantly increased in 500 and 1,000 mg/kg males and all dosed groups of females (Table 16, Table C-4, and Table D-3). Regions of stomach ulceration were localized to the forestomach and characterized by focal or multifocal loss of the entire thickness of the squamous epithelium (Figure 21 to Figure 24). Ulceration was considered the primary lesion and there were a few secondary lesions (epithelium hyperplasia, inflammation, mononuclear cell infiltrate) that formed in response to the ulcer. Squamous epithelium adjacent to the ulcer was often hyperplastic. Areas of ulceration were often accompanied by varying degrees of inflammation that ranged from primarily neutrophilic to a mixed population of

neutrophils, macrophages, lymphocytes, and plasma cells. Inflammatory infiltrates were admixed with eosinophilic necrotic and keratin debris and variable numbers of bacteria. A robust immune response was present within the mucosa, submucosa, and tunica muscularis underlying areas of ulceration, and was diagnosed as mononuclear cell infiltration. The lesion was characterized by multifocal to coalescing lymphocytes and low numbers of plasma cells that, in severe cases, formed follicle-like structures.

Table 16. Incidences of Selected Non-neoplastic Lesions in Mice in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Male				
Kidney[a]	50	50	50	48
Renal Tubule, Cytoplasmic Alteration[b]	0	20** (1.9)[c]	47** (2.4)	46** (2.6)
Nephropathy	41 (1.3)	30** (1.9)	32** (1.3)	42 (1.9)
Stomach, Forestomach	50	49	50	49
Ulcer	9 (1.8)	9 (2.4)	19* (2.2)	28** (2.4)
Infiltration Cellular, Mononuclear Cell	5 (1.6)	8 (1.8)	21** (2.1)	27** (2.3)
Inflammation	9 (1.3)	10 (1.7)	20* (2.2)	26** (2.3)
Epithelium, Hyperplasia	10 (1.7)	13 (2.2)	27** (2.8)	28** (2.7)
Female				
Stomach, Forestomach	50	50	50	48
Ulcer	2 (2.0)	15** (2.0)	40** (2.2)	38** (2.1)
Infiltration Cellular, Mononuclear Cell	2 (3.0)	13** (2.2)	33** (2.4)	28** (1.8)
Inflammation	2 (3.0)	14** (1.4)	41** (2.0)	37** (2.2)
Epithelium, Hyperplasia	4 (2.5)	16** (2.6)	39** (3.0)	39** (2.3)

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Bone: The incidences of fibro-osseous lesion were significantly decreased in all dosed groups of females (Table D-3).

Ovary: The incidences of follicle cyst were slightly decreased in all dosed groups of females (7/50, 6/50, 4/50, 1/47; Table D-3).

Genetic Toxicology

Tetrabromobisphenol A was tested for bacterial mutagenicity in two independent assays and results were negative in both assays. In the first assay, tetrabromobisphenol A (100 to 10,000 µg/plate) showed no evidence of mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation from induced hamster or rat liver S9[77] (Table E-1). In the second assay, conducted with the same lot of

tetrabromobisphenol A that was used in the 2-year studies, no mutagenic activity was detected in *S. typhimurium* strains TA98 or TA100, or in *Escherichia coli* strain WP2 *uvrA/*pKM101; all tests were conducted with and without rat liver S9, and the highest concentration tested was 6,000 µg/plate (Table E-2). In vivo, no increases in micronucleated normochromatic erythrocytes were observed in male or female B6C3F1/N mice following 3 months of administration of tetrabromobisphenol A by gavage over a dose range of 10 to 1,000 mg/kg (Table E-3). In addition, no significant changes in the percentage of circulating polychromatic (immature) erythrocytes were observed in dosed mice, suggesting that tetrabromobisphenol A did not induce bone marrow toxicity over the dose range tested.

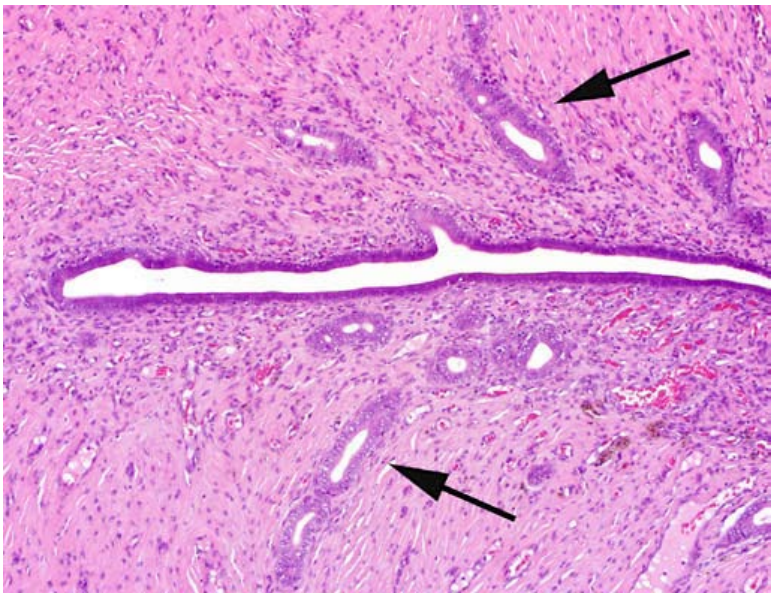

Figure 9. Normal Uterus in a Vehicle Control Female Wistar Han Rat in the Two-year Gavage Study of Tetrabromobisphenol A (H&E)

Note the normal simple tubular endometrial glands (arrows).

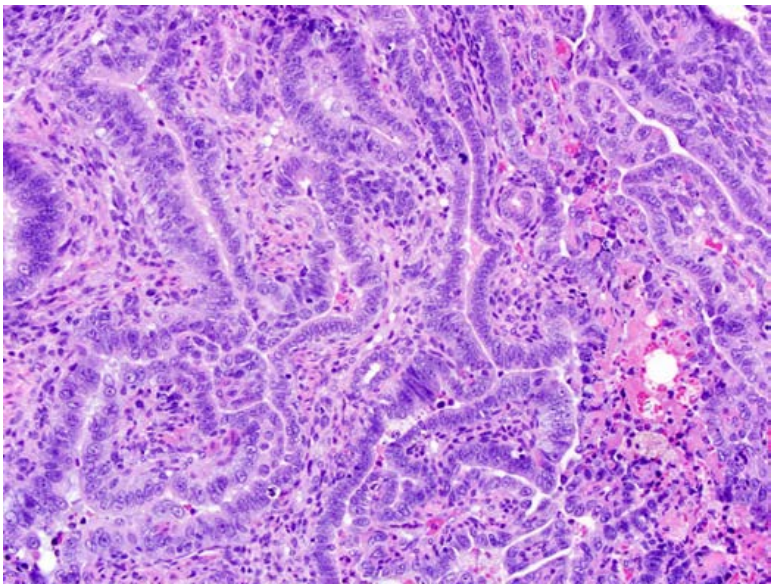

Figure 10. Uterine Adenocarcinoma in a Female Wistar Han Rat Administered 250 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

Cords and acinar structures are lined by one or multiple cell layers of pleomorphic neoplastic cells. The cells are cuboidal to columnar with varying amounts of slightly basophilic cytoplasm. Cell nuclei are centrally to basally located, round to oval, with multiple prominent nucleoli. Inflammation and cell debris are common within the fibrous stroma.

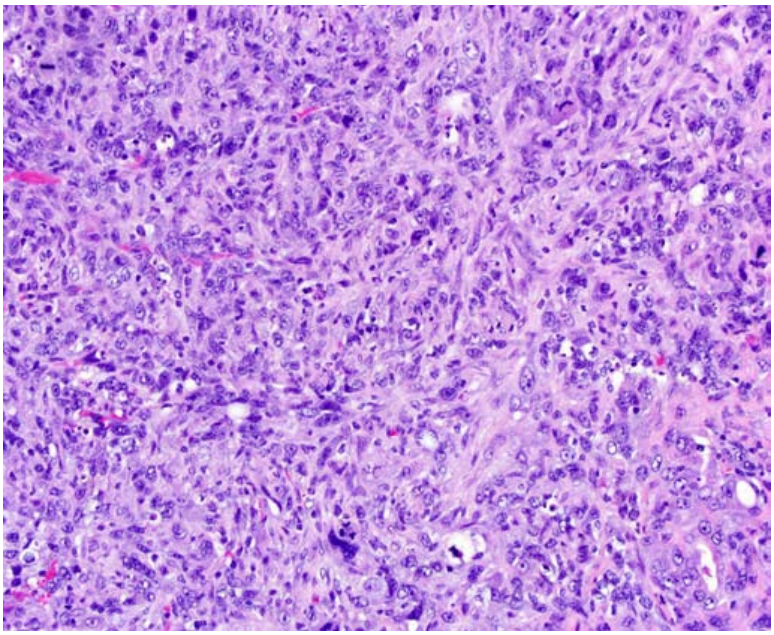

Figure 11. Malignant Mixed Müllerian Tumor in the Uterus of a Female Wistar Han Rat Administered 250 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

This area of the neoplasm has a more solid pattern than an adenocarcinoma with a mixture of both neoplastic epithelial cells and neoplastic mesenchymal cells. The neoplastic cells are disorganized. Individual neoplastic cells are atypical with variation in size and shape and have pleomorphic nuclei containing vesicular chromatin.

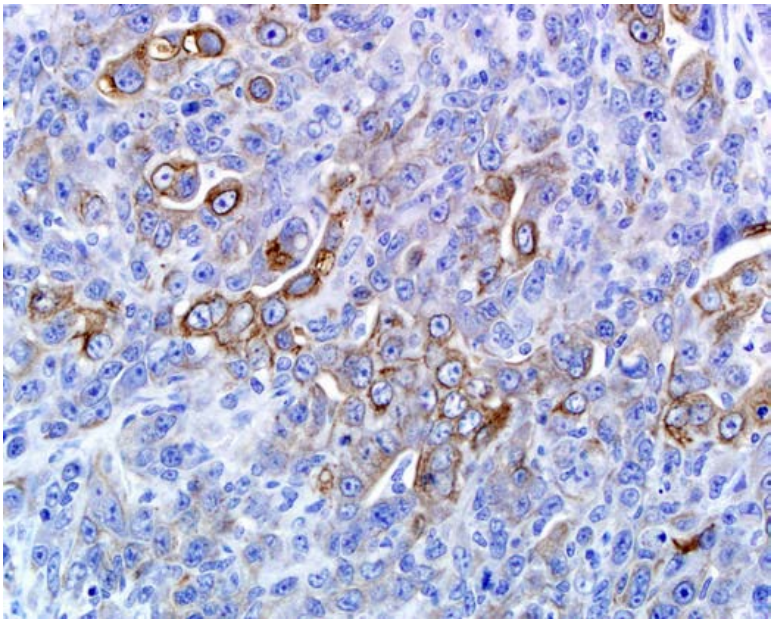

Figure 12. Malignant Mixed Müllerian Tumor in the Uterus of a Female Wistar Han Rat Administered 250 mg/kg Tetrabromobisphenol A by Gavage for Two Years

A subset of the neoplastic cells show cytokeratin-positive cytoplasmic staining. Cytokeratin antibody is an immunohistochemical stain for cells of epithelial origin.

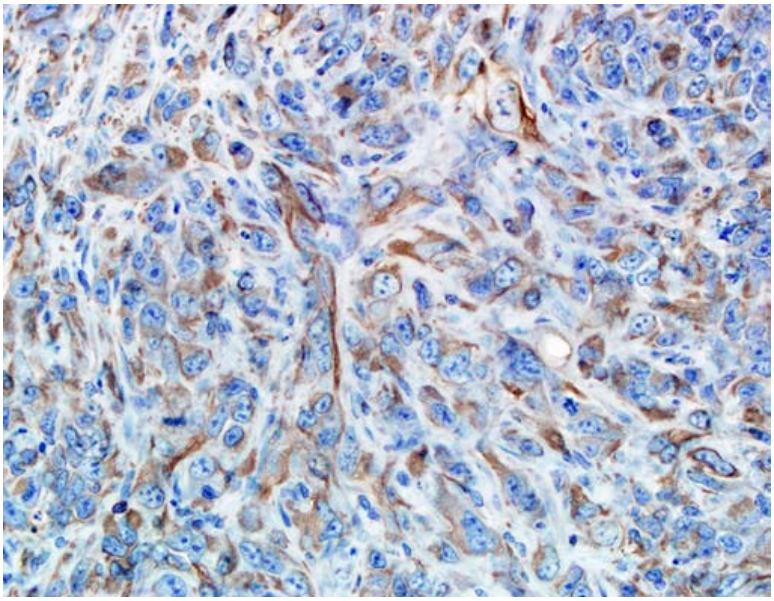

Figure 13. Malignant Mixed Müllerian Tumor in the Uterus of a Female Wistar Han Rat Administered 250 mg/kg Tetrabromobisphenol A by Gavage for Two Years

A subset of the neoplastic cells show vimentin-positive cytoplasmic staining. Vimentin antibody is an immunohistochemical stain for cells of mesenchymal origin.

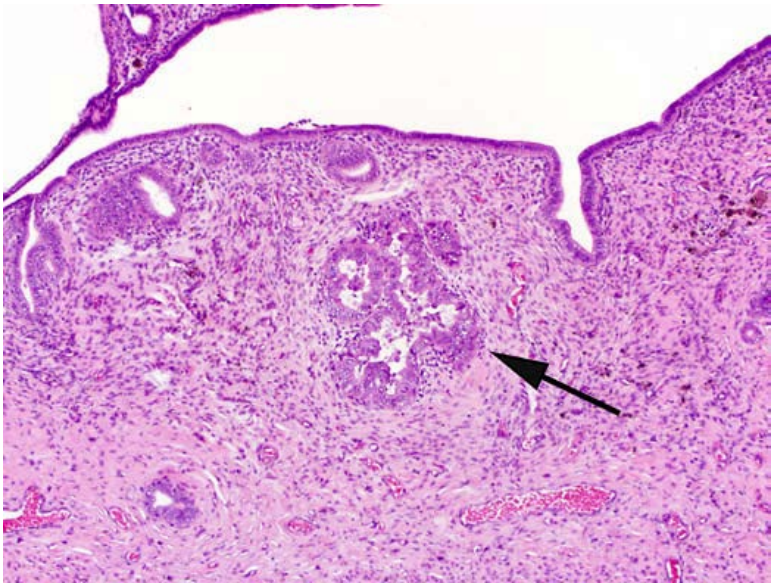

Figure 14. Uterus from a Female Wistar Han Rat Administered 250 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

Note the focal cluster of endometrial glands with atypical hyperplasia (arrow).

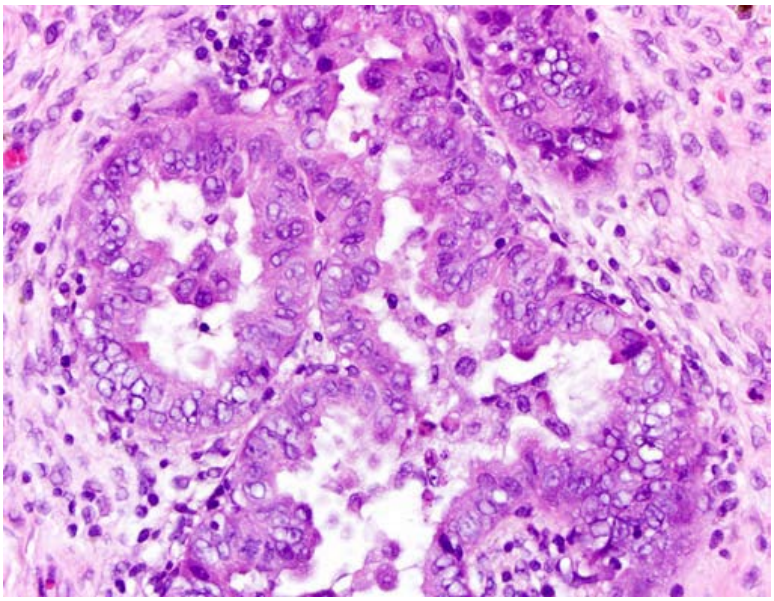

Figure 15. Higher Magnification of the Endometrial Glands in Figure 14 (H&E)

The glands are separated by scant stroma and lined by multiple layers of disorganized epithelium. The thickened epithelium projects into the glandular lumens forming multiple thickened infoldings and projections. Epithelial cells lining the glands show loss of nuclear polarization, karyomegaly, and cellular pleiomorphism.

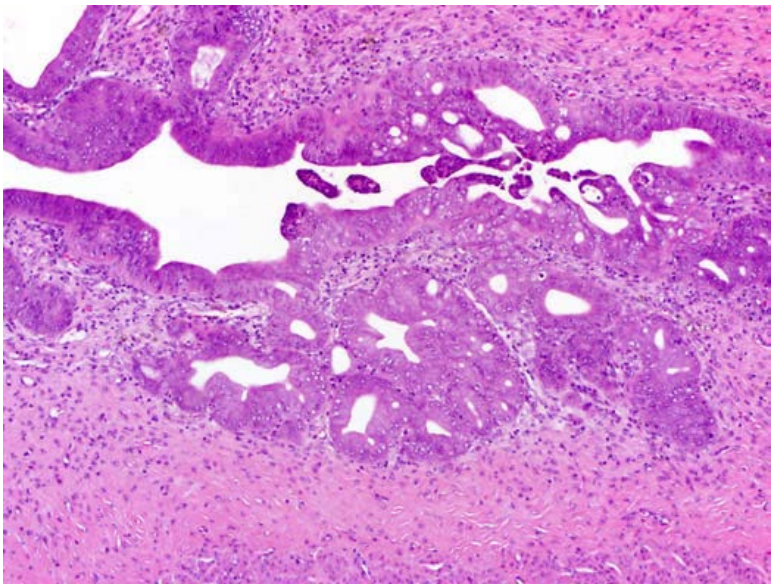

Figure 16. Uterus from a Female Wistar Han Rat Administered 1,000 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

This is an example of atypical hyperplasia of both the glandular epithelium and the uterine luminal epithelium.

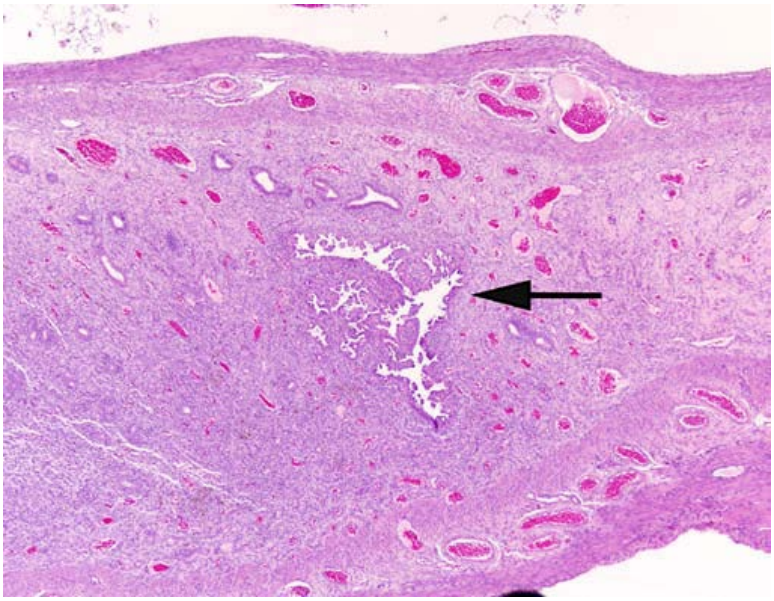

Figure 17. Uterus from a Female Wistar Han Rat Administered 500 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

This is an example of a papillary type of endometrial gland atypical hyperplasia (arrow).

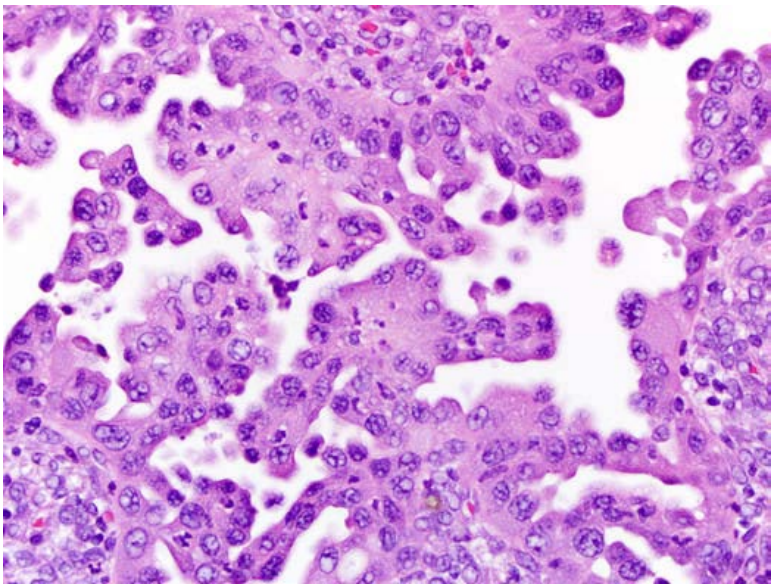

Figure 18. Higher Magnification of the Lesion in Figure 17 (H&E)

The papillary type of atypical hyperplasia consists of numerous small branching projections of epithelium extending into the uterine gland, occasionally on small fibrovascular stalks. Epithelial blebbing and loss of nuclear polarization are also present.

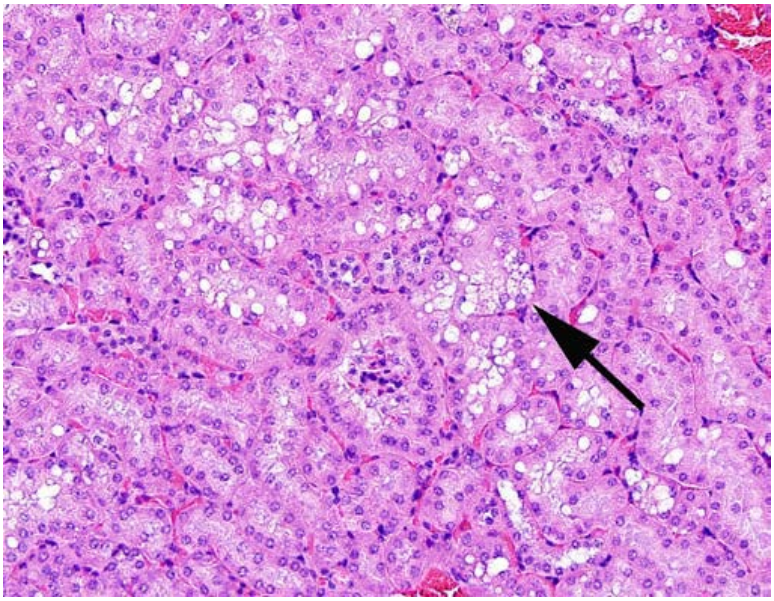

Figure 19. Normal Renal Cortex in a Vehicle Control Male B6C3F1/N Mouse in the Two-year Gavage Study of Tetrabromobisphenol A (H&E)

Note the clear autophagic vacuoles in the proximal epithelial cells (arrow).

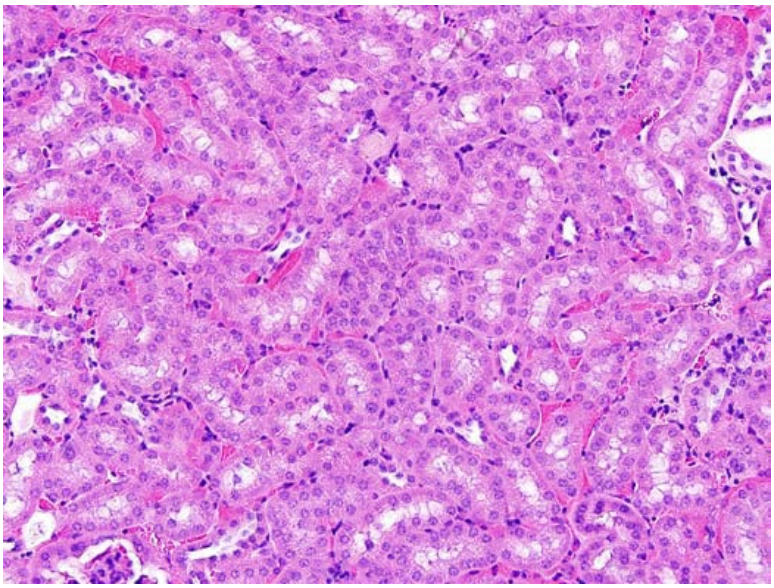

Figure 20. Cytoplasmic Alteration in the Kidney of a Male B6C3F1/N Mouse Administered 1,000 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

There is an absence of clear vacuoles in the proximal tubule epithelial cells, diagnosed as “cytoplasmic alteration.”

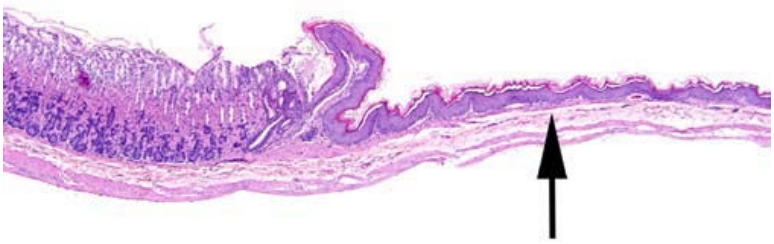

Figure 21. Normal Stomach in a Vehicle Control Male B6C3F1/N Mouse in the Two-year Gavage Study of Tetrabromobisphenol A (H&E)

Glandular stomach is on the left and forestomach is on the right (arrow).

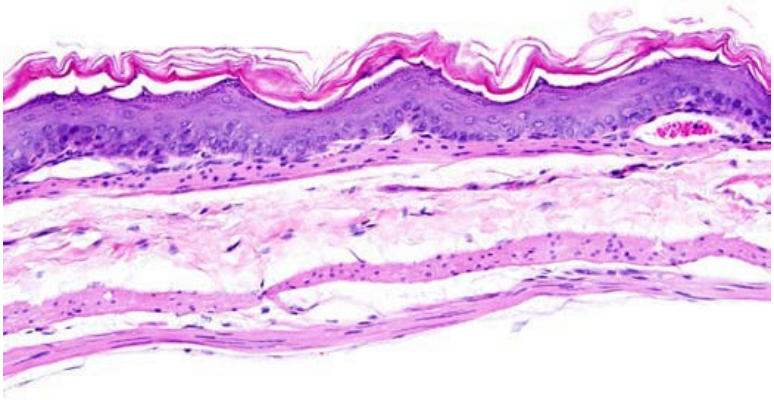

Figure 22. Higher magnification of the normal forestomach in Figure 21 (H&E)

The thin keratinized stratified squamous epithelial layer is two to three cell layers thick.

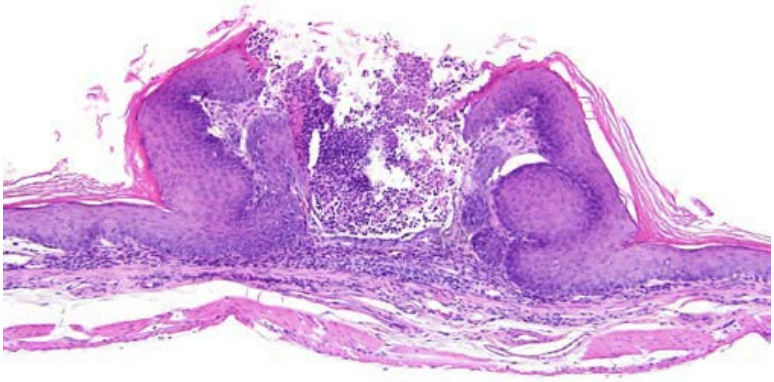

Figure 23. Ulcer in the Forestomach of a Male B6C3F1/N Mouse Administered 1,000 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

A focal area of ulceration is characterized by loss of the entire thickness of squamous epithelium. There is secondary inflammation within the lesion and hyperplasia of the adjacent epithelium.

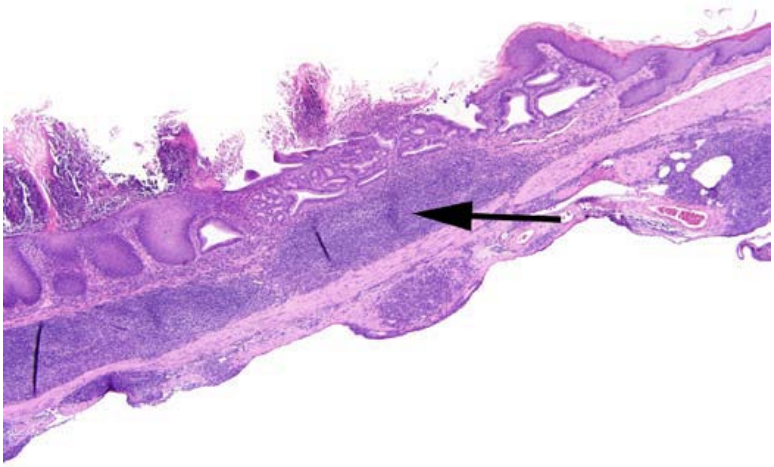

Figure 24. Ulcer in the Forestomach of a Male B6C3F1/N Mouse Administered 1,000 mg/kg Tetrabromobisphenol A by Gavage for Two Years (H&E)

There is a focal area of prior ulceration with healing. Underlying the area of injury is a mononuclear cell infiltrate (arrow) indicative of a robust immune response.

Discussion

These tetrabromobisphenol A rodent studies were performed to evaluate the toxic and carcinogenic potential of this widely used flame retardant. 3-month studies were conducted in F344/NTac rats and B6C3F1/N mice, and 2-year studies were conducted in Wistar Han rats and B6C3F1/N mice. A special 3-month interim evaluation using Wistar Han rats was conducted as part of the 2-year study in order to compare the results in this rat strain with those from the 3-month F344/NTac rat study.

In the 3-month tetrabromobisphenol A studies, there was no treatment-related mortality in F344/NTac rats or B6C3F1/N mice, and final mean body weights of dosed groups were similar to those of the vehicle controls. Liver weights of 500 and 1,000 mg/kg male mice and male and female rats and 1,000 mg/kg female mice were increased (increases of 9% to 14%). Increases in liver CYP2B (PROD) activity were seen at 500 and 1,000 mg/kg and treatment-related decreases in thyroxine (T_4) concentration were seen in male and female rats. There were no treatment-related lesions in rats or mice in the 3-month studies other than an increase in the incidences of renal tubule cytoplasmic alteration in 500 and 1,000 mg/kg male mice. The results of the 3-month interim evaluation in the 2-year Wistar Han rat study (vehicle control and 1,000 mg/kg groups) were similar to those in the 3-month F344/NTac rat study, where there was no treatment-related mortality, mean body weights in the treated groups were similar to those of the vehicle control groups, and there were no treatment related lesions. Liver weights at 1,000 mg/kg were 4% to 7% greater than those of the vehicle controls.

Thyroid hormones have been shown to play an integral role in testicular development[122] and also to affect ovarian follicular maturation[10], although, in the current NTP studies, there was no evidence for a tetrabromobisphenol A-associated disruption of reproductive evaluations in the 3-month studies. In addition, although in vitro studies suggest tetrabromobisphenol A could increase estrogen activity, reproductive studies with tetrabromobisphenol A have not demonstrated a significant estrogen-mediated response (e.g., accelerated vaginal opening) consistent with either estrogen receptor agonist activity or increased circulating estrogen levels due to inhibition of estradiol sulfation.

The high dose selected for the 2-year rat and mouse studies was 1,000 mg/kg because, although there were some increases in liver weights and alterations in clinical pathology endpoints in rats and/or mice in the 3-month studies, these effects were not considered to be severe enough to compromise the conduct of a 2-year study.

In the 2-year studies, survival of male and female Wistar Han rats in the treated groups was comparable to the vehicle controls. Survival of 1,000 mg/kg male and female mice and final mean body weight of 1,000 mg/kg female mice were reduced. While gastrointestinal toxic lesions were not present at 3 months in mice, gastrointestinal toxicity was found in treated mice in the 2-year study, although the severities of the lesions did not increase with dose. The ability of tetrabromobisphenol A to cause oxidative damage[30] may have contributed to this toxicity. The reduced capacity of animals to repair oxidative damage as they age may account for the occurrence of gastrointestinal toxicity at 2 years but not at 3 months[123-127].

There was tetrabromobisphenol A carcinogenic activity in the uterus of female rats and the liver of male mice. The occurrence of testicular tumors in male rats and large intestine tumors and

hemangiosarcoma in male mice may have been related to tetrabromobisphenol A administration. Chemical induction of uterine tumors in rats was considered to be an important finding, not present in most of the previous NTP 2-year chemical carcinogenesis studies.

No treatment-related lesions were found in the uterus at 3 months in the F344/NTac rat, Wistar Han rat, or B6C3F1/N mouse, but treatment-related neoplastic and non-neoplastic uterine lesions were found in the 2-year Wistar Han rat study. These lesions included increased incidences of atypical endometrium hyperplasia, uterine adenocarcinoma, and malignant mixed Müllerian tumor. The occurrence of uterine epithelial tumors (predominantly adenocarcinoma) was considered to be clear evidence for carcinogenic activity because the incidences of malignant uterine epithelial tumors were significantly increased in the 500 and 1,000 mg/kg groups by pairwise comparison and by the trend test ($P < 0.001$). In addition, the incidences of the malignant uterine eithelial tumors exceeded the historical control ranges in all treatment groups. The predominant tumor type in rats was uterine adenocarcinoma, which is also the predominant uterine tumor type in humans[128; 129].

The initial uterine neoplastic findings in the 2-year Wistar Han study were based on the traditional NTP histopathology review of a transverse section through each uterine horn 0.5 cm from the cervix of the uterus. Cervix and vagina were not present in the original transverse review, except in several cases where a large mass was identified during necropsy. The extended residual tissue review involved trimming, embedding, and sectioning the remaining uterine tissue, cervix, and vagina longitudinally. The reasons for the residual longitudinal review were 1) a need to determine the site of origin for the cervical and vaginal tumors, 2) the need to have a complete review of cervices for stromal hyperplasia and stromal fibrosis, and 3) to look for additional neoplasms. In this residual longitudinal review, additional non-neoplastic and neoplastic uterine lesions were found that supported the initial findings. In several cases, the additional neoplasms were found in an animal already diagnosed with uterine cancer during the original transverse review. During the residual longitudinal review, atypical endometrial hyperplasia was diagnosed that was not present in the original transverse sections. This is a potentially preneoplastic lesion and the increased incidences in all treated groups were statistically significant. Cystic endometrial hyperplasia was also identified in the original transverse and residual longitudinal reviews. In the original transverse review, this appeared to be a treatment-related lesion, however, after the residual longitudinal review, additional lesions were identified in all groups, and the differences were no longer statistically significant.

Morphologically similar atypical endometrial hyperplasia has been diagnosed in women and rats. In women, this is considered a preneoplastic lesion and is diagnosed as simple or complex, depending on the architectural changes in the lesion[130; 131]. In both types, there are atypical changes in glandular cells, including cell stratification, tufting, loss of nuclear polarity, enlarged nuclei, and an increase in mitotic activity. These changes are similar to those seen in cancer cells, but atypical hyperplasia does not show invasion into the surrounding connective tissue. Most cases of atypical hyperplasia in women result from high levels of estrogens with insufficient levels of progesterone-like hormones. Risk factors include obesity, polycystic ovary syndrome, estrogen producing tumors (e.g., granulosa cell tumor), and some estrogen replacement therapies. Atypical endometrial hyperplasia is considered a significant risk factor for the development or coexistence of endometrial cancer. Among patients with atypical endometrial hyperplasia, 22% will eventually develop cancer[132].

The occurrence of uterine adenocarcinomas was supported by increases in the incidences of uterine adenomas, significant by the trend statistic (P = 0.010). Uterine adenomas were well-circumscribed endometrium masses with no evidence of invasion into the myometrium. In contrast, uterine adenocarcinomas were less well-circumscribed than adenomas and showed evidence of invasion into the myometrium in some cases and metastasis to other tissues (e.g., lung). Uterine tumor metastases were found in the intestine, liver, mesentery, pancreas, glandular stomach, adrenal cortex, lymph nodes, spleen, thymus, skeletal muscle, lung, kidney, and urinary bladder. In humans, uterine endometrial carcinomas may be staged according to tumor size, involvement of adjacent organ systems (e.g., vagina), and involvement of nonadjacent tissues and lymph nodes[133-136].

The malignant mixed Müllerian cell tumors seen in Wistar Han rats treated with tetrabromobisphenol A are uncommon tumors thought to arise from a pluripotent Müllerian duct cell[137]. Dysregulation of the cell cycle and apoptotic regulatory proteins have been reported to be involved in malignant mixed Müllerian tumor neoplasia[138]. In humans, these tumors account for about 5% of all malignant tumors derived from the body of the uterus, and they are highly malignant and associated with a poor prognosis[139; 140]. Risk factors are similar to those of adenocarcinomas and include obesity, exogenous estrogen therapies, nulliparity, tamoxifen therapy, and pelvic irradiation. There are two types of malignant mixed Müllerian cell tumors that can display differentiation along multiple pathways[137]: the homologous type contains a sarcomatous component that is made up of tissues found in the uterus such as endometrial, fibrous, or smooth muscle tissues; the heterologous type is made up of tissue not found in the uterus such as cartilage, skeletal muscle, or bone. Both types were seen in this study. For statistical analysis, malignant mixed Müllerian cell tumors were combined with the uterine adenomas and adenocarcinomas because, based on our knowledge of histogenesis of this tumor from the human literature, the epithelial component is considered to be the “driving force” of the tumor and the mesenchymal component is considered to be derived from the epithelial component. Evidence for this histogenesis theory includes clinical, histopathologic, immunohistochemical, ultrastructural, tissue culture, and molecular data[141]. As an example, the behavior of human malignant mixed Müllerian cell tumors is more related to the type and grade of the epithelium rather than the mesenchymal component. For this reason, treatments are generally aimed at the epithelial component. Moreover, metastases tend to be the epithelial component, as they all were in this study.

The Wistar Han rat is responsive to chemical induction of uterine tumors by other chemicals. Tamoxifen treatment (given on days 2 to 5 after birth at 1 mg/kg body weight per day) caused uterine adenocarcinomas in Wistar Han rats at 24 to 35 months[142]. This induction of uterine tumors in the Wistar Han rat by tamoxifen has been confirmed by the finding that tamoxifen treatment caused uterine adenocarcinomas in humans; the IARC has classified tamoxifen as a Group 1 carcinogen (carcinogenic to humans)[143].

Tetrabromobisphenol A may interfere with complex gene regulation systems[144]. Alterations in proto-oncogenes, tumor suppressor genes, apoptosis genes, and DNA repair genes are central to the process of carcinogenesis, and the study of these alterations has revealed mechanistic insights into the process of chemical carcinogenesis[145]. Chemical exposure (or exposure to chemical metabolites) may induce direct alterations in DNA, leading to chemical-DNA adduct formation, or may induce mutations indirectly through secondary mechanisms such as oxidative stress, cytotoxicity, or regenerative proliferation. Changes in the frequency or type of DNA mutation in

chemically exposed animals may reveal chemical-related alterations that may drive carcinogenesis, or promote endogenous tumorigenic events.

The *Tp53* tumor suppressor gene is responsible for cell cycle checkpoint maintenance, apoptosis, and genomic stability[146]. *Tp53* mutation results in the generation of a mutant protein that has lost normal tumor suppressor function and has additional oncogenic properties including promotion of cell survival and increased cell proliferation[147]. Loss of cell cycle checkpoint control due to *Tp53* mutation also results in inadequate DNA repair, which contributes to the generation of additional mutations in the genome. A number of "hot spot" regions in the central DNA binding domain of human *Tp53* are more prone to mutational events[147; 148], and the location and type of mutation in corresponding regions of the rat *Tp53* gene (exons 5 to 8) are commonly used to study chemical-induced carcinogenesis and may reflect exposure to specific carcinogens[149].

Alterations in *Tp53* signaling due to mutation or dysregulation of the *Tp53* signaling pathway are important events in the pathogenesis of many different types of cancer in rodents and humans, including aggressive endometrial cancer[148; 150-154]. *Tp53* mutations in human endometrial cancer are associated with advanced disease and a poor prognosis[154]. They occur at a high rate in high grade tumors (80% to 90%)[154-157] and are thought to possibly occur as a late event in the development of aggressive endometrial cancer.

In the current study, NTP's primary objective was to evaluate spontaneous adenocarcinomas and adenocarcinomas from tetrabromobisphenol A-dosed rats for alterations in the frequencies of *Tp53* mutations to determine if the incidences of these mutations in treated rats difered from spontaneous tumors (Appendix M). Exons 5 to 8 of the rat *Tp53* gene were examined for mutations in spontaneous uterine adenocarcinomas and adenocarcinomas from tetrabromobisphenol A-dosed rats. A statistically significant increase in the incidence of *Tp53* mutations was observed in uterine adenocarcinomas from tetrabromobisphenol A-dosed rats compared to spontaneous tumors from control rats ($P < 0.05$). There was no difference between the mutation spectra of spontaneous tumors and those from tetrabromobisphenol A-dosed animals although two treated rats harbored multiple *Tp53* mutations per tumor.

The increased incidence of *Tp53* mutations observed in uterine adenocarcinomas from tetrabromobisphenol A-dosed rats compared to spontaneous tumors suggests that uterine carcinogenesis in tetrabromobisphenol A-dosed animals may be at least partly driven by alterations in the *Tp53* signaling pathway. It is unclear whether or not tetrabromobisphenol A exposure induced a direct genotoxic event leading to *Tp53* mutations, or if the increased incidence of mutations was a result of a secondary nongenotoxic event. Tetrabromobisphenol A was not mutagenic in bacterial studies[77] nor did it induce chromosomal damage in the form of micronuclei in progenitor red blood cells in the bone marrow (Appendix E). Although positive results in bacterial mutagenicity assays and rodent micronucleus tests are highly predictive of rodent carcinogenicity, negative results in these assays are not good predictors of noncarcinogenicity[117; 121; 158].

The incidences of testicular interstitial cell adenoma occurred with a significant positive trend in male Wistar Han rats (0 mg/kg, 0/50; 250 mg/kg, 0/50; 500 mg/kg, 1/50; 1,000 mg/kg, 3/50), and the incidence at the high dose exceeded the historical control incidence for all routes (4/150). However, the incidence in the vehicle control group was at the low end of the historical control range for this tumor and the incidence in the high dose group was only one greater than that in

some of the historical studies; thus, these tumors were considered to be equivocal evidence for carcinogenic activity.

The incidences of hepatoblastoma in 250 and 500 mg/kg male mice were significantly greater than that in the concurrent vehicle controls and exceeded the historical control ranges both for corn oil gavage and for all routes of administration. In addition, there was supportive evidence from increased incidences of liver foci and multiple hepatocellular adenomas in treated groups of male mice. Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma are considered to represent a biological and morphological continuum[159]. Hepatoblastomas are uncommon spontaneous neoplasms that may occur after chemical administration (primarily in mice) and have previously been seen as a treatment-related effect in mice in several NTP studies (benzofuran, ethylene thiourea, *o*-nitroanisole, coumarin, oxazepam, methyl-phenidate hydrochloride, 1-amino-2,4-dibromoanthra-quinone, pyridine, primidone, goldenseal root powder, and G*inkgo biloba* extract)[160-170]. The incidences of hepatoblastoma in this study were considered some evidence of carcinogenic activity, but not clear evidence, because the combined incidences of hepatocellular carcinomas and hepatoblastomas were significant only in the 250 mg/kg group and the trend test was not significant.

The incidences of renal tubule cytoplasmic alteration were increased in the treated groups of male mice in the 2-year study. Cytoplasmic alteration of the renal tubule is a lesion that is defined as the reduction or loss of normal vacuoles in the proximal tubules of the outer cortex in male mice. These vacuoles have been shown to be autophagic vacuoles[171]. They are part of the normal sequestration and degradation of organelles and membrane trafficking and recycling in the renal proximal tubule cells. This morphologic sexual dimorphism of the mouse kidney is also accompanied by an enzymatic dimorphism. A greater kidney acid hydrolase activity is correlated with an expansive lysosomal-vacuolar system in the proximal tubule cells of male mice. This sexual dimorphism has been shown to be dependent on endogenous testosterone. Following orchiectomy, there is a marked decrease in kidney enzymes and urinary excretion of hydrolases and protein. Even greater increases in kidney enzymes, lysosomal enzymuria, and proteinuria have been induced in female mice and orchiectomized male mice by testosterone administration. Thus, testosterone stimulates RNA and protein synthesis, modulates the structural and functional properties of mitochondria, and increases the activity of the lysosomal-vacuolar system in proximal tubule cells by augmenting intracellular autophagy.

The combined occurrence of large intestine tumors (cecum or colon) in male mice (one cecum carcinoma, one colon carcinoma, and one colon adenoma) at 500 mg/kg was considered to be equivocal evidence of carcinogenic activity because the occurrence of these intestinal tumors was significant by the trend statistic ($P = 0.039$), and the incidence in the 500 mg/kg group exceeded the historical control ranges for corn oil gavage studies and for all routes of administration. The occurrence of these intestinal tumors was not considered to be some evidence of a carcinogenic effect because the increased incidence of these tumors at 500 mg/kg was low, there was no supportive evidence of a carcinogenic effect in the female mice, and the increase at 500 mg/kg was not significant by the pairwise Poly-3 statistic.

The occurrence of hemangiosarcoma (all organs) in male mice was considered to be equivocal evidence of a carcinogenic effect because the increased incidence at 500 mg/kg was significant and the trend test for incidences of this tumor was significant. This was not considered to be some evidence for a carcinogenic effect because the incidence of hemangiosarcoma in the

vehicle controls was at the low end of the historical control ranges for corn oil gavage studies and all routes of administration (2%-18% for corn oil gavage studies and 2%-18% for all routes of administration) and the incidence at 500 mg/kg (16%) was within both historical control ranges.

In the current 2-year study, the incidences of bone fibro-osseous lesion, a background lesion in female mice, were significantly decreased in treated groups of females. These decreases may have occurred, in part, due to the early deaths of 1,000 mg/kg mice from gastrointestinal toxicity and/or decreased incidences of cystic ovarian lesions that could have altered the hormonal status of the dosed females. The etiology of fibro-osseous lesion is unknown but it is more prevalent in females and an association with cystic ovaries and cystic endometrial hyperplasia suggests that there may have been an altered estrogen or sex hormone status in the dosed females.

Uterine tumors have been attributed to both estrogenic and nonestrogenic effects[172]. In the current study, the occurrence of uterine tumors may be related to both the ability of tetrabromobisphenol A-derived metabolites to disrupt hormone signaling and the potential of tetrabromobisphenol A to cause oxidative damage[30; 61; 62]. Glucuronidases in the uterus or other organs[173; 174] may work to release free tetrabromobisphenol A from its conjugated form, thus increasing the potential for free radical formation at target sites. Conjugation is the major biotransformation pathway for tetrabromobisphenol A in rodents and this pathway is shared by estrogen and its potentially genotoxic catechol metabolite[175]. Competition for glucuronosyltransferases and/or sulfotransferases by tetrabromobisphenol A could result in higher circulating levels of estrogen and increased formation of estrogen-derived reactive species, especially following exposure to high concentrations of the chemical. Either process may contribute to tumorigenesis in the uterus.

Tetrabromobisphenol A may disrupt endocrine signaling through direct interaction with endocrine receptors or indirectly, through binding to estradiolsulfotransferase, thereby preventing sulfation of estradiol and its subsequent elimination[55; 176]. Tetrabromobisphenol A has a low IC_{50} (12 to 33 nM) sulfotransferase enzyme (SULT1E1) inhibition level[177]. Crystallography studies show that tetrabromobisphenol A can bind to SULT1E1 and that the phenolic ring is critical for stable binding[178].

Conclusions

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity*[a] of tetrabromobisphenol A in male Wistar Han rats based on the occurrence of testicular adenoma. There was *clear evidence of carcinogenic activity* of tetrabromobisphenol A in female Wistar Han rats based on increased incidences of uterine epithelial tumors (predominantly uterine adenocarcinoma). There was *some evidence of carcinogenic activity* of tetrabromobisphenol A in male B6C3F1/N mice based on increased incidences of hepatoblastoma. The increased incidences of large intestine neoplasms and hemangiosarcoma (all organs) may have been related to chemical administration. There was *no evidence of carcinogenic activity* of tetrabromobisphenol A in female B6C3F1/N mice administered 250 or 500 mg/kg.

Administration of tetrabromobisphenol A resulted in increased incidences of non-neoplastic lesions of the uterus and ovary in female rats, the liver and kidney in male mice, and the forestomach in male and female mice.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. See summary of the peer review panel comments and the public discussion on this Technical Report in Appendix N.

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Appendix A. Summary of Lesions in Male Wistar Han Rats in the Two-Year Gavage Study of Tetrabromobisphenol A

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month Interim Evaluation	10	–	–	10
Early deaths				
Accidental deaths	1	–	–	3
Moribund	14	18	8	6
Natural deaths	2	4	4	2
Survivors				
Died last week of study	1	–	–	–
Terminal kill	32	28	38	39
Animals examined microscopically	60	50	50	60
Systems Examined at 3 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, blood vessel	1 (2%)	–	–	–
Intestine small, jejunum	(50)	(50)	(50)	(50)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Hepatocellular adenoma	–	1 (2%)	–	–
Mesentery	(3)	(3)	(0)	(2)
Pancreas	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Acinus, adenoma	1 (2%)	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(49)
Myoepithelioma	1 (2%)	–	–	–
Sublingual gland, adenoma	–	–	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Leiomyosarcoma	–	1 (2%)	–	–
Squamous cell papilloma	–	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Adventitia, hemangiosarcoma	1 (2%)	–	–	–
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney	–	–	–	1 (2%)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Adenoma	–	1 (2%)	–	1 (2%)
Adrenal medulla	(49)	(50)	(49)	(50)
Pheochromocytoma benign	–	–	1 (2%)	–
Pheochromocytoma malignant	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	2 (4%)	2 (4%)
Parathyroid gland	(45)	(48)	(47)	(49)
Adenoma	–	–	1 (2%)	–
Pituitary gland	(50)	(49)	(50)	(48)
Pars distalis, adenoma	20 (40%)	24 (49%)	13 (26%)	13 (27%)
Pars distalis, adenoma, multiple	1 (2%)	–	1 (2%)	3 (6%)
Pars intermedia, adenoma	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Bilateral, C-cell, adenoma	–	–	1 (2%)	–
C-cell, adenoma	4 (8%)	8 (16%)	7 (14%)	5 (10%)
C-cell, adenoma, multiple	1 (2%)	–	–	–
C-cell, carcinoma	–	1 (2%)	–	–
Follicular cell, adenoma	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Follicular cell, carcinoma	–	1 (2%)	–	–
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Bilateral, interstitial cell, adenoma	–	–	1 (2%)	–
Interstitial cell, adenoma	–	–	–	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(0)	(2)	(1)
Pancreatic, hemangiosarcoma, metastatic, blood vessel	1 (33%)	–	–	–
Lymph node, mandibular	(49)	(50)	(50)	(48)
Fibrous histiocytoma, metastatic, skin	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)	–	–	–
Hemangiosarcoma	1 (2%)	2 (4%)	1 (2%)	
Hemangiosarcoma, metastatic, blood vessel	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Thymus	(49)	(49)	(49)	(50)
Fibrous histiocytoma, metastatic, skin	–	–	1 (2%)	–
Schwannoma malignant	–	–	1 (2%)	–
Thymoma benign	1 (2%)	1 (2%)	–	3 (6%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Integumentary System				
Mammary gland	(47)	(50)	(50)	(50)
Fibroadenoma	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	2 (4%)	1 (2%)	–	–
Basal cell carcinoma	1 (2%)	–	–	–
Fibroma	–	–	2 (4%)	–
Fibrous histiocytoma	–	–	1 (2%)	–
Keratoacanthoma	4 (8%)	–	1 (2%)	2 (4%)
Schwannoma malignant	–	–	–	2 (4%)
Squamous cell papilloma	1 (2%)	–	1 (2%)	1 (2%)
Subcutaneous tissue, fibroma	–	1 (2%)	–	–
Subcutaneous tissue, fibrous histiocytoma	–	1 (2%)	–	1 (2%)
Subcutaneous tissue, schwannoma malignant	2 (4%)	1 (2%)	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skeletal muscle	1 (2%)	–	–	–
Skeletal muscle	(1)	(0)	(0)	(1)
Rhabdomyosarcoma	–	–	–	1 (100%)
Sarcoma	1 (100%)	–	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign	1 (2%)	–	–	1 (2%)
Meningioma malignant	–	1 (2%)	–	–
Peripheral nerve	(0)	(2)	(1)	(0)
Spinal cord	(0)	(2)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	–	–	–	1 (2%)
Carcinoma, metastatic, kidney	–	–	–	1 (2%)
Carcinoma, metastatic, thyroid gland	–	1 (2%)	–	–
Fibrous histiocytoma, metastatic, skin	–	1 (2%)	1 (2%)	–
Mediastinum, lipoma	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(49)	(50)	(50)	(50)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, carcinoma	–	–	–	1 (2%)
Transitional epithelium, carcinoma	–	–	–	1 (2%)
Urethra	(1)	(0)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma	1 (2%)	–	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	1 (2%)	–	–	–
Lymphoma malignant	–	–	–	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
Two-year study	34	37	34	33
Total primary neoplasms				
Two-year study	54	50	44	48
Total animals with benign neoplasms				
Two-year study	30	32	29	28
Total benign neoplasms				
Two-year study	46	41	39	40
Total animals with malignant neoplasms				
Two-year study	8	8	5	8
Total malignant neoplasms				
Two-year study	8	9	5	8
Total animals with metastatic neoplasms				
Two-year study	2	2	1	2
Total metastatic neoplasms				
Two-year study	4	2	8	3

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table A-2. Statistical Analysis of Primary Neoplasms in Male Rats in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate[a]	21/50 (42%)	24/49 (49%)	14/50 (28%)	16/48 (33%)
Adjusted rate[b]	47.4%	52.4%	29.8%	37.0%
Terminal rate[c]	11/33 (33%)	10/28 (36%)	9/38 (24%)	14/38 (37%)
First incidence (days)	485	397	511	608
Poly-3 test[d]	P = 0.084N	P = 0.395	P = 0.063N	P = 0.221N
Pituitary Gland (Pars Intermedia): Adenoma				
Overall rate	2/50 (4%)	1/49 (2%)	3/50 (6%)	2/48 (4%)
Adjusted rate	5.0%	2.4%	6.6%	4.7%
Terminal rate	2/33 (6%)	1/28 (4%)	3/38 (8%)	2/38 (5%)
First incidence (days)	727 (T)	727 (T)	727 (T)	727 (T)
Poly-3 test	P = 0.534	P = 0.488N	P = 0.559	P = 0.669N
Skin: Keratoacanthoma				
Overall rate	4/50 (8%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.1%	0.0%	2.2%	4.5%
Terminal rate	4/33 (12%)	0/28 (0%)	0/38 (0%)	2/39 (5%)
First incidence (days)	727 (T)	–[e]	723	727 (T)
Poly-3 test	P = 0.362N	P = 0.053N	P = 0.142N	P = 0.289N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	5/50 (10%)	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adjusted rate	12.6%	0.0%	4.4%	6.7%
Terminal rate	5/33 (15%)	0/28 (0%)	0/38 (0%)	2/39 (5%)
First incidence (days)	727 (T)	–	673	605
Poly-3 test	P = 0.434N	P = 0.026N	P = 0.165N	P = 0.295N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	7/50 (14%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	17.6%	2.4%	4.4%	6.7%
Terminal rate	6/33 (18%)	0/28 (0%)	0/38 (0%)	2/39 (5%)
First incidence (days)	695	596	673	605
Poly-3 test	P = 0.159N	P = 0.023N	P = 0.051N	P = 0.114N
Skin: Fibroma or Fibrous Histiocytoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	4.8%	6.5%	2.3%
Terminal rate	0/33 (0%)	1/28 (4%)	2/38 (5%)	1/39 (3%)
First incidence (days)	–	694	393	727 (T)
Poly-3 test	P = 0.508	P = 0.249	P = 0.148	P = 0.521

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Testes: Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.2%	6.8%
Terminal rate	0/33 (0%)	0/28 (0%)	1/38 (3%)	3/39 (8%)
First incidence (days)	–	–	727 (T)	727 (T)
Poly-3 test	P = 0.023	–[f]	P = 0.526	P = 0.138
Thymus: Thymoma Benign				
Overall rate	1/49 (2%)	1/49 (2%)	0/49 (0%)	3/50 (6%)
Adjusted rate	2.5%	2.4%	0.0%	6.7%
Terminal rate	0/33 (0%)	0/28 (0%)	0/38 (0%)	2/39 (5%)
First incidence (days)	502	678	–	517
Poly-3 test	P = 0.180	P = 0.752N	P = 0.476N	P = 0.349
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	7.5%	2.4%	6.6%	4.5%
Terminal rate	2/33 (6%)	1/28 (4%)	2/38 (5%)	2/39 (5%)
First incidence (days)	622	727 (T)	673	727 (T)
Poly-3 test	P = 0.487N	P = 0.290N	P = 0.604N	P = 0.456N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	7.5%	4.8%	6.6%	4.5%
Terminal rate	2/33 (6%)	2/28 (7%)	2/38 (5%)	2/39 (5%)
First incidence (days)	622	727 (T)	673	727 (T)
Poly-3 test	P = 0.415N	P = 0.481N	P = 0.604N	P = 0.456N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	5/50 (10%)	8/50 (16%)	8/50 (16%)	5/50 (10%)
Adjusted rate	12.3%	18.9%	17.7%	11.3%
Terminal rate	3/33 (9%)	7/28 (25%)	8/38 (21%)	5/39 (13%)
First incidence (days)	496	596	727 (T)	727 (T)
Poly-3 test	P = 0.397N	P = 0.297	P = 0.346	P = 0.579N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	9/50 (18%)	8/50 (16%)	5/50 (10%)
Adjusted rate	12.3%	21.2%	17.7%	11.3%
Terminal rate	3/33 (9%)	7/28 (25%)	8/38 (21%)	5/39 (13%)
First incidence (days)	496	596	727 (T)	727 (T)
Poly-3 test	P = 0.355N	P = 0.212	P = 0.346	P = 0.579N

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.5%	4.8%	2.2%	0.0%
Terminal rate	2/33 (6%)	1/28 (4%)	0/38 (0%)	0/39 (0%)
First incidence (days)	687	673	654	–
Poly-3 test	P = 0.053N	P = 0.476N	P = 0.260N	P = 0.101N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	9.9%	4.8%	2.2%	0.0%
Terminal rate	2/33 (6%)	1/28 (4%)	0/38 (0%)	0/39 (0%)
First incidence (days)	502	673	654	–
Poly-3 test	P = 0.025N	P = 0.320N	P = 0.146N	P = 0.050N
All Organs: Benign Neoplasms				
Overall rate	30/50 (60%)	32/50 (64%)	29/50 (58%)	28/50 (56%)
Adjusted rate	66.3%	68.1%	60.8%	61.1%
Terminal rate	19/33 (58%)	16/28 (57%)	22/38 (58%)	24/39 (62%)
First incidence (days)	244	397	511	517
Poly-3 test	P = 0.276N	P = 0.514	P = 0.370N	P = 0.382N
All Organs: Malignant Neoplasms				
Overall rate	8/50 (16%)	8/50 (16%)	5/50 (10%)	8/50 (16%)
Adjusted rate	18.7%	18.8%	10.6%	17.3%
Terminal rate	2/33 (6%)	4/28 (14%)	1/38 (3%)	4/39 (10%)
First incidence (days)	196	625	393	447
Poly-3 test	P = 0.443N	P = 0.607	P = 0.215N	P = 0.540N
All Organs: Benign or Malignant Neoplasms				
Overall rate	34/50 (68%)	37/50 (74%)	34/50 (68%)	33/50 (66%)
Adjusted rate	70.8%	77.6%	68.5%	68.7%
Terminal rate	19/33 (58%)	18/28 (64%)	23/38 (61%)	25/39 (64%)
First incidence (days)	196	397	393	447
Poly-3 test	P = 0.335N	P = 0.298	P = 0.491N	P = 0.501N

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for pituitary gland, testes, thymus, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal kill.
[d]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.

Table A-3. Historical Incidence of Adenoma of the Testis in Control Male Wistar Han Rats[a]

	Incidence in Controls
Overall Historical Incidence: All Routes	
Total (%)	4/150 (2.7%)
Mean ± standard deviation	2.7% ± 2.3%
Range	0%–4%

[a]Data as of June 2013.

Table A-4. Summary of the Incidence of Non-neoplastic Lesions in Male Rats in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month Interim Evaluation	10	–	–	10
Early deaths				
Accidental deaths	1	–	–	3
Moribund	14	18	8	6
Natural deaths	2	4	4	2
Survivors				
Died last week of study	1	–	–	–
Terminal kill	32	28	38	39
Animals examined microscopically	60	50	50	60
Alimentary System				
Stomach, glandular	(10)	–	–	(10)
Cardiomyopathy	1 (10%)	–	–	–
Cardiovascular System				
Heart	(10)	–	–	(10)
Inflammation, chronic	1 (10%)	–	–	1 (10%)
Endocrine System				
Pituitary gland	(10)	–	–	(10)
Cyst	1 (10%)	–	–	1 (10%)
Thyroid gland	(10)	–	–	(10)
Cyst	–	–	–	1 (10%)
Ectopic thymus	1 (10%)	–	–	–
Genital System				
Preputial gland	(10)	–	–	(10)
Inflammation, chronic	1 (10%)	–	–	2 (20%)
Prostate	(10)	–	–	(10)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Hyperplasia	–	–	–	1 (10%)
Inflammation	–	–	–	1 (10%)
Seminal vesicle	(10)	–	–	(10)
Inflammation	1 (10%)	–	–	–
Hematopoietic System				
Lymph node, mesenteric	(10)	–	–	(10)
Hyperplasia, lymphoid	4 (40%)	–	–	3 (30%)
Necrosis	1 (10%)			–
Respiratory System				
Lung	(10)	–	–	(10)
Infiltration cellular, histiocyte	1 (10%)	–	–	–
Inflammation, chronic	–	–	–	1 (10%)
Perivascular, inflammation, chronic active	1 (10%)	–	–	2 (20%)
Nose	(10)	–	–	(10)
Olfactory epithelium, accumulation, hyaline droplet	1 (10%)	–	–	3 (30%)
Olfactory epithelium, inflammation, chronic active	–	–	–	1 (10%)
Respiratory epithelium, accumulation, hyaline droplet	1 (10%)	–	–	1 (10%)
Special Senses System				
Harderian gland	(10)	–	–	(10)
Inflammation, chronic	–	–	–	1 (10%)
Urinary System				
Kidney	(10)	–	–	(10)
Hydronephrosis	1 (10%)	–	–	–
Nephropathy	2 (20%)	–	–	1 (10%)
Systems Examined at 3 Months with No Lesions Observed				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Ulcer	–	–	1 (2%)	–
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Ulcer	2 (4%)	–	–	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Parasite metazoan	1 (2%)	1 (2%)	1 (2%)	–
Ulcer	1 (2%)	–	1 (2%)	–
Intestine small, duodenum	(50)	(50)	(50)	(50)
Diverticulum	–	–	–	1 (2%)
Metaplasia, osseous	–	–	–	1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Hyperplasia	–	–	–	1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	2 (4%)	–	1 (2%)
Basophilic focus	19 (38%)	26 (52%)	24 (48%)	13 (26%)
Clear cell focus	34 (68%)	33 (66%)	43 (86%)	41 (82%)
Congestion	–	–	1 (2%)	–
Eosinophilic focus	–	3 (6%)	–	3 (6%)
Fatty change	28 (56%)	35 (70%)	30 (60%)	27 (54%)
Hepatodiaphragmatic nodule	1 (2%)	–	2 (4%)	–
Inflammation, suppurative	–	–	1 (2%)	–
Mixed cell focus	9 (18%)	10 (20%)	12 (24%)	16 (32%)
Necrosis	1 (2%)	–	–	–
Thrombosis	–	1 (2%)	–	–
Artery, vein, necrosis	–	1 (2%)	–	–
Bile duct, hyperplasia	3 (6%)	5 (10%)	7 (14%)	7 (14%)
Oval cell, hyperplasia	–	1 (2%)	–	–
Mesentery	(3)	(3)	(0)	(2)
Inflammation, chronic	1 (33%)	–	–	–
Fat, necrosis	2 (67%)	3 (100%)	–	2 (100%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	1 (2%)	1 (2%)	2 (4%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Necrosis	1 (2%)	–	–	–
Acinus, hyperplasia	1 (2%)	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst	–	–	1 (2%)	–
Inflammation, chronic	6 (12%)	5 (10%)	–	1 (2%)
Mineralization	1 (2%)	–	–	–
Necrosis	1 (2%)	–	–	–
Ulcer	1 (2%)	1 (2%)	2 (4%)	–
Epithelium, hyperplasia	–	–	1 (2%)	–
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Mineralization	–	3 (6%)	4 (8%)	1 (2%)
Necrosis	1 (2%)	–	–	–
Ulcer	1 (2%)	–	1 (2%)	1 (2%)
Epithelium, glands, hyperplasia	–	1 (2%)	–	1 (2%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Adventitia, aorta, hemorrhage	1 (2%)	–	–	–
Aorta, inflammation, chronic	1 (2%)	–	–	–
Aorta, mineralization	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	33 (66%)	23 (46%)	23 (46%)	30 (60%)
Mineralization	–	–	–	1 (2%)
Atrium, epicardium, inflammation, chronic	1 (2%)	–	–	–
Endocardium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Epicardium, fibrosis	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Cytoplasmic alteration	–	–	1 (2%)	–
Degeneration, cystic	1 (2%)	–	–	–
Hemorrhage	–	1 (2%)	–	–
Hyperplasia	12 (24%)	5 (10%)	6 (12%)	9 (18%)
Metaplasia, osseous	–	–	1 (2%)	–
Necrosis	–	2 (4%)	1 (2%)	–
Vacuolization cytoplasmic	17 (34%)	17 (34%)	15 (31%)	16 (32%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Adrenal medulla	(49)	(50)	(49)	(50)
Hyperplasia	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Necrosis	–	1 (2%)	1 (2%)	–
Vacuolization cytoplasmic	–	–	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	–	–
Parathyroid gland	(45)	(48)	(47)	(49)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Pituitary gland	(50)	(49)	(50)	(48)
Cyst	1 (2%)	3 (6%)	–	3 (6%)
Pars distalis, hyperplasia	8 (16%)	8 (16%)	9 (18%)	7 (15%)
Pars intermedia, hyperplasia	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	–	–	–
C-cell, hyperplasia	16 (32%)	27 (54%)	26 (52%)	19 (38%)
Follicle, hyperplasia	3 (6%)		2 (4%)	2 (4%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	–	–
Granuloma sperm	–	1 (2%)	–	–
Preputial gland	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	6 (12%)	10 (20%)	3 (6%)
Prostate	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	–
Fibrosis	–	–	–	1 (2%)
Hyperplasia	2 (4%)	–	–	–
Inflammation	12 (24%)	19 (38%)	12 (24%)	14 (28%)
Artery, inflammation	1 (2%)	–	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	–
Inflammation	3 (6%)	5 (10%)	2 (4%)	1 (2%)
Testes	(50)	(50)	(50)	(50)
Edema	35 (70%)	32 (64%)	37 (74%)	36 (72%)
Arteriole, necrosis, fibrinoid	–	–	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Germinal epithelium, atrophy	–	4 (8%)	1 (2%)	2 (4%)
Germinal epithelium, mineralization	–	–	–	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(0)	(2)	(1)
Mediastinal, hyperplasia, lymphoid	1 (33%)	–	–	–
Renal, ectasia	–	–	1 (50%)	–
Lymph node, mandibular	(49)	(50)	(50)	(48)
Atrophy	–	1 (2%)	–	–
Ectasia	–	1 (2%)	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	–
Ectasia	–	–	1 (2%)	4 (8%)
Hyperplasia, lymphoid	–	1 (2%)	–	–
Necrosis	–	1 (2%)	–	–
Spleen	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Congestion	–	1 (2%)	–	–
Hematopoietic cell proliferation	8 (16%)	15 (30%)	9 (18%)	10 (20%)
Necrosis	1 (2%)	–	–	–
Capsule, fibrosis	–	1 (2%)	–	–
Lymphoid follicle, atrophy	6 (12%)	5 (10%)	–	2 (4%)
Thymus	(49)	(49)	(49)	(50)
Atrophy	39 (80%)	46 (94%)	44 (90%)	45 (90%)
Hyperplasia	1 (2%)	–	–	–
Inflammation, chronic	1 (2%)	–	–	–
Integumentary System				
Mammary gland	(47)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	–
Ulcer	10 (20%)	7 (14%)	3 (6%)	6 (12%)
Epidermis, hyperplasia	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosclerosis	–	–	–	1 (2%)
Skeletal muscle	(1)	(0)	(0)	(1)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	12 (24%)	9 (18%)	5 (10%)
Gliosis	–	–	–	1 (2%)
Peripheral nerve	(0)	(2)	(1)	(0)
Axon, degeneration	–	1 (50%)	1 (100%)	–
Spinal cord	(0)	(2)	(1)	(0)
Axon, degeneration		1 (50%)	1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body	–	–	–	1 (2%)
Hemorrhage	–	1 (2%)	–	–
Inflammation, granulomatous	2 (4%)	2 (4%)	–	3 (6%)
Inflammation, chronic	8 (16%)	6 (12%)	6 (12%)	6 (12%)
Metaplasia, osseous	–	–	–	3 (6%)
Alveolar epithelium, necrosis	–	1 (2%)	–	–
Alveolus, inflammation	1 (2%)	–	–	–
Arteriole, thrombosis	–	1 (2%)	–	–
Bronchiole, hyperplasia	3 (6%)	2 (4%)	1 (2%)	4 (8%)
Vein, necrosis	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation	8 (16%)	5 (10%)	7 (14%)	8 (16%)
Ulcer	1 (2%)	–	–	–
Goblet cell, hyperplasia	1 (2%)	–	–	1 (2%)
Olfactory epithelium, degeneration	–	–	2 (4%)	
Trachea	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	–	–	–
Inflammation, chronic	–	1 (2%)	–	–
Perforation	–	–	–	1 (2%)
Peritracheal tissue, inflammation	1 (2%)	–	–	–
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	–	–	1 (2%)	–
Inflammation	1 (2%)	–	–	–
Inflammation, acute	–	1 (2%)	–	–
Retina, atrophy	1 (2%)	1 (2%)	3 (6%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Harderian gland	(49)	(50)	(50)	(50)
Hyperplasia	–	1 (2%)	1 (2%)	2 (4%)
Inflammation	1 (2%)	–	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Hydronephrosis	–	2 (4%)	1 (2%)	1 (2%)
Infarct	–	–	–	1 (2%)
Inflammation, suppurative, multifocal	–	1 (2%)	–	–
Inflammation, suppurative	–	–	–	1 (2%)
Inflammation, chronic	–	–	–	2 (4%)
Metaplasia, lipocyte	–	–	1 (2%)	–
Metaplasia, osseous	–	–	–	1 (2%)
Nephropathy	39 (78%)	30 (60%)	35 (70%)	31 (62%)
Pelvis, inflammation, suppurative	4 (8%)	2 (4%)	2 (4%)	5 (10%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	–	1 (2%)	–
Renal tubule, dilatation	–	–	1 (2%)	–
Urethra	(1)	(0)	(0)	(0)
Inflammation, chronic	1 (100%)	–	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Transitional epithelium, hyperplasia	–	1 (2%)	–	–

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix B. Summary of Lesions in Female Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month Interim Evaluation	10	–	–	10
Early deaths				
Accidental deaths	3	–	–	4
Moribund	8	14	15	10
Natural deaths	4	2	6	3
Survivors				
Died last week of study	1	–	–	1
Terminal kill	34	34	29	32
Animals examined microscopically	60	50	50	60
Systems Examined at 3 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(49)	(50)	(50)
Intestine large, cecum	(50)	(49)	(50)	(50)
Leiomyoma	–	–	1 (2%)	–
Intestine large, colon	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small	(0)	(1)	(0)	(0)
Leiomyosarcoma, metastatic, uterus	–	1 (100%)	–	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Intestine small, duodenum	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	–	–
Intestine small, ileum	(50)	(49)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyoma	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	3 (6%)	2 (4%)
Hepatocellular adenoma	1 (2%)	2 (4%)	–	1 (2%)
Malignant mixed Müllerian tumor, metastatic, uterus	–	2 (4%)	–	–
Mesentery	(2)	(8)	(5)	(4)
Adenocarcinoma, metastatic, uterus	–	1 (13%)	2 (40%)	2 (50%)
Leiomyosarcoma, metastatic, stomach, glandular	–	–	1 (20%)	–
Leiomyosarcoma, metastatic, uterus	–	1 (13%)	–	–
Malignant mixed Müllerian tumor, metastatic, uterus	–	2 (25%)	–	–
Pancreas	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	3 (6%)	1 (2%)	3 (6%)
Granulosa cell tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Leiomyosarcoma, metastatic, uterus	–	1 (2%)	–	–
Malignant mixed Müllerian tumor, metastatic, uterus	–	3 (6%)	–	–
Salivary glands	(50)	(48)	(49)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	1 (2%)	–
Malignant mixed Müllerian tumor, metastatic, uterus	–	1 (2%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocardium, schwannoma benign	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Adenoma	1 (2%)	–	–	1 (2%)
Carcinoma	1 (2%)	–	–	–
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)	–	–	–
Pheochromocytoma malignant	–	–	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Parathyroid gland	(48)	(39)	(48)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	20 (40%)	25 (50%)	18 (36%)	16 (32%)
Pars distalis, adenoma, multiple	1 (2%)	2 (4%)	–	–
Pars distalis, carcinoma	–	–	–	1 (2%)
Pars intermedia, adenoma	4 (8%)	1 (2%)	–	1 (2%)
Thyroid gland	(50)	(48)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)	–	–	–
Bilateral, follicular cell, adenoma	1 (2%)	–	–	–
C-cell, adenoma	6 (12%)	9 (19%)	5 (10%)	3 (6%)
Follicular cell, adenoma	2 (4%)	3 (6%)	2 (4%)	–
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Ovary	(50)	(49)	(50)	(49)
Adenocarcinoma	–	–	–	1 (2%)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	1 (2%)	1 (2%)
Granulosa cell tumor malignant	1 (2%)	–	–	–
Malignant mixed Müllerian tumor, metastatic, uterus	–	1 (2%)	–	–
Sex cord stromal tumor, benign, mixed cell	2 (4%)	–	–	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma	2 (4%)	3 (6%)	7 (14%)	9 (18%)
Adenocarcinoma, multiple	1 (2%)	–	1 (2%)	
Adenoma	–	–	3 (6%)	4 (8%)
Leiomyosarcoma	–	1 (2%)	–	–
Malignant mixed Müllerian tumor	–	4 (8%)	–	2 (4%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Polyp stromal	2 (4%)	4 (8%)	3 (6%)	1 (2%)
Sarcoma stromal	–	2 (4%)	–	1 (2%)
Cervix, sarcoma stromal	–	–	1 (2%)	–
Cervix, squamous cell carcinoma	–	1 (2%)	–	–
Vagina	(1)	(1)	(1)	(1)
Granular cell tumor malignant	–	1 (100%)	–	–
Leiomyoma	1 (100%)	–	–	–
Polyp	–	–	–	1 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(0)	(1)	(1)	(0)
Lymph node, mandibular	(50)	(48)	(49)	(49)
Lymph node, mediastinal	(0)	(0)	(1)	(0)
Adenocarcinoma, metastatic, uterus	–	–	1 (100%)	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	1 (2%)	1 (2%)
Malignant mixed Müllerian tumor, metastatic, uterus	–	1 (2%)	–	–
Thymus	(50)	(50)	(49)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Sarcoma	1 (2%)	–	–	–
Thymoma benign	1 (2%)	–	2 (4%)	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenocarcinoma	1 (2%)	–	–	3 (6%)
Adenoma	3 (6%)	5 (10%)	2 (4%)	–
Adenoma, multiple	1 (2%)	–	–	–
Fibroadenoma	7 (14%)	12 (24%)	6 (12%)	11 (22%)
Fibroadenoma, multiple	1 (2%)	3 (6%)	2 (4%)	–
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	–	1 (2%)	–	–
Basal cell carcinoma	–	1 (2%)	–	–
Fibroma	–	1 (2%)	–	–
Squamous cell papilloma	–	1 (2%)	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Subcutaneous tissue, adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Subcutaneous tissue, hemangiosarcoma	1 (2%)	–	–	–
Subcutaneous tissue, lipoma	–	–	1 (2%)	–
Subcutaneous tissue, malignant mixed Müllerian tumor, metastatic, uterus	–	2 (4%)	–	–
Subcutaneous tissue, sarcoma	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(2)	(1)	(1)
Adenocarcinoma, metastatic, uterus	–	1 (50%)	1 (100%)	–
Leiomyosarcoma, metastatic, uterus	–	1 (50%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Ependymoma malignant	–	–	1 (2%)	–
Glioma malignant, mixed cell	–	–	2 (4%)	–
Granular cell tumor benign	–	1 (2%)	–	–
Peripheral nerve	(0)	(1)	(0)	(0)
Spinal cord	(0)	(1)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	1 (2%)	4 (8%)	1 (2%)	4 (8%)
Alveolar/bronchiolar adenoma	–	–	–	2 (4%)
Carcinoma, metastatic, adrenal cortex	1 (2%)	–	–	–
Granulosa cell tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Malignant mixed Müllerian tumor, metastatic, uterus	–	1 (2%)	–	–
Olfactory neuroblastoma, metastatic, nose	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Olfactory neuroblastoma	1 (2%)	–	–	–
Trachea	(50)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(48)	(49)	(49)
Harderian gland	(50)	(48)	(49)	(49)
Urinary System				

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Kidney	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	2 (4%)	–	1 (2%)
Lipoma	–	–	1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Granulosa cell tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Malignant mixed Müllerian tumor, metastatic, uterus	–	1 (2%)	–	–
Transitional epithelium, papilloma	1 (2%)	–	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	–	–	1 (2%)	–
Neoplasm Summary				
Total animals with primary neoplasms[c]				
Two-year study	40	43	39	38
Total primary neoplasms				
Two-year study	71	84	62	61
Total animals with benign neoplasms				
Two-year study	38	38	34	30
Total benign neoplasms				
Two-year study	59	70	48	43
Total animals with malignant neoplasms				
Two-year study	10	13	12	14
Total malignant neoplasms				
Two-year study	12	14	14	18
Total animals with metastatic neoplasms				
Two-year study	4	7	4	4
Total metastatic neoplasms				
Two-year study	6	36	12	14

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table B-2. Statistical Analysis of Primary Neoplasms in Female Rats in the Two-year Gavage Study of Tetrabromobisphenol A

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Mammary Gland: Fibroadenoma				
Overall rate[a]	8/50 (16%)	15/50 (30%)	8/50 (16%)	11/50 (22%)
Adjusted rate[b]	18.7%	34.0%	19.2%	25.4%
Terminal rate[c]	6/34 (18%)	12/34 (35%)	3/29 (10%)	7/33 (21%)
First incidence (days)	713	658	243	462
Poly-3 test[d]	P = 0.477	P = 0.082	P = 0.585	P = 0.310
Mammary Gland: Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted rate	9.3%	11.3%	5.2%	0.0%
Terminal rate	3/34 (9%)	1/34 (3%)	1/29 (3%)	0/33 (0%)
First incidence (days)	726	624	637	–[e]
Poly-3 test	P = 0.028N	P = 0.522	P = 0.385N	P = 0.063N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	12/50 (24%)	20/50 (40%)	10/50 (20%)	11/50 (22%)
Adjusted rate	28.0%	44.5%	23.8%	25.4%
Terminal rate	9/34 (27%)	13/34 (38%)	4/29 (14%)	7/33 (21%)
First incidence (days)	713	624	243	462
Poly-3 test	P = 0.191N	P = 0.079	P = 0.423N	P = 0.489N
Mammary Gland: Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	0.0%	0.0%	7.1%
Terminal rate	0/34 (0%)	0/34 (0%)	0/29 (0%)	2/33 (6%)
First incidence (days)	726	–	–	625
Poly-3 test	P = 0.080	P = 0.497N	P = 0.522N	P = 0.300
Mammary Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	2/50 (4%)	3/50 (6%)
Adjusted rate	9.3%	11.3%	5.2%	7.1%
Terminal rate	3/34 (9%)	1/34 (3%)	1/29 (3%)	2/33 (6%)
First incidence (days)	726	624	637	625
Poly-3 test	P = 0.339N	P = 0.522	P = 0.385N	P = 0.508N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	12/50 (24%)	20/50 (40%)	10/50 (20%)	14/50 (28%)
Adjusted rate	28.0%	44.5%	23.8%	32.1%
Terminal rate	9/34 (27%)	13/34 (38%)	4/29 (14%)	9/33 (27%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
First incidence (days)	713	624	243	462
Poly-3 test	P = 0.448N	P = 0.079	P = 0.423N	P = 0.429
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	21/50 (42%)	27/50 (54%)	18/50 (36%)	16/50 (32%)
Adjusted rate	44.3%	58.1%	42.8%	36.4%
Terminal rate	10/34 (29%)	18/34 (53%)	9/29 (31%)	11/33 (33%)
First incidence (days)	364	492	488	531
Poly-3 test	P = 0.119N	P = 0.127	P = 0.527N	P = 0.288N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	21/50 (42%)	27/50 (54%)	18/50 (36%)	17/50 (34%)
Adjusted rate	44.3%	58.1%	42.8%	38.7%
Terminal rate	10/34 (29%)	18/34 (53%)	9/29 (31%)	12/33 (36%)
First incidence (days)	364	492	488	531
Poly-3 test	P = 0.172N	P = 0.127	P = 0.527N	P = 0.369N
Pituitary Gland (Pars Intermedia): Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	9.4%	2.3%	0.0%	2.4%
Terminal rate	4/34 (12%)	1/34 (3%)	0/29 (0%)	1/33 (3%)
First incidence (days)	728 (T)	728 (T)	–	728 (T)
Poly-3 test	P = 0.109N	P = 0.173N	P = 0.075N	P = 0.185N
Skin: Squamous Cell Papilloma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.8%	2.6%	0.0%
Terminal rate	0/34 (0%)	2/34 (6%)	0/29 (0%)	0/33 (0%)
First incidence (days)	–	383	636	–
Poly-3 test	P = 0.376N	P = 0.125	P = 0.480	–[f]
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	3/48 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.0%	7.1%	5.2%	0.0%
Terminal rate	2/34 (6%)	3/34 (9%)	1/29 (3%)	0/33 (0%)
First incidence (days)	662	728 (T)	639	–
Poly-3 test	P = 0.077N	P = 0.657	P = 0.549N	P = 0.123N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	7/50 (14%)	9/48 (19%)	5/50 (10%)	3/50 (6%)
Adjusted rate	16.3%	21.0%	12.6%	7.1%
Terminal rate	5/34 (15%)	7/34 (21%)	3/29 (10%)	2/33 (6%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
First incidence (days)	713	624	496	614
Poly-3 test	P = 0.074N	P = 0.393	P = 0.436N	P = 0.162N
Uterus: Stromal Polyp (Original Transverse Review)				
Overall rate	2/50 (4%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.7%	9.2%	7.8%	2.4%
Terminal rate	2/34 (6%)	3/34 (9%)	3/29 (10%)	0/33 (0%)
First incidence (days)	728 (T)	693	728 (T)	614
Poly-3 test	P = 0.304N	P = 0.346	P = 0.450	P = 0.505N
Uterus: Stromal Polyp (Residual Longitudinal Review)				
Overall rate	5/50 (10%)	7/50 (14%)	8/50 (16%)	8/50 (16%)
Adjusted rate	11.7%	15.9%	20.5%	18.5%
Terminal rate	4/34 (12%)	5/34 (15%)	6/29 (21%)	5/33 (15%)
First incidence (days)	725	636	607	442
Poly-3 test	P = 0.241	P = 0.398	P = 0.216	P = 0.282
Uterus: Stromal Polyp (Original Transverse and Residual Longitudinal Reviews)				
Overall rate	5/50 (10%)	9/50 (18%)	9/50 (18%)	8/50 (16%)
Adjusted rate	11.7%	20.5%	23.0%	18.5%
Terminal rate	4/34 (12%)	7/34 (21%)	7/29 (24%)	5/33 (15%)
First incidence (days)	725	636	607	442
Poly-3 test	P = 0.307	P = 0.206	P = 0.141	P = 0.282
Uterus: Stromal Polyp, Stromal Sarcoma, or Leiomyosarcoma (Original Transverse Review)				
Overall rate	2/50 (4%)	7/50 (14%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.7%	15.7%	7.8%	4.7%
Terminal rate	2/34 (6%)	4/34 (12%)	3/29 (10%)	0/33 (0%)
First incidence (days)	728 (T)	527	728 (T)	614
Poly-3 test	P = 0.332N	P = 0.089	P = 0.450	P = 0.691
Uterus: Stromal Polyp, Stromal Sarcoma, or Leiomyosarcoma (Residual Longitudinal Review)				
Overall rate	5/50 (10%)	7/50 (14%)	8/50 (16%)	8/50 (16%)
Adjusted rate	11.7%	15.9%	20.5%	18.5%
Terminal rate	4/34 (12%)	5/34 (15%)	6/29 (21%)	5/33 (15%)
First incidence (days)	725	636	607	442
Poly-3 test	P = 0.241	P = 0.398	P = 0.216	P = 0.282
Uterus: Stromal Polyp, Stromal Sarcoma, or Leiomyosarcoma (Original Transverse and Residual Longitudinal Reviews)				
Overall rate	5/50 (10%)	12/50 (24%)	9/50 (18%)	9/50 (18%)
Adjusted rate	11.7%	26.7%	23.0%	20.7%

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Terminal rate	4/34 (12%)	8/34 (24%)	7/29 (24%)	5/33 (15%)
First incidence (days)	725	527	607	442
Poly-3 test	P = 0.314	P = 0.064	P = 0.141	P = 0.199
Uterus: Adenoma (Original Transverse Review)				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	7.8%	9.4%
Terminal rate	0/34 (0%)	0/34 (0%)	3/29 (10%)	2/33 (6%)
First incidence (days)	–	–	728 (T)	625
Poly-3 test	P = 0.010	–	P = 0.100	P = 0.059
Uterus: Adenoma (Residual Longitudinal Review)				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	7.0%	4.5%	2.6%	7.0%
Terminal rate	1/34 (3%)	1/34 (3%)	1/29 (3%)	1/33 (3%)
First incidence (days)	668	548	728 (T)	442
Poly-3 test	P = 0.556	P = 0.489N	P = 0.347N	P = 0.662
Uterus: Adenoma (Original Transverse and Residual Longitudinal Reviews)				
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	6/50 (12%)
Adjusted rate	7.0%	4.5%	10.4%	13.9%
Terminal rate	1/34 (3%)	1/34 (3%)	4/29 (14%)	3/33 (9%)
First incidence (days)	668	548	728 (T)	442
Poly-3 test	P = 0.103	P = 0.489N	P = 0.437	P = 0.242
Uterus: Adenocarcinoma (Original Transverse Review)				
Overall rate	3/50 (6%)	3/50 (6%)	8/50 (16%)	9/50 (18%)
Adjusted rate	7.0%	6.7%	19.8%	20.9%
Terminal rate	2/34 (6%)	0/34 (0%)	4/29 (14%)	5/33 (15%)
First incidence (days)	713	548	321	607
Poly-3 test	P = 0.016	P = 0.644N	P = 0.078	P = 0.058
Uterus: Adenocarcinoma (Residual Longitudinal Review)				
Overall rate	4/50 (8%)	9/50 (18%)	15/50 (30%)	15/50 (30%)
Adjusted rate	9.3%	19.9%	36.4%	33.8%
Terminal rate	3/34 (9%)	4/34 (12%)	9/29 (31%)	10/33 (30%)
First incidence (days)	713	548	321	442
Poly-3 test	P = 0.003	P = 0.137	P = 0.002	P = 0.005
Uterus: Adenocarcinoma (Original Transverse and Residual Longitudinal Reviews)				
Overall rate	4/50 (8%)	10/50 (20%)	15/50 (30%)	16/50 (32%)
Adjusted rate	9.3%	22.0%	36.4%	35.9%

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Terminal rate	3/34 (9%)	4/34 (12%)	9/29 (31%)	10/33 (30%)
First incidence (days)	713	548	321	442
Poly-3 test	P = 0.002	P = 0.089	P = 0.002	P = 0.002
Uterus: Malignant Mixed Müllerian Tumor (Original Transverse Review)				
Overall rate	0/50 (0%)	4/50 (8%)	0/50 (0%)	2/50 (4%)[g]
Adjusted rate	0.0%	9.0%	0.0%	4.7%
Terminal rate	0/34 (0%)	1/34 (3%)	0/29 (0%)	1/33 (3%)
First incidence (days)	–	656	–	615
Poly-3 test	P = 0.433	P = 0.064	–	P = 0.234
Uterus: Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor (Original Transverse Review)				
Overall rate	3/50 (6%)	7/50 (14%)	11/50 (22%)	13/50 (26%)
Adjusted rate	7.0%	15.4%	27.3%	29.9%
Terminal rate	2/34 (6%)	1/34 (3%)	7/29 (24%)	7/33 (21%)
First incidence (days)	713	548	321	607
Poly-3 test	P = 0.003	P = 0.181	P = 0.013	P = 0.005
Uterus: Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor (Residual Longitudinal Review)				
Overall rate	6/50 (12%)	10/50 (20%)	16/50 (32%)	16/50 (32%)
Adjusted rate	13.9%	22.1%	38.8%	35.8%
Terminal rate	3/34 (9%)	5/34 (15%)	10/29 (35%)	10/33 (30%)
First incidence (days)	668	548	321	442
Poly-3 test	P = 0.008	P = 0.237	P = 0.007	P = 0.015
Uterus: Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor (Original Transverse and Residual Longitudinal Reviews)				
Overall rate	6/50 (12%)	11/50 (22%)	16/50 (32%)	19/50 (38%)
Adjusted rate	13.9%	24.2%	38.8%	42.2%
Terminal rate	3/34 (9%)	5/34 (15%)	10/29 (35%)	11/33 (33%)
First incidence (days)	668	548	321	442
Poly-3 test	P = 0.001	P = 0.168	P = 0.007	P = 0.002
All Organs: Benign Neoplasms				
Overall rate	38/50 (76%)	38/50 (76%)	34/50 (68%)	30/50 (60%)
Adjusted rate	80.2%	81.7%	75.3%	65.5%
Terminal rate	26/34 (77%)	28/34 (82%)	20/29 (69%)	20/33 (61%)
First incidence (days)	364	492	243	462
Poly-3 test	P = 0.032N	P = 0.528	P = 0.373N	P = 0.080N
All Organs: Malignant Neoplasms				

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Overall rate	10/50 (20%)	13/50 (26%)	12/50 (24%)	14/50 (28%)
Adjusted rate	22.5%	27.5%	28.6%	32.2%
Terminal rate	5/34 (15%)	4/34 (12%)	5/29 (17%)	8/33 (24%)
First incidence (days)	546	383	321	607
Poly-3 test	P = 0.195	P = 0.378	P = 0.345	P = 0.217
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	43/50 (86%)	39/50 (78%)	38/50 (76%)
Adjusted rate	83.4%	87.6%	81.8%	81.2%
Terminal rate	27/34 (79%)	28/34 (82%)	21/29 (72%)	25/33 (76%)
First incidence (days)	364	383	243	462
Poly-3 test	P = 0.342N	P = 0.383	P = 0.524N	P = 0.497N

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for pituitary gland and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal kill.
[d]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.
[g]One additional malignant mixed Müllerian tumor was found during the residual longitudinal review in an animal that already had this tumor diagnosed during the original transverse review.

Table B-3. Historical Incidence of Uterus Neoplasms in Control Female Wistar Han Rats[a]

	Adenoma	Adenocarcinoma[b]	Malignant Mixed Müllerian Tumor	Adenoma, Adenocarcinoma or Malignant Mixed Müllerian Tumor[b]
Overall Historical Incidence: All Routes				
Total (%)	0/150	7/150 (4.7%)	0/150	7/150 (4.7%)
Mean ± standard deviation	–	4.7% ± 2.3%	–	4.7% ± 2.3%
Range	–	2%–6%	–	2%–6%

[a]Data as of June 2013.
[b]Includes one endometrium carcinoma.

Table B-4. Summary of the Incidence of Non-neoplastic Lesions in Female Rats in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month Interim Evaluation	10	–	–	10
Early deaths				
Accidental deaths	3	–	–	4
Moribund	8	14	15	10
Natural deaths	4	2	6	3
Survivors				
Died last week of study	1	–	–	1
Terminal kill	34	34	29	32
Animals examined microscopically	60	50	50	60
Three-month Interim Evaluation				
Alimentary System				
Intestine, large, rectum	(10)	–	–	(10)
Lymphoid tissue, hyperplasia	1 (10%)	–	–	1 (10%)
Pancreas	(10)	–	–	(10)
Acinus, atrophy	–	–	–	1 (10%)
Endocrine System				
Adrenal cortex	(10)	–	–	(10)
Hypertrophy	1 (10%)	–	–	–
Pituitary gland	(10)	–	–	(10)
Cyst	1 (10%)	–	–	–
Hematopoietic System				
Lymph node, mesenteric	(10)	–	–	(10)
Hyperplasia, lymphoid	3 (30%)	–	–	3 (30%)
Respiratory System				
Lung	(10)	–	–	(10)
Infiltration cellular, histiocyte	1 (10%)	–	–	–
Perivascular, inflammation, chronic active	1 (10%)	–	–	1 (10%)
Nose	(10)	–	–	(10)
Olfactory epithelium, accumulation, hyaline droplet	–	–	–	2 (20%)
Olfactory epithelium, necrosis	–	–	–	1 (10%)
Respiratory epithelium, accumulation, hyaline droplet	–	–	–	1 (10%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Respiratory epithelium, necrosis	–			1 (10%)
Urinary System				
Kidney	(10)	–	–	(10)
Cyst	–	–	–	1 (10%)
Nephropathy	2 (20%)	–	–	2 (20%)
Systems Examined at 3 Months with No Lesions Observed				
Cardiovascular System				
General Body System				
Genital System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(49)	(50)	(50)
Inflammation	1 (2%)	–	–	–
Inflammation, acute	–	–	–	1 (2%)
Perforation	3 (6%)	–	–	3 (6%)
Intestine large, cecum	(50)	(49)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Inflammation, suppurative		1 (2%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	1 (2%)	–	–
Parasite metazoan	–	1 (2%)	–	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Intestine small	(0)	(1)	(0)	(0)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(49)	(50)	(50)
Parasite metazoan	1 (2%)	–	1 (2%)	–
Lymphoid tissue, hyperplasia	–	–	–	1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	–	–
Basophilic focus	47 (94%)	38 (76%)	40 (80%)	47 (94%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Clear cell focus	24 (48%)	19 (38%)	19 (38%)	18 (36%)
Congestion	1 (2%)	–	–	–
Cyst	–	1 (2%)	–	–
Eosinophilic focus	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Fatty change	11 (22%)	12 (24%)	7 (14%)	7 (14%)
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hepatodiaphragmatic nodule	1 (2%)	–	1 (2%)	–
Inflammation, suppurative		1 (2%)	–	–
Mixed cell focus	13 (26%)	22 (44%)	12 (24%)	20 (40%)
Necrosis	–	1 (2%)	4 (8%)	2 (4%)
Pigmentation	–	1 (2%)	–	–
Bile duct, cyst	2 (4%)	–	3 (6%)	1 (2%)
Bile duct, hyperplasia	11 (22%)	29 (58%)	21 (42%)	20 (40%)
Centrilobular, necrosis	–	–	1 (2%)	–
Oval cell, hyperplasia	–	–	–	1 (2%)
Mesentery	(2)	(8)	(5)	(4)
Fat, necrosis	2 (100%)	3 (38%)	2 (40%)	2 (50%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	4 (8%)	4 (8%)	1 (2%)	–
Duct, cyst	2 (4%)	–	–	–
Salivary glands	(50)	(48)	(49)	(49)
Duct, hyperplasia	1 (2%)	–	–	–
Duct, metaplasia, squamous	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	1 (2%)	–	–
Inflammation, chronic	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Ulcer	1 (2%)	–	1 (2%)	–
Epithelium, hyperplasia	–	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	1 (2%)	–	–
Inflammation, acute	–	–	1 (2%)	–
Inflammation, chronic	1 (2%)	2 (4%)	–	–
Mineralization	6 (12%)	1 (2%)	1 (2%)	2 (4%)
Ulcer	–	1 (2%)	2 (4%)	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Mineralization	1 (2%)	–	–	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	9 (18%)	10 (20%)	3 (6%)	7 (14%)
Congestion	1 (2%)	–	–	–
Endocardium, hyperplasia	2 (4%)	2 (4%)	1 (2%)	–
Myocardium, mineralization	1 (2%)	–	–	–
Pericardium, inflammation, acute	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	12 (24%)	7 (14%)	6 (12%)	9 (18%)
Degeneration, cystic	1 (2%)	1 (2%)	1 (2%)	–
Fibrosis	–	–	1 (2%)	–
Hyperplasia	7 (14%)	7 (14%)	7 (14%)	11 (22%)
Inflammation, suppurative	–	–	–	1 (2%)
Mineralization	–	–	–	1 (2%)
Necrosis	–	1 (2%)	–	–
Vacuolization cytoplasmic	2 (4%)	3 (6%)	5 (10%)	2 (4%)
Capsule, fibrosis	1 (2%)	–	–	–
Adrenal medulla	(49)	(50)	(50)	(50)
Hemorrhage	–	–	–	2 (4%)
Hyperplasia	2 (4%)	2 (4%)	–	2 (4%)
Inflammation, suppurative	–	–	–	1 (2%)
Thrombosis	–	–	1 (2%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(48)	(39)	(48)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Cyst	1 (2%)	1 (2%)	1 (2%)	–
Pars distalis, hyperplasia	16 (32%)	13 (26%)	14 (28%)	19 (38%)
Pars intermedia, hyperplasia	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Thyroid gland	(50)	(48)	(50)	(50)
C-cell, hyperplasia	32 (64%)	37 (77%)	39 (78%)	36 (72%)
Follicle, hyperplasia	2 (4%)	4 (8%)	3 (6%)	1 (2%)
Follicular cell, hyperplasia	1 (2%)	–	–	–
General Body System				

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	–
Inflammation	4 (8%)	2 (4%)	4 (8%)	5 (10%)
Ovary	(50)	(49)	(50)	(49)
Cyst	1 (2%)	4 (8%)	4 (8%)	6 (12%)
Inflammation, acute	1 (2%)	–	–	–
Stromal hyperplasia, mixed cell	1 (2%)	–	–	–
Bilateral, cyst	1 (2%)	–	–	–
Bursa, dilatation	4 (8%)	2 (4%)	5 (10%)	8 (16%)
Rete ovarii, cyst	1 (2%)	–	6 (12%)	6 (12%)
Uterus	(50)	(50)	(50)	(50)
Adenomyosis	–	–	–	2 (4%)
Cyst	1 (2%)	–	–	1 (2%)
Dilatation	1 (2%)	4 (8%)	3 (6%)	2 (4%)
Hyperplasia, glandular, focal	–	–	1 (2%)	–
Inflammation, suppurative	7 (14%)	3 (6%)	2 (4%)	3 (6%)
Ulcer	–	–	–	1 (2%)
Cervix, hyperplasia, stromal	–	1 (2%)	4 (8%)	2 (4%)
Endometrium, hyperplasia, adenomatous	–	–	–	1 (2%)
Endometrium, hyperplasia, cystic	8 (16%)	13 (26%)	11 (22%)	18 (36%)
Vagina	(1)	(1)	(1)	(1)
Cyst	–	–	1 (100%)	–
Necrosis	1 (100%)	–	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(0)	(1)	(1)	(0)
Mediastinal, congestion	–	1 (100%)	–	–
Mediastinal, ectasia	–	–	1 (100%)	–
Lymph node, mandibular	(50)	(48)	(49)	(49)
Lymph node, mediastinal	(0)	(0)	(1)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hyperplasia, plasma cell	–	1 (2%)	–	–
Inflammation, suppurative	–	1 (2%)	–	–
Spleen	(50)	(50)	(50)	(50)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Hematopoietic cell proliferation	26 (52%)	30 (60%)	26 (52%)	26 (52%)
Inflammation, suppurative	–	1 (2%)	–	–
Lymphoid follicle, atrophy	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Red pulp, atrophy	–	1 (2%)	–	–
Thymus	(50)	(50)	(49)	(50)
Atrophy	43 (86%)	43 (86%)	40 (82%)	45 (90%)
Cyst	–	–	1 (2%)	–
Hemorrhage	–	–	–	1 (2%)
Hyperplasia	1 (2%)	2 (4%)	–	–
Epithelial cell, hyperplasia		1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Hyperplasia	1 (2%)	2 (4%)	–	–
Inflammation, suppurative	–	–	–	1 (2%)
Skin	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Ulcer	–	–	1 (2%)	–
Epidermis, hyperplasia	–	–	–	1 (2%)
Vein, cyst	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture	–	–	–	1 (2%)
Hyperplasia	–	–	1 (2%)	–
Skeletal muscle	(0)	(2)	(1)	(1)
Inflammation, acute	–	–	–	1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	11 (22%)	10 (20%)	9 (18%)	6 (12%)
Hippocampus, necrosis	1 (2%)	–	–	–
Meninges, inflammation, acute	1 (2%)	–	–	–
Peripheral nerve	(0)	(1)	(0)	(0)
Spinal cord	(0)	(1)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	–	–	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Inflammation, granulomatous	1 (2%)	2 (4%)	–	4 (8%)
Inflammation, chronic	2 (4%)	–	3 (6%)	1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)	–
Bronchiole, hyperplasia	1 (2%)	1 (2%)	–	1 (2%)
Serosa, inflammation, suppurative	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation	11 (22%)	8 (16%)	7 (14%)	9 (18%)
Trachea	(50)	(49)	(50)	(50)
Inflammation	2 (4%)	–	–	–
Special Senses System				
Eye	(50)	(48)	(49)	(49)
Cataract	–	–	2 (4%)	1 (2%)
Degeneration	1 (2%)	–	–	1 (2%)
Malformation	1 (2%)	–	–	–
Cornea, inflammation	–	1 (2%)	–	–
Retina, atrophy	–	2 (4%)	3 (6%)	4 (8%)
Harderian gland	(50)	(48)	(49)	(49)
Inflammation	–	1 (2%)	–	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	–	–	1 (2%)
Cyst	2 (4%)	1 (2%)	2 (4%)	–
Hydronephrosis	1 (2%)	1 (2%)	–	2 (4%)
Infarct	–	1 (2%)	1 (2%)	–
Inflammation, suppurative	–	2 (4%)	–	1 (2%)
Inflammation, chronic	1 (2%)	–	–	–
Nephropathy	9 (18%)	15 (30%)	13 (26%)	9 (18%)
Thrombosis	–	2 (4%)	–	–
Pelvis, inflammation, suppurative	2 (4%)	4 (8%)	3 (6%)	1 (2%)
Renal tubule, autolysis	1 (2%)	1 (2%)	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	1 (2%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix C. Summary of Lesions in Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	25 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	–	1
Moribund	9	10	6	12
Natural deaths	8	14	5	25
Survivors				
Died last week of study	1	1	–	–
Terminal kill	32	25	39	12
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(46)	(50)	(49)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Intestine large, cecum	(47)	(44)	(47)	(38)
Carcinoma	–	–	1 (2%)	–
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Intestine large, colon	(47)	(46)	(50)	(40)
Adenoma	–	–	1 (2%)	–
Carcinoma	–	–	1 (2%)	–
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Intestine large, rectum	(47)	(46)	(50)	(41)
Intestine small, duodenum	(47)	(41)	(48)	(31)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Intestine small, ileum	(47)	(43)	(50)	(40)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Intestine small, jejunum	(47)	(44)	(49)	(38)
Adenoma	–	–	–	1 (3%)
Adenoma, multiple	1 (2%)	–	–	–
Carcinoma	1 (2%)	1 (2%)	2 (4%)	1 (3%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	2 (4%)	–	–
Hemangioma	2 (4%)	–	–	1 (2%)
Hemangiosarcoma	–	4 (8%)	3 (6%)	2 (4%)
Hepatoblastoma	2 (4%)	11 (22%)	8 (16%)	3 (6%)

	Vehicle Control	25 mg/kg	500 mg/kg	1,000 mg/kg
Hepatocellular adenoma	20 (40%)	13 (26%)	10 (20%)	9 (18%)
Hepatocellular adenoma, multiple	12 (24%)	20 (40%)	28 (56%)	12 (24%)
Hepatocellular carcinoma	9 (18%)	11 (22%)	12 (24%)	7 (14%)
Hepatocellular carcinoma, multiple	2 (4%)	4 (8%)	5 (10%)	2 (4%)
Osteosarcoma, metastatic, skin	–	1 (2%)	–	–
Rhabdomyosarcoma, metastatic, skeletal muscle	–	1 (2%)	–	–
Sarcoma	–	1 (2%)	–	–
Mesentery	(3)	(3)	(4)	(2)
Carcinoma, metastatic, pancreas	–	1 (33%)	–	–
Hemangiosarcoma	–	–	–	1 (50%)
Fat, hepatocellular carcinoma, metastatic, liver	–	–	1 (25%)	–
Pancreas	(50)	(50)	(50)	(50)
Acinus, carcinoma	–	2 (4%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(50)	(49)
Squamous cell papilloma	5 (10%)	–	1 (2%)	–
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Tooth	(14)	(9)	(9)	(2)
Odontoma	2 (14%)	1 (11%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma complex	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(45)	(43)	(48)	(42)
Pituitary gland	(50)	(48)	(48)	(50)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	–	1 (2%)	–	–

	Vehicle Control	25 mg/kg	500 mg/kg	1,000 mg/kg
General Body System				
Peritoneum	(0)	(2)	(0)	(0)
Carcinoma, metastatic, pancreas	–	1 (50%)	–	–
Genital System				
Coagulating gland	(3)	(4)	(1)	(0)
Adenoma	–	1 (25%)	–	–
Carcinoma, metastatic, pancreas	–	2 (50%)	–	–
Granular cell tumor	1 (33%)	–	–	–
Sarcoma, metastatic, skin	1 (33%)	–	–	–
Epididymis	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	2 (4%)	–	–
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Testes	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	2 (4%)	1 (2%)	–
Lymph node	(3)	(0)	(2)	(0)
Hepatocellular carcinoma, metastatic, liver	–	–	1 (50%)	–
Renal, sarcoma, metastatic, skin	1 (33%)	–	–	–
Lymph node, mandibular	(50)	(50)	(50)	(49)
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Carcinoma, metastatic, pancreas	–	2 (4%)	–	–
Hemangiosarcoma, metastatic, spleen	–	–	1 (2%)	–
Spleen	(50)	(48)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	–	–	–
Carcinoma, metastatic, pancreas	–	2 (4%)	–	–
Hemangioma	–	–	1 (2%)	–
Hemangiosarcoma	1 (2%)	3 (6%)	4 (8%)	3 (6%)
Rhabdomyosarcoma, metastatic, skeletal muscle	–	1 (2%)	–	–
Thymus	(47)	(45)	(41)	(48)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–

	Vehicle Control	25 mg/kg	500 mg/kg	1,000 mg/kg
Rhabdomyosarcoma, metastatic, skeletal muscle	–	1 (2%)	–	–
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Fibrous histiocytoma	–	1 (2%)	–	1 (2%)
Hemangiosarcoma	–	–	2 (4%)	–
Keratoacanthoma	1 (2%)	–	–	–
Melanoma benign	–	–	1 (2%)	–
Osteosarcoma	–	1 (2%)	–	–
Rhabdomyosarcoma, metastatic, skeletal muscle	–	1 (2%)	–	–
Sarcoma	1 (2%)	–	–	–
Pinna, fibrous histiocytoma	–	1 (2%)	–	–
Subcutaneous tissue, lipoma	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Vertebra, hemangiosarcoma	–	–	1 (2%)	–
Skeletal muscle	(0)	(1)	(1)	(0)
Rhabdomyosarcoma	–	1 (100%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(0)	(0)	(0)	(2)
Spinal cord	(0)	(0)	(0)	(2)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	5 (10%)	2 (4%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	–	–	–
Alveolar/bronchiolar carcinoma	4 (8%)	4 (8%)	7 (14%)	2 (4%)
Carcinoma, metastatic, Harderian gland	1 (2%)	–	–	–
Carcinoma, metastatic, pancreas	–	2 (4%)	–	–
Hepatoblastoma, metastatic, liver	1 (2%)	2 (4%)	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	5 (10%)	5 (10%)	4 (8%)	2 (4%)
Osteosarcoma, metastatic, skin	–	1 (2%)	–	–
Rhabdomyosarcoma, metastatic, skeletal muscle	–	1 (2%)	–	–
Sarcoma, metastatic, skin	1 (2%)	–	–	–
Serosa, hemangiosarcoma	–	–	1 (2%)	–

	Vehicle Control	25 mg/kg	500 mg/kg	1,000 mg/kg
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	3 (6%)	7 (14%)	9 (18%)
Carcinoma	1 (2%)	1 (2%)	2 (4%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	–	–	–
Hemangiosarcoma	–	–	–	1 (2%)
Sarcoma, metastatic, skin	1 (2%)	–	–	–
Renal tubule, adenoma	–	1 (2%)	1 (2%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	1 (2%)	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	2 (4%)	–
Leukemia granulocytic	–	–	–	1 (2%)
Lymphoma malignant	3 (6%)	1 (2%)	2 (4%)	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	48	48	33
Total primary neoplasms	82	95	106	62
Total animals with benign neoplasms	38	35	41	28
Total benign neoplasms	58	45	52	38
Total animals with malignant neoplasms	21	33	34	18
Total malignant neoplasms	24	50	54	24
Total animals with metastatic neoplasms	9	10	7	2
Total metastatic neoplasms	13	40	8	2

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg
Harderian Gland: Adenoma			
Overall rate[b]	7/50 (14%)	3/50 (6%)	7/50 (14%)
Adjusted rate[c]	16.1%	7.0%	15.3%
Terminal rate[d]	6/33 (18%)	1/25 (4%)	6/39 (15%)
First incidence (days)	673	426	668
Poly-3 test[e]	P = 0.534N	P = 0.161N	P = 0.572N
Harderian Gland: Carcinoma			
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.3%	2.4%	4.4%
Terminal rate	0/33 (0%)	0/25 (0%)	2/39 (5%)
First incidence (days)	555	708	730 (T)
Poly-3 test	P = 0.393	P = 0.750	P = 0.514
Harderian Gland: Adenoma or Carcinoma			
Overall rate	8/50 (16%)	4/50 (8%)	9/50 (18%)
Adjusted rate	18.2%	9.3%	19.7%
Terminal rate	6/33 (18%)	1/25 (4%)	8/39 (21%)
First incidence (days)	555	426	668
Poly-3 test	P = 0.470	P = 0.186N	P = 0.537
Large Intestine (Cecum or Colon): Adenoma or Carcinoma			
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	6.5%
Terminal rate	0/33 (0%)	0/25 (0%)	3/39 (5%)
First incidence (days)	–[f]	–	513
Poly-3 test	P = 0.039	–[g]	P = 0.131
Liver: Hemangiosarcoma			
Overall rate	0/50 (0%)	4/50 (8%)	3/50 (6%)
Adjusted rate	0.0%	9.5%	6.6%
Terminal rate	0/33 (0%)	2/25 (8%)	3/39 (8%)
First incidence (days)	–	602	730 (T)
Poly-3 test	P = 0.134	P = 0.057	P = 0.128
Liver: Hepatocellular Adenoma			
Overall rate	32/50 (64%)	33/50 (66%)	38/50 (76%)
Adjusted rate	70.1%	73.4%	79.2%
Terminal rate	25/33 (76%)	19/25 (76%)	32/39 (82%)
First incidence (days)	374	470	522
Poly-3 test	P = 0.172	P = 0.451	P = 0.208

	Vehicle Control	250 mg/kg	500 mg/kg
Liver: Hepatocellular Carcinoma			
Overall rate	11/50 (22%)	15/50 (30%)	17/50 (34%)
Adjusted rate	24.5%	34.1%	35.3%
Terminal rate	6/33 (18%)	7/25 (28%)	9/39 (23%)
First incidence (days)	521	589	513
Poly-3 test	P = 0.160	P = 0.224	P = 0.182
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	39/50 (78%)	39/50 (78%)	43/50 (86%)
Adjusted rate	82.8%	84.3%	87.0%
Terminal rate	28/33 (85%)	21/25 (84%)	33/39 (85%)
First incidence (days)	374	470	513
Poly-3 test	P = 0.324	P = 0.539	P = 0.380
Liver: Hepatoblastoma			
Overall rate	2/50 (4%)	11/50 (22%)	8/50 (16%)
Adjusted rate	4.6%	25.6%	17.6%
Terminal rate	1/33 (3%)	7/25 (28%)	7/39 (18%)
First incidence (days)	619	535	722
Poly-3 test	P = 0.065	P = 0.006	P = 0.052
Liver: Hepatocellular Carcinoma or Hepatoblastoma			
Overall rate	12/50 (24%)	24/50 (48%)	20/50 (40%)
Adjusted rate	26.8%	52.8%	41.5%
Terminal rate	7/33 (21%)	12/25 (48%)	12/39 (31%)
First incidence (days)	521	535	513
Poly-3 test	P = 0.099	P = 0.008	P = 0.099
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma			
Overall rate	39/50 (78%)	42/50 (84%)	43/50 (86%)
Adjusted rate	82.8%	88.8%	87.0%
Terminal rate	28/33 (85%)	22/25 (88%)	33/39 (85%)
First incidence (days)	374	470	513
Poly-3 test	P = 0.325	P = 0.284	P = 0.380
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	6/50 (12%)	5/50 (10%)	2/50 (4%)
Adjusted rate	13.8%	11.7%	4.4%
Terminal rate	4/33 (12%)	2/25 (8%)	2/39 (5%)
First incidence (days)	661	470	730 (T)
Poly-3 test	P = 0.093N	P = 0.512N	P = 0.119N
Lung: Alveolar/bronchiolar Carcinoma			

	Vehicle Control	250 mg/kg	500 mg/kg
Overall rate	4/50 (8%)	4/50 (8%)	7/50 (14%)
Adjusted rate	9.0%	9.4%	15.4%
Terminal rate	2/33 (6%)	2/25 (8%)	7/39 (18%)
First incidence (days)	448	613	730 (T)
Poly-3 test	P = 0.215	P = 0.620	P = 0.277
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	10/50 (20%)	9/50 (18%)	9/50 (18%)
Adjusted rate	22.4%	20.6%	19.8%
Terminal rate	6/33 (18%)	4/25 (16%)	9/39 (23%)
First incidence (days)	448	470	730 (T)
Poly-3 test	P = 0.431N	P = 0.524N	P = 0.482N
Spleen: Hemangiosarcoma			
Overall rate	1/50 (2%)	3/48 (6%)	4/50 (8%)
Adjusted rate	2.3%	7.3%	8.8%
Terminal rate	0/33 (0%)	2/25 (8%)	4/39 (10%)
First incidence (days)	645	602	730 (T)
Poly-3 test	P = 0.149	P = 0.283	P = 0.193
Stomach (Forestomach): Squamous Cell Papilloma			
Overall rate	5/50 (10%)	0/50 (0%)	1/50 (2%)
Adjusted rate	11.4%	0.0%	2.2%
Terminal rate	3/33 (9%)	0/25 (0%)	1/39 (3%)
First incidence (days)	448	–	730 (T)
Poly-3 test	P = 0.033N	P = 0.035N	P = 0.094N
All Organs: Hemangiosarcoma			
Overall rate	1/50 (2%)	5/50 (10%)	8/50 (16%)
Adjusted rate	2.3%	11.9%	17.6%
Terminal rate	0/33 (0%)	3/25 (12%)	8/39 (21%)
First incidence (days)	645	602	730 (T)
Poly-3 test	P = 0.014	P = 0.093	P = 0.019
All Organs: Hemangioma or Hemangiosarcoma			
Overall rate	3/50 (6%)	5/50 (10%)	9/50 (18%)
Adjusted rate	6.9%	11.9%	19.8%
Terminal rate	2/33 (6%)	3/25 (12%)	9/39 (23%)
First incidence (days)	645	602	730 (T)
Poly-3 test	P = 0.047	P = 0.338	P = 0.069
All Organs: Malignant Lymphoma			
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)

	Vehicle Control	250 mg/kg	500 mg/kg
Adjusted rate	6.9%	2.4%	4.4%
Terminal rate	2/33 (6%)	0/25 (0%)	2/39 (5%)
First incidence (days)	699	526	730 (T)
Poly-3 test	P = 0.386N	P = 0.314N	P = 0.477N
All Organs: Benign Neoplasms			
Overall rate	38/50 (76%)	35/50 (70%)	41/50 (82%)
Adjusted rate	81.7%	76.5%	85.1%
Terminal rate	29/33 (88%)	20/25 (80%)	34/39 (87%)
First incidence (days)	374	426	522
Poly-3 test	P = 0.369	P = 0.353N	P = 0.428
All Organs: Malignant Neoplasms			
Overall rate	21/50 (42%)	33/50 (66%)	34/50 (68%)
Adjusted rate	44.3%	69.5%	70.6%
Terminal rate	10/33 (30%)	15/25 (60%)	26/39 (67%)
First incidence (days)	448	470	513
Poly-3 test	P = 0.004	P = 0.009	P = 0.006
All Organs: Benign or Malignant Neoplasms			
Overall rate	47/50 (94%)	48/50 (96%)	48/50 (96%)
Adjusted rate	95.3%	97.6%	97.2%
Terminal rate	31/33 (94%)	25/25 (100%)	38/39 (97%)
First incidence (days)	374	426	513
Poly-3 test	P = 0.401	P = 0.465	P = 0.517

(T) Terminal kill.
[a]Due to early mortality, lesion incidences for the 1,000 mg/kg group are not presented.
[b]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.
[c]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[d]Observed incidence at terminal kill.
[e]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f]Not applicable; no neoplasms in animal group.
[g]Value of statistic cannot be computed.

Table C-3. Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies				
Ginkgo biloba extract (March 2005)	31/50	22/50	3/50	24/50
Indole-3-carbinol (April 2007)	26/50	12/50	3/50	15/50
Kava kava extract (August 2004)	27/50	20/50	0/50	20/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	29/50	22/50	1/50	22/50
Tetrabromobisphenol A (August 2007)	32/50	11/50	2/50	12/50
Total (%)	145/250 (58.0%)	87/250 (34.8%)	9/250 (3.6%)	93/250 (37.2%)
Mean ± standard deviation	58.0% ± 5.1%	34.8% ± 10.9%	3.6% ± 2.6%	37.2% ± 10.0%
Range	52%-64%	22%-44%	0%-6%	24%-48%
Overall Historical Incidence: All Routes				
Total (%)	594/949 (62.6%)	348/949 (36.7%)	40/949 (4.2%)	371/949 (39.1%)
Mean ± standard deviation	62.6% ± 9.1%	36.7% ± 11.4%	4.2% ± 3.5%	39.1% ± 11.6%
Range	48%-78%	22%-56%	0%-12%	22%-54%

[a]Data as of June 2013.

Table C-4. Historical Incidence of Large Intestine (Cecum or Colon) Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies	
Ginkgo biloba extract (March 2005)	0/50
Indole-3-carbinol (April 2007)	0/50
Kava kava extract (August 2004)	0/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	0/50
Tetrabromobisphenol A (August 2007)	0/50
Total	0/250
Overall Historical Incidence: All Routes	
Total (%)	4/950 (0.4%)
Mean ± standard deviation	0.4% ± 0.8%
Range	0%-2%

[a]Data as of June 2013.

Table C-5. Historical Incidence of Hemangioma and Hemangiosarcoma in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hemangioma	Hemangiosarcoma	Hemangioma or Hemangiosarcoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	0/50	9/50	9/50
Indole-3-carbinol (April 2007)	0/50	4/50	4/50
Kava kava extract (August 2004)	2/50	6/50	8/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	0/50	8/50	8/50
Tetrabromobisphenol A (August 2007)	2/50	1/50	3/50
Total (%)	4/250 (1.6%)	28/250 (11.2%)	32/250 (12.8%)
Mean ± standard deviation	1.6% ± 2.2%	11.2% ± 6.4%	12.8% ± 5.4%
Range	0%–4%	2%–18%	6%–18%
Overall Historical Incidence: All Routes			
Total (%)	13/950 (1.4%)	92/950 (9.7%)	105/950 (11.1%)
Mean ± standard deviation	1.4% ± 1.5%	9.7% ± 4.5%	11.1% ± 4.2%
Range	0%–4%	2%–18%	4%–18%

[a]Data as of June 2013.

Table C-6. Summary of the Incidence of Non-neoplastic Lesions in Male Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	–	1
Moribund	9	10	6	12
Natural deaths	8	14	5	25
Survivors				
Died last week of study	1	1	–	–
Terminal kill	32	25	39	12
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Gallbladder	(49)	(46)	(50)	(49)
Pigmentation, hematoidin	1 (2%)	–	–	–
Intestine large, cecum	(47)	(44)	(47)	(38)
Intestine large, colon	(47)	(46)	(50)	(40)
Diverticulum	–	–	1 (2%)	–
Inflammation, chronic active	–	1 (2%)	–	–
Intestine large, rectum	(47)	(46)	(50)	(41)
Intestine small, duodenum	(47)	(41)	(48)	(31)
Intestine small, ileum	(47)	(43)	(50)	(40)
Hyperplasia	1 (2%)	–	–	–
Intestine small, jejunum	(47)	(44)	(49)	(38)
Diverticulum	–	1 (2%)	–	–
Peyer’s patch, hyperplasia, lymphoid	–	–	1 (2%)	1 (3%)
Liver	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)	–	–	1 (2%)
Angiectasis	–	–	2 (4%)	1 (2%)
Basophilic focus	9 (18%)	9 (18%)	6 (12%)	9 (18%)
Clear cell focus	11 (22%)	10 (20%)	25 (50%)	8 (16%)
Eosinophilic focus	20 (40%)	33 (66%)	40 (80%)	14 (28%)
Fatty change	1 (2%)	2 (4%)	1 (2%)	–
Fatty change, focal	–	1 (2%)	2 (4%)	1 (2%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Fibrosis	–	1 (2%)	–	–
Hemorrhage, chronic	–	–	1 (2%)	–
Inflammation	1 (2%)	–	–	–
Inflammation, granulomatous		–	–	1 (2%)
Mixed cell focus	7 (14%)	8 (16%)	12 (24%)	6 (12%)
Necrosis	1 (2%)	1 (2%)	–	6 (12%)
Pigmentation	1 (2%)	–	–	–
Tension lipidosis	3 (6%)	–	3 (6%)	2 (4%)
Bile duct, cyst	–	–	3 (6%)	4 (8%)
Bile duct, cyst, multiple	–	–	–	1 (2%)
Hepatocyte, atrophy	–	1 (2%)	–	–
Hepatocyte, hypertrophy	2 (4%)	–	–	–
Hepatocyte, necrosis	–	2 (4%)	1 (2%)	–
Kupffer cell, pigmentation	–	–	2 (4%)	–
Oval cell, hyperplasia	–	–	1 (2%)	–
Periportal, vacuolization cytoplasmic	–	–	1 (2%)	2 (4%)
Serosa, inflammation	–	–	1 (2%)	–
Mesentery	(3)	(3)	(4)	(2)
Hemorrhage	–	–	1 (25%)	–
Fat, necrosis	2 (67%)	2 (67%)	2 (50%)	1 (50%)
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	–	–	1 (2%)	–
Acinus, atrophy	–	1 (2%)	1 (2%)	–
Arteriole, fibrosis	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(50)	(49)
Hyperkeratosis	–	–	1 (2%)	1 (2%)
Infiltration cellular, mononuclear cell	5 (10%)	8 (16%)	21 (42%)	27 (55%)
Inflammation	9 (18%)	10 (20%)	20 (40%)	26 (53%)
Ulcer	9 (18%)	9 (18%)	19 (38%)	28 (57%)
Epithelium, hyperplasia	10 (20%)	13 (27%)	27 (54%)	28 (57%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	1 (2%)	–	–	–
Hyperplasia	1 (2%)	–	2 (4%)	1 (2%)
Hyperplasia, focal	–	–	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Tooth	(14)	(9)	(9)	(2)
Dysplasia	11 (79%)	8 (89%)	9 (100%)	2 (100%)
Inflammation	1 (7%)	1 (11%)	1 (11%)	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	2 (4%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	24 (48%)	20 (40%)	18 (36%)	8 (16%)
Inflammation	–	–	–	1 (2%)
Mineralization	–	2 (4%)	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Atrium, thrombosis	–	1 (2%)	1 (2%)	–
Myocardium, necrosis	1 (2%)	–	–	–
Pericardium, fibrosis	1 (2%)	–	–	–
Valve, degeneration	–	–	1 (2%)	–
Valve, inflammation	2 (4%)	2 (4%)	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)	–	–	1 (2%)
Hyperplasia	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hypertrophy	2 (4%)	–	–	–
Vacuolization cytoplasmic	–	1 (2%)	–	–
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Hyperplasia	1 (2%)	2 (4%)	–	–
Parathyroid gland	(45)	(43)	(48)	(42)
Pituitary gland	(50)	(48)	(48)	(50)
Thyroid gland	(50)	(50)	(50)	(50)
Fibrosis	–	–	–	1 (2%)
Inflammation	1 (2%)	–	–	–
Follicle, cyst	–	1 (2%)	1 (2%)	–
General Body System				
Peritoneum	(0)	(2)	(0)	(0)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Inflammation, suppurative	–	1 (50%)	–	–
Genital System				
Coagulating gland	(3)	(4)	(1)	(0)
Inflammation	–	–	1 (100%)	–
Inflammation, chronic active	–	1 (25%)	–	–
Epididymis	(50)	(50)	(50)	(50)
Degeneration	1 (2%)	–	–	–
Granuloma sperm	–	–	1 (2%)	–
Inflammation, chronic active	–	1 (2%)	–	–
Preputial gland	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	–
Ectasia	2 (4%)	2 (4%)	1 (2%)	5 (10%)
Inflammation	3 (6%)	2 (4%)	3 (6%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	1 (2%)	–
Inflammation	–	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	–	1 (2%)	–	–
Epithelium, hyperplasia	1 (2%)	–	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, degeneration	5 (10%)	3 (6%)	5 (10%)	3 (6%)
Interstitial cell, hyperplasia	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	1 (2%)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Lymph node	(3)	(0)	(2)	(0)
Lymph node, mandibular	(50)	(50)	(50)	(49)
Hyperplasia, lymphoid	1 (2%)	–	–	–
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Atrophy	–	–	–	1 (2%)
Hemorrhage	1 (2%)	1 (2%)	–	–
Hyperplasia, lymphoid	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Infiltration cellular, histiocyte	–	–	2 (4%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Inflammation	–	1 (2%)	–	2 (4%)
Necrosis	–	1 (2%)	–	–
Necrosis, lymphoid	–	–	–	1 (2%)
Spleen	(50)	(48)	(50)	(49)
Amyloid deposition	1 (2%)	–	–	1 (2%)
Angiectasis	–	–	–	1 (2%)
Fibrosis	–	–	–	1 (2%)
Hematopoietic cell proliferation	1 (2%)	3 (6%)	–	5 (10%)
Hyperplasia, lymphoid	–	1 (2%)	–	2 (4%)
Pigmentation, hemosiderin	–	2 (4%)	–	–
Lymphoid follicle, atrophy	3 (6%)	2 (4%)	1 (2%)	6 (12%)
Thymus	(47)	(45)	(41)	(48)
Atrophy	41 (87%)	42 (93%)	40 (98%)	40 (83%)
Cyst	1 (2%)	–	–	–
Hyperplasia, lymphoid	1 (2%)	–	–	–
Thrombosis	–	1 (2%)	–	–
Epithelial cell, hyperplasia	–	1 (2%)	–	–
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Ulcer	2 (4%)	4 (8%)	3 (6%)	4 (8%)
Subcutaneous tissue, necrosis	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	1 (2%)	–	–	1 (2%)
Fibrosis	–	–	–	1 (2%)
Fibrous osteodystrophy	1 (2%)	–	–	–
Femur, callus	–	2 (4%)	–	–
Joint, degeneration	–	–	–	4 (8%)
Vertebra, fracture	–	–	1 (2%)	–
Skeletal muscle	(0)	(1)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	1 (2%)	–	–
Peripheral nerve	(0)	(0)	(0)	(2)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Axon, sciatic, degeneration	–	–	–	1 (50%)
Spinal cord	(0)	(0)	(0)	(2)
Axon, degeneration	–	–	–	2 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	–	–	–
Infiltration cellular, histiocyte	1 (2%)	–	1 (2%)	–
Inflammation	1 (2%)	–	–	2 (4%)
Pigmentation, hemosiderin	–	–	1 (2%)	–
Thrombosis	1 (2%)	–	–	–
Alveolar epithelium, hyperplasia	5 (10%)	1 (2%)	6 (12%)	2 (4%)
Alveolar epithelium, hypertrophy	2 (4%)	–	1 (2%)	1 (2%)
Arteriole, thrombosis	–	–	1 (2%)	–
Bronchiole, hyperplasia	–	1 (2%)	–	–
Interstitium, fibrosis	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Inflammation	5 (10%)	2 (4%)	3 (6%)	2 (4%)
Polyp, inflammatory	–	1 (2%)	–	–
Respiratory epithelium, hyperplasia	27 (54%)	25 (50%)	20 (40%)	12 (24%)
Respiratory epithelium, necrosis	–	–	1 (2%)	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	1 (2%)	–
Cataract	2 (4%)	–	1 (2%)	1 (2%)
Inflammation	1 (2%)	–	–	1 (2%)
Cornea, inflammation	1 (2%)	1 (2%)	1 (2%)	–
Harderian gland	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	–	–	–
Hyperplasia	1 (2%)	1 (2%)	–	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(48)
Cyst	1 (2%)	–	–	–
Hydronephrosis	–	3 (6%)	–	1 (2%)
Infarct	–	1 (2%)	–	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Infiltration cellular, lymphocyte	–	1 (2%)	2 (4%)	–
Nephropathy	41 (82%)	30 (60%)	32 (64%)	42 (88%)
Glomerulus, amyloid deposition	1 (2%)	–	–	1 (2%)
Papilla, mineralization	–	–	1 (2%)	–
Papilla, necrosis	3 (6%)	1 (2%)	–	–
Pelvis, inflammation	–	–	1 (2%)	1 (2%)
Renal tubule, cyst	6 (12%)	2 (4%)	5 (10%)	6 (13%)
Renal tubule, cyst, multiple	–	–	–	1 (2%)
Renal tubule, cytoplasmic alteration	–	20 (40%)	47 (94%)	46 (96%)
Renal tubule, inflammation	–	–	–	4 (8%)
Renal tubule, mineralization	1 (2%)	–	1 (2%)	2 (4%)
Renal tubule, necrosis	–	–	–	4(8%)
Renal tubule, pigmentation	–	5 (10%)	1 (2%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)	–	1 (2%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix D. Summary of Lesions in Female Mice in the Two-year Gavage Study of Tetrabromobisphenol A

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths	–	–	–	–
Accidental death	–	–	–	1
Moribund	6	8	3	7
Natural deaths	4	11	11	38
Survivors				
Terminal kill	40	31	36	4
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(47)	(49)	(50)	(50)
Intestine large, cecum	(48)	(46)	(45)	(21)
Leiomyoma	–	1 (2%)	–	–
Intestine large, colon	(50)	(48)	(50)	(43)
Intestine large, rectum	(50)	(50)	(50)	(41)
Adenoma	–	–	1 (2%)	–
Intestine small, duodenum	(47)	(46)	(42)	(18)
Adenoma	1 (2%)	–	–	–
Carcinoma	–	1 (2%)	–	–
Intestine small, ileum	(48)	(46)	(45)	(19)
Intestine small, jejunum	(48)	(47)	(43)	(18)
Liver	(50)	(50)	(49)	(49)
Hemangioma	–	–	2 (4%)	–
Hemangiosarcoma	–	1 (2%)	–	–
Hepatocellular adenoma	12 (24%)	9 (18%)	11 (22%)	1 (2%)
Hepatocellular adenoma, multiple	1 (2%)	4 (8%)	4 (8%)	–
Hepatocellular carcinoma	2 (4%)	3 (6%)	5 (10%)	1 (2%)
Hepatocellular carcinoma, multiple	–	1 (2%)	–	–
Squamous cell carcinoma, metastatic, stomach, forestomach	–	1 (2%)	–	–
Mesentery	(3)	(8)	(7)	(0)
Oral mucosa	(1)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)	–	–	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Pancreas	(50)	(49)	(50)	(50)
Salivary glands	(50)	(48)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(48)
Squamous cell carcinoma	–	1 (2%)	–	–
Squamous cell carcinoma, multiple	–	1 (2%)	–	–
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(0)	(0)
Squamous cell papilloma	1 (100%)	–	–	–
Tooth	(1)	(1)	(1)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Adrenal medulla	(50)	(50)	(49)	(50)
Pheochromocytoma benign	1 (2%)	–	–	–
Islets, pancreatic	(50)	(49)	(50)	(50)
Parathyroid gland	(34)	(41)	(42)	(43)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	1 (2%)	–	–	–
Pars intermedia, adenoma	–	–	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, carcinoma	–	–	1 (2%)	–
Follicular cell, adenoma	–	–	1 (2%)	–
General Body System				
Peritoneum	(0)	(1)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(49)	(48)
Ovary	(50)	(50)	(50)	(47)
Cystadenoma	2 (4%)	–	1 (2%)	2 (4%)
Granulosa cell tumor malignant	–	–	1 (2%)	–
Luteoma	–	1 (2%)	–	–
Oviduct	(1)	(0)	(1)	(0)
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	–	1 (2%)	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Sarcoma stromal	1 (2%)	–	1 (2%)	–
Cervix, sarcoma stromal	1 (2%)	–	–	–
Vagina	(0)	(1)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(1)	(5)	(3)	(1)
Pancreatic, granulosa cell tumor malignant, metastatic, ovary	–	–	1 (33%)	–
Lymph node, mandibular	(50)	(48)	(48)	(46)
Lymph node, mesenteric	(50)	(50)	(50)	(47)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Thymus	(50)	(50)	(48)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	–	–	1 (2%)	–
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	–	–	–
Fibrosarcoma	1 (2%)	–	–	–
Fibrous histiocytoma		1 (2%)	–	–
Hemangioma	1 (2%)	–	–	–
Schwannoma malignant	–	–	1 (2%)	–
Squamous cell papilloma	–	–	–	1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	–	–	–
Subcutaneous tissue, sarcoma		–	–	1 (2%)
Subcutaneous tissue, schwannoma malignant	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibroma	1 (2%)	–	–	–
Osteosarcoma	–	1 (2%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	2 (4%)	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Alveolar/bronchiolar carcinoma	1 (2%)	–	–	–
Schwannoma malignant, metastatic, skin	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	2 (4%)	6 (12%)	3 (6%)	1 (2%)
Carcinoma	1 (2%)	1 (2%)	2 (4%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(47)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)	–	–
Leukemia mononuclear	–	1 (2%)	–	–
Lymphoma malignant	9 (18%)	4 (8%)	4 (8%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	32	32	28	6
Total primary neoplasms	50	42	42	8
Total animals with benign neoplasms	22	23	21	5
Total benign neoplasms	28	24	27	5
Total animals with malignant neoplasms	18	17	15	2
Total malignant neoplasms	22	18	15	3
Total animals with metastatic neoplasms	–	2	2	–
Total metastatic neoplasms	–	2	2	–

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg
Harderian Gland: Adenoma			
Overall rate[b]	2/50 (4%)	6/50 (12%)	3/50 (6%)
Adjusted rate[c]	4.2%	13.0%	6.7%
Terminal rate[d]	1/40 (3%)	4/31 (13%)	2/36 (6%)
First incidence (days)	438	556	669
Poly-3 test[e]	P = 0.378	P = 0.122	P = 0.469
Harderian Gland: Adenoma or Carcinoma			
Overall rate	3/50 (6%)	7/50 (14%)	4/50 (8%)
Adjusted rate	6.3%	15.2%	8.8%
Terminal rate	2/40 (5%)	5/31 (16%)	2/36 (6%)
First incidence (days)	438	556	486
Poly-3 test	P = 0.393	P = 0.143	P = 0.472
Liver: Hepatocellular Adenoma			
Overall rate	13/50 (26%)	13/50 (26%)	15/49 (31%)
Adjusted rate	27.3%	28.2%	33.8%
Terminal rate	9/40 (23%)	9/31 (29%)	14/36 (39%)
First incidence (days)	663	619	688
Poly-3 test	P = 0.289	P = 0.552	P = 0.326
Liver: Hepatocellular Carcinoma			
Overall rate	2/50 (4%)	4/50 (8%)	5/49 (10%)
Adjusted rate	4.3%	8.8%	11.1%
Terminal rate	2/40 (5%)	3/31 (10%)	3/36 (8%)
First incidence (days)	729 (T)	718	552
Poly-3 test	P = 0.154	P = 0.322	P = 0.200
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	15/50 (30%)	14/50 (28%)	18/49 (37%)
Adjusted rate	31.5%	30.4%	39.7%
Terminal rate	11/40 (28%)	10/31 (32%)	15/36 (42%)
First incidence (days)	663	619	552
Poly-3 test	P = 0.237	P = 0.543N	P = 0.271
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	10.4%	4.4%	2.3%
Terminal rate	3/40 (8%)	2/31 (7%)	1/36 (3%)
First incidence (days)	563	729 (T)	729 (T)
Poly-3 test	P = 0.070N	P = 0.239N	P = 0.120N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			

	Vehicle Control	250 mg/kg	500 mg/kg
Overall rate	6/50 (12%)	2/50 (4%)	1/50 (2%)
Adjusted rate	12.5%	4.4%	2.3%
Terminal rate	3/40 (8%)	2/31 (7%)	1/36 (3%)
First incidence (days)	563	729 (T)	729 (T)
Poly-3 test	P = 0.034N	P = 0.152N	P = 0.069N
All Organs: Malignant Lymphoma			
Overall rate	9/50 (18%)	4/50 (8%)	4/50 (8%)
Adjusted rate	19.1%	8.8%	8.9%
Terminal rate	9/40 (23%)	3/31 (10%)	3/36 (8%)
First incidence (days)	729 (T)	694	669
Poly-3 test	P = 0.089N	P = 0.128N	P = 0.135N
All Organs: Benign Neoplasms			
Overall rate	22/50 (44%)	23/50 (46%)	21/50 (42%)
Adjusted rate	44.6%	49.2%	46.4%
Terminal rate	15/40 (38%)	17/31 (55%)	18/36 (50%)
First incidence (days)	438	556	624
Poly-3 test	P = 0.467	P = 0.404	P = 0.514
All Organs: Malignant Neoplasms			
Overall rate	18/50 (36%)	17/50 (34%)	15/50 (30%)
Adjusted rate	37.3%	36.0%	32.0%
Terminal rate	13/40 (33%)	8/31 (26%)	9/36 (25%)
First incidence (days)	606	526	486
Poly-3 test	P = 0.333N	P = 0.530N	P = 0.371N
All Organs: Benign or Malignant Neoplasms			
Overall rate	32/50 (64%)	32/50 (64%)	28/50 (56%)
Adjusted rate	64.0%	66.2%	59.3%
Terminal rate	22/40 (55%)	19/31 (61%)	21/36 (58%)
First incidence (days)	438	526	486
Poly-3 test	P = 0.359N	P = 0.495	P = 0.392N

(T) Terminal kill.
[a]Due to early mortality, lesion incidences for the 1,000 mg/kg group are not presented.
[b]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.
[c]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[d]Observed incidence at terminal kill.
[e]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

Table D-3. Summary of the Incidence of Non-neoplastic Lesions in Female Mice in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths	–	–	–	–
Accidental death	–	–	–	1
Moribund	6	8	3	7
Natural deaths	4	11	11	38
Survivors	–	–	–	–
Terminal kill	40	31	36	4
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Periesophageal tissue, inflammation	–	–	–	1 (2%)
Gallbladder	(47)	(49)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Intestine large, cecum	(48)	(46)	(45)	(21)
Lymphoid tissue, hyperplasia	–	–	1 (2%)	1 (5%)
Intestine large, colon	(50)	(48)	(50)	(43)
Serosa, inflammation	1 (2%)	–	–	–
Intestine large, rectum	(50)	(50)	(50)	(41)
Intestine small, duodenum	(47)	(46)	(42)	(18)
Perforation	–	–	–	1 (6%)
Epithelium, vacuolization cytoplasmic	1 (2%)	–	–	–
Intestine small, ileum	(48)	(46)	(45)	(19)
Ulcer	1 (2%)	–	1 (2%)	–
Intestine small, jejunum	(48)	(47)	(43)	(18)
Diverticulum	1 (2%)	–	–	–
Epithelium, vacuolization cytoplasmic	1 (2%)	–	–	–
Peyer’s patch, hyperplasia	2 (4%)	–	–	–
Liver	(50)	(50)	(49)	(49)
Angiectasis	–	1 (2%)	3 (6%)	–
Basophilic focus	8 (16%)	3 (6%)	3 (6%)	1 (2%)
Clear cell focus	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Eosinophilic focus	11 (22%)	16 (32%)	11 (22%)	1 (2%)
Fatty change	6 (12%)	1 (2%)	1 (2%)	2 (4%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Fatty change, focal	–	1 (2%)	3 (6%)	–
Fibrosis	–	–	–	1 (2%)
Hematopoietic cell proliferation	–	2 (4%)	1 (2%)	–
Infiltration cellular, lymphocyte	1 (2%)	–	–	–
Infiltration cellular, polymorphonuclear	–	1 (2%)	–	–
Inflammation, chronic active	–	5 (10%)	1 (2%)	–
Mineralization	–	–	1 (2%)	–
Mixed cell focus	4 (8%)	3 (6%)	3 (6%)	–
Tension lipidosis	4 (8%)	3 (6%)	4 (8%)	2 (4%)
Hepatocyte, atrophy	–	1 (2%)	–	–
Hepatocyte, hypertrophy	–	–	1 (2%)	–
Hepatocyte, necrosis	3 (6%)	1 (2%)	1 (2%)	–
Mesentery	(3)	(8)	(7)	(0)
Degeneration, cystic	1 (33%)	–	–	–
Inflammation, focal	–	1 (13%)	–	–
Fat, inflammation	1 (33%)	–	–	–
Fat, necrosis	2 (67%)	6 (75%)	7 (100%)	–
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(49)	(50)	(50)
Basophilic focus	–	–	1 (2%)	–
Infiltration cellular, lymphocyte	–	–	1 (2%)	–
Inflammation	1 (2%)	–	–	–
Acinus, atrophy	–	–	–	2 (4%)
Salivary glands	(50)	(48)	(50)	(50)
Atrophy	–	–	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(48)
Foreign body	–	–	–	1 (2%)
Hyperkeratosis	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, mononuclear cell	2 (4%)	13 (26%)	33 (66%)	28 (58%)
Inflammation	2 (4%)	14 (28%)	41 (82%)	37 (77%)
Ulcer	2 (4%)	15 (30%)	40 (80%)	38 (79%)
Epithelium, dysplasia	–	–	2 (4%)	–
Epithelium, hyperplasia	4 (8%)	16 (32%)	39 (78%)	39 (81%)
Epithelium, metaplasia, glandular	–	–	2 (4%)	–
Stomach, glandular	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	–	1 (2%)	–	–
Mineralization	1 (2%)	–	–	1 (2%)

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Epithelium, dysplasia	–	–	1 (2%)	–
Serosa, infiltration cellular, lymphocyte	1 (2%)	–	–	–
Tongue	(1)	(0)	(0)	(0)
Tooth	(1)	(1)	(1)	(0)
Dysplasia	1 (100%)	1 (100%)	1 (100%)	–
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	2 (4%)	5 (10%)	5 (10%)	–
Mineralization	–	2 (4%)	1 (2%)	–
Epicardium, inflammation	2 (4%)	–	–	–
Valve, inflammation	1 (2%)	1 (2%)	1 (2%)	–
Valve, pigmentation, hemosiderin	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Atrophy	–	1 (2%)	–	–
Degeneration, cystic	1 (2%)	–	–	–
Hematopoietic cell proliferation	–	1 (2%)	–	–
Hyperplasia	1 (2%)	–	–	–
Hypertrophy	1 (2%)	–	–	–
Adrenal medulla	(50)	(50)	(49)	(50)
Hyperplasia	1 (2%)	–	1 (2%)	–
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)	–	–	1 (2%)
Parathyroid gland	(34)	(41)	(42)	(43)
Hyperplasia, focal	–	–	1 (2%)	–
Pituitary gland	(50)	(50)	(50)	(49)
Pigmentation, hemosiderin	1 (2%)	–	–	–
Pars distalis, hyperplasia	–	1 (2%)	–	1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	–	–	1 (2%)	–
C-cell, hyperplasia	–	1 (2%)	–	–
Follicle, cyst	–	1 (2%)	–	–
Follicular cell, hyperplasia	1 (2%)	–	–	–
General Body System				
Peritoneum	(0)	(1)	(0)	(0)
Inflammation, suppurative	–	1 (100%)	–	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Genital System				
Clitoral gland	(50)	(50)	(49)	(48)
Ovary	(50)	(50)	(50)	(47)
Angiectasis	–	3 (6%)	1 (2%)	1 (2%)
Cyst	–	–	2 (4%)	–
Hemorrhage	2 (4%)	–	–	–
Inflammation	–	2 (4%)	2 (4%)	–
Thrombosis	–	1 (2%)	1 (2%)	–
Bursa, cyst	2 (4%)		1 (2%)	1 (2%)
Follicle, cyst	7 (14%)	6 (12%)	4 (8%)	1 (2%)
Periovarian tissue, necrosis	1 (2%)	–	–	–
Oviduct	(1)	(0)	(1)	(0)
Inflammation	1 (100%)	–	1 (100%)	
Uterus	(50)	(50)	(50)	(50)
Dilatation	–	–	1 (2%)	–
Inflammation	2 (4%)	1 (2%)	–	–
Thrombosis	1 (2%)	–	1 (2%)	–
Cervix, inflammation	–	1 (2%)	–	–
Endometrium, hyperplasia, cystic	35 (70%)	35 (70%)	29 (58%)	22 (44%)
Vagina	(0)	(1)	(0)	(0)
Epithelium, necrosis	–	1 (100%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	3 (6%)	2 (4%)	
Myeloid cell, hyperplasia	–	3 (6%)	–	–
Lymph node	(1)	(5)	(3)	(1)
Mediastinal, hyperplasia, lymphoid	1 (100%)	1 (20%)	–	–
Mediastinal, inflammation	–	–	–	1 (100%)
Mediastinal, necrosis, lymphoid	–	–	–	1 (100%)
Renal, ectasia	–	–	1 (33%)	–
Renal, hemorrhage	–	–	1 (33%)	–
Lymph node, mandibular	(50)	(48)	(48)	(46)
Atrophy	–	–	–	1 (2%)
Infiltration cellular, plasma cell	1 (2%)	–	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(47)
Atrophy	–	–	1 (2%)	–
Ectasia	–	–	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Hyperplasia, lymphoid	1 (2%)	–	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	2 (4%)	–
Hematopoietic cell proliferation	5 (10%)	4 (8%)	3 (6%)	2 (4%)
Hyperplasia, lymphoid	10 (20%)	4 (8%)	6 (12%)	4 (8%)
Pigmentation, hemosiderin	1 (2%)	1 (2%)	–	–
Lymphoid follicle, atrophy	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Thymus	(50)	(50)	(48)	(50)
Atrophy	29 (58%)	24 (48%)	21 (44%)	29 (58%)
Hyperplasia, lymphoid	1 (2%)	–	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Skin	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	31 (62%)	19 (38%)	10 (20%)	6 (12%)
Osteopetrosis	1 (2%)	–	–	–
Osteosclerosis	–	–	1 (2%)	–
Joint, degeneration	3 (6%)	1 (2%)	1 (2%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	–	–	1 (2%)	–
Cerebrum, neuron, necrosis	–	1 (2%)	–	–
Meninges, infiltration cellular, lymphocyte	–	–	1 (2%)	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	–	–	–
Hyperplasia, lymphoid	–	1 (2%)	–	–
Infiltration cellular, histiocyte	2 (4%)	–	–	–
Infiltration cellular, lymphocyte	1 (2%)	–	1 (2%)	–
Inflammation	1 (2%)	1 (2%)	1 (2%)	–
Pigmentation, hemosiderin	–	1 (2%)	–	–
Alveolar epithelium, hyperplasia	1 (2%)	–	2 (4%)	1 (2%)
Interstitium, fibrosis	–	1 (2%)	–	–
Serosa, hyperplasia	–	–	1 (2%)	–

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Serosa, inflammation	1 (2%)	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	3 (6%)	2 (4%)	–
Respiratory epithelium, hyperplasia	8 (16%)	3 (6%)	1 (2%)	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	–
Cataract	–	–	1 (2%)	–
Hemorrhage	–	1 (2%)	–	–
Synechia	–	–	1 (2%)	–
Cornea, inflammation	–	2 (4%)	2 (4%)	–
Harderian gland	(50)	(49)	(50)	(50)
Hyperplasia	–	1 (2%)	1 (2%)	–
Epithelium, hyperplasia	1 (2%)	–	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(47)
Angiectasis	–	–	1 (2%)	–
Infarct	1 (2%)	–	–	–
Infiltration cellular, lymphocyte	2 (4%)	–	1 (2%)	–
Metaplasia, osseous	1 (2%)	–	–	–
Nephropathy	18 (36%)	11 (22%)	23 (46%)	26 (55%)
Papilla, mineralization	1 (2%)	–	1 (2%)	–
Papilla, necrosis	2 (4%)	–	–	–
Renal tubule, cyst	–	–	2 (4%)	2 (4%)
Renal tubule, mineralization	–	3 (6%)	1 (2%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix E. Genetic Toxicology

Table of Contents

Tables

E.1. Bacterial Mutagenicity Test Protocol

Tetrabromobisphenol A was tested for bacterial mutagenicity in two independent tests. In the first, testing was performed as reported by Mortelmans et al.[77] using a different lot of chemical than was used in the NTP animal studies. Briefly, tetrabromobisphenol A was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37ºC. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37ºC.

In the second bacterial mutagenicity test, a sample of lot M032607KA of tetrabromobisphenol A that was used in the 2-year studies was sent to the testing laboratory for assessment of mutagenicity in *S. typhimurium* strains TA98 and TA100 and in *Escherichia coli* strain WP2 *uvrA*/pKM101. Incubation in either buffer or S9 mix (from induced Sprague Dawley rat liver) and plating on minimal glucose agar plates was carried out as described above. Histidine-independent (for the *S. typhimurium* strains) or tryptophan-independent (for the *E. coli* strain) mutant colonies arising on these plates were counted following incubation for 2 days at 37ºC.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of tetrabromobisphenol A. The high dose was limited by experimental design to 10,000 (first test) or 6,000 (second test) µg/plate. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold-increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

E.2. Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[179]. At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group. In addition, the percentage of polychromatic (immature) erythrocytes (PCEs) in a population of 1,000 erythrocytes in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells

among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

E.3. Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

E.4. Results

Tetrabromobisphenol A was tested for bacterial mutagenicity in two independent assays and results were negative in both assays. In the first assay, tetrabromobisphenol A (100 to 10,000 µg/plate) showed no evidence of mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation from induced hamster or rat liver S9[77] (Table E-1). In the second assay, conducted with the same lot of tetrabromobisphenol A that was used in the 2-year studies, no mutagenic activity was detected in *S. typhimurium* strains TA98 or TA100 or in *E. coli* strain WP2 *uvrA*; all tests were conducted with and without rat liver S9, and the highest concentration tested was 6,000 µg/plate (Table E-2). In vivo, no increases in micronucleated NCEs were observed in male or female B6C3F1/N mice following 3 months of administration of tetrabromobisphenol A by gavage over a dose range of 10 to 1,000 mg/kg (Table E-3). In addition, no significant changes in the percentage of circulating polychromatic (immature) erythrocytes were observed in dosed mice, suggesting that tetrabromobisphenol A did not induce bone marrow toxicity over the dose range tested.

Table E-1. Mutagenicity of Tetrabromobisphenol A in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 10% hamster S9	With 10% rat S9	With 10% rat S9
TA100							
	0	105 ± 5	93 ± 4	104 ± 3	87 ± 3	112 ± 2	94 ± 9
	100	99 ± 13	99 ± 13	95 ± 5	107 ± 4	125 ± 7	115 ± 9
	333	110 ± 6[b]	74 ± 2[b]	91 ± 20	85 ± 6	114 ± 6	93 ± 10
	1,000	90 ± 9[b]	78 ± 3[b]	86 ± 8[b]	69 ± 4[b]	97 ± 4[b]	84 ± 3[b]
	3,333	88 ± 3[b]	79 ± 5[b]	79 ± 11[b]	79 ± 4[b]	92 ± 3[b]	96 ± 6[b]
	10,000	100 ± 2[b]	76 ± 3[b]	98 ± 9[b]	77 ± 9[b]	78 ± 8[b]	90 ± 5[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		372 ± 10	345 ± 18	1,521 ± 83	1,133 ± 40	645 ± 9	441 ± 9
TA1535							
	0	27 ± 3	28 ± 4	10 ± 2	6 ± 0	10 ± 1	10 ± 1
	100	23 ± 2	26 ± 1	12 ± 1	9 ± 2	8 ± 2	8 ± 2
	333	22 ± 1[b]	24 ± 3[b]	9 ± 2	8 ± 1	7 ± 1	7 ± 1
	1,000	14 ± 2[b]	20 ± 1[b]	8 ± 1[b]	8 ± 1[b]	5 ± 2[b]	4 ± 0[b]
	3,333	18 ± 1[b]	25 ± 3[b]	8 ± 3[b]	7 ± 1[b]	7 ± 1[b]	6 ± 1[b]
	10,000	17 ± 3[b]	24 ± 2[b]	7 ± 1[b]	6 ± 1[b]	6 ± 0b	9 ± 2[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		274 ± 13	324 ± 21	344 ± 9	452 ± 10	159 ± 20	187 ± 10
TA1537							
	0	4 ± 1	7 ± 3	5 ± 1	6 ± 2	6 ± 1	3 ± 1
	100	4 ± 1	5 ± 0	6 ± 2	10 ± 3	8 ± 1	10 ± 3
	333	4 ± 1[b]	4 ± 0[b]	4 ± 1	5 ± 1	8 ± 1	8 ± 1
	1,000	3 ± 0[b]	4 ± 1[b]	4 ± 1[b]	5 ± 2[b]	3 ± 0[b]	7 ± 1[b]
	3,333	4 ± 1[b]	4 ± 1[b]	3 ± 0[b]	4 ± 1[b]	3 ± 0[b]	4 ± 0[b]
	10,000	4 ± 1[b]	3 ± 1[b]	4 ± 0[b]	7 ± 1[b]	5 ± 1[b]	6 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		192 ± 6	154 ± 24	342 ± 12	339 ± 18	108 ± 4	105 ± 6
TA98							
	0	19 ± 1	15 ± 2	17 ± 1	30 ± 1	25 ± 2	25 ± 4
	100	18 ± 1	14 ± 1	28 ± 3	35 ± 3	26 ± 7	38 ± 3
	333	16 ± 1[b]	12 ± 3[b]	24 ± 2	18 ± 1	24 ± 3	20 ± 5
	1,000	12 ± 2[b]	12 ± 1[b]	13 ± 0[b]	20 ± 3[b]	17 ± 3[b]	20 ± 3[b]
	3,333	15 ± 3[b]	12 ± 2[b]	11 ± 1[b]	15 ± 2[b]	15 ± 2[b]	15 ± 3[b]
	10,000	16 ± 2[b]	11 ± 0[b]	14 ± 3[b]	23 ± 3[b]	16 ± 1[b]	13 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		844 ± 37	354 ± 31	1,665 ± 37	1,444 ± 62	541 ± 11	404 ± 30

[a]Study performed at SRI International. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol and these data are presented by Mortelmans et al.[77]. 0 µg/plate was the solvent control.
[b]Precipitate on plate.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-2. Mutagenicity of Tetrabromobisphenol A in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	69 ± 3	87 ± 7	91 ± 9	87 ± 1
	50	62 ± 2	59 ± 4	74 ± 3	–
	100	54 ± 2	48 ± 4	70 ± 2	85 ± 4
	250	37 ± 2[b]	42 ± 7	62 ± 7	68 ± 4
	500	37 ± 8[b]	33 ± 5[b]	43 ± 4	55 ± 3
	1,000	14 ± 0[c]	26 ± 4[b]	52 ± 5	49 ± 3
	3,000	20 ± 7[c]	49 ± 3[b]	53 ± 8[b]	33 ± 4[b]
	6,000	–	–	–	58 ± 6[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control[d]		470 ± 10	524 ± 14	750 ± 21	491 ± 34
TA98					
	0	18 ± 4	20 ± 2	23 ± 4	16 ± 2
	50	15 ± 1	21 ± 2	24 ± 4	–
	100	11 ± 1	16 ± 3	23 ± 4	24 ± 3
	250	13 ± 1	13 ± 2	21 ± 1	18 ± 4
	500	6 ± 1[b]	12 ± 1[b]	15 ± 1	15 ± 1
	1,000	9 ± 2[b]	9 ± 4[b]	21 ± 2	14 ± 2
	3,000	8 ± 1[b]	12 ± 5[b]	12 ± 1[b]	8 ± 1[b]
	6,000	–	–	–	8 ± 0[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		528 ± 31	629 ± 17	1,878 ± 79	1,078 ± 55
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	138 ± 8	128 ± 8	118 ± 6	187 ± 11
	50	–	166 ± 13	119 ± 5	–
	100	112 ± 2	155 ± 13	113 ± 9	165 ± 4
	250	114 ± 3[b]	161 ± 9	103 ± 11	184 ± 13
	500	112 ± 15[b]	127 ± 27	121 ± 12[b]	159 ± 6
	1,000	95 ± 4[b]	102 ± 4	78 ± 6[b]	131 ± 3
	3,000	120 ± 9[b]	94 ± 8[b]	98 ± 8[b]	117 ± 8[b]
	6,000	143 ± 2[b]	–	–	122 ± 9[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,450 ± 73	1,123 ± 65	1,399 ± 20	1,286 ± 39

[a]Study was performed at ILS, Inc., using lot M032607KA. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[b]Precipitate on plate.
[c]Slight toxicity and precipitate on plate.
[d]The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Tetrabromobisphenol A by Gavage for Three Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	1.70 ± 0.75	–	2.54 ± 0.31
Tetrabromobisphenol A	10	5	1.20 ± 0.30	0.7426	2.98 ± 0.38
	50	5	1.70 ± 0.82	0.5000	2.72 ± 0.25
	100	5	2.90 ± 0.68	0.1072	3.04 ± 0.32
	500	5	2.50 ± 0.76	0.1932	3.88 ± 0.40
	1,000	5	1.90 ± 0.24	0.4075	2.70 ± 0.37
			P = 0.334[e]		
Female					
Corn oil	0	5	1.00 ± 0.27	–	3.16 ± 0.25
Tetrabromobisphenol A	10	5	1.60 ± 0.51	0.1195	2.90 ± 0.47
	50	5	1.20 ± 0.41	0.3348	3.34 ± 0.56
	100	5	1.10 ± 0.29	0.4136	2.84 ± 0.14
	500	5	1.60 ± 0.19	0.1195	2.98 ± 0.16
	1,000	5	1.20 ± 0.41	0.3348	2.30 ± 0.31
			P = 0.431		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[179]. NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant at $P \leq 0.005$.
[d]Vehicle control.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 23	53.1 ± 1.6	53.5 ± 1.8	50.4 ± 1.3	52.9 ± 1.4	47.7 ± 0.7**	47.8 ± 0.9**
Week 14	46.9 ± 0.3	47.6 ± 0.4	47.5 ± 0.5	46.8 ± 0.5	46.3 ± 0.4	45.7 ± 0.5
Hemoglobin (g/dL)						
Day 23	16.4 ± 0.5	16.5 ± 0.5	15.5 ± 0.3	16.3 ± 0.4	14.8 ± 0.1**	14.9 ± 0.2**
Week 14	14.6 ± 0.1	14.6 ± 0.1	14.6 ± 0.2	14.6 ± 0.2	14.4 ± 0.1	14.0 ± 0.1**
Erythrocytes (10^6/μL)						
Day 23	8.78 ± 0.23	8.86 ± 0.26	8.36 ± 0.20	8.74 ± 0.21	8.05 ± 0.08**	8.04 ± 0.13**
Week 14	9.10 ± 0.06	9.22 ± 0.09	9.27 ± 0.08	9.13 ± 0.12	9.04 ± 0.07	8.81 ± 0.09*
Reticulocytes (10^6/μL)						
Day 23	0.32 ± 0.02	0.31 ± 0.02	0.33 ± 0.01	0.36 ± 0.02	0.27 ± 0.02	0.34 ± 0.01
Week 14	0.21 ± 0.00	0.22 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.22 ± 0.01
Mean cell volume (fL)						
Day 23	60.4 ± 0.4	60.3 ± 0.3	60.2 ± 0.3	60.5 ± 0.3	59.2 ± 0.4	59.5 ± 0.4
Week 14	51.6 ± 0.2	51.7 ± 0.2	51.2 ± 0.2	51.2 ± 0.3	51.3 ± 0.2	51.9 ± 0.2
Mean cell hemoglobin (pg)						
Day 23	18.6 ± 0.2	18.7 ± 0.1	18.5 ± 0.1	18.7 ± 0.1	18.4 ± 0.1	18.5 ± 0.1
Week 14	16.1 ± 0.1	15.9 ± 0.1	15.7 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	15.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 23	30.9 ± 0.2	31.0 ± 0.2	30.8 ± 0.2	30.8 ± 0.1	31.1 ± 0.3	31.1 ± 0.2
Week 14	31.2 ± 0.2	30.8 ± 0.2	30.7 ± 0.2	31.2 ± 0.2	31.0 ± 0.1	30.7 ± 0.2
Platelets (10^3/μL)						
Day 23	975 ± 33	1,034 ± 23	1,023 ± 24	939 ± 46	994 ± 23	956 ± 16
Week 14	783 ± 32	845 ± 15	841 ± 29	852 ± 11	794 ± 20	858 ± 12
Leukocytes (10^3/μL)						
Day 23	8.47 ± 0.38	8.53 ± 0.27	8.35 ± 0.28	8.19 ± 0.20	8.11 ± 0.27	7.32 ± 0.32*
Week 14	8.62 ± 0.27	9.24 ± 0.48	9.64 ± 0.42	8.63 ± 0.32	8.80 ± 0.34	8.95 ± 0.40
Segmented neutrophils (10^3/μL)						
Day 23	1.44 ± 0.11	1.66 ± 0.09	1.58 ± 0.11	1.47 ± 0.06	1.48 ± 0.08	1.20 ± 0.09
Week 14	1.64 ± 0.17	1.44 ± 0.09	1.55 ± 0.11	1.46 ± 0.07	1.56 ± 0.12	1.32 ± 0.12
Lymphocytes (10^3/μL)						
Day 23	6.59 ± 0.30	6.41 ± 0.20	6.35 ± 0.21	6.25 ± 0.22	6.24 ± 0.28	5.80 ± 0.25

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Week 14	6.64 ± 0.26	7.50 ± 0.41	7.75 ± 0.40	6.87 ± 0.29	6.90 ± 0.30	7.32 ± 0.31
Monocytes (10^3/μL)						
Day 23	0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.25 ± 0.02	0.19 ± 0.01
Week 14	0.20 ± 0.02	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.03	0.22 ± 0.01	0.22 ± 0.02
Basophils (10^3/μL)						
Day 23	0.138 ± 0.021	0.157 ± 0.021	0.126 ± 0.016	0.138 ± 0.023	0.100 ± 0.013	0.088 ± 0.009
Week 14	0.048 ± 0.003	0.051 ± 0.005	0.058 ± 0.007	0.042 ± 0.004	0.048 ± 0.005	0.052 ± 0.006
Eosinophils (10^3/μL)						
Day 23	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.04 ± 0.00
Week 14	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.08 ± 0.02	0.05 ± 0.01**
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	8.4 ± 0.5	9.6 ± 0.6	10.0 ± 0.5*	10.6 ± 0.5**[b]	10.9 ± 0.6**	10.2 ± 0.4**[b]
Day 23	10.3 ± 0.3[c]	11.0 ± 0.6[b]	10.7 ± 0.5	10.6 ± 0.6[b]	11.5 ± 0.3	11.8 ± 0.9
Week 14	12.6 ± 0.7	12.9 ± 0.7	11.9 ± 0.5	12.1 ± 0.4	11.5 ± 0.5	11.1 ± 0.7
Creatinine (mg/dL)						
Day 4	0.49 ± 0.01	0.50 ± 0.02	0.51 ± 0.01	0.49 ± 0.01[b]	0.49 ± 0.01	0.50 ± 0.00[b]
Day 23	0.59 ± 0.02[b]	0.64 ± 0.04[b]	0.59 ± 0.01	0.61 ± 0.03	0.56 ± 0.02	0.57 ± 0.02
Week 14	0.65 ± 0.02	0.68 ± 0.02	0.64 ± 0.02	0.68 ± 0.01	0.63 ± 0.02	0.62 ± 0.01
Glucose (mg/dL)						
Day 4	130 ± 1	124 ± 2	133 ± 2	133 ± 3[b]	133 ± 3	137 ± 2[b]
Day 23	195 ± 8[c]	176 ± 6[b]	180 ± 5	195 ± 4[b]	177 ± 6	185 ± 7
Week 14	230 ± 8	223 ± 8	235 ± 5	246 ± 5	217 ± 8	214 ± 8
Total protein (g/dL)						
Day 4	5.7 ± 0.0	5.7 ± 0.1	5.8 ± 0.1	5.8 ± 0.1[b]	5.7 ± 0.1	5.8 ± 0.1[b]
Day 23	6.2 ± 0.1[c]	6.6 ± 0.1[b]	6.3 ± 0.1	6.4 ± 0.0[b]	6.2 ± 0.0	6.3 ± 0.1
Week 14	7.1 ± 0.0	7.3 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	7.4 ± 0.1**	7.3 ± 0.1**
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.1 ± 0.0[b]	4.1 ± 0.0	4.2 ± 0.1[b]
Day 23	4.2 ± 0.1[c]	4.4 ± 0.1*[b]	4.3 ± 0.0	4.3 ± 0.0[b]	4.2 ± 0.0	4.3 ± 0.0
Week 14	4.6 ± 0.0	4.7 ± 0.0	4.6 ± 0.0	4.6 ± 0.1	4.9 ± 0.0**	4.9 ± 0.0**
Cholesterol (mg/dL)						
Day 4	91 ± 2	94 ± 2	91 ± 2	89 ± 4	93 ± 2	99 ± 3

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Day 23	88 ± 4	94 ± 4	86 ± 3	95 ± 3	88 ± 2	89 ± 3
Week 14	76 ± 1	76 ± 2	74 ± 3	79 ± 2	79 ± 1	75 ± 2
Alanine aminotransferase (IU/L)						
Day 4	64 ± 1	66 ± 3	65 ± 2	64 ± 2	70 ± 2	67 ± 2
Day 23	60 ± 2	55 ± 3	52 ± 1*	57 ± 3	50 ± 1**	53 ± 2
Week 14	87 ± 5b	89 ± 6[b]	77 ± 5[b]	62 ± 3**	57 ± 2**	56 ± 2**
Alkaline phosphatase (IU/L)						
Day 4	647 ± 11	674 ± 33	639 ± 10	644 ± 16	678 ± 15	661 ± 18
Day 23	507 ± 19[b]	538 ± 21[b]	507 ± 9	543 ± 14	505 ± 9	508 ± 16
Week 14	283 ± 4	281 ± 6	278 ± 5	260 ± 6	363 ± 11**	360 ± 11**
Creatine kinase (IU/L)						
Day 4	353 ± 70	450 ± 111	343 ± 34	304 ± 31[b]	439 ± 59	282 ± 36[b]
Day 23	252 ± 18[c]	259 ± 21[b]	214 ± 21	223 ± 15[b]	248 ± 26	236 ± 29
Week 14	264 ± 125	283 ± 120	237 ± 103	116 ± 8	153 ± 19	156 ± 17
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 1	14 ± 1	15 ± 1	15 ± 1	14 ± 3	12 ± 1
Day 23	14 ± 1[b]	14 ± 1	12 ± 1	13 ± 1	13 ± 0	12 ± 1
Week 14	30 ± 1[b]	31 ± 1[b]	28 ± 1[b]	27 ± 1*	23 ± 1**	20 ± 1**
Bile acids (µmol/L)						
Day 4	13.5 ± 1.2	13.7 ± 1.6	16.0 ± 2.4	11.9 ± 1.0	25.7 ± 2.5**	31.6 ± 5.6**
Day 23	6.4 ± 0.5[b]	8.7 ± 1.0[b]	8.4 ± 1.8	7.0 ± 0.8	11.9 ± 2.6*	16.8 ± 2.4**
Week 14	15.2 ± 1.7	22.9 ± 3.5	19.5 ± 2.2	23.7 ± 2.6	14.7 ± 4.0	8.6 ± 2.6
Total thyroxine (µg/dL)						
Day 4	6.13 ± 0.18	5.94 ± 0.19	6.12 ± 0.14	5.56 ± 0.17	4.78 ± 0.18**	4.49 ± 0.30**
Day 23	5.11 ± 0.31	5.71 ± 0.34	5.52 ± 0.27	4.72 ± 0.22	3.35 ± 0.19**	3.78 ± 0.22**[d]
Week 14	4.66 ± 0.16	4.78 ± 0.25	4.61 ± 0.13	3.67 ± 0.21**	3.08 ± 0.12**	2.80 ± 0.13**
Total triiodothyronine (µg/dL)						
Day 23	151.2 ± 6.2	190.9 ± 14.7	167.6 ± 6.8	184.4 ± 7.5*	164.4 ± 9.7	199.6 ± 10.6**[d]
Week 14	105.9 ± 5.6[c]	109.4 ± 8.2[b]	106.7 ± 6.7[b]	96.7 ± 5.5	97.6 ± 4.5	102.4 ± 5.2
Thyroid stimulating hormone (ng/dL)						
Day 4	5.37 ± 0.39[b]	5.70 ± 0.29	5.06 ± 0.43	4.80 ± 0.35[b]	4.84 ± 0.35[b]	4.78 ± 0.22[b]
Day 23	7.41 ± 0.43	8.10 ± 0.54[b]	8.49 ± 0.49	6.95 ± 0.41	6.22 ± 0.30	6.50 ± 0.33[d]
Week 14	8.04 ± 0.42	7.94 ± 0.49	8.19 ± 0.37	7.83 ± 0.42	5.99 ± 0.29**	7.38 ± 0.34*
Female						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Day 23	56.0 ± 2.0	56.2 ± 1.8	56.0 ± 2.1	52.2 ± 1.6	51.6 ± 0.8	53.4 ± 1.7
Week 14	46.3 ± 0.5	45.7 ± 0.6	46.4 ± 0.3	45.9 ± 0.5	44.5 ± 0.4**	44.7 ± 0.4**
Hemoglobin (g/dL)						
Day 23	17.7 ± 0.6	17.7 ± 0.5	17.5 ± 0.6	16.5 ± 0.5	16.1 ± 0.2	16.7 ± 0.5
Week 14	14.5 ± 0.1	14.6 ± 0.2	14.5 ± 0.1	14.4 ± 0.2	13.9 ± 0.1**	14.0 ± 0.1**
Erythrocytes (10^6/μL)						
Day 23	9.49 ± 0.32	9.52 ± 0.28	9.43 ± 0.32	8.86 ± 0.28	8.87 ± 0.16	9.05 ± 0.27
Week 14	8.57 ± 0.07	8.44 ± 0.12	8.51 ± 0.05	8.37 ± 0.09	8.21 ± 0.06**	8.31 ± 0.06*
Reticulocytes (10^6/μL)						
Day 23	0.20 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.22 ± 0.01
Week 14	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
Mean cell volume (fL)						
Day 23	59.0 ± 0.2	59.0 ± 0.3	59.3 ± 0.4	59.0 ± 0.2	58.2 ± 0.2	59.0 ± 0.3
Week 14	54.1 ± 0.2	54.1 ± 0.2	54.6 ± 0.2	54.8 ± 0.2	54.2 ± 0.1	53.8 ± 0.3
Mean cell hemoglobin (pg)						
Day 23	18.6 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.2 ± 0.1*	18.5 ± 0.1
Week 14	17.0 ± 0.1	17.3 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	16.9 ± 0.1	16.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 23	31.6 ± 0.1	31.5 ± 0.1	31.3 ± 0.2	31.6 ± 0.1	31.2 ± 0.1	31.3 ± 0.2
Week 14	31.4 ± 0.2	31.9 ± 0.2	31.3 ± 0.1	31.3 ± 0.2	31.2 ± 0.1	31.3 ± 0.2
Platelets (10^3/μL)						
Day 23	862 ± 41	866 ± 48	837 ± 38	910 ± 30	924 ± 19	918 ± 51
Week 14	862 ± 12	853 ± 16	832 ± 28	862 ± 28	875 ± 20	856 ± 27
Leukocytes (10^3/μL)						
Day 23	8.84 ± 0.43	9.39 ± 0.47	9.08 ± 0.51	8.46 ± 0.37	9.58 ± 0.57	7.19 ± 0.37
Week 14	8.55 ± 0.34	8.29 ± 0.32	8.18 ± 0.49	9.19 ± 0.38	8.41 ± 0.37	8.44 ± 0.43
Segmented neutrophils (10^3/μL)						
Day 23	1.35 ± 0.08	1.45 ± 0.11	1.36 ± 0.11	1.12 ± 0.10	1.33 ± 0.13	1.09 ± 0.07
Week 14	1.34 ± 0.10	1.39 ± 0.08	1.36 ± 0.08	1.67 ± 0.11	1.35 ± 0.07	1.30 ± 0.11
Lymphocytes (10^3/μL)						
Day 23	6.98 ± 0.38	7.45 ± 0.38	7.26 ± 0.54	6.95 ± 0.37	7.79 ± 0.48	5.75 ± 0.30
Week 14	6.84 ± 0.26	6.52 ± 0.27	6.47 ± 0.42	7.08 ± 0.32	6.72 ± 0.33	6.78 ± 0.31
Monocytes (10^3/μL)						
Day 23	0.22 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	0.19 ± 0.03	0.22 ± 0.02	0.17 ± 0.03
Week 14	0.23 ± 0.01	0.23 ± 0.02	0.23 ± 0.02	0.28 ± 0.02	0.24 ± 0.02	0.22 ± 0.02
Basophils (10^3/μL)						
Day 23	0.206 ± 0.023	0.191 ± 0.028	0.145 ± 0.027	0.118 ± 0.018	0.164 ± 0.025	0.109 ± 0.011**

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Week 14	0.071 ± 0.010	0.070 ± 0.005	0.060 ± 0.007	0.079 ± 0.012	0.044 ± 0.004	0.059 ± 0.010
Eosinophils ($10^3/\mu L$)						
Day 23	0.09 ± 0.01	0.07 ± 0.00	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.01
Week 14	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.01
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	8	10	10	9	10	10
Week 14	10	9	10	10	9	10
Urea nitrogen (mg/dL)						
Day 4	11.0 ± 0.7[e]	8.8 ± 0.7	9.8 ± 0.7[b]	8.9 ± 0.7[c]	7.8 ± 0.5*[f]	10.1 ± 0.7[e]
Day 23	13.6 ± 0.5	11.7 ± 0.7[e]	13.4 ± 0.5[e]	11.6 ± 0.6	13.0 ± 0.6[c]	12.8 ± 0.7[g]
Week 14	13.0 ± 0.4	14.7 ± 0.3	12.5 ± 0.5	13.2 ± 0.4	11.6 ± 0.6	13.2 ± 0.4
Creatinine (mg/dL)						
Day 4	0.44 ± 0.03[e]	0.45 ± 0.02	0.41 ± 0.03[b]	0.44 ± 0.02[c]	0.48 ± 0.03[f]	0.43 ± 0.04[e]
Day 23	0.55 ± 0.02	0.51 ± 0.01[e]	0.54 ± 0.02[e]	0.52 ± 0.01	0.54 ± 0.02[c]	0.56 ± 0.02[g]
Week 14	0.68 ± 0.01	0.67 ± 0.02	0.66 ± 0.02	0.66 ± 0.02	0.68 ± 0.01	0.69 ± 0.01
Glucose (mg/dL)						
Day 4	121 ± 4[e]	125 ± 2	120 ± 3[b]	123 ± 2[c]	132 ± 5[f]	124 ± 3[e]
Day 23	179 ± 5	181 ± 8[e]	187 ± 5[d]	174 ± 5	182 ± 6[c]	173 ± 6[g]
Week 14	213 ± 5	214 ± 7	221 ± 6	200 ± 7	204 ± 6	191 ± 6*
Total protein (g/dL)						
Day 4	6.0 ± 0.1[e]	5.7 ± 0.1[b]	5.9 ± 0.1[b]	5.8 ± 0.1[c]	5.8 ± 0.1[e]	5.9 ± 0.1[e]
Day 23	6.3 ± 0.1	6.3 ± 0.1[e]	6.5 ± 0.1[e]	6.2 ± 0.1	6.2 ± 0.1[c]	6.3 ± 0.2[g]
Week 14	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.4 ± 0.1**
Albumin (g/dL)						
Day 4	4.4 ± 0.1[e]	4.2 ± 0.1	4.3 ± 0.1[b]	4.3 ± 0.1[c]	4.3 ± 0.1[f]	4.3 ± 0.1[e]
Day 23	4.5 ± 0.1	4.5 ± 0.1[e]	4.7 ± 0.1[e]	4.5 ± 0.1	4.5 ± 0.1[c]	4.6 ± 0.1[g]
Week 14	4.9 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.1 ± 0.1
Cholesterol (mg/dL)						
Day 4	105 ± 3	98 ± 2	96 ± 8	102 ± 2	99 ± 2	107 ± 2
Day 23	95 ± 4	95 ± 5[c]	95 ± 4[c]	88 ± 3	88 ± 2	93 ± 3[b]
Week 14	82 ± 2	79 ± 2	83 ± 2	78 ± 2	83 ± 3	85 ± 2
Alanine aminotransferase (IU/L)						
Day 4	58 ± 2	59 ± 2	55 ± 2	61 ± 2	61 ± 3	62 ± 1
Day 23	49 ± 1	49 ± 1[c]	47 ± 2[c]	46 ± 1	52 ± 1	52 ± 1[b]
Week 14	68 ± 4	65 ± 6	57 ± 2	56 ± 2*	47 ± 1**	58 ± 6**

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Alkaline phosphatase (IU/L)						
Day 4	589 ± 11	588 ± 15	593 ± 17	627 ± 22	665 ± 23*	665 ± 20**
Day 23	423 ± 10	441 ± 12[c]	419 ± 17[c]	409 ± 8	443 ± 13	472 ± 10*[b]
Week 14	300 ± 10	314 ± 17	259 ± 7	238 ± 8**	272 ± 10	282 ± 12
Creatine kinase (IU/L)						
Day 4	426 ± 49[e]	614 ± 209	426 ± 104[b]	363 ± 49[c]	443 ± 99[f]	369 ± 40[e]
Day 23	163 ± 18	167 ± 14[e]	209 ± 16[e]	173 ± 19	176 ± 16[c]	215 ± 18[g]
Week 14	155 ± 37	105 ± 14	91 ± 8	120 ± 28	134 ± 24	203 ± 75
Sorbitol dehydrogenase (IU/L)						
Day 4	11 ± 1	12 ± 1	14 ± 1	13 ± 1	14 ± 1	14 ± 1
Day 23	13 ± 1	11 ± 1c	13 ± 1[c]	11 ± 0	11 ± 1	13 ± 1[b]
Week 14	22 ± 1	20 ± 2	17 ± 1*	19 ± 1	15 ± 1**	21 ± 3
Bile acids (µmol/L)						
Day 4	7.2 ± 0.7	10.9 ± 1.4	13.9 ± 1.7	8.7 ± 1.1	40.5 ± 7.3**	26.6 ± 7.7**
Day 23	8.1 ± 1.4	9.3 ± 1.4[c]	6.0 ± 1.0[c]	7.9 ± 2.5	18.1 ± 2.9	12.0 ± 1.0[b]
Week 14	30.3 ± 3.0	26.0 ± 3.4	27.4 ± 4.0	19.3 ± 2.5*	20.2 ± 2.5*	14.7 ± 1.8**
Total thyroxine (µg/dL)						
Day 4	5.52 ± 0.16	5.63 ± 0.12	5.18 ± 0.22	4.52 ± 0.18**	4.05 ± 0.27**	3.87 ± 0.30**
Day 23	4.26 ± 0.25[d]	4.51 ± 0.26	4.05 ± 0.25	3.75 ± 0.30[d]	2.56 ± 0.25**	2.64 ± 0.21**
Week 14	3.33 ± 0.22	3.58 ± 0.17[d]	3.07 ± 0.20	2.76 ± 0.19	1.83 ± 0.15**[d]	1.66 ± 0.10**
Total triiodothyronine (µg/dL)						
Day 23	180.4 ± 8.1[d]	177.5 ± 11.9	180.5 ± 12.1	167.1 ± 5.5[d]	143.8 ± 3.7**	168.1 ± 7.2
Week 14	116.2 ± 6.9[b]	115.8 ± 8.5	115.9 ± 10.7	128.3 ± 8.3	117.7 ± 7.2	113.1 ± 8.2[b]
Thyroid stimulating hormone (ng/dL)						
Day 4	4.95 ± 0.48	5.00 ± 0.36	4.65 ± 0.29	4.77 ± 0.27	4.26 ± 0.18	3.89 ± 0.09*
Day 23	5.26 ± 0.29[b]	6.46 ± 0.42[b]	5.85 ± 0.33	5.16 ± 0.17[d]	5.06 ± 0.23	4.89 ± 0.18
Week 14	7.36 ± 0.39	7.47 ± 0.69[d]	7.79 ± 0.47	8.87 ± 0.64	7.65 ± 0.39[d]	7.00 ± 0.46

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b]n = 9.
[c]n = 8.
[d]n = 10.
[e]n = 7.
[f]n = 4.
[g]n = 5.

Table F-2. Hematology Data for Mice in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
n	**10**	**10**	**10**	**10**	**10**	**10**
Male						
Hematocrit (%)	47.3 ± 0.9	45.7 ± 0.6	45.5 ± 0.7	46.1 ± 0.4	45.2 ± 0.3	**!K1 Is Not In Table** ± 0.3
Hemoglobin (g/dL)	15.9 ± 0.3	15.5 ± 0.2	15.3 ± 0.2	15.6 ± 0.2	15.3 ± 0.1	15.3 ± 0.1
Erythrocytes (10^6/µL)	10.63 ± 0.18	10.30 ± 0.14	10.18 ± 0.14	10.42 ± 0.09	10.26 ± 0.09	10.39 ± 0.05
Reticulocytes (10^6/µL)	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	**!K1 Is Not In Table** ± 0.01
Mean cell volume (fL)	44.5 ± 0.2	44.3 ± 0.1	44.6 ± 0.3	44.2 ± 0.2	44.1 ± 0.2	44.0 ± 0.1
Mean cell hemoglobin (pg)	15.0 ± 0.0	15.0 ± 0.1	15.0 ± 0.1	15.0 ± 0.1	14.9 ± 0.1	14.8 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.1	33.9 ± 0.1	33.7 ± 0.1	34.0 ± 0.1	33.8 ± 0.1	33.6 ± 0.1
Platelets (10^3/µL)	1,053 ± 70	1,128 ± 51	1,086 ± 26	1,036 ± 67	1,213 ± 25*	1,230 ± 20**
Leukocytes (10^3/µL)	0.00 ± 0.25	4.25 ± 0.20	4.25 ± 0.34	4.26 ± 0.38	4.62 ± 0.47	5.09 ± 0.44
Segmented neutrophils (10^3/µL)	0.70 ± 0.05	0.68 ± 0.05	0.63 ± 0.06	0.63 ± 0.06	0.69 ± 0.05	0.69 ± 0.07
Lymphocytes (10^3/µL)	3.68 ± 0.22	3.40 ± 0.17	3.43 ± 0.28	3.44 ± 0.35	3.72 ± 0.39	4.21 ± 0.37
Monocytes (10^3/µL)	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.09 ± 0.01
Basophils (10^3/µL)	0.017 ± 0.003	0.015 ± 0.003	0.011 ± 0.002	0.017 ± 0.003	0.015 ± 0.003	0.019 ± 0.003
Eosinophils (10^3/µL)	0.11 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Female						
Hematocrit (%)	48.2 ± 0.7	50.2 ± 1.1	47.7 ± 0.6	49.4 ± 0.6	49.5 ± 1.0	47.6 ± 1.0
Hemoglobin (g/dL)	16.4 ± 0.2	17.1 ± 0.3	16.2 ± 0.2	16.7 ± 0.2	16.9 ± 0.3	16.1 ± 0.3
Erythrocytes (10^6/µL)	10.67 ± 0.18	11.13 ± 0.22	10.57 ± 0.15	10.96 ± 0.11	**!I1 Is Not In Table** ± 0.22	10.67 ± 0.19
Reticulocytes (10^6/µL)	0.27 ± 0.01	0.35 ± 0.01**	0.31 ± 0.02	0.29 ± 0.01	0.28 ± 0.01	0.27 ± 0.02
Mean cell volume (fL)	45.2 ± 0.3	45.1 ± 0.2	45.1 ± 0.2	45.1 ± 0.2	45.0 ± 0.1	44.6 ± 0.2
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.1 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.2	34.1 ± 0.2	34.0 ± 0.1	33.9 ± 0.1	34.2 ± 0.2	33.8 ± 0.1
Platelets (10^3/µL)	697 ± 59	677 ± 80	796 ± 77	668 ± 41	709 ± 83	849 ± 91
Leukocytes (10^3/µL)	2.81 ± 0.26	2.94 ± 0.21	3.42 ± 0.29	3.38 ± 0.27	3.38 ± 0.42	3.28 ± 0.34

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Segmented neutrophils (10^3/µL)	0.30 ± 0.07	0.29 ± 0.03	0.40 ± 0.09	0.37 ± 0.05	0.32 ± 0.06	0.33 ± 0.04
Lymphocytes (10^3/µL)	2.39 ± 0.20	2.56 ± 0.18	2.84 ± 0.21	2.87 ± 0.23	2.91 ± 0.35	2.85 ± 0.30
Monocytes (10^3/µL)	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
Basophils (10^3/µL)	0.007 ± 0.002	0.009 ± 0.003	0.013 ± 0.003	0.010 ± 0.004	0.012 ± 0.003	0.008 ± 0.002
Eosinophils (10^3/µL)	0.07 ± 0.03	0.04 ± 0.02	0.11 ± 0.03	0.08 ± 0.03	0.08 ± 0.02	0.04 ± 0.02

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Appendix G. Liver Enzyme Results

Tables

Table G-1. Liver Enzyme Activities for F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
n	**10**	**10**	**10**	**10**	**10**	**10**
Male						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 23	0.967 ± 0.033	0.995 ± 0.043	0.693 ± 0.060**	0.561 ± 0.016**	0.671 ± 0.015**	0.673 ± 0.029**
Week 14	0.781 ± 0.028	0.802 ± 0.027	0.613 ± 0.040	0.629 ± 0.034	0.787 ± 0.039	0.997 ± 0.046
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Day 23	42.4 ± 1.9	41.1 ± 2.7	28.0 ± 4.0*	21.7 ± 1.0**	24.2 ± 0.8**	22.8 ± 1.6**
Week 14	36.8 ± 1.6	36.6 ± 1.1	27.5 ± 1.8	38.1 ± 0.8	50.3 ± 1.8**	66.5 ± 2.4**
7-Pentoxyresorufin-*O*-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
Day 23	8.5 ± 0.2	8.3 ± 0.4	5.8 ± 0.5**	5.1 ± 0.3**	6.2 ± 0.5*	10.1 ± 1.7
Week 14	8.4 ± 0.3	7.8 ± 0.3	6.2 ± 0.5	7.2 ± 0.3	108.6 ± 7.4*	196.7 ± 11.2**
UDP-Glucuronosyl transferase (pmol/minute per mg microsomal protein)						
Day 23	4.75 ± 0.15	3.50 ± 0.14**	3.06 ± 0.25**	2.63 ± 0.16**	2.92 ± 0.14**	3.16 ± 0.26**
Week 14	3.34 ± 0.13	3.55 ± 0.15	2.83 ± 0.14	3.02 ± 0.19	3.94 ± 0.20	4.20 ± 0.21
Female						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 23	1.125 ± 0.044	1.013 ± 0.045	0.633 ± 0.033**	0.613 ± 0.026**	0.795 ± 0.050**	0.841 ± 0.046**
Week 14	0.873 ± 0.030	0.856 ± 0.021	0.664 ± 0.020**	0.629 ± 0.038**	0.855 ± 0.040	0.961 ± 0.038
7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Day 23	70.8 ± 1.9	64.8 ± 2.3	39.7 ± 2.5**	54.1 ± 1.8**	64.6 ± 3.1	62.1 ± 2.7
Week 14	80.2 ± 2.8	75.3 ± 2.1	59.4 ± 1.3**	53.1 ± 2.7**	77.6 ± 4.7	84.1 ± 4.2
7-Pentoxyresorufin-O-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
Day 23	8.0 ± 0.3	7.2 ± 0.3	4.4 ± 0.2**	5.5 ± 0.2**	7.3 ± 0.5	9.4 ± 1.6
Week 14	7.1 ± 0.4	6.9 ± 0.3	5.3 ± 0.2	5.3 ± 0.3	26.8 ± 4.2**	56.0 ± 6.5**
UDP-Glucuronosyl transferase (pmol/minute per mg microsomal protein)						
Day 23	3.77 ± 0.14	3.34 ± 0.16	2.48 ± 0.13**	2.23 ± 0.07**	2.41 ± 0.09**	2.21 ± 0.10**
Week 14	4.03 ± 0.16	3.95 ± 0.09	3.45 ± 0.11**	2.96 ± 0.08**	2.86 ± 0.07**	2.76 ± 0.05**

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are given as mean ± standard error. Statistical tests are performed on unrounded data.

Table G-2. Liver Enzyme Activities for Mice in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
n	**10**	**10**	**10**	**10**	**10**	**10**
Male						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
	0.952 ± 0.096	0.886 ± 0.071	0.883 ± 0.089	0.787 ± 0.058	0.719 ± 0.045*	0.665 ± 0.061*
7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein)						
	207.5 ± 14.4	203.5 ± 9.3	204.4 ± 13.4	195.7 ± 7.7	167.1 ± 7.0*	131.1 ± 9.2**
7-Pentoxyresorufin-O-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
	10.5 ± 0.8	9.4 ± 0.4	9.2 ± 0.9	9.0 ± 0.6	7.8 ± 0.4**	6.4 ± 0.4**
UDP-Glucuronosyl transferase (pmol/minute per mg microsomal protein)						
	2.27 ± 0.19	2.12 ± 0.10	1.97 ± 0.12	2.02 ± 0.12	1.95 ± 0.13	2.49 ± 0.13
Female						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
	0.543 ± 0.042	0.609 ± 0.032	0.483 ± 0.031	0.523 ± 0.020	0.627 ± 0.023	0.527 ± 0.029
7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein)						
	98.5 ± 5.5	110.4 ± 6.1	86.2 ± 4.4	96.2 ± 2.0	109.0 ± 5.4	88.8 ± 4.7
7-Pentoxyresorufin-O-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
	13.9 ± 0.7	15.4 ± 0.6	12.6 ± 0.5	12.4 ± 0.5	12.8 ± 0.6	9.9 ± 0.7**
UDP-Glucuronosyl transferase (pmol/minute per mg microsomal protein)						
	2.95 ± 0.22	2.59 ± 0.15	2.66 ± 0.13*	2.89 ± 0.10	2.87 ± 0.15	2.84 ± 0.18

*Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are given as mean ± standard error. Statistical tests are performed on unrounded data.

Appendix H. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

Table H-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
n	**10**	**10**	**10**	**10**	**10**	**10**
Male						
Necropsy body wt	345 ± 5	354 ± 6	350 ± 7	352 ± 7	339 ± 5	337 ± 6
Heart						
Absolute	0.95 ± 0.02	1.01 ± 0.03	1.02 ± 0.02	0.95 ± 0.02	0.97 ± 0.02	0.97 ± 0.02
Relative	2.75 ± 0.04	2.83 ± 0.06	2.91 ± 0.04	2.70 ± 0.05	2.88 ± 0.04	2.89 ± 0.06
R. Kidney						
Absolute	0.94 ± 0.02	0.95 ± 0.02	0.96 ± 0.02	0.92 ± 0.02	0.92 ± 0.03	0.92 ± 0.02
Relative	2.72 ± 0.04	2.67 ± 0.05	2.74 ± 0.03	2.61 ± 0.05	2.71 ± 0.05	2.72 ± 0.05
Liver						
Absolute	11.88 ± 0.26	12.04 ± 0.34	11.74 ± 0.30	11.89 ± 0.28	12.98 ± 0.22**	13.24 ± 0.27**
Relative	34.40 ± 0.35	33.93 ± 0.48	33.55 ± 0.30	33.81 ± 0.33	38.31 ± 0.23**	39.25 ± 0.30**
Lung						
Absolute	1.92 ± 0.06	2.11 ± 0.13	2.26 ± 0.12	2.02 ± 0.09	2.01 ± 0.11	1.91 ± 0.09
Relative	5.56 ± 0.18	5.99 ± 0.40	6.46 ± 0.28	5.77 ± 0.27	5.92 ± 0.27	5.65 ± 0.20
Spleen						
Absolute	0.660 ± 0.011	0.673 ± 0.017	0.655 ± 0.010	0.647 ± 0.008	0.584 ± 0.011**	0.602 ± 0.017**
Relative	1.92 ± 0.03	1.90 ± 0.03	1.87 ± 0.03	1.84 ± 0.03	1.72 ± 0.02**	1.79 ± 0.04**
R. Testis						
Absolute	1.414 ± 0.025	1.460 ± 0.029	1.422 ± 0.023	1.443 ± 0.016	1.413 ± 0.019	1.438 ± 0.029
Relative	4.102 ± 0.074	4.123 ± 0.060	4.072 ± 0.056	4.111 ± 0.061	4.175 ± 0.056	4.267 ± 0.061
Thymus						
Absolute	0.338 ± 0.015	0.340 ± 0.013	0.370 ± 0.013	0.337 ± 0.011	0.327 ± 0.010	0.291 ± 0.012*
Relative	0.979 ± 0.041	0.959 ± 0.027	1.056 ± 0.026	0.957 ± 0.026	0.969 ± 0.033	0.863 ± 0.037
Female						
Necropsy body wt	185 ± 2	189 ± 4	191 ± 2	186 ± 5	189 ± 4	187 ± 3
Heart						
Absolute	0.66 ± 0.01	0.63 ± 0.01	0.67 ± 0.02	0.65 ± 0.02	0.63 ± 0.02	0.63 ± 0.01
Relative	3.60 ± 0.08	3.36 ± 0.05	3.50 ± 0.11	3.48 ± 0.09	3.31 ± 0.06*	3.34 ± 0.04
R. Kidney						
Absolute	0.57 ± 0.01	0.57 ± 0.01	0.59 ± 0.01	0.59 ± 0.02	0.56 ± 0.01	0.57 ± 0.01
Relative	3.09 ± 0.03	3.04 ± 0.03	3.12 ± 0.04	3.15 ± 0.06	2.97 ± 0.03	3.02 ± 0.04

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Liver						
Absolute	5.82 ± 0.10	6.04 ± 0.18	6.08 ± 0.11	6.01 ± 0.18	6.44 ± 0.14**	6.60 ± 0.10**
Relative	31.48 ± 0.31	31.93 ± 0.44	31.84 ± 0.32	32.34 ± 0.35	34.13 ± 0.42**	35.25 ± 0.36**
Lung						
Absolute	1.42 ± 0.03	1.40 ± 0.04	1.36 ± 0.08	1.34 ± 0.05	1.24 ± 0.05	1.31 ± 0.06
Relative	7.70 ± 0.19	7.45 ± 0.31	7.15 ± 0.42	7.23 ± 0.29	6.56 ± 0.28*	6.95 ± 0.24
Spleen						
Absolute	0.462 ± 0.010	0.456 ± 0.012	0.478 ± 0.010	0.489 ± 0.017	0.447 ± 0.008	0.443 ± 0.008
Relative	2.50 ± 0.04	2.41 ± 0.04	2.51 ± 0.05	2.64 ± 0.09	2.37 ± 0.06	2.37 ± 0.05
Thymus						
Absolute	0.253 ± 0.013	0.243 ± 0.011	0.268 ± 0.010	0.247 ± 0.012	0.242 ± 0.011	0.238 ± 0.004
Relative	1.371 ± 0.067	1.287 ± 0.062	1.402 ± 0.043	1.332 ± 0.061	1.280 ± 0.045	1.274 ± 0.030

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table H-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Wistar Han Rats at the Three-month Interim Evaluation in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	1,000 mg/kg
n	**10**	**10**
Male		
Necropsy body wt	399 ± 8	394 ± 14
Heart		
Absolute	1.25 ± 0.05	1.24 ± 0.05
Relative	3.14 ± 0.10	3.17 ± 0.11
R. Kidney		
Absolute	1.27 ± 0.02	1.27 ± 0.04
Relative	3.19 ± 0.05	3.24 ± 0.05
Liver		
Absolute	15.59 ± 0.52	16.73 ± 0.67
Relative	39.04 ± 0.76	42.50 ± 0.78**
Lung		
Absolute	2.37 ± 0.14	2.14 ± 0.11
Relative	5.93 ± 0.31	5.49 ± 0.30
R. Testis		
Absolute	1.871 ± 0.052	1.895 ± 0.040

	Vehicle Control	1,000 mg/kg
Relative	4.704 ± 0.151	4.865 ± 0.188
Thymus		
Absolute	0.468 ± 0.023	0.377 ± 0.012**
Relative	1.178 ± 0.063	0.970 ± 0.046*
Female		
Necropsy body wt	242 ± 7	236 ± 5
Heart		
Absolute	0.77 ± 0.03	0.78 ± 0.03
Relative	3.18 ± 0.05	3.29 ± 0.07
R. Kidney		
Absolute	0.84 ± 0.03	0.80 ± 0.03
Relative	3.49 ± 0.06	3.38 ± 0.10
Liver		
Absolute	8.39 ± 0.28	8.73 ± 0.25
Relative	34.77 ± 0.81	36.92 ± 0.43*
Lung		
Absolute	1.47 ± 0.07	1.38 ± 0.08
Relative	6.05 ± 0.13	5.83 ± 0.24
Thymus		
Absolute	0.383 ± 0.023	0.311 ± 0.016*
Relative	1.579 ± 0.072	1.317 ± 0.060*

*Significantly different ($P \leq 0.05$) from the vehicle control group by a t-test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table H-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
n	**10**	**10**	**10**	**10**	**10**	**10**
Male						
Necropsy body wt	37.4 ± 0.9	34.7 ± 0.7	38.4 ± 0.6	36.2 ± 1.0	37.1 ± 0.9	35.2 ± 1.2
Heart						
Absolute	0.17 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Relative	4.52 ± 0.19	4.71 ± 0.20	4.89 ± 0.19	4.73 ± 0.16	4.22 ± 0.13	4.43 ± 0.15
R. Kidney						
Absolute	0.28 ± 0.00	0.27 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.27 ± 0.01	0.24 ± 0.01**
Relative	7.42 ± 0.15	7.75 ± 0.14	7.54 ± 0.14	7.91 ± 0.30	7.22 ± 0.23	6.72 ± 0.16*
Liver						
Absolute	1.44 ± 0.06	1.42 ± 0.04	1.59 ± 0.03	1.43 ± 0.03	1.61 ± 0.03**	1.60 ± 0.06*
Relative	38.33 ± 0.74	40.91 ± 0.50*	41.42 ± 0.57*	39.65 ± 0.67*	43.66 ± 1.02**	45.43 ± 0.81**
Lung						
Absolute	0.26 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.02
Relative	6.95 ± 0.51	7.17 ± 0.47	6.97 ± 0.41	6.65 ± 0.48	6.00 ± 0.35	6.56 ± 0.30
Spleen						
Absolute	0.064 ± 0.001	0.063 ± 0.002	0.069 ± 0.002	0.066 ± 0.002	0.069 ± 0.002*	0.069 ± 0.002*
Relative	1.71 ± 0.05	1.81 ± 0.06	1.80 ± 0.04	1.85 ± 0.07	1.88 ± 0.06	1.98 ± 0.08**
R. Testis						
Absolute	0.118 ± 0.002	0.112 ± 0.002	0.112 ± 0.002	0.118 ± 0.002	0.121 ± 0.002	0.113 ± 0.002
Relative	3.180 ± 0.075	3.247 ± 0.081	2.915 ± 0.061	3.274 ± 0.087	3.269 ± 0.094	3.236 ± 0.076
Thymus						
Absolute	0.049 ± 0.003	0.041 ± 0.002	0.055 ± 0.003	0.046 ± 0.004	0.047 ± 0.002	0.047 ± 0.003
Relative	1.323 ± 0.070	1.171 ± 0.050	1.419 ± 0.069	1.276 ± 0.088	1.263 ± 0.051	1.339 ± 0.070
Female						
Necropsy body wt	27.5 ± 0.6	29.3 ± 1.0	28.6 ± 0.7	26.2 ± 0.7	29.2 ± 0.7	27.7 ± 0.6
Heart						
Absolute	0.14 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
Relative	5.26 ± 0.22	5.38 ± 0.17	4.91 ± 0.25	5.67 ± 0.16	5.08 ± 0.21	5.49 ± 0.28
R. Kidney						
Absolute	0.16 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.16 ± 0.00	0.17 ± 0.01	0.17 ± 0.00
Relative	5.89 ± 0.12	5.87 ± 0.17	5.88 ± 0.15	6.15 ± 0.13	5.80 ± 0.11	5.96 ± 0.08

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Liver						
Absolute	1.10 ± 0.03	1.15 ± 0.04	1.16 ± 0.03	1.09 ± 0.04	1.18 ± 0.03	1.24 ± 0.04*
Relative	40.18 ± 1.05	39.31 ± 0.75	40.77 ± 1.06	41.76 ± 0.40	40.34 ± 0.59	44.85 ± 0.64**
Lung						
Absolute	0.29 ± 0.01	0.29 ± 0.01	0.27 ± 0.02	0.29 ± 0.02	0.31 ± 0.01	0.28 ± 0.02
Relative	10.61 ± 0.33	10.03 ± 0.58	9.47 ± 0.51	11.23 ± 0.47	10.61 ± 0.64	10.00 ± 0.64
Spleen						
Absolute	0.079 ± 0.002	0.089 ± 0.005	0.084 ± 0.003	0.078 ± 0.002	0.088 ± 0.003	0.091 ± 0.005
Relative	2.86 ± 0.08	3.05 ± 0.15	2.96 ± 0.13	2.97 ± 0.06	3.00 ± 0.05	3.27 ± 0.12*
Thymus						
Absolute	0.048 ± 0.002	0.052 ± 0.003	0.051 ± 0.002	0.047 ± 0.002	0.052 ± 0.004	0.050 ± 0.002
Relative	1.749 ± 0.054	1.761 ± 0.109	1.792 ± 0.057	1.796 ± 0.055	1.779 ± 0.124	1.818 ± 0.049

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Appendix I. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Figures

Table I-1. Summary of Reproductive Tissue Evaluations for Male F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	100 mg/kg	500 mg/kg	1,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	345 ± 5	352 ± 7	339 ± 5	337 ± 6
L. Cauda epididymis	0.1394 ± 0.0025	0.1428 ± 0.0036	0.1358 ± 0.0036	0.1397 ± 0.0046
L. Epididymis	0.4201 ± 0.0069	0.4277 ± 0.0039	0.4194 ± 0.0064	0.4192 ± 0.0062
L. Testis	1.4766 ± 0.0242	1.5395 ± 0.0191	1.4783 ± 0.0247	1.4904 ± 0.0200
Spermatid measurements				
Spermatid heads (10^6/testis)	160.25 ± 8.04	175.25 ± 5.68	173.25 ± 7.85	168.63 ± 4.16
Spermatid heads (10^6/g testis)	131.76 ± 7.01	138.33 ± 4.52	144.09 ± 5.29	140.47 ± 3.31
Epididymal spermatozoal measurements				
Sperm motility (%)	82.0 ± 0.7	82.5 ± 0.4	82.8 ± 0.6	82.1 ± 0.5
Sperm (10^6/cauda epididymis)	89.7 ± 7.4	83.5 ± 5.0	88.8 ± 4.7	82.9 ± 6.7
Sperm (10^6/g cauda epididymis)	644 ± 53	585 ± 35	653 ± 31	596 ± 50

[a]Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table I-2. Estrous Cycle Characterization for Female F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	100 mg/kg	500 mg/kg	1,000 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	185 ± 2	186 ± 5	189 ± 4	187 ± 3
Proportion of regular cycling females[b]	10/10	10/10	10/10	10/10
Estrous cycle length (days)	5.0 ± 0.00	4.9 ± 0.07	4.9 ± 0.08	5.0 ± 0.00
Estrous stages (% of cycle)				
Diestrus	61.7	57.5	55.8	60.8
Proestrus	16.7	15.0	13.3	15.0
Estrus	20.8	25.0	26.7	23.3
Metestrus	0.8	1.7	4.2	0.8
Uncertain diagnoses	0.0	0.8	0.0	0.0

[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated dosed females spent significantly more time in extended estrus than did females in the vehicle control group (100 mg/kg, $P = 0.008$; 500 and 1,000 mg/kg, $P < 0.001$).
[b]Number of females with a regular cycle/number of females cycling.

Table I-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	0.124	–
Overall Tests	100 mg/kg vs. Vehicle Controls	0.208	–
Overall Tests	500 mg/kg vs. Vehicle Controls	0.34	–
Overall Tests	1,000 mg/kg vs. Vehicle Controls	0.095	–
Extended Estrus	Overall	<0.001	–
Extended Estrus	100 mg/kg vs. Vehicle Controls	0.008	–
Extended Estrus	500 mg/kg vs. Vehicle Controls	<0.001	–
Extended Estrus	1,000 mg/kg vs. Vehicle Controls	<0.001	–
Extended Diestrus	Overall	0.0275	–
Extended Diestrus	100 mg/kg vs. Vehicle Controls	0.318	N
Extended Diestrus	500 mg/kg vs. Vehicle Controls	0.2	N
Extended Diestrus	1,000 mg/kg vs. Vehicle Controls	0.366	N
Extended Metestrus	Overall	1	–
Extended Metestrus	100 mg/kg vs. Vehicle Controls	1	–
Extended Metestrus	500 mg/kg vs. Vehicle Controls	1	–
Extended Metestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Extended Proestrus	Overall	1	–
Extended Proestrus	100 mg/kg vs. Vehicle Controls	1	–
Extended Proestrus	500 mg/kg vs. Vehicle Controls	1	–
Extended Proestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Skipped Estrus	Overall	1	–
Skipped Estrus	100 mg/kg vs. Vehicle Controls	1	–
Skipped Estrus	500 mg/kg vs. Vehicle Controls	1	–
Skipped Estrus	1,000 mg/kg vs. Vehicle Controls	1	–
Skipped Diestrus	Overall	1	–
Skipped Diestrus	100 mg/kg vs. Vehicle Controls	1	–
Skipped Diestrus	500 mg/kg vs. Vehicle Controls	1	–
Skipped Diestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Summary of Significant Groups			
Extended Estrus	100 mg/kg vs. Vehicle Controls	0.008	–
Extended Estrus	500 mg/kg vs. Vehicle Controls	<0.001	–
Extended Estrus	1,000 mg/kg vs. Vehicle Controls	<0.001	–

[a]N means that the dosed group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

Table I-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	100 mg/kg	500 mg/kg	1,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.4 ± 0.9	36.2 ± 1.0	37.1 ± 0.9	35.2 ± 1.2
L. Cauda epididymis	0.0159 ± 0.0009	0.0159 ± 0.0009	0.0171 ± 0.0014	0.0183 ± 0.0030
L. Epididymis	0.0446 ± 0.0014	0.0438 ± 0.0014	0.0445 ± 0.0011	0.0454 ± 0.0026
L. Testis	0.1114 ± 0.0021	0.1104 ± 0.0016	0.1130 ± 0.0021	0.1075 ± 0.0020
Spermatid measurements				
Spermatid heads (10^6/testis)	21.53 ± 0.81	21.90 ± 0.69	22.92 ± 0.67	21.26 ± 0.94
Spermatid heads (10^6/g testis)	223.35 ± 7.23	218.24 ± 8.63	217.09 ± 6.38	212.52 ± 8.03
Epididymal spermatozoal measurements				
Sperm motility (%)	83.8 ± 0.4	85.3 ± 0.4	84.3 ± 1.0	84.5 ± 0. 5
Sperm (10^6/cauda epididymis)	18.3 ± 1.7	15.7 ± 2.1	15.7 ± 2.8	14.8 ± 1.9
Sperm (10^6/g cauda epididymis)	1,152 ± 100	1,005 ± 137	976 ± 174	909 ± 154

[a]Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table I-5. Estrous Cycle Characterization for Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	100 mg/kg	500 mg/kg	1,000 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	27.5 ± 0.6	26.2 ± 0.7	29.2 ± 0.7	27.7 ± 0.6
Proportion of regular cycling females[b]	9/10	8/10	9/9	9/10
Estrous cycle length (days)	4.2 ± 0.12	3.9 ± 0.14	3.9 ± 0.06[c]	4.1 ± 0.11
Estrous stages (% of cycle)				
Diestrus	30.0	35.8	31.7	37.5
Proestrus	0.0	0.0	0.0	0.0
Estrus	47.5	41.7	44.2	40.0
Metestrus	21.7	21.7	24.2	22.5
Uncertain diagnoses	0.8	0.8	0.0	0.0

[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated dosed females did not have significantly more extended estrus or diestrus than the vehicle controls.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

Table I-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	0.042	–
Overall Tests	100 mg/kg vs. Vehicle Controls	0.232	–
Overall Tests	500 mg/kg vs. Vehicle Controls	0.039	N
Overall Tests	1,000 mg/kg vs. Vehicle Controls	0.159	N
Extended Estrus	Overall	0.581	
Extended Estrus	100 mg/kg vs. Vehicle Controls	0.77	N
Extended Estrus	500 mg/kg vs. Vehicle Controls	0.351	N
Extended Estrus	1,000 mg/kg vs. Vehicle Controls	0.351	N
Extended Diestrus	Overall	0.202	–
Extended Diestrus	100 mg/kg vs. Vehicle Controls	0.703	–
Extended Diestrus	500 mg/kg vs. Vehicle Controls	0.05	–
Extended Diestrus	1,000 mg/kg vs. Vehicle Controls	0.405	–
Extended Metestrus	Overall	1	–
Extended Metestrus	100 mg/kg vs. Vehicle Controls	1	–
Extended Metestrus	500 mg/kg vs. Vehicle Controls	1	–
Extended Metestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Extended Proestrus	Overall	1	–
Extended Proestrus	100 mg/kg vs. Vehicle Controls	1	–
Extended Proestrus	500 mg/kg vs. Vehicle Controls	1	–
Extended Proestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Skipped Estrus	Overall	1	–
Skipped Estrus	100 mg/kg vs. Vehicle Controls	1	–
Skipped Estrus	500 mg/kg vs. Vehicle Controls	1	–
Skipped Estrus	1,000 mg/kg vs. Vehicle Controls	1	–
Skipped Diestrus	Overall	1	–
Skipped Diestrus	100 mg/kg vs. Vehicle Controls	1	–
Skipped Diestrus	500 mg/kg vs. Vehicle Controls	1	–
Skipped Diestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Summary of Significant Groups			
Overall Tests	500 mg/kg vs. Vehicle Controls	0.039	N

[a]N means that the dosed group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

Dose (mg/kg)																			
0		D	D	D	P	E	D		D	P	E	D	D						
0			D	D	P	E	D	D	D	P	E	D	D	D					
0						E	D	D	D	P	E	D	D	D	P	E	D		
0					P	E	D	D	D	P	E	D	D	D	P	E			
0			D	D	D	P	E	D	D	D	E	E	D	D					
0		D	D	D	P	E	D	D	D	P	E	D	D						
0						E	D	D	D	P	E	D	D	D	P	E	D		
0			D	D	P	E	D	D	D	P	E	D	D	D					
0					P	E	D	D	D	D	E	M	D	D	P	E			
0				D	P	E	D	D	D	P	E	D	D	D	D				
100		D	D	D	P	E	D	D	D	P	E	D	D						
100						E	D	D	D	P	E	D	D	D	P	E	D		
100				IC	P	E	D	D	D	P	E	D	D	D	P				
100							E	M	D	D	E	D	D	D	P	E	D	D	
100						E	D	D	D	P	E	D	D	D	E	E	D		
100					P	E	D	D	D	P	E	D	D	D	P	E			
100					P	E	D	D	D	P	E	D	D	D	P	E			
100		D	D	D	P	E	D	D	D	P	E	D	D						
100						E	E	D	D	D	E	M	D	D	E	E	D		
100		D	D	D	E	E	D	D	D	P	E	D	D						
500				D	D	E	E	D	D	D	E	E	D	D	D				
500				D	P	E	M	D	D	P	E	D	D	D	E				
500						P	E	D	D	D	E	E	D	D	D	E	E		
500		D	D	D	P	E	D	D	D	P	E	D	D						
500				D	D	P	E	D	D	D	E	E	M	D	D				
500					P	E	D	D	D	P	E	D	D	D	P	E			
500						P	E	D	D	D	E	M	D	D	P	E	D		
500							E	M	D	D	E	M	D	D	E	E	D	D	
500			D	D	P	E	D	D	D	P	E	D	D	D					
500					P	E	D	D	D	P	E	D	D	D	P	E			
1,000				D	D	E	E	D	D	D	E	E	D	D	D				
1,000					D	P	E	D	D	D	E	E	M	D	D	P			
1,000				D	P	E	D	D	D	P	E	D	D	D	P				
1,000		D	D	D	P	E	D	D	D	P	E	D	D						
1,000					P	E	D	D	D	P	E	D	D	D	P	E			
1,000				D	D	P	E	D	D	D	E	E	D	D	D				
1,000			D	D	P	E	D	D	D	P	E	D	D	D					
1,000			D	D	D	P	E	D	D	D	E	E	D	D					
1,000		D	D	D	E	E	D	D	D	P	E	D	D						
1,000					P	E	D	D	D	P	E	D	D	D	P	E			

Figure I-1. Vaginal Cytology Plots for Female F344/NTac Rats in the Three-month Gavage Study of Tetrabromobisphenol A

D = diestrus, P = proestrus, E = estrus, M = metestrus, IC = insufficient number of cells to determine stage.

Dose (mg/kg)																							
0									D	E	E	M	D	E	E	M	D	E	E	M			
0								IC	D	E	E	M	D	E	E	D	D	E	E				
0							M	D	E	E	E	M	D	E	E	E	M	D					
0											E	M	D	E	E	M	D	E	E	M	D	D	
0											E	M	D	E	E	M	D	E	E	M	D	D	
0									D	E	E	M	D	E	E	M	D	E	E	M			
0										E	M	D	D	E	E	M	D	E	E	M	D		
0									D	E	E	M	D	E	E	M	D	D	E	E			
0									D	E	E	M	D	E	E	M	D	D	E	E			
0											E	M	D	E	E	M	D	E	E	M	D	E	
100										E	E	M	D	E	E	M	D	E	E	M	D		
100			D	D	D	D	D	D	D	E	E	M	D	E									
100										E	M	D	D	E	E	M	D	E	M	D	D		
100											E	M	D	E	M	D	D	E	E	M	D	E	
100											E	M	D	E	E	M	D	E	E	M	D	E	
100											E	M	D	E	M	D	D	E	E	M	D	E	
100								D	E	E	M	D	D	E	M	D	D	D	E				
100									IC	E	M	D	D	E	E	M	D	E	E	M			
100								D	E	E	E	M	D	E	E	M	D	E	E				
100										E	E	M	D	E	E	M	D	E	M	D	D		
500								M	D	E	E	M	D	E	E	M	D	E	E				
500									D	E	E	M	D	E	E	M	D	E	E	M			
500											E	M	D	E	E	M	D	E	E	M	D	E	
500									D	E	E	M	D	E	E	M	D	E	E	M			
500									D	E	E	M	D	E	E	M	D	E	E	M			
500									D	E	E	M	D	E	E	M	D	E	E	M			
500										E	E	M	D	E	E	M	D	E	M	D	D		
500								M	D	E	E	M	D	E	M	D	D	E	M				
500		E	E	M	D	D	D	D	D	D	D	D	D										
500										E	E	M	D	E	E	M	D	E	E	M	D		
1,000											E	M	D	E	M	D	D	E	E	M	D	E	
1,000										E	E	M	D	E	E	M	D	E	E	M	D		
1,000								D	D	E	E	M	D	E	E	M	D	E	E				
1,000									D	E	E	M	D	E	E	M	D	E	E	M			
1,000			D	D	D	D	D	D	D	E	M	D	D	E									
1,000									D	E	M	D	D	E	E	M	D	E	M	D			
1,000						M	D	D	E	E	M	D	D	E	E	M	D						
1,000								M	D	E	E	M	D	E	M	D	D	E	E				
1,000									D	E	M	D	D	E	E	M	D	E	M	D			
1,000									D	E	E	M	D	E	E	M	D	E	E	M			

Figure I-2. Vaginal Cytology Plots for Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A

D = diestrus, E = estrus, M = metestrus, IC = insufficient number of cells to determine stage.

Appendix J. Chemical Characterization and Dose Formulation Studies

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Tables

Figures

J.1. Procurement and Characterization

J.1.1. Tetrabromobisphenol A

Tetrabromobisphenol A was obtained from Albemarle Corporation (Baton Rouge, LA) in three lots (25317K-1, C16263X, and 25337XX-8). Lot 25317K-1 was used during the 3-month studies; lots 25317K-1 and C16263X were combined into one lot and renamed lot M032607KA, which was used in the 2-year studies; lot 25337XX-8 was used for dose formulation development studies performed at the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO) and was not used in any of the animal studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, and identity was confirmed by the study laboratory at Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the tetrabromobisphenol A studies are on file at the National Institute of Environmental Health Sciences.

Lots 25317K-1 and M032607KA of the test chemical, a white, crystalline powder, were identified as tetrabromobisphenol A by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy; identity was confirmed by the study laboratory using IR spectroscopy. All spectra were consistent with the literature spectra[180; 181] and the structure of tetrabromobisphenol A. Representative IR and NMR spectra are presented in Figure J-1 and Figure J-2.

For lot 25317K-1 and combined lot M032607KA, the analytical chemistry laboratory determined the melting points using differential scanning calorimetry (DSC) with a Perkin-Elmer diamond differential scanning calorimeter (Perkin-Elmer, Norwalk, CT); the purity was determined using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection by system A.

A) The system included an HPLC instrument (Waters Corporation, Milford, MA) with UV detection, an Alltech Nucleosil C_{18} column, (250 mm × 4.6 mm, 5 μm particle size), (Alltech, Inc., Deerfield, IL), a mobile phase of A) aqueous 18 mM ammonium acetate with 0.5% acetic acid and B) acetonitrile with 0.5% acetic acid, isocratic, 30%A:70%B, UV detection at 254 and 290 nm, and a flow rate of 1.0 mL/minute.

For lot 25317K-1, the DSC results indicated high purity and were in agreement with the manufacturer's certificate of analysis; HPLC/UV analysis indicated one major peak and one impurity detected at both 254 and 290 nm with areas of 0.7% and 0.8% relative to the total peak area, respectively. The overall purity of lot 25317K-1 was determined to be greater than 99%.

For lot M032607KA, the DSC results indicated high purity and were in agreement with the manufacturer's certificate of analysis; HPLC/UV analysis indicated one major peak and one impurity detected at both 254 and 290 nm with areas of 0.8% and 1.1% relative to the total peak area, respectively. The impurity was determined to be tribromobisphenol A by liquid chromatography/mass spectrometry, based on the isotopic pattern in the mass spectrum indicating the presence of three bromine atoms and the m/z of 460.9 ($[M\text{-}H]^-$), consistent with a mass of 461.8. However, the positions of bromination were not determined. Tribromobisphenol A is listed as an impurity in the US Patent issued to Albemarle Corporation for the manufacturing of tetrabromobisphenol A; the positions of bromination are not specified in the patent. The overall purity of lot M032607KA was determined to be approximately 99%.

To ensure stability, the bulk chemical was stored in sealed glass bottles protected from light at room temperature. Periodic reanalyses of the bulk chemical were performed by the study laboratory twice during the 3-month studies and seven times during the 2-year studies using HPLC/UV by system B. No degradation of the test chemical was detected.

> B) The system included an HPLC UV instrument (Waters Corporation or Agilent Inc., Palo Alto, CA), a Nucleosil C_{18} column, (250 mm × 4.6 mm, 5 µm particle size) (Alltech, Inc.), a mobile phase of 70:30 acetonitrile:water (containing 0.5% acetic acid); isocratic, with UV detection at 290 nm, and a flow rate of 1.0 mL/minute.

J.1.2. Corn Oil

National Formulary-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and from Sigma-Aldrich (St. Louis, MO) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than the rejection level of 3 mEq/kg.

J.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing tetrabromobisphenol A with corn oil to give the required concentrations (Table J-1). The dose formulations were prepared monthly during the 3-month studies and approximately every 6 weeks during the 2-year studies. Dose formulations were stored in sealed glass bottles for up to 42 days at room temperature.

Homogeneity studies of 0.5 and 600 mg/mL formulations and stability studies of a 0.5 mg/mL formulation were performed by the analytical chemistry laboratory using HPLC/UV by a system similar to system B, without acetic acid in the mobile phase. Homogeneity was confirmed, and stability was confirmed for at least 42 days for formulations stored in sealed glass vials, protected from light, at temperatures up to 25°C, and for at least 3 hours under simulated animal room conditions.

Prior to the 3-month studies, homogeneity studies of 1, 2, 50, 100, 200, and 400 mg/mL formulations were performed by the study laboratory using HPLC/UV by system B; gavageability studies of 200 and 400 mg/mL formulations were also performed. Homogeneity was confirmed for all of the formulations; gavageability was confirmed only for the 200 mg/mL formulation. Additional homogeneity studies were performed on 10 mg/mL dose formulations and gavageability studies on 100 and 200 mg/mL dose formulations. Homogeneity and gavageability were confirmed. Prior to the 2-year studies, homogeneity studies of 25, 50, and 100 mg/mL dose formulations were performed by the study laboratory using HPLC/UV by system B. Homogeneity was confirmed.

Periodic analyses of the dose formulations of tetrabromobisphenol A were conducted by the study laboratory using HPLC/UV by a system similar to system B. During the 3-month studies, the dose formulations were analyzed three times; all 15 of the dose formulations for rats and all 15 for mice were within 10% of the target concentrations (Table J-2). Animal room samples of these dose formulations were also analyzed; all 15 for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed

approximately every 3 months (Table J-3); of the dose formulations analyzed and used during the studies, all 72 for rats and all 45 for mice were within 10% of the target concentrations. Animal room samples were also analyzed; seven of nine animal room samples for rats and eight of nine for mice were within 10% of the target concentrations.

Table J-1. Preparation and Storage of Dose Formulations in the Gavage Studies of Tetrabromobisphenol A

Three-month Studies	Two-year Studies
Preparation	
Dose formulations were prepared by adding the appropriate amount of tetrabromobisphenol A directly into a calibrated glass beaker, adding enough corn oil to wet the test article, and stirring with a spatula to form a smooth slurry. The spatula and sides of the beaker were rinsed with corn oil, diluted to the final volume with corn oil, and stirred using an overhead stirrer with a vigorous vortex for approximately 15 minutes and scraping the bottom of the beaker with a spatula. Dose formulations were prepared monthly.	Same as the 3-month studies. Dose formulations were prepared approximately every 6 weeks.
Chemical Lot Number	
25317K-1	M032607KA
Maximum Storage Time	
42 days	42 days
Storage Conditions	
Stored in sealed glass bottles protected from light at room temperature.	Stored in sealed glass bottles protected from light at room temperature.
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

Table J-2. Results of Analyses of Dose Formulations Administered to F344/NTac Rats and B6C3F1/N Mice in the Three-month Gavage Studies of Tetrabromobisphenol A

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
November 30, 2005	December 1-2, 2005	2	1.954	–2
		10	9.552[b]	–5
		20	19.31	–3
		100	96.12	–4
	December 5-6, 2005	200	198.8[c]	–1
	January 10-11, 2006[d]	2	1.915	–4
		10	9.933	–1

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		20	19.60	–2
		100	96.14	–4
		200	199.2	0
January 19, 2006	January 24-25, 2006	2	1.914	–4
		10	9.816	–2
		20	19.15	–4
		100	93.12	–7
		200	197.2	–1
	February 27-28, 2006[d]	2	1.937	–3
		10	9.591	–4
		20	19.56	–2
		100	97.30	–3
		200	197.0	–2
February 13, 2006	February 14-16, 2006	2	1.918	–4
		10	9.768	–2
		20	19.45	–3
		100	92.97	–7
		200	197.7	–1
	March 23-24, 2006[d]	2	1.890	–6
		10	9.506	–5
		20	19.07	–5
		100	94.15	–6
		200	190.5	–5
Mice				
November 30, 2005	December 1-2, 2005	1	0.9492	–5
		5	4.658	–7
		10	9.552[b]	–5
		50	48.45	–3
		100	96.12	–4
	January 10-11, 2006[d]	1	0.9626	–4
		5	4.762	–5
		10	9.906	–1
		50	49.32	–1
		100	97.53	–3
January 19, 2006	January 24-25, 2006	1	0.9433	–6

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		5	4.754	–5
		10	9.816	–2
		50	48.83	–2
		100	93.12	–7
	February 27-28, 2006[d]	1	0.9385	–6
		5	4.756	–5
		10	9.669	–3
		50	48.12	–4
		100	95.91	–4
February 13, 2006	February 14-16, 2006	1	0.9025	–10
		5	4.519	–10
		10	9.768	–2
		50	48.46	–3
		100	92.97	–7
	March 23-24, 2006[d]	1	0.9655	–4
		5	4.795	–4
		10	9.594	–4
		50	49.00	–2
		100	96.53	–3

[a]Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 2 mg/mL=10 mg/kg, 10 mg/mL=50 mg/kg, 20 mg/mL=100 mg/kg, 100 mg/mL=500 mg/kg, 200 mg/mL=1,000 mg/kg. For mice, dosing volume=10 mL/kg; 1 mg/mL=10 mg/kg, 5 mg/mL=50 mg/kg, 10 mg/mL=100 mg/kg, 50 mg/mL=500 mg/kg, and 100 mg/mL=1,000 mg/kg.
[b]Results of twelve analyses.
[c]Results of four analyses.
[d]Animal room samples.

Table J-3. Results of Analyses of Dose Formulations Administered to Wistar Han Rats and B6C3F1/N Mice in the Two-year Gavage Studies of Tetrabromobisphenol A

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
July 12, 2007	July 13, 2007	50	48.2	–4
		50	47.7	–5
		100	94.9	–5
		100	93.9	–6
		200	195	–3
		200	192	–4
	August 21, 2007[b]	50	47.2	–6

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		100	118	18
		200	195	–3
July 19, 2007	July 23, 2007	200	191	–5
		200	198	–1
October 9, 2007	October 10, 2007	50	50.6	+1
		50	47.6	–5
		100	92.1	–8
		100	96.3	–4
		200	193	–4
		200	193	–4
		200	192	–4
		200	188	–6
January 2, 2008	January 3, 2008	50	47.5	–5
		50	47.0	–6
		100	96.5	–4
		100	96.1	–4
		200	193	–4
		200	201	+1
		200	202	+1
		200	198	–1
April 22, 2008	April 23, 2008	50	47.6	–5
		50	46.8	–6
		200	192	–4
		200	184	–8
		200	187	–7
		200	187	–7
	May 29, 2008[b]	50	47.2	–6
		200	192	–4
April 28, 2008	April 28, 2008	100	98.3	–2
		100	96.8	–3
	May 30, 2008[b]	100	94.4	–6
July 15, 2008	July 16, 2008	50	48.1	–4
		50	45.8	–8
		100	91.4	–9
		100	91.9	–8
		200	194	–3

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		200	197	–2
		200	189	–6
		200	190	–5
October 7, 2008	October 8, 2008	50	46.3	–7
		100	91.7	–8
		100	92.9	–7
		200	216	+8
		200	210	+5
		200	219	+10
October 20, 2008	October 22, 2008	50	47.3	–5
		200	212	+6
January 27, 2009	January 28, 2009	50	47.7	–5
		50	46.2	–8
		100	93.7	–6
		100	94.2	–6
		200	199	–1
		200	200	0
		200	194	–3
		200	207	+4
	March 4-5, 2009[b]	50	48.3	–3
		100	92.3	–8
		200	225	+13
April 21, 2009	April 23, 2009	50	47.5	–5
		50	47.6	–5
		100	93.9	–6
		100	96.5	–4
		200	197	–2
		200	199	–1
		200	196	–2
		200	198	–1
July 1, 2009	July 2, 2009	50	46.7	–7
		50	47.6	–5
		100	93.6	–6
		100	91.8	–8
		200	193	–4
		200	193	–4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		200	190	–5
		200	193	–3
Mice				
July 12, 2007	July 13, 2007	25	24.3	–3
		50	48.2	–4
		50	47.7	–5
		100	94.9	–5
		100	93.9	–6
	August 21, 2007[b]	25	25.2	+1
		50	48.5	–3
		100	94.8	–5
October 9, 2007	October 10, 2007	25	24.9	0
		50	50.6	+1
		50	47.6	–5
		100	92.1	–8
		100	96.3	–4
January 2, 2008	January 3, 2008	25	24.3	–3
		50	47.5	–5
		50	47.0	–6
		100	96.5	–4
		100	96.1	–4
April 22, 2008	April 23, 2008	25	23.8	–5
		50	47.6	–5
		50	46.8	–6
	May 29, 2008[b]	25	24.3	–3
		50	48.7	–3
April 28, 2008	April 28, 2008	100	98.3	–2
		100	96.8	–3
	May 30, 2008[b]	100	96.2	–4
July 15, 2008	July 16, 2008	25	24.1	–4
		50	48.1	–4
		50	45.8	–8
		100	91.4	–9
		100	91.9	–8
October 7, 2008	October 8, 2008	25	23.4	–6
		50	46.3	–7

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		100	91.7	–8
		100	92.9	–7
October 20, 2008	October 22, 2008	50	47.3	–6
January 27, 2009	January 28, 2009	25	24.3	–3
		50	47.7	–5
		50	46.2	–8
		100	93.7	–6
		100	94.2	–6
	March 4-5, 2009[b]	25	23.4	–6
		50	47.1	–6
		100	89.3	–11
April 21, 2009	April 23, 2009	25	24.3	–3
		50	47.5	–5
		50	47.6	–5
		100	93.9	–6
		100	96.5	–4
July 1, 2009	July 2, 2009	25	23.9	–4
		50	46.7	–7
		50	47.6	–5
		100	93.6	–6
		100	91.8	–8

[a]Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 50 mg/mL=250 mg/kg, 100 mg/mL=500 mg/kg, 200 mg/mL=1,000 mg/kg. For mice, dosing volume=10 mL/kg; 25 mg/mL=250 mg/kg, 50 mg/mL=500 mg/kg, and 100 mg/mL=1,000 mg/kg.
[b]Animal room samples.

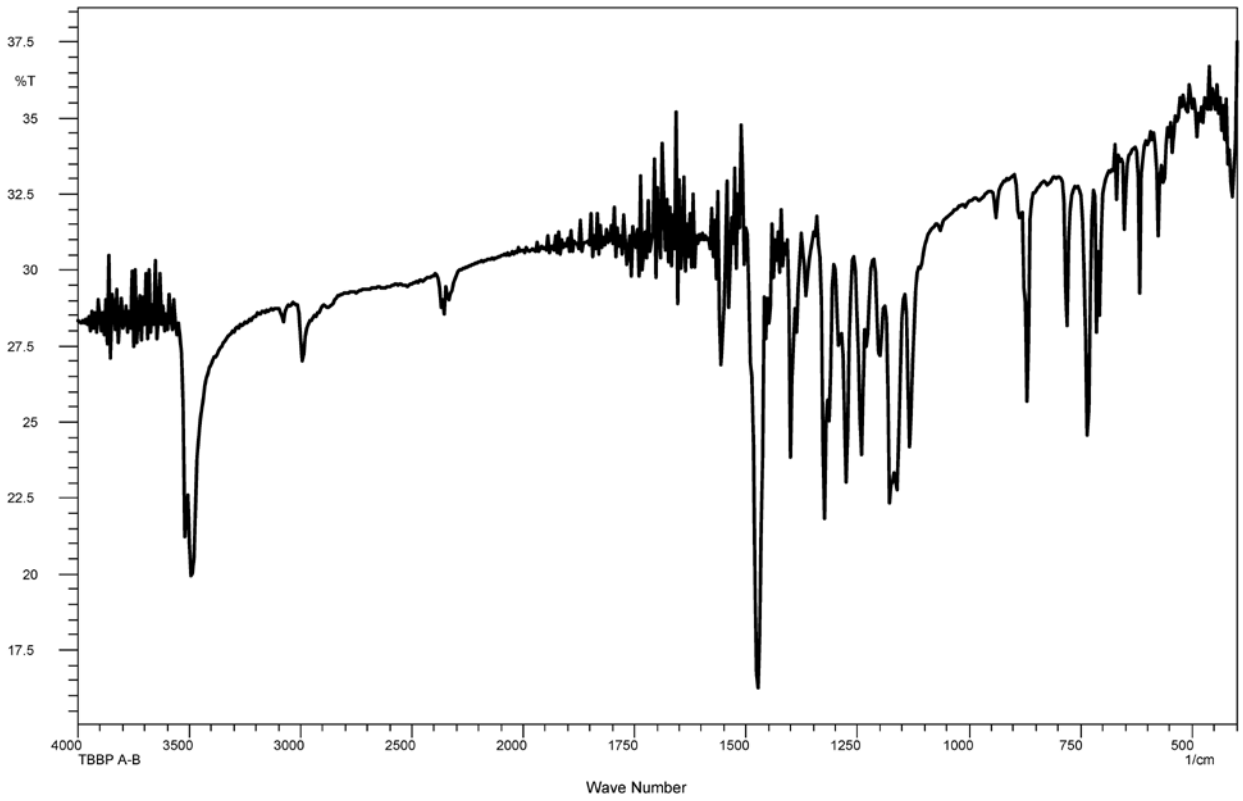

Figure J-1. Infrared Absorption Spectrum of Tetrabromobisphenol A

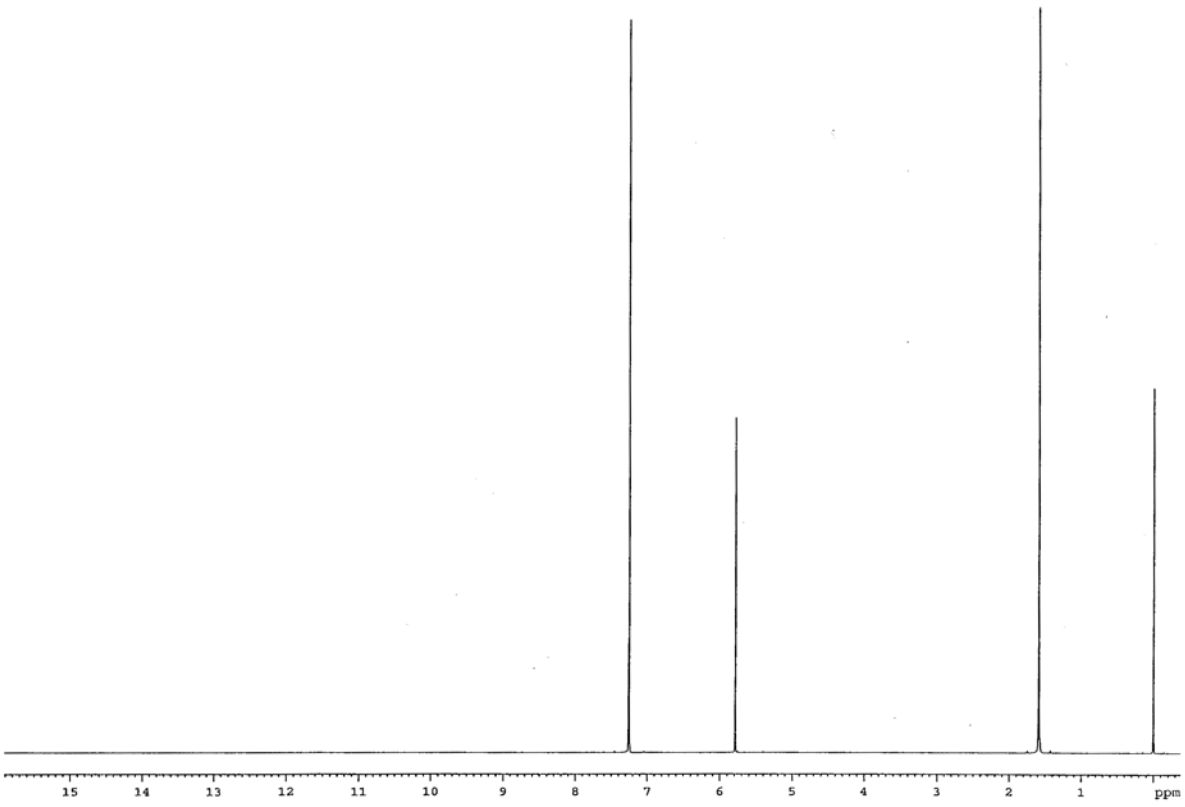

Figure J-2. Proton Nuclear Magnetic Resonance Spectrum of Tetrabromobisphenol A

Appendix K. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table K-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table K-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	–
Niacin	23 mg	–
Folic acid	1.1 mg	–
d-Pantothenic acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table K-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.64	13.7–15.9	24
Crude fat (% by weight)	8.2 ± 0.28	7.7–8.8	24
Crude fiber (% by weight)	9.1 ± 0.52	8.2–10.3	24
Ash (% by weight)	5.1 ± 0.21	4.4–5.4	24
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.070	0.67–0.97	22
Cystine	0.220 ± 0.024	0.15–0.25	22
Glycine	0.701 ± 0.041	0.62–0.80	22
Histidine	0.352 ± 0.077	0.27–0.68	22
Isoleucine	0.546 ± 0.044	0.43–0.66	22
Leucine	1.095 ± 0.067	0.96–1.24	22
Lysine	0.711 ± 0.114	0.31–0.86	22
Methionine	0.409 ± 0.046	0.26–0.49	22
Phenylalanine	0.628 ± 0.040	0.54–0.72	22
Threonine	0.505 ± 0.043	0.43–0.61	22
Tryptophan	0.150 ± 0.028	0.11–0.20	22
Tyrosine	0.401 ± 0.061	0.28–0.54	22
Valine	0.665 ± 0.043	0.55–0.73	22
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.259	3.49–4.55	22
Linolenic	0.30 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	3,689 ± 82	2,350–5,720	24
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm)[b]	6.9 ± 1.10	5.1–9.0	24
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic acid (ppm)	26.9 ± 12.63	17.4–81.0	22
Pyridoxine (ppm)[b]	9.54 ± 1.99	6.44–13.7	22
Folic acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	22
Vitamin B12 (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm)[b]	2,846 ± 485	1,820–3,790	22

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.918 ± 0.049	0.808–1.02	24
Phosphorus (%)	0.554 ± 0.066	0.471–0.822	24
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.016	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.490	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	186 ± 39.2	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.562	3.21–16.3	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.276	0.330–1.380	22
Cobalt (ppm)	0.256 ± 0.164	0.098–0.864	22

[a]From formulation.
[b]As hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table K-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 ± 0.040	0.16–0.32	24
Cadmium (ppm)	0.06 ± 0.010	0.05–0.10	24
Lead (ppm)	0.10 ± 0.020	0.07–0.16	24
Mercury (ppm)	<0.02	–	24
Selenium (ppm)	0.23 ± 0.172	0.14–1.02	24
Aflatoxins (ppb)	<5.00	–	24
Nitrate nitrogen (ppm)[c]	20.81 ± 8.90	10.0–42.3	24
Nitrite nitrogen (ppm)[c]	<0.61	–	24
BHA (ppm)[d]	<1.0	–	24
BHT (ppm)[d]	<1.0	–	24
Aerobic plate count (CFU/g)	10 ± 0	10.0	24
Coliform (MPN/g)	3.0 ± 0	3.0	24
Escherichia coli (MPN/g)	<10	–	24
Salmonella (MPN/g)	Negative	–	24
Total nitrosoamines (ppb)[e]	10.6 ± 6.12	2.0–28.0	24
N-Nitrosodimethylamine (ppb)[e]	3.1 ± 3.28	0.9–11.1	24
N-Nitrosopyrrolidine (ppb)[e]	8.0 ± 4.55	1.0–17.7	24
Pesticides (ppm)			
α-BHC	<0.01	–	24
β-BHC	<0.02	–	24
γ-BHC	<0.01	–	24
δ-BHC	<0.01	–	24
Heptachlor	<0.01	–	24
Aldrin	<0.01	–	24
Heptachlor epoxide	<0.01	–	24
DDE	<0.01	–	24
DDD	<0.01	–	24
DDT	<0.01	–	24
HCB	<0.01	–	24
Mirex	<0.01	–	24
Methoxychlor	<0.05	–	24
Dieldrin	<0.01	–	24
Endrin	<0.01	–	24

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	24
Chlordane	<0.05	–	24
Toxaphene	<0.10	–	24
Estimated PCBs	<0.20	–	24
Ronnel	<0.01	–	24
Ethion	<0.02	–	24
Trithion	<0.05	–	24
Diazinon	<0.10	–	24
Methyl chlorpyrifos	0.079 ± 0.072	0.020–0.300	24
Methyl parathion	<0.02	–	24
Ethyl parathion	<0.02	–	24
Malathion	0.065 ± 0.056	0.020–0.234	24
Endosulfan I	<0.01	–	24
Endosulfan II	<0.01	–	24
Endosulfan sulfate	<0.03	–	24

[a]All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride.
[b]For values less than the limit of detection, the detection limit is given as the mean.
[c]Sources of contamination: alfalfa, grains, and fish meal.
[d]Sources of contamination: soy oil and fish meal.
[e]All values were corrected for percent recovery.

Appendix L. Sentinel Animal Program

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Tables

L.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera and feces from extra (sentinel) or dosed animals in the study rooms. These sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) or the Research Animal Diagnostic Laboratory at the University of Missouri (Columbia, MO) for determination of the presence of pathogens. The laboratory methods and viral agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood and fecal (mice) samples were collected from five animals per sex at the time points indicated below.

Table L-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-month Study	
ELISA	
PVM (pneumonia virus of mice)	End of quarantine, 4 weeks, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	End of quarantine, 4 weeks, study termination
Sendai	End of quarantine, 4 weeks, study termination
Immunofluorescence Assay	
Parvovirus	End of quarantine, 4 weeks, study termination
Sendai	Study termination
Two-year Study	
ELISA	
PVM	4 weeks
RCV/SDA	4 weeks
RPV (rat parvovirus)	4 weeks
Sendai	4 weeks
Immunofluorescence Assay	
Parvovirus	4 weeks
Multiplex Fluorescent Immunoassay	
H-1 (Toolan's H-1 virus)	6, 12, and 18 months, study termination

Method and Test	Time of Collection
KRV (Kilham rat virus)	6, 12, and 18 months, study termination
Mycoplasma pulmonis	6, 12, and 18 months, study termination
Parvovirus NS-1	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
RMV (rat minute virus)	6, 12, and 18 months, study termination
RPV	6, 12, and 18 months, study termination
RTV (rat theilovirus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
TMEV (Theiler's murine encephalomyelitis virus)	6, 12, and 18 months, study termination
Mice	
Three-month Study	
ELISA	
Ectromelia virus	End of quarantine, 4 weeks, study termination
EDIM (epizootic diarrhea of infant mice)	End of quarantine, 4 weeks, study termination
GDVII (mouse poliovirus)	End of quarantine, 4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	End of quarantine, 4 weeks, study termination
Mouse adenoma virus-FL	End of quarantine, 4 weeks, study termination
MHV (mouse hepatitis virus)	End of quarantine, 4 weeks, study termination
MMV VP2 (mouse minute virus)	End of quarantine, 4 weeks, study termination
MPV VP2 (mouse parvovirus)	End of quarantine, 4 weeks, study termination
PVM	End of quarantine, 4 weeks, study termination
Reovirus	End of quarantine, 4 weeks, study termination
Sendai	End of quarantine, 4 weeks, study termination
Immunofluorescence Assay	
GDVII	End of quarantine
MHV	End of quarantine
MPV VP2	Study termination
Two-year Study	
ELISA	
Ectromelia virus	4 weeks
EDIM	4 weeks
GDVII	4 weeks
LCM	4 weeks
Mouse adenoma virus-FL	4 weeks
MHV	4 weeks

Method and Test	Time of Collection
MMV VP2	4 weeks
MPV VP2	4 weeks
PVM	4 weeks
Reovirus	4 weeks
Sendai	4 weeks
Immunofluorescence Assay	
PVM	4 weeks
Multiplex Fluorescent Immunoassay	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
M. pulmonis	6, 12, and 18 months, study termination
MHV	6, 12, and 18 months, study termination
MNV (mouse norovirus)	6, 12, and 18 months, study termination
MPV	6, 12, and 18 months, study termination
MMV	6, 12, and 18 months, study termination
Parvo NS-1	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
TMEV, strain GDVII	6, 12, and 18 months, study termination
Reovirus	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter spp.	18 months

L.2. Results

All test results were negative.

Appendix M. Analysis of *Tp53* Mutations in Wistar Han Rat Uterine Carcinomas Resulting from Chronic Tetrabromobisphenol A Exposure by Gavage

J.B. Harvey[1], H.L. Hong[1], S. Bhusari[1], A.R. Pandiri[1], J.K. Dunnick[2], S.A. Elmore[1], R.C. Sills[1], and M.J. Hoenerhoff[1]

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Tables

M.1. Introduction

Uterine adenocarcinomas occur infrequently in Wistar Han rats in reported National Toxicology Program studies (2% incidence in inhalation studies, 4.67% incidence all routes of exposure), according to the current NTP Wistar Han rat historical control values. Investigation of the molecular alterations that occur in tumors from animals exposed to compounds provides valuable mechanistic information on the pathogenesis of chemically induced tumors, and may aid in distinguishing chemically induced tumors from spontaneous tumors. This study compares *Tp53* mutations in female Wistar Han rat uterine carcinomas occurring either spontaneously in vehicle controls or due to chronic exposure to tetrabromobisphenol A. *Tp53* is one of the most commonly altered tumor suppressor genes in multiple types of cancers including uterine carcinomas. In this study, formalin-fixed, paraffin embedded (FFPE) uterine carcinomas were evaluated for mutations within the hotspot regions (exons 5 to 8) of the *Tp53* gene.

M.2. Materials and Methods

M.2.1. Uterine Neoplasms

Sixteen FFPE uterine adenocarcinomas from tetrabromobisphenol A-dosed female Wistar Han rats from the 2-year study (three, seven, and six tumors from the 250, 500, and 1,000 mg/kg groups, respectively) and 9 FFPE spontaneous uterine adenocarcinomas from female control animals from various NTP studies using Wistar Han rats [tetrabromobisphenol A (two), polybrominated diethyl ether mixture (one), green tea extract (two), Cimstar 3800 (one), and antimony trioxide (three)] were available for analysis. Uterine adenocarcinomas were selected for molecular biology analysis based on their overall size and viability (minimal to no necrosis/hemorrhage microscopically) in order to maximize the amount and quality of DNA obtained from FFPE sections. DNA quality was measured using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) to calculate the 260/280 nm absorbance ratio, and DNA samples with a purity range of 1.7 to 2.0 nm were used for analysis. Five FFPE normal uteri from vehicle control females in the concurrent tetrabromobisphenol A study were used as controls.

M.2.2. Statistical Analysis of Mutation Incidence

To compare exon-specific and total mutation incidence in each dosed group compared to the incidence in controls, a one-sided Fisher's exact test was used. Exact one-sided Cochran-Armitage trend tests were used to test for dose-related trends in the total mutations across all groups.

M.2.3. DNA Isolation, Polymerase Chain Reaction Amplification, and Autosequencing

DNA was isolated from 16 FFPE tetrabromobisphenol A-induced uterine adenocarcinomas and 9 spontaneous uterine adenocarcinomas from control animals with the DNeasy® Tissue Kit (Qiagen, Valencia, CA). Amplification reactions were carried by seminested polymerase chain reaction (PCR) using the designed primer sets (Table M-1) for *Tp53* exons 5 to 8. Controls lacking DNA were run with all sets of reactions. PCR products were purified using a QIAquick® Gel Extraction Kit (Qiagen). The purified PCR products were cycled with Terminal Ready

Reaction Mix-Big Dye® (PerkinElmer, Inc., Foster City, CA), and the extension products were purified using the DyeEx® 2.0 Spin Kit (Qiagen). The PCR products were sequenced with an automatic sequencer (Perkin-Elmer ABI Model 3100). Electropherograms from normal uterus from vehicle controls and uterine adenocarcinomas from controls and tetrabromobisphenol A-dosed animals were used for comparison.

M.3. Results

There was a significant increase in *Tp53* mutations in uterine adenocarcinomas from tetrabromobisphenol A-dosed animals (10/16, 63%) compared to spontaneous uterine adenocarcinomas from control Wistar Han rats (1/9, 11%). Mutations resulting in synonymous amino acid substitutions (in animal number: 225, 374, 412, 430) were not considered significant since they do not alter the amino acid in the *Tp53* protein and have no functional significance (Table M-2). Hence these silent mutations were not summarized in Table M-3. Uterine adenocarcinomas from the tetrabromobisphenol A-dosed Wistar Han rats had not only higher incidences of *Tp53* mutations, but they also harbored multiple mutations per tumor. *Tp53* mutations in spontaneous uterine adenocarcinomas were observed in only one exon (exon 6), whereas two uterine adenocarcinomas from tetrabromobisphenol A-dosed animals harbored mutations in multiple exons, one animal with mutations in exons 6 and 7, and another had mutations in exons 6 and 8 (Table M-3). Although there was no difference in exon-specific mutation frequencies between tetrabromobisphenol A-dosed rats and the control group, there was a statistically significant difference between the incidences of total *Tp53* mutations in uterine adenocarcinomas from tetrabromobisphenol A-dosed rats (10/16) compared to controls (1/9) by the Fisher exact test ($P < 0.05$) (Table M-3).

M.4. Discussion

Uterine adenocarcinomas from Wistar Han rats chronically exposed to tetrabromobisphenol A had a significantly higher incidence of *Tp53* mutations compared to those arising spontaneously in controls. The *Tp53* tumor suppressor gene is responsible for cell cycle checkpoint maintenance, regulation of apoptosis, and genomic stability[146], and loss of this tumor suppressor function via mutation or dysregulation of the *Tp53* signaling pathway is an important event in the pathogenesis of many different types of cancer in rodents and humans[148; 150-153]. Mutant TP53 protein resulting from mutation of the hotspot region in this gene has an increased half-life compared to wildtype TP53, which is rapidly degraded in normal cells[146]. Mutant TP53 is nonfunctional and results in loss of cell cycle checkpoint control, resulting in uncontrolled cell growth and proliferation, leading to carcinogenesis. In this study, the high rate of *Tp53* mutations in uterine adenocarcinomas from tetrabromobisphenol A-dosed Wistar Han rats compared to spontaneous uterine adenocarcinomas suggests that the increased incidence of uterine adenocarcinomas in tetrabromobisphenol A-dosed animals may be driven at least in part through a *Tp53*-mediated mechanism.

Table M-1. Primers Used to Amplify Hotspot Regions of Rat *Tp53*

Exon	Codon	Primer	Strand	Sequence
5	124-184	*p53*Ex5OF1366	Sense	5′-CCTAGTTGGCTTGTCCG-3′
		*p53*Ex5OR1671	Antisense	5′-AGCAAGAATAAGTCAGAGGC-3′
		*p53*Ex5IF1382	Sense	5′-CGCTGACCTTTGATTCTTTCTCC-3′
		*p53*Ex5IR1639	Antisense	5′-GACAACCAGTTCTAAACCCCACAG-3′
6	185-259	*p53*Ex6OF1620	Sense	5′-TGGGGTTAGAACTGGTTG-3′
		*p53*Ex6OR1963	Antisense	5′-GAACAAAAACAGGCCGAG-3′
		*p53*Ex6IF1645	Sense	5′-TCTCCCGGCCTCTGACTTATTC-3′
		*p53*Ex6IR1927	Antisense	5′-CAGCCCAACCTGGCACAC-3′
7	260-304	*p53*Ex7OF2101	Sense	5′-AGCTCCAGATAGGACAAG-3′
		*p53*Ex7OR2434	Antisense	5′-TGGGCAGTGCTATGGAAG-3′
		*p53*Ex7IF2166	Sense	5′-AGCTTTCTTACTGCCTTGTG-3′
		*p53*Ex7IR2402	Antisense	5′-TGACTTTGGGGTGAAGCTG-3′
8	305-329	*p53*Ex8OF2333	Sense	5′-GGAGTGCAAAGAGAGGTG-3′
		*p53*Ex8OR2602	Antisense	5′-TGCGCTCTGACGATAATG-3′
		*p53*Ex8IF2386	Sense	5′-GCTTCACCCCAAAGTCAC-3′
		*p53*Ex8IR2549	Antisense	5′-GCGTTTTGTGTCCTAGACTTAG-3′

Table M-2. *Tp53* Mutations in Uterine Carcinomas from Female Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A[a]

Animal Number	Dose (mg/kg)	Exon 5	Exon 6	Exon 7	Exon 8
249	0	NM	NM	NM	NM
262	0	NM	NM	NM	NM
238	0	NM	Cdn 246 CGC→TGC (Arg→Cys)	NM	NM
225	0	NM	Cdn 232 TAC→TAT (Tyr→Tyr)	NM	NM
231	0	NM	NM	NM	NM
138	0	NM	NM	NM	NM
110	0	NM	NM	NM	NM
130	0	NM	NM	NM	NM
157	0	NM	NM	NM	NM
302	250	Cdn 173 CGC→CAC (Arg→His)	NM	NM	NM
316	250	NM	Cdn 247 CGC→CAG	NM	NM

Animal Number	Dose (mg/kg)	Exon 5	Exon 6	Exon 7	Exon 8
			(Arg→Gln)		
323	250	NM	Cdn 231 CAC→TAC (His→Tyr)	Cdn 299 CCA→TCA (Pro→Ser)	NM
336	500	NM	NM	Cdn 271 CGT→TGT (Arg→Cys)	NM
337	500	NM	NM	NM	NM
356	500	NM	NM	NM	NM
374	500	NM	Cdn 207 AGG→AGA (Arg→Arg)	NM	NM
376	500	NM	NM	NM	Cdn 307 CCC→CTC (Pro→Leu)
397	500	NM	NM	NM	NM
388	500	Cdn 173 CGC→CAC (Arg→His)	NM	NM	NM
400	1,000	NM	NM	NM	Cdn 307 CCC→CTC (Pro→Leu)
412	1,000	NM	Cdn 248 CCC→CCT (Pro→Pro)	NM	NM
417	1,000	Cdn 173 CGC→CAC (Arg→His)	NM	NM	NM
418	1,000	NM	NM	NM	NM
426	1,000	NM	Cdn 211 CGG→TGG (Arg→Trp)	NM	NM
430	1,000	NM	Cdn 211 CGG→TGG (Arg→Trp)	NM	Cdn 318 AAA→GAA (Lys→Glu)
			Cdn 248 CCC→CCT (Pro→Pro)		

aFemale Wistar Han rats were administered 0, 250, 500, or 1,000 mg/kg tetrabromobisphenol A in corn oil by gavage for 2 years. Due to the paucity of spontaneous uterine carcinomas from control Wistar Han rats in the tetrabromobisphenol A study, they were sourced from control Wistar Han rats from various NTP studies (Animal number 249, 262: tetrabromobisphenol A, 2-year corn oil gavage study; 238, 231: green tea extract, 2-year corn oil gavage study; 138: Cimstar 3800, 2-year inhalation study; 110, 130, 157: antimony trioxide, 2-year inhalation study; 225: polybrominated diethyl ether mixture, 2-year corn oil gavage study). NM = no mutation.

Table M-3. Pattern of *Tp53* Mutations in Uterine Carcinomas from Female Wistar Han Rats in the Two-year Gavage Study of Tetrabromobisphenol A[a]

	Mutation Frequency (%)	Exon 5	Exon 6	Exon 7	Exon 8
Control					
Total incidence	1/9 (11%)	0	1	0	0
Tetrabromobisphenol A-dosed					
250	3/3 (100%)	1	2[b]	1[b]	0
500	3/7 (43%)	1	0	1	1
1,000	4/6 (67%)	1	2[b]	0	2[b]
Total incidence	10/16* (63%)	3	4[b]	2[b]	3[b]

*Significantly different ($P < 0.05$) from total control incidence.
[a]Female Wistar Han rats were administered 0, 250, 500, or 1,000 mg/kg tetrabromobisphenol A in corn oil by gavage for 2 years. Silent mutations are not included.
[b]Includes at least one animal with double mutations.

Appendix N. Summary of Peer Review Panel Comments

On October 29, 2013, the draft Technical Report on the toxicology and carcinogenesis studies of tetrabromobisphenol A received review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the studies on tetrabromobisphenol A, a high production-volume flame retardant widely used in plastics, paper, electronics, textiles, and adhesive materials. Three-month oral gavage toxicology studies were conducted in F344/NTac rats and B6C3F1/N mice. Two-year oral gavage toxicology and carcinogenesis studies were conducted in Wistar Han rats and B6C3F1/N mice. There was a 3-month interim evaluation in Wistar Han rats for comparison to the 3-month F344/NTac rat study. Genetic toxicity studies were negative.

The proposed conclusions for the 2-year studies were *equivocal evidence of carcinogenic activity* of tetra-bromobisphenol A in male Wistar Han rats, *clear evidence of carcinogenic activity* of tetrabromobisphenol A in female Wistar Han rats, *some evidence of carcinogenic activity* of tetrabromobisphenol A in male B6C3F1/N mice, and *no evidence of carcinogenic activity* of tetrabromobisphenol A in female B6C3F1/N mice administered 250 or 500 mg/kg.

Dr. S.A. Elmore, NIEHS, described the pathology review of female rat reproductive tissues and reported that the residual tissue review of the remaining formalin fixed cervix, vagina, and uterine remnants sectioned longitudinally revealed additional adenomas and adenocarcinomas, which supported the clear evidence call. Atypical hyperplastic lesions were also found that were not present in the original slides. This is the first report of malignant mixed Müllerian tumors (MMMTs) in an NTP study; these very rare tumors are considered more aggressive than adenocarcinomas. They were found in the original transverse review due to their large size and were combined with adenomas and adenocarcinomas because the current histogenesis theory and epithelial metastases indicate that the epithelial component is the driving force in their production. Atypical hyperplasia, a rare and potentially preneoplastic lesion seen in the uteri of the rats, was treatment related. It was not found in the original transverse review due to small lesion size. Renal tubule cytoplasmic alteration found in the kidneys of male mice in the 3-month and 2-year studies was considered to be treatment related and may be associated with altered hormonal status.

Dr. Cullen noted receipt and distribution to the panel of written comments from Dr. J. Popp, Stratoxon LLC, on behalf of the American Chemistry Council's North American Flame Retardant Alliance.

The first public commenter was Dr. M. Hardy, Albemarle Corporation, who spoke by telephone. She provided background information about tetrabromobisphenol A and its regulatory history. She noted the draft Technical Report relies on the peer-reviewed literature, with underrepresentation of unpublished data from guideline/GLP-compliant studies; therefore, she said the draft Techncial Report does not present a clear and comprehensive overview of tetrabromobisphenol A toxicology. She described relevant unpublished data. She noted that tetrabromobisphenol A kinetics and metabolism are critically important in evaluating and interpreting the studies and listed several kinetic and metabolic elements. She made several comments related to use of the Wistar Han rat in the 2-year study and called for more infomation

on dose selection for the 2-year study, selection of gavage as the route of administration, the change to Wistar Han rats, the discontinuance of Wister Han rats, NTP's historical control data in the Wistar Han model, and the possible association of rat strain and the observed uterine adenocarcinomas. She said the Introduction section also needed revision.

The second public commenter, Dr. D. Wikoff, ToxStrategies, Inc., spoke on behalf of the American Chemistry Council's North American Flame Retardants Alliance, reflecting her own and Dr. James Popp's written comments. She reported that Dr. Popp reviewed the hepatoblastomas in male mice and suggested the level of carcinogenic activity should be equivocal evidence, not some evidence. Dr. Wikoff presented some of Dr. Popp's key findings related to hepatoblastomas. Citing shortcomings in the comparison of uterine tumor incidences to those in historical controls, she asked for clarifications related to historical control data and for all historical control data to be made available in the report. She also described limitations in the analysis and interpretation of the *Tp53* mutation data. She noted the limited relevance and unclear impact of the NTP study dose levels. She remarked that even the lowest doses tested were substantially higher than human exposure, making it difficult to accurately extrapolate the study findings to humans. She asked that these issues be addressed in the Discussion section.

Dr. Cory-Slechta asked Dr. Wikoff about the issue of human-relevant dosing and why human-relevant doses would be used when testing in a mouse or a rat. She noted that such extrapolations between species are commonly done in terms of therapeutic compounds. Dr. Wikoff replied that use of human-relevant doses would help to better characterize responses in humans. Dr. Hardy added that it was her understanding that for most pharmaceuticals, toxicology tests are run at multiple, potentially effective doses. In toxicology, dose levels are set very differently from pharmaceuticals.

Dr. Barlow, the first primary reviewer, suggested that the rats in the study were not dosed high enough to potentially drive a carcinogenic effect. In the highest dose, there was no effect on mortality, no body weight changes, and no histologic changes in the 3-month study; yet, the highest dose for the 2-year study stayed at 1,000 mg/kg. Also, the half-life is less than 5 hours, and there was low bioavailability and no accumulation. He said there should be more elaboration on the statement in the Materials and Methods that formulation limitations precluded doses higher than 1,000 mg/kg. He suggested the dose could have been pushed higher. He agreed with the conclusion of clear evidence of uterine epithelial tumors in female rats, and questioned the combination of MMMTs with adenomas and adenocarcinomas. He proposed that MMMTs be considered separate neoplasms that may have been related to exposure and called for a better explanation in the report of how the uterine findings were handled. In general, he agreed with the calls as listed, except that the MMMTs should be separated out and characterized as "may have been related to exposure."

Dr. Regan, the second primary reviewer, suggested that the cervix and vagina deserved added attention as important structures in the female reproductive tract. She asked if there might have been a location bias regarding the atypical hyperplasias. She asked for clarification about the metastases from the uterine adenocarcinomas and MMMTs in the treated and vehicle control animals. She noted there should be a clearer distinction between metastases and local invasions. She was not surprised to see that carcinogenicity was found in the 2-year study despite the fact that there was none detected in the 3-month study. She supported the proposed conclusions.

Dr. Parker, the third primary reviewer, said he understood the reasoning behind looking at the mutations from the coding regions in lieu of considering "silent" mutations, with respect to the *Tp53* mutation data. He proposed that use of the term "hot spot" was an exaggeration, at least with respect to the human data available. He said it would be useful in the future to sequence the length of a gene for tumor suppressors such as *Tp53*, or at least all of the exons. He said the number of *Tp53* mutations in the study was severely underestimated, which may have hurt the study by limiting power, rendering P values marginal.

Dr. Dunnick responded to Dr. Barlow's comments. Regarding his questions about the highest dose used, she said 1,000 mg/kg was the maximum dose that could be used in the study due to solubility and gavagability. The 5-days-per-week regimen was employed to mimic worker exposure. Dr. Elmore responded to Dr. Barlow's question regarding a separate call for the MMMTs. She reiterated that, based on NTP knowledge of the histogenesis of MMMTs, the epithelial compo°nent is considered to be the primary component in the MMMTs and the mesenchymal component is derived from the carcinoma. In the current study, all the metastases were carcinomas, which supports this hypothesis. For this reason, the MMMTs were combined with the epithelial tumors.

Dr. Dunnick said the historical data are limited in Wistar Han rats because few studies using this strain have been conducted. She said the cervix and vagina were studied in the residual longitudinal review, and she would provide more data in the report.

Regarding points raised by Dr. Parker on the mutation analysis, Dr. M.J. Hoenerhoff, NIEHS, said *Tp53* was screened because it is one of the most commonly deleted or mutated tumor suppressor genes in human and rodent cancers. He agreed that a more compre-hensive evaluation of the entire sequence of the gene would be beneficial. He also agreed with Dr. Parker's point regarding silent vs. coding mutations. He said the number of *Tp53* mutations might have been underestimated, and agreed that additional exome sequencing or a broader analysis could address that issue.

Dr. Cattley asked whether NTP has a defined practice for when to combine hepatoblastomas with other hepatocellular neoplasms. Dr. D.E. Malarkey, NIEHS, cited two publications that have served as guidance (by Drs. Amy Brix and Eugene McConnell). He said it is acceptable to combine them, but not required, as there is some evidence that they are individual types of tumors genetically. Dr. Cullen noted that there is flexibility on the issue, but asked for some discussion in the report about the decision to combine and the consequences of not combining.

Dr. Cullen called for a motion to accept the conclusions in the draft report as written. Dr. Regan so moved, and Dr. Gordon seconded. The Peer Review Panel voted (4 yes and 1 no) to accept the conclusions on tetrabromobisphenol A as written. Dr. Barlow explained his negative vote as being based on his opinion that the uterine epithelial tumors and MMMTs should not have been combined.

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