NTP
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U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (CASRN 21850-44-2) Administered by Gavage to F344/NTac Rats and B6C3F1/N Mice

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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 Toxicity Study Report series began in 1991. The studies described in the Toxicity Study Report series are designed and conducted to characterize and evaluate the toxicologic potential of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in the Toxicity Study 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 toxic 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.

NTP Toxicity Study Reports are indexed in National Center for Biotechnology Information (NCBI) Bookshelf and are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov).

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This report has been reformatted to meet new NTP publishing requirements; its content has not changed.

About This Report

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Peer Review

The draft *NTP Technical Report on the Toxicity Studies of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (CASRN 21850-44-2) Administered by Gavage to F344/NTac Rats and B6C3F1/N Mice* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this NTP Toxicity Study Report presented the experimental results and conclusions fully and clearly.

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Abstract

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is used as a flame retardant in electronics, building and construction materials, and automotive materials. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was nominated for toxicology and in vivo genotoxicity study by the National Institute of Environmental Health Sciences because, although human exposure potential may be low, there was concern that this chemical has carcinogenic potential and has not been adequately studied. The compound was also selected for study because dibromo-1-propanol (the core structure of the 2,3-dibromopropyl ether side chain) has been studied by NTP and found to be carcinogenic. Male and female F344/NTac rats and B6C3F1/N mice were administered tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (approximately 94% pure) in corn oil by gavage for 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

Groups of 10 male and 10 female F344/NTac rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg tetrabromobisphenol A-bis(2,3-dibromopropyl ether)/kg body weight in corn oil by gavage, 5 days per week for 14 weeks, and additional groups of 10 male and 10 female rats were administered the same doses for 23 days. Groups of 10 male and 10 female mice were administered 0, 125, 250, 500, 1,000, or 2,000 mg/kg for 14 weeks. Two 62.5 mg/kg male rats died during week 6 (one dosing accident), and one vehicle control female rat died during week 8. All mice survived to the end of the study. Final mean body weights and body weight gains of male and female rats and mice were similar to those of the vehicle controls except the final mean body weight of 250 mg/kg female mice was 11% more than that of the vehicle controls. There were no treatment-related clinical findings. Microsomal protein levels were increased in treated rats and mice. There were no chemical-related changes in absolute or relative organ weights in male or female rats or mice. There were no gross or histologic lesions in rats or mice that were considered treatment related. There were no chemical-related hematology or clinical chemistry findings in rats or mice.

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, or TA102, with or without rat liver S9 metabolic activation enzymes. In vivo, no significant increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood samples from male or female B6C3F1/N mice in the 3-month study. In addition, no significant changes in the percentage of polychromatic erythrocytes were seen in these mice, suggesting that tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did not cause bone marrow toxicity.

Under the conditions of these 3-month gavage studies, there were no clinical findings or treatment-related lesions in male or female F344/NTac rats or B6C3F1/N mice administered tetrabromobisphenol A-bis(2,3-dibromopropyl ether) at 0, 62.5, 125, 250, 500, or 1,000 mg/kg in rats or 0, 125, 250, 500, 1,000, or 2,000 mg/kg in mice. Final mean body weights of treated rats and mice were generally within 10% of vehicle controls, and there were no treatment-related effects on organ weights.

Synonyms: 2,2-Bis[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl]propane; 2,2-bis[3,5-dibromo-4-(2,3-dibromopropyloxy)phenyl]propane; 2,2-bis[4-(2,3-dibromopropoxy)-3,5-dibromophenyl]propane; 2,2-bis[4-(2,3-dibromopropyloxy)-3,5-dibromophenyl]propane; bis(2,3-dibromopropoxy)tetrabromobisphenol A; bis(2,3-dibromopropyl)tetrabromobisphenol A; 1,1′-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene]; 1,1′-(1-

methylethylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)]benzene; TBBPA-DBPE; 3,3′,5,5′-tetrabromobisphenol A bis(2,3-dibromopropyl) ether; tetrabromobisphenol A dibromopropyl ether

Trade names: 403AF, Bromcal 66.8, Bromkal 66-8, CHEMPACIFIC 34721, D 5532, EcoFlame B-943, FG 3100, Fire Guard 3100, Flame Cut 121K, Flame Cut 121R, FR 720, GX 5532, HP-800 AG, HP-800 AGC, PE-68, Pyroguard SR 720, SAYTEX® HP-800 A, SR 720

Summary of Findings Considered to be Toxicologically Relevant in Rats and Mice Administered Tetrabromobisphenol A-bis(dibromopropyl ether) by Gavage for Three Months

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in corn oil	0, 62.5, 125, 250, 500, or 1,000 mg/kg	0, 62.5, 125, 250, 500, or 1,000 mg/kg	0, 125, 250, 500, 1,000, or 2,000 mg/kg	0, 125, 250, 500, 1,000, or 2,000 mg/kg
Survival rates	10/10, 8/10, 10/10, 10/10, 10/10, 10/10	9/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	250 mg/kg group 11% more than the vehicle control group
Clinical findings	None[a]	None	None	None
Organ weights	None	None	None	None
Hematology/clinical chemistry [hematology includes: rats (day 23), rats and mice (week 14); clinical chemistry includes: rats (days 4, 23, week 14)]	None	None	None	None
Sperm parameters and vaginal cytology	None	None	None	None
Nonneoplastic effects	None	None	None	None
Genetic toxicology				
Bacterial gene mutations:			Negative in *S. typhimurium* strains TA98, TA100, and TA102 with or without S9	
Micronucleated erythrocytes				
Mouse peripheral blood in vivo:			Negative in males and females	

[a]None = no treatment-related effects for this endpoint.

Introduction

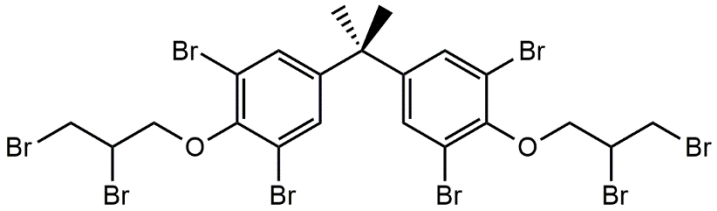

Figure 1. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (CASRN 21850-44-2; Chemical Formula: $C_{21}H_{20}Br_8O_2$; Molecular Weight: 943.6)

Synonyms: 2,2-Bis[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl]propane; 2,2-bis[3,5-dibromo-4-(2,3-dibromopropyloxy)phenyl]propane; 2,2-bis[4-(2,3-dibromopropoxy)-3,5-dibromophenyl]propane; 2,2-bis[4-(2,3-dibromopropyloxy)-3,5-dibromophenyl]propane; bis(2,3-dibromopropoxy)tetrabromobisphenol A; bis(2,3-dibromopropyl)tetrabromobisphenol A; 1,1′-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene]; 1,1′-(1-methylethylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)]benzene; TBBPA-DBPE; 3,3′,5,5′-tetrabromobisphenol A bis(2,3-dibromopropyl) ether; tetrabromobisphenol A dibromopropyl ether.
Trade names: 403AF, Bromcal 66.8, Bromkal 66-8, CHEMPACIFIC 34721, D 5532, EcoFlame B-943, FG 3100, Fire Guard 3100, Flame Cut 121K, Flame Cut 121R, FR 720, GX 5532, HP-800 AG, HP-800 AGC, PE-68, Pyroguard SR 720, SAYTEX® HP-800 A, SR 720.

Chemical and Physical Properties

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is a white to off-white crystalline solid with a slight odor. Decomposition takes place at temperatures greater than 270°C. The relative molecular mass is 943.9; the melting point is 90° to 100°C (95°C); and the specific gravity is 0.7 to 0.9 g/cm^3. The bromine content is 68% and the solubility is 1 g per liter water at 25°C[1].

Production, Use, and Human Exposure

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is used as a flame retardant in electronics, building and construction materials, and automotive materials[2]. It is an additive flame retardant for polyolefins, polymers, and high- and low-density polyethylene. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) may be used as a flame retardant in plastic products such as pipes, water barriers, kitchen hoods, and electronics[3].

The U.S. Environmental Protection Agency (USEPA)[4] reports the production volume in the United States is estimated to be 1 to 10 million pounds. The USEPA notes that 100 to 1,000 workers are likely to be exposed to the chemical during its manufacturing and processing.

Quantitative measurement of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) exposure in the United States is limited, and it is not part of the Centers for Disease Control and Prevention NHANES survey of chemical exposure in the United States population. However, human exposure may occur during its manufacture and use as a flame retardant. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) has been detected in dust samples from a primary school in the United Kingdom at concentrations of 200 µg/kg[3].

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is hydrophobic and thus may bind various particles in the environment[3]. Studies reported in the literature indicate that tetrabromobisphenol A-bis(2,3-dibromopropyl ether) can persist in the environment[5; 6]. The USEPA[7] has also

predicted that tetrabromobisphenol A-bis(2,3-dibromopropyl ether) would have high persistence in the environment based on an analysis of unpublished degradation tests submitted for review. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) has been found in sewage sludge in China at up to 8,950 µg/kg dry weight[3].

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was identified in environmental samples (i.e., soil, sediments, rice hulls, and earthworms) taken near a brominated flame retardant manufacturing plant in China[6]. The concentration of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) ranged from 0.7 to 293 ng/g dry weight in these samples. Mollusks collected from the Chinese Bhohai Sea were found to contain tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in 32% of the samples[6].

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is listed as an alternative flame retardant for decabromodiphenyl ether by the USEPA[7]. It was considered by the EPA for an alternative to the flame retardant hexabromocyclododecane (CASRNs 25637-99-4 and 3194-55-6) in expanded and extruded polystyrene foam insulation but was excluded from consideration[7]. Another alternative tetrabromobisphenol A-bis(2,3-dibromopropyl ether) derivative was considered as a possible alternative flame retardant for hexabromocyclododecane[2].

Exposure to flame retardants, including tetrabromobisphenol A-bis(2,3-dibromopropyl ether), could occur by inhalation, ingestion, or dermal exposure. Of particular concern is exposure to children in the home by the oral route[8; 9].

Regulatory Status

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is listed on the Toxic Substances Control Act Inventory, but no test rules have been issued for this chemical[7]. This chemical is listed by the USEPA as requiring contaminant testing (EPA-1)[10] and export notification (EPA-2)[11].

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

There are limited data on the metabolism and disposition of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in rodents. In studies conducted by the National Toxicology Program, following gavage administration of 20 mg/kg [^{14}C]-tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in fasted F344 rats, 89% of the administered dose was excreted in feces within the first 24 hours; the total dose excreted in feces at 96 hours following dosing was approximately 95%[12; 13]. Excretion via urine was minimal (≤0.1%). When a similar dose was administered in bile duct-cannulated rats, approximately 1% of the dose was recovered in bile within 24 hours suggesting that the majority of fecal radioactivity was due to unabsorbed dose. The total dose in non-gastrointestinal (GI) tract tissues at 96 hours was approximately 1%, with liver, adipose, and muscle showing the highest concentrations. The percent of dose in the liver at 6, 24, 72, and 96 hours following gavage administration was 4.8, 0.9, 0.6, and 0.2, respectively, whereas the level in adipose did not change with time. Following a 20 mg/kg intravenous dose in fasted rats, fecal excretion was slower than following gavage administration, with approximately 30% of the administered dose excreted in 24 hours; the total dose recovered in feces at 96 hours was 71%. Urinary excretion accounted for approximately 0.2% of the dose. Tissues (non-GI

tract) contained approximately 14% of the administered dose with liver, adipose, and muscle having the highest concentrations. Disposition was also investigated in nonfasted male rats following one, five, or 10 doses, with sacrifice 24 hours after the last dose. The pattern of disposition was similar to that in fasted rats except that the total dose in tissues at 24 hours, mainly in the liver, was higher in nonfasted rats (6.7%) than in fasted rats (0.9 %), demonstrating a higher absorption in non-fasted rats. There was no difference in the pattern of disposition between single or repeated (5 or 10) doses. Blood toxicokinetic parameters were estimated following a single gavage dose of [^{14}C]-tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in fasted male F344 rats. The time to reach the maximum blood concentration (C_{max}) of 0.6 µg/mL was 7.4 hours following dosing. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was eliminated slowly from blood with an elimination half-life of 13.9 hours. The estimated absolute bioavailability was 2.2%. These data suggest that tetrabromobisphenol A-bis(2,3-dibromopropyl ether) is poorly absorbed and slowly eliminated following gavage administration in rodents.

Analysis of bile by radiochromatography showed two peaks that did not co-elute with the parent compound either before or after deconjugation experiments, suggesting formation of metabolites other than direct conjugates of the parent. Analysis of fecal extracts also showed some evidence of metabolism of tetrabromobisphenol A-bis(2,3-dibromopropyl ether). Approximately 5% of the radioactivity in feces was associated with a metabolite peak; however, metabolite identification was not attempted. Approximately 90% of the fecal radioactivity eluted at the retention time of the parent. In vitro studies using F344 rat hepatocytes or liver microsomes indicated very little metabolism of tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[12; 13].

Humans

There are no absorption, distribution, metabolism, or excretion studies of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in humans reported in the peer reviewed literature, and no information on tissue levels of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in humans were found in the literature.

Toxicity

Experimental Animals

The acute LD_{50} for tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in mice was greater than 20 g/kg when given in feed and observed for 14 days; the acute dermal LD_{50} for mice was greater than 20 g/kg when applied to skin for 24 hours and observed for 14 days[1]. An inhalation LC_{50} in mice is estimated at 87 mg/L[7]. There are no 14-day or 90-day toxicity studies of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) reported in the scientific literature[1; 7].

The World Health Organization[1] reviewed unpublished studies of tetrabromobisphenol A dibromopropyl ether [another name for tetrabromobisphenol A-bis(2,3-dibromopropyl ether)] and reported that, when it was administered to mice at 200 or 2,000 mg/kg in the diet for 90 days, there were no treatment-related deaths or gross pathologic changes[1].

Humans

No in vivo studies on the toxic potential of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in humans were found in the peer reviewed literature.

In vitro studies found that tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did not inhibit CYP17 catalytic activity in human H295R adrenocortical carcinoma cells[14]. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did not exhibit agonistic or antagonistic activity with aryl hydrocarbon, androgen, progesterone, or estrogen receptors in a series of chemically activated luciferase gene expression assays[14]. However, tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did displace the thyroid hormone precursor thyroxine from the plasma transport protein in transthyretin binding assays, and it inhibited estradiol binding in estradiol sulfotransferase assays[14]. These assays were run at concentrations of 0.01 µM to 10 µM.

Reproductive and Developmental Toxicity

No information on the reproductive or developmental toxicity of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in experimental animals or humans was found in a review of the literature.

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was found to inhibit estradiol-sulfotransferase and thereby may have an effect on sulfation of estradiol and its subsequent elimination by conjugation[14]. A USEPA[7] review of this chemical noted a potential for alkylation and a potential for reproductive effects from tetrabromobisphenol A-bis(2,3-dibromopropyl ether) exposure.

Carcinogenicity

Experimental Animals

No information of the carcinogenic potential of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in experimental animals was found in a review of the literature.

Humans

No epidemiology studies of the carcinogenic potential of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in humans were found in the peer-reviewed literature.

Genetic Toxicity

No genetic toxicology studies of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) were found in the peer-reviewed literature.

Study Rationale

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (TBBPA-DBPE) was nominated by the National Institute of Environmental Health Sciences for toxicology and in vivo genotoxicity study because this is a widely used flame retardant with little or no toxicity data reported in the literature, and because there is potential for human exposure.

Materials and Methods

Procurement and Characterization

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was obtained from Great Lakes Chemical Corporation (West Lafayette, IN) in one lot (534106) that was used during the 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO), and the study laboratory at Battelle Columbus Operations (Columbus, OH) conducted additional identity analyses and confirmed purity (Appendix G). Reports on analyses performed in support of the tetrabromobisphenol A-bis(2,3-dibromopropyl ether) studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white powder, was identified as tetrabromobisphenol A-bis(2,3-dibromopropyl ether) using infrared and proton nuclear magnetic resonance spectroscopy. Melting point analysis by differential scanning calorimetry indicated that lot 534106 of the test article contained impurities. The purity of lot 534106 was determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection; 12 reportable impurities with a total relative peak area of approximately 5.8% were detected. One impurity (approximately 2% relative to the total peak area) was tentatively identified as 1,3-dibromo-2-{[(2E)-3-bromoprop-2-en-1-yl]oxy}-5-{1-[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl]-1-methylethyl}benzene using liquid chromatography coupled with mass spectrometry. The overall purity of lot 534106 was determined to be approximately 94%.

To ensure stability, the bulk chemical was stored at room temperature (~25°C) in amber glass bottles. Periodic reanalyzes of the bulk chemical were performed by the study laboratory during the 3-month studies using HPLC/UV, and no degradation of the test article was detected.

Corn Oil

Corn oil was obtained in two lots (UU0854 and UW0493) from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 3-month 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 Analyses of Dose Formulations

The dose formulations were prepared four times by mixing tetrabromobisphenol A-bis(2,3-dibromopropyl ether) with corn oil to give the required concentrations (Table G-1). Homogeneity studies of 1.0 and 600 mg/mL formulations and stability studies of the 1.0 mg/mL formulation were performed by the analytical chemistry laboratory using HPLC/UV; the study laboratory used the same analytical system to perform additional homogeneity studies of 12.5, 25, 50, 400, and 800 mg/mL formulations and a gavageability study of a 200 mg/mL dose formulation. Homogeneity was deemed acceptable for all of the formulations; stability was confirmed for at least 42 days for dose formulations stored in sealed glass containers at temperatures up to 25°C, and for 3 hours under simulated animal room conditions. Gavageability of the 200 mg/mL dose formulation was confirmed by the study laboratory.

Analyses of the dose formulations of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) were conducted three times by the study laboratory using HPLC/UV; animal room samples of these dose formulations were also analyzed (Table G-2). Fourteen of the 15 dose formulations for rats and mice were within 10% of the target concentrations; 14 of 18 animal room samples for rats and 12 of 15 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), and B6C3F1/N mice were obtained from the NTP colony at Taconic Farms, Inc. 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 rat 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 to evaluate different rat models. The F344/NTac rat was used in four subchronic and two chronic studies between 2005 and 2006[15].

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 the 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

On receipt, the rats were 3 to 4 weeks old and the mice were 4 to 5 weeks old. Animals were quarantined for 11 to 14 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 using the protocols of the NTP Sentinel Animal Program (Appendix I); all test results were negative.

Recommended doses for the NTP subchronic studies in rats and mice were 0, 125, 250, 500, and 1,000 mg/kg (corn oil gavage). These doses were based on the relatively low toxicity reported for this chemical in the literature[1] and the NTP finding that the maximum amount of chemical that could be gavaged was 1,000 mg/kg due to viscosity. Corn oil gavage was selected as the route of administration because oral exposure is a likely route of exposure in humans, and to allow for comparison of results with those of other flame retardants administered by this route.

Core study groups of 10 male and 10 female rats were administered tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in corn oil by gavage at doses of 0, 62.5, 125, 250, 500, or

1,000 mg/kg body weight 5 days per week for 14 weeks, and additional groups of 10 male and 10 female special study rats were administered the same doses for 23 days for hematology, clinical chemistry, and liver enzyme level determinations. Groups of 10 male and 10 female mice were administered 0, 125, 250, 500, 1,000, or 2,000 mg/kg for 14 weeks. 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 postdosing on day 1, weekly, and at the end of the studies; animals were weighed on day 1, 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 the retroorbital venous sinus of mice for hematology analyses, and from the retroorbital plexus of special study rats and 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 or vaginal cytology evaluations on rats in the 0, 250, 500, and 1,000 mg/kg groups and mice in the 0, 500, 1,000, and 2,000 mg/kg groups. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal euthanasia, 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 Tyrone'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 all core study rats and mice at the end of the studies for cytochrome P450 and uridine diphosphate-glucuronosyltransferase (UDP-GT) activity determinations. Microsomal suspensions were

prepared as described by Battelle[16; 17]. 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 (CYP2B1) activities were determined spectrofluorimetrically[18], acetanilide-4-hydroxylase (CYP1A2) activity was determined by HPLC with ultraviolet detection[19-21], and UDP-GT activity toward T_4 was determined by quantifying the amount of ^{125}I-T_4-glucuronide produced[22].

Necropsies were performed on all core study rats and mice. 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 (eyes were first fixed in Davidson's solution); testes, vaginal tunics of testes, and epididymides were first fixed 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 (H&E). Complete histopathologic examinations were performed by the study laboratory pathologist on 0 and 1,000 mg/kg rats and 0 and 2,000 mg/kg mice. In addition, the liver, lung, and mesenteric lymph node were examined in the remaining dosed groups of rats because lesions in these tissues were identified in the high dose groups. When present, gross lesions were examined in all dosed groups. 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 Peer Review (PPR) 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 PPR or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s) and the PPR coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[23] and Boorman et al.[24].

To determine the nature of the pulmonary granulomatous and chronic active inflammatory lesions, special microscopic reviews (Reviews 1 through 5) were performed on respiratory system tissues of rats and mice in the current 3-month corn oil gavage study of tetrabromobisphenol A-bis(2,3-dibromopropyl ether), on lung tissue of rats from the 3-month interim evaluation in the 2-year corn oil gavage study of tetrabromobisphenol A[25], and on lung tissue of rats from the 3-month feed study of *p*-toluenesulfonamide[26]. For Review 1, H&E stained sections of the nasal cavity, larynx, and trachea from all vehicle control and treated F344/NTac rats in the current study were examined to determine if gavage-related reflux may have caused granulomatous lesions in the lung. For Review 2, Sudan black stained sections of the lung from three vehicle control and three treated F344/NTac rats in the current study were examined to determine if corn oil droplets were present and whether their presence correlated with the occurrence of granulomatous inflammation. For Review 3, H&E and Sudan black stained sections of the lung of five vehicle control and five high dose Wistar Han rats at the 3-month interim evaluation in the 2-year corn oil gavage study of tetrabromobisphenol A[25] were evaluated for the presence of granulomatous inflammation and corn oil droplets. For Review 4, H&E and Sudan black stained sections of the lung of five control and five high dose F344/NTac rats from the 3-month feed study of *p*-toluenesulfonamide were evaluated for the presence of granulomatous inflammation and corn oil droplets. For Review 5, Sudan black stained sections of the lung from three vehicle control and three high dose B6C3F1/N mice in the current study

were examined to determine if corn oil droplets were present and whether their presence correlated with the occurrence of granulomatous lesions.

Table 1. Experimental Design and Materials and Methods in the Three-month Gavage Studies of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Three-month Studies
Study Laboratory
Battelle Columbus Operations (Columbus, OH)
Strain and Species
F344/NTac rats B6C3F1/N mice
Animal Source
Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies
Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days
Average Age When Studies Began
Rats: 5 to 6 weeks Mice: 6 to 7 weeks
Date of First Dose
Rats: January 9 (males) or 10 (females), 2006 Mice: January 11 (females) or 12 (males), 2006
Duration of Dosing
5 days per week for 14 weeks
Rats: April 10 (males) or 11 (females), 2006 Mice: April 12 (females) or 13 (males), 2006
Necropsy Dates
Rats: April 11 (males) or 12 (females), 2006 Mice: April 13 (females) or 14 (males), 2006
Average Age at Necropsy
Rats: 18 to 19 weeks Mice: 19 to 20 weeks
Size of Study Groups
Core study animals: 10 males and 10 females (rats and mice)
Special study animals: 10 males and 10 females (rats only)
Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.
Animals per Cage
5 (rats and female mice) or 1 (male mice)

Three-month Studies

Method of Animal Identification

Tail tattoo

Diet

Irradiated NTP-2000 open formula wafer feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*; changed at least weekly

Water

Tap water (City of Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages

Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly (male mice) or twice weekly (rats and female mice)

Bedding

Irradiated Sani Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (male mice) or twice weekly (rats and female mice)

Rack Filters

Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH) changed every 2 weeks

Racks

Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks

Animal Room Environment

Temperature: 72° ± 3°F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: at least 10/hour

Doses

Rats: 0, 62.5, 125, 250, 500, or 1,000 mg/kg in corn oil (dosing volume, 5 mL/kg)
Mice: 0, 125, 250, 500, 1,000, or 2,000 mg/kg in corn oil (dosing volume, 10 mL/kg)

Type and Frequency of Observation

Observed twice daily; animals were weighed and clinical findings were recorded on day 1, weekly thereafter, and at the end of the studies.

Method of Euthanasia

Carbon dioxide asphyxiation (special study rats and mice) or exsanguination by severing portal vein (core study rats)

Necropsy

Necropsies were performed on all core study rats and mice. Organs weighed were heart, right kidney, liver, lung, spleen, 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 (except triiodothyronine) and 23 and at the end of the study.

Three-month Studies

Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; 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

Liver Protein and Enzyme Levels

Liver samples were collected from special study rats on day 23 and from core study rats and mice at study termination for determination of microsomal protein concentration and acetanilide-4-hydroxylase, 7-ethoxyresorufin-*O*-deethylase, 7-pentoxyresorufin-*O*-dealkylase, and uridine diphosphate-glucuronosyltransferase activities.

Histopathology

Complete histopathology was performed on core study rats in the 0 and 1,000 mg/kg groups, mice in the 0 and 2,000 mg/kg groups, and all animals that died early. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with mainstem bronchus), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. The liver, lung, and mesenteric lymph nodes were also examined in the remaining dosed groups of rats. Special microscopic reviews were performed on respiratory system tissues of rats and mice.

Sperm Motility and Vaginal Cytology

At the end of the studies, sperm samples were collected from male rats in the 0, 250, 500, and 1,000 mg/kg groups and male mice in the 0, 500, 1,000, and 2,000 mg/kg groups. The following parameters were evaluated: spermatid heads per gram testis and per testis, sperm motility, and sperm per gram cauda epididymis and per 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 in the 0, 250, 500, and 1,000 mg/kg groups and female mice in the 0, 500, 1,000, and 2,000 mg/kg groups.

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test[27], a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle 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[28] and Williams[29; 30]. Hematology, clinical chemistry, thyroid hormone, microsomal protein, cytochrome P450, UDP-GT, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[31] (as modified by Williams[32]) and

Dunn[33]. Jonckheere's test[34] 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[35] 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 vehicle control group using the Fisher exact test[27]. 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[36]. 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 vehicle control group and each dosed group was tested using chi-square statistics.

Quality Assurance Methods

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[37]. In addition, as records from the 3-month 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 Toxicity Study 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 Toxicity Study Report.

Genetic Toxicology

Salmonella typhimurium Mutagenicity Test Protocol

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (lot 324811, Great Lakes Chemical Corp.) was tested for mutagenicity in three strains of *Salmonella typhimurium* following protocols reported by Zeiger et al.[38]. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was sent to the testing laboratory as a coded sample. It was incubated with the *S. typhimurium* tester strains TA98, TA100, and TA102 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver) for 20 minutes at 37º C. 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 37º C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of tetrabromobisphenol A-bis(2,3-dibromopropyl ether). In the absence of toxicity, the highest concentration tested was 10,000 µg/plate.

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.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[39]. At the termination 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 micronucleated cells in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group. In addition, the percentage of polychromatic erythrocytes in a population of 1,000 erythrocytes was determined 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 using 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. After the statistical analysis of the test data has been completed, the scientific staff determines the final call for the assay, considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

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 results presented in the Abstract of this Toxicity Study Report represent a scientific judgment of the overall evidence for activity of the chemical in an assay.

Results

Three-month Study in Rats

All rats survived to the end of the study with the exception of two 62.5 mg/kg males and one vehicle control female; one male death was due to a dosing accident (Table 2). Final mean body weights and body weight gains of male and female rats were similar to those of the vehicle controls (Table 2; Figure 2). No treatment-related clinical findings or treatment-related gross lesions were identified in males or females.

Table 2. Survival and Body Weights of Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[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	85 ± 3	330 ± 9	244 ± 9	
62.5	8/10[c]	85 ± 2	344 ± 8	258 ± 8	104
125	10/10	85 ± 3	329 ± 7	245 ± 7	100
250	10/10	84 ± 2	342 ± 6	257 ± 6	104
500	10/10	86 ± 3	333 ± 5	247 ± 6	101
1,000	10/10	86 ± 3	320 ± 8	234 ± 8	97
Female					
0	9/10[d]	83 ± 2	191 ± 4	108 ± 4	
62.5	10/10	83 ± 1	195 ± 4	112 ± 4	102
125	10/10	83 ± 2	188 ± 4	106 ± 4	98
250	10/10	83 ± 1	194 ± 4	111 ± 4	101
500	10/10	84 ± 2	188 ± 3	104 ± 3	98
1,000	10/10	83 ± 2	190 ± 4	106 ± 3	99

[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. 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.
[c]One 62.5 mg/kg male died early of a natural death on test day 37 and had lesions of minimal cardiomyopathy, minimal chronic liver inflammation, minimal liver cytoplasmic vacuolization, and acute and minimal pulmonary inflammation. Another 62.5 mg/kg male died of a dosing accident on test day 40 and had lesions of minimal cardiomyopathy, minimal renal nephropathy and mineralization, minimal chronic liver cytoplasmic vacuolization, mesenteric lymph node moderate histiocytic cellular infiltration, and acute mild pulmonary inflammation.
[d]Week of death: 8.

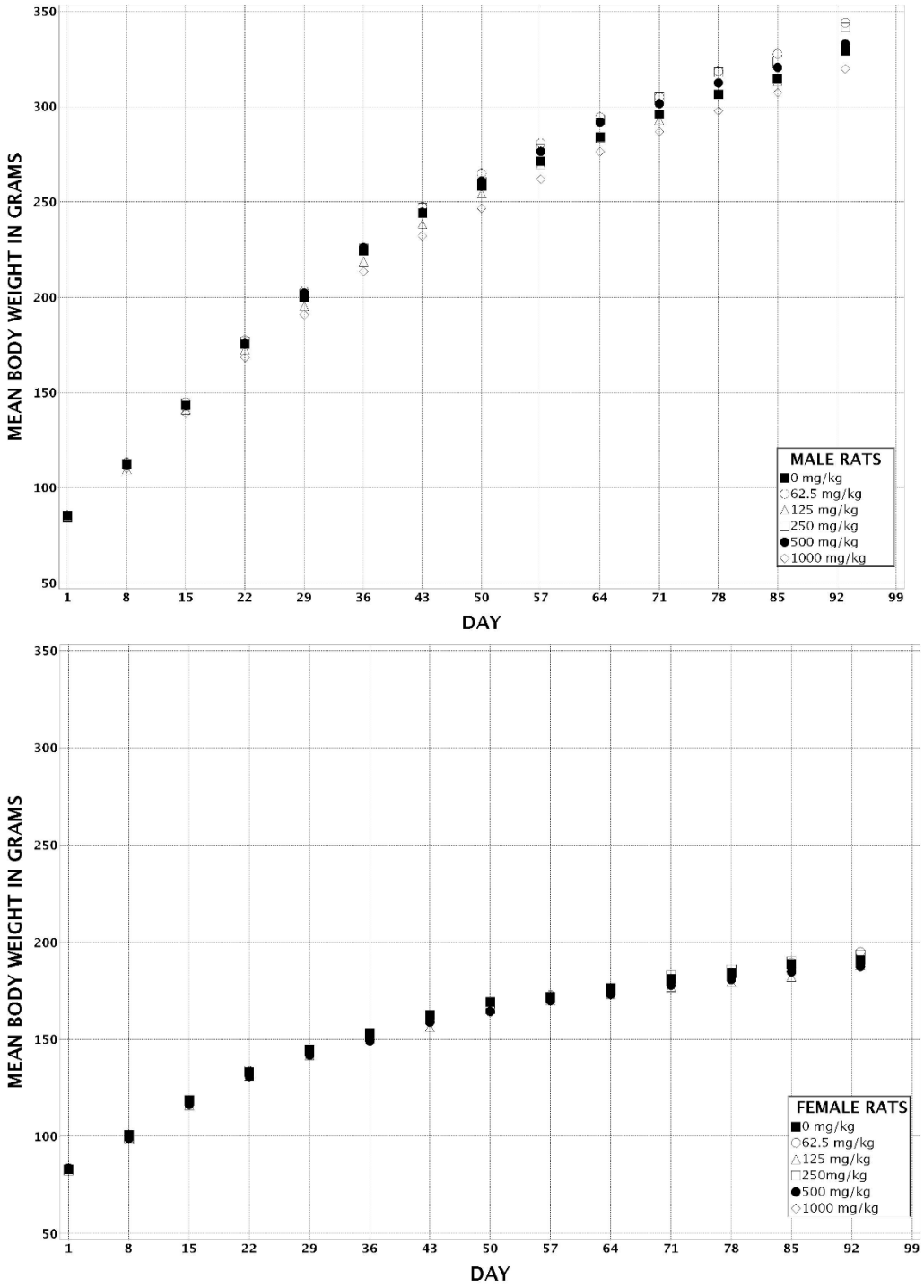

Figure 2. Growth Curves for Rats Administered Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) by Gavage for Three Months

Small (≤15%) decreases in cholesterol concentrations occurred in the 250 mg/kg or greater male and female rats (Table B-1). This effect was observed in one or more of the higher dose groups at all time points, albeit inconsistently within a dosed group. Only the 500 mg/kg female rats demonstrated decreases at all time points. The toxicologic relevance of these small, inconsistent

decreases in circulating cholesterol concentrations is unknown, but could suggest some small alteration in cholesterol metabolism related to treatment.

Liver enzyme levels showed an apparent decrease in treated groups of males and females when normalized on a microsomal protein level (Table F-1). However, this decrease in liver enzyme levels was not considered to be biologically relevant because the absolute values for the liver enzymes did not increase with treatment.

There were no chemical-related effects on absolute or relative organ weights in males or females (Table C-1).

There were no changes in the number of sperm or spermatids, sperm motility, or testis or epididymis weights of dosed males (Table D-1). There were no estrous cycle changes in dosed females (Table D-2, Table D-3, Figure D-1). Under the conditions of this study, gavage administration of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did not exhibit the potential to be a reproductive toxicant in male or female rats.

In the lung, incidences of granulomatous inflammation in 62.5, 125, 250, and 500 mg/kg males and in females administered 125 mg/kg or greater were significantly greater than those in the vehicle control groups (Table 3, Table A-1, Table A-2). The incidences of chronic active inflammation were significantly increased in 250 mg/kg or greater females; the incidence of this lesion was significantly decreased in 62.5 mg/kg males.

Granulomatous inflammation in the lung of male and female rats was characterized by foci of vacuolated mononuclear and occasionally multinucleated macrophages within the alveoli lumens, and less often in the terminal bronchioles, with minimal to no perivascular involvement. Some macrophages contained linear clear spaces consistent with sterol clefts and others contained small refractile globules, consistent with oil droplets.

Chronic active inflammation in the lung of male and female rats was characterized by dense perivascular accumulations of lymphocytes with fewer macrophages, granulocytes, and erythrocytes. This lesion is morphologically consistent with *Pneumocystis carinii* infection[40].

Table 3. Incidences of Nonneoplastic Lesions of the Lung in Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Number Examined						
Microscopically	10	10	10	10	10	10
Inflammation, Granulomatous[a]	0	4* (1.5)[b]	8** (1.6)	8** (1.4)	6** (1.0)	2 (1.5)
Inflammation, Chronic Active	7 (1.1)	1** (1.0)	9 (2.3)	6 (1.0)	8 (1.1)	7 (1.1)
Female						
Number Examined						
Microscopically	10	10	10	10	10	10

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Inflammation, Granulomatous	0	3 (1.3)	6** (1.5)	6** (1.5)	6** (1.0)	5* (1.4)
Inflammation, Chronic Active	1 (1.0)	4 (1.0)	3 (1.7)	6* (1.2)	6* (1.2)	8** (1.4)

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

Three-month Study in Mice

All mice survived to the end of the study (Table 4). The final mean body weights and body weight gains of all dosed groups of males were similar to those of the vehicle control group; the final mean body weight of 250 mg/kg females was 11% greater than that of the vehicle controls, and the mean body weight gain of this group was significantly increased (Table 4; Figure 3). No treatment-related clinical findings or treatment-related gross lesions were identified in males or females.

No changes attributable to administration of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) occurred in the hematology data for mice (Table B-2).

Liver enzyme levels showed an apparent decrease in treated groups of males and females when normalized on a microsomal protein level (Table F-2). However, this decrease in liver enzyme levels was not considered to be biologically relevant because the absolute values for the liver enzymes did not increase with treatment.

Table 4. Survival and Body Weights of Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[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	23.3 ± 0.4	36.8 ± 1.0	13.5 ± 0.9	
125	10/10	23.2 ± 0.4	39.0 ± 0.9	15.9 ± 0.6	106
250	10/10	23.0 ± 0.3	38.3 ± 1.0	15.3 ± 0.7	104
500	10/10	23.4 ± 0.4	39.4 ± 1.5	16.0 ± 1.1	107
1,000	10/10	23.0 ± 0.3	38.2 ± 0.9	15.2 ± 0.7	104
2,000	10/10	22.8 ± 0.3	39.1 ± 1.1	16.3 ± 0.9	106
Female					
0	10/10	18.4 ± 0.4	29.2 ± 1.0	10.9 ± 0.7	
125	10/10	18.7 ± 0.3	31.6 ± 1.3	12.9 ± 1.0	108
250	10/10	18.6 ± 0.3	32.4 ± 0.7	13.8 ± 0.5*	111
500	10/10	18.5 ± 0.3	31.0 ± 0.8	12.5 ± 0.7	106
1,000	10/10	18.8 ± 0.3	31.0 ± 0.8	12.2 ± 0.7	106
2,000	10/10	18.5 ± 0.3	30.2 ± 0.9	11.7 ± 0.7	103

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test.
[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group.

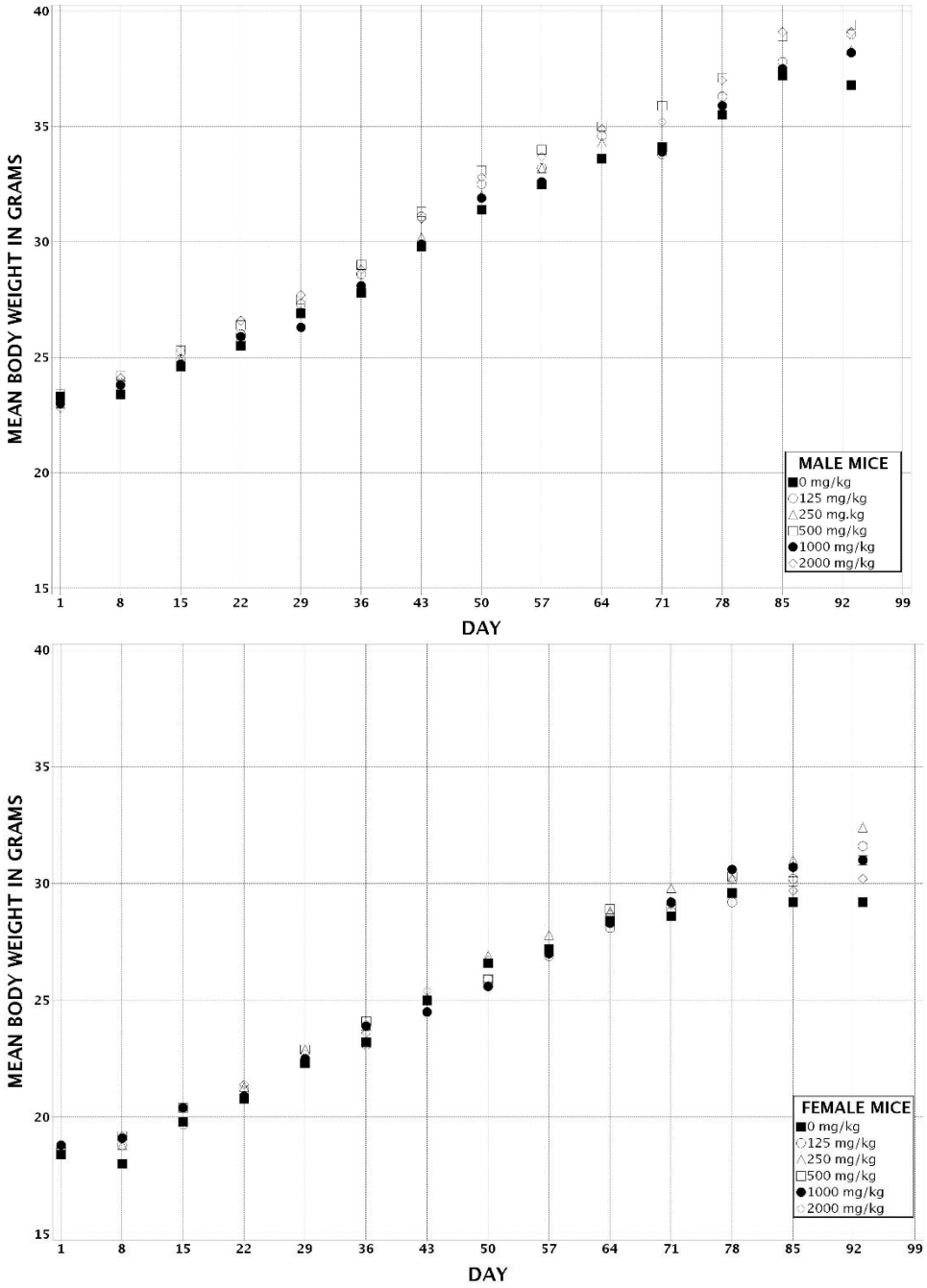

Figure 3. Growth Curves for Mice Administered Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) by Gavage for Three Months

There were no chemical-related effects on absolute or relative organ weights in males or females (Table C-2).

There were no changes in the number of sperm or spermatids, sperm motility, or testis or epididymis weights of dosed males (Table D-4). There were no estrous cycle changes in dosed females (Table D-5, Table D-6, Figure D-2). Under the conditions of this study, gavage

administration of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did not exhibit the potential to be a reproductive toxicant in male or female mice.

There were no gross or histologic lesions that were considered treatment related (Table A-3, Table A-4).

Special Respiratory System Microscopic Review

Special NTP reviews (1 through 5) of respiratory system tissues of rats and mice were conducted to better define and determine the nature of the pulmonary granulomatous and chronic active inflammatory lesions that occurred in the lungs of rats in the current study.

In Review 1, histologic evaluation of H&E stained sections of the nasal cavity, larynx, and trachea from all male and female vehicle control and treated rats in the current study was conducted to determine if gavage-related reflux could have caused the granulomatous lesions[41]. No significant treatment-related histologic lesions were noted in any of the tissues examined.

In Review 2, Sudan black stained sections of the lung from three vehicle control and three dosed F344/NTac rats in the current study were evaluated to determine if corn oil could be present in the lung and, if so, was it present within the granulomatous lesions. Sudan black is a diazo dye used for the staining of some lipoproteins in paraffin sections[42]. The presence of granulomatous inflammation correlated with the presence of Sudan black positive material within the distal alveolar spaces. This positive staining correlated with the small round globules, consistent with oil droplets, that were noted with H&E stain.

In Review 3, H&E and Sudan black stained sections of lung from five vehicle control Wistar Han rats and five high dose Wistar Han rats at the 3-month interim evaluation in the 2-year corn oil gavage study of tetrabromobisphenol A[25] were examined for granulomatous inflammation and corn oil droplets. Animals from that tetrabromobisphenol A study were used because that study was conducted at the same laboratory at approximately the same time as the current tetrabromobisphenol A-bis(2,3-dibromopropyl ether) study, tetrabromobisphenol A is a related chemical, and the route of administration was the same. Granulomatous lesions similar to those that occurred in F344/NTac rats in the current tetrabromobisphenol A-bis(2,3-dibromopropyl ether) study were not present in any of the tetrabromobisphenol A Wistar Han rat lungs examined, but occasional similar pale yellow, small refractile, round globules (consistent with oil droplets) were present without an inflammatory response. In addition, there was a correlation between the Sudan black positive material in the alveolar spaces with the presence of this pale yellow material on H&E staining in the lung tissues from Wistar Han rats in the tetrabromobisphenol A study.

Review 4 involved an evaluation of select H&E and Sudan black stained sections of lungs from five control and five high dose F344/NTac rats from the 3-month feed study of *p*-toluenesulfonamide[26] for similar granulomatous lesions. The rationale for comparing animals from a feed study to those from the current gavage study was to determine if the granulomatous lung lesions in the current study were due to corn oil aspiration. There was an absence of granulomatous lesions similar to those observed in the lungs of rats treated with tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in the current study, and no evidence of Sudan black positive material in any of the animals examined. In addition, there were fewer

numbers of alveolar macrophages and intra-alveolar debris in the rats from the feed study compared to those from the current gavage study.

In Review 5, Sudan black stained sections of lung from six select vehicle control (i.e., corn oil) and six high dose B6C3F1/N mice in the current gavage study of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) were examined. The rationale for using tetrabromobisphenol A-bis(2,3-dibromopropyl ether) treated mice was to determine if corn oil was present in mice treated with tetrabromobisphenol A-bis(2,3-dibromopropyl ether) as was found in tetrabromobisphenol A-bis(2,3-dibromopropyl ether) treated rats. Only one mouse had questionable Sudan black positive material, however, granulomatous lesions similar to those present in the rat lungs did not occur.

Genetic Toxicology

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was tested for mutagenicity in three strains of *Salmonella typhimurium* (TA98, TA100, and TA102), with and without 10% rat liver S9 mix. The sample of tetrabromobis-phenol A-bis(2,3-dibromopropyl ether) (lot 324811) that was used in the mutagenicity studies was not the same lot that was used in the 3-month gavage studies in rats and mice. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (dose range of 100 to 10,000 µg/plate) showed no evidence of mutagenic activity in any of the three tester strains, with or without S9 (Table E-1).

In vivo, no significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male or female mice from the 3-month gavage study (Table E-2). Furthermore, no significant changes in the percentage of polychromatic erythrocytes among total red blood cells were seen in these mice, suggesting that tetrabromobisphenol A-bis(2,3-dibromopropyl ether) did not induce bone marrow toxicity under the conditions of this study.

Discussion

The current tetrabromobisphenol A-bis(2,3-dibromopropyl ether) 3-month F344/NTac rat and B6C3F1/N mouse studies were performed to evaluate the toxic potential of this flame retardant and to compare the toxicity of the test chemical to that of the structurally related flame retardant tetrabromobisphenol A[25].

In these 3-month tetrabromobisphenol A-bis(2,3-dibromopropyl ether) studies, there were no treatment-related mortality or clinical signs in F344/NTac rats or B6C3F1/N mice, and final mean body weights of dosed groups were within 10% of those of the vehicle controls, except for 250 mg/kg female mice.

Two types of lung lesions were seen in rats treated with tetrabromobisphenol A-bis(2,3-dibromopropyl ether). The first lung lesion, granulomatous inflammation, was seen in all treated rat groups, but the severity of this lesion did not increase with increased tetrabromobisphenol A-bis(2,3-dibromopropyl ether) exposure. This lung lesion was characterized by foci of vacuolated mononuclear and occasionally multinucleated macrophages within the alveolar lumens and less often in the terminal bronchioles. At times, these macrophages had linear clear spaces consistent with sterol clefts and/or small, pale yellow, round refractile globules, which may have been corn oil. Special studies were performed to determine the pathogenesis of this lesion. Evaluation of the nasal cavity, larynx, and trachea revealed no histologic lesions, ruling out gavage-related reflux as a potential etiology[41]. Because H&E evaluation revealed foreign material consistent with corn oil within the granulomatous foci, Sudan black was used to better characterize this lesion. Sudan black is a dye that is commonly used to stain lipids. In the animals evaluated, this foreign material did stain with Sudan black, indicating that it was most likely the corn oil vehicle. Select lung tissue from Wistar Han rats at the 3-month interim evaluation in the 2-year corn oil gavage study of tetrabromobisphenol A[25] were also evaluated with Sudan black. Although no similar granulomatous lesions were present, there was occasional foreign material, morphologically consistent with corn oil, which was Sudan black positive. This suggests that small amounts of corn oil can be aspirated and not necessarily result in histologic lesions. Select lung tissue from rats in the 3-month *p*-toluenesulfonamide feed study[26] were evaluated as well, and not surprisingly, there were no granulomatous lesions, no similar foreign material (small pale yellow refractile globules) and no Sudan black positive foreign material. One additional study looked at lungs from mice in the current study of tetrabromobisphenol A-bis(2,3-dibromopropyl ether), and there were no granulomatous lesions, no foreign material, and no conclusive Sudan black positivity. The reason for this lack of similar effects in mice is unknown and may be multifactorial. Potential factors may include superior gavage techniques in the mouse study and/or the smaller gavage needle bore/diameters used for the mice.

Based on the presence of a Sudan black positive foreign lipid material associated with the granulomatous lesions in the affected rats in this study, it is suggested that this material was corn oil vehicle aspirated during the gavage process and was not due to a systemic effect of tetrabromobisphenol A-bis(2,3-dibromopropyl ether). However, because corn oil was seen in the lungs of some rats from the tetrabromobisphenol A study without an inflammatory component[25], and this granulomatous lesion did not occur in the vehicle control rats from the current tetrabromobisphenol A-bis(2,3-dibromopropyl ether) study, it may be that test article was also present in the aspirated material and this may have caused the granulomatous response in the

lung. Accordingly, the occurrence of this granulomatous lung lesion was most likely caused by the procedure of administering the test article to rats that resulted in a small amount of gavage material (corn oil and test article) remaining on the gavage needle during removal resulting in a localized, topical contact with the chemical. High viscosity of the corn oil gavage solution may have caused the material to adhere to the gavage needles. Because oral intubation of mice is somewhat more difficult compared to rats, the rate of dose administration for mice is accordingly slower. The slower rate of administration (and, therefore, no deposition of the gavage material in the lung) could explain the lack of pulmonary lesions in the mice.

The second lung lesion seen in the rats was chronic inflammation, and it was seen in vehicle control and treated rats. This lesion was morphologically different from the lesion associated with aspiration of gavage solution and was in a different location within the lung. The granulomatous lesions were within the alveoli lumens, and less often in the terminal bronchioles, with minimal to no perivascular involvement. The chronic inflammatory lesions were confined to the perivascular regions. No Sudan black positive material was identified within these perivascular chronic inflammatory lesions.

The chronic inflammatory lung lesions were characterized by dense perivascular accumulations of mostly lymphocytes with fewer macrophages, granulocytes, and erythrocytes. The morphology of this rat lung lesion is characteristic of *Pneumocystis carinii* infection[40]. In immunocompetent rats, infection with the fungus *P. carinii* causes an infectious interstitial pneumonia characterized by dense perivascular cuffs of primarily lymphocytes and a lymphohistiocytic interstitial pneumonia. This lesion has been reported in research rats since the 1990s and was previously attributed to "rat respiratory virus" with an unknown etiology. In 2011, Livingston et al.[40] determined that the etiologic agent was *P. carinii*. In immunocompetent animals, there are usually no clinical signs associated with this infection and it is considered self-limiting, resolving by about 20 weeks of age. Historically, this organism had been undetectable with silver stains and thus was undiagnosed in rat colonies until 2011 when Livingston et al.[40] used polymerase chain reaction (PCR) to detect the presence of this specific fungus. Since that time, both PCR and serological tests have been used to detect *P. carinii*. The exact etiology of the lung lesions in the current study remains uncertain because the NTP rat colonies and sentinel animals were not tested for this organism. At the time this study was performed the causative agent for "rat respiratory virus" had not been determined, hence there was no assay for the disease. However, due to the morphology of the lesions and the age of the rats, the most likely etiology is *P. carinii*.

In the current NTP study, there were no tetrabromobisphenol A-bis(2,3-dibromopropyl ether) treatment-related changes in thyroxine, triiodothyronine, or thyroid stimulating hormone levels in rats (not measured in mice), while with tetrabromobisphenol A[25], there were decreases in thyroxine levels in F344/NTac rats after 4, 23, and 93 days of chemical administration.

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was negative in bacterial mutagenicity tests (Table E-1) consistent with what has been seen previously with tetrabromobisphenol A and other brominated flame retardants[25; 43]. In tests with other brominated chemicals, cancer target organs are not always identified in 3-month studies. For example, tetrabromobisphenol A induced uterine adenocarcinomas in the 2-year rat study, but uterus was not identified as a target organ in the 3-month rat study, based on the absence of treatment-related lesions[25]. Similarly, in a 2-year 2,3-dibromo-1-propanol study, intestinal tumors were seen in rats, but the intestine was

not designated a target organ in the 3-month study[44]. Thus, while the current tetrabromobisphenol A-bis(2,3-dibromopropyl ether) study did not result in treatment-related target organ toxicity in either rats or mice, it is uncertain if this result would predict positive or negative carcinogenic activity after longer term administration of tetrabromobisphenol A-bis(2,3-dibromopropyl ether).

Under the conditions of these 3-month gavage studies, there were no clinical findings or treatment-related lesions in male or female F344/NTac rats or B6C3F1/N mice administered tetrabromobisphenol A-bis(2,3-dibromopropyl ether) at 0, 62.5, 125, 250, 500, or 1,000 mg/kg in rats or 0, 125, 250, 500, 1,000, or 2,000 mg/kg in mice. Final mean body weights of treated rats and mice were generally within 10% of vehicle controls, and there were no treatment-related effects on organ weights.

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Appendix A. Summary of Nonneoplastic Lesions in Rats and Mice

Tables

Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths[b]						
Accidental death	–	1	–	–	–	–
Natural death	–	1	–	–	–	–
Survivors						
Terminal euthanasia	10	8	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(2)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(1)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(2)	(0)	(0)	(0)	(10)
Serosa, parasite metazoan	–	–	–	–	–	1 (10%)
Intestine large, rectum	(10)	(2)	(0)	(0)	(0)	(10)
Parasite metazoan	–	–	–	–	–	2 (20%)
Intestine small, duodenum	(10)	(1)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(1)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(1)	(0)	(0)	(0)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Vacuolization cytoplasmic	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mesentery	(0)	(0)	(0)	(0)	(0)	(1)
Fat, necrosis	–	–	–	–	–	1 (100%)
Oral mucosa	(0)	(0)	(0)	(0)	(1)	(0)
Cyst, focal	–	–	–	–	1 (100%)	–
Pancreas	(10)	(2)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)	–	–	–	–	–
Acinus, atrophy	2 (20%)	–	–	–	–	1 (10%)
Arteriole, inflammation	1 (10%)	–	–	–	–	–
Salivary glands	(10)	(2)	(0)	(0)	(0)	(10)
Stomach, forestomach	(10)	(2)	(0)	(0)	(0)	(10)
Stomach, glandular	(10)	(2)	(0)	(0)	(0)	(10)
Muscularis, mineralization	–	–	–	–	–	1 (10%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Cardiovascular System						
Blood vessel	(10)	(2)	(0)	(0)	(0)	(10)
Heart	(10)	(2)	(0)	(0)	(0)	(10)
Cardiomyopathy	10 (100%)	2 (100%)	–	–	–	10 (100%)
Infiltration cellular, mixed cell	1 (10%)	–	–	–	–	–
Pigmentation	1 (10%)	–	–	–	–	–
Endocrine System						
Adrenal cortex	(10)	(2)	(0)	(0)	(0)	(10)
Adrenal medulla	(10)	(2)	(0)	(0)	(0)	(10)
Islets, pancreatic	(10)	(2)	(0)	(0)	(0)	(10)
Parathyroid gland	(7)	(2)	(0)	(0)	(0)	(10)
Pituitary gland	(10)	(2)	(0)	(0)	(0)	(10)
Thyroid gland	(10)	(2)	(0)	(0)	(0)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	(2)	(0)	(0)	(0)	(10)
Inflammation, chronic active	5 (50%)	–	–	–	–	–
Preputial gland	(10)	(2)	(0)	(0)	(0)	(10)
Inflammation	3 (30%)	–	–	–	–	3 (30%)
Prostate	(10)	(2)	(0)	(0)	(0)	(10)
Inflammation, chronic active	1 (10%)	–	–	–	–	–
Seminal vesicle	(10)	(2)	(0)	(0)	(0)	(10)
Testes	(10)	(2)	(0)	(0)	(0)	(10)
Hematopoietic System						
Bone marrow	(10)	(2)	(0)	(0)	(0)	(10)
Lymph node, mandibular	(10)	(2)	(0)	(0)	(0)	(10)
Lymph node, mesenteric	(10)	(9)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	10 (100%)	9 (100%)	10 (100%)	10 (100%)	1 (100%)	10 (100%)
Spleen	(10)	(2)	(0)	(0)	(0)	(10)
Thymus	(10)	(2)	(0)	(0)	(0)	(10)
Integumentary System						
Mammary gland	(10)	(2)	(0)	(0)	(0)	(10)
Skin	(10)	(2)	(0)	(0)	(0)	(10)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Musculoskeletal System						
Bone	(10)	(2)	(0)	(0)	(0)	(10)
Nervous System						
Brain	(10)	(2)	(0)	(0)	(0)	(10)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	1 (10%)	1 (10%)	–	–	1 (10%)	1 (10%)
Inflammation, granulomatous	–	4 (40%)	8 (80%)	8 (80%)	6 (60%)	2 (20%)
Inflammation, acute	–	2 (20%)	–	–	–	–
Inflammation, chronic active	7 (70%)	1 (10%)	9 (100%)	6 (60%)	8 (80%)	7 (70%)
Metaplasia, osseous	1 (10%)	2 (20%)	–	–	–	–
Nose	(10)	(2)	(0)	(0)	(0)	(10)
Inflammation, chronic active	–	–	–	–	–	1 (10%)
Trachea	(10)	(2)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)	–	–	–	–	2 (20%)
Special Senses System						
Eye	(10)	(3)	(0)	(0)	(0)	(10)
Harderian gland	(10)	(2)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	2 (20%)	–	–	–	–	–
Inflammation, chronic active	–	–	–	–	–	1 (10%)
Bilateral, inflammation, chronic active	–	–	–	–	–	1 (10%)
Urinary System						
Kidney	(10)	(2)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)	–	–	–	–	2 (20%)
Mineralization	8 (80%)	1 (50%)	–	–	–	6 (60%)
Nephropathy	10 (100%)	1 (50%)	–	–	–	10 (100%)
Urinary bladder	(10)	(2)	(0)	(0)	(0)	(10)
Inflammation, chronic	–	–	–	–	–	1 (10%)
Muscularis, mineralization	–	–	–	–	–	1 (10%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.
[b]One 62.5 mg/kg rat died a natural death on test day 37 and another 62.5 mg/kg rat died from a dosing accident on day 40; these early death animals were examined for histopathologic lesions.

Table A-2. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Natural death	1	–	–	–	–	–
Survivors						
Terminal euthanasia	9	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, rectum	(10)	(0)	(0)	(0)	(0)	(10)
Parasite metazoan	–	–	–	–	–	1 (10%)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(0)	(0)	(0)	(0)	(10)
Liver	(10)	(8)	(8)	(9)	(10)	(10)
Inflammation, chronic	8 (80%)	8 (100%)	8 (100%)	9 (100%)	10 (100%)	10 (100%)
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Salivary glands	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, forestomach	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, glandular	(10)	(0)	(0)	(0)	(0)	(10)
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	9 (90%)	–	–	–	–	9 (90%)
Inflammation, chronic active	1 (10%)	–	–	–	–	–
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Adrenal medulla	(10)	(0)	(0)	(0)	(0)	(10)
Islets, pancreatic	(10)	(0)	(0)	(0)	(0)	(10)
Parathyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
Pituitary gland	(10)	(0)	(0)	(0)	(0)	(10)
Cyst	–	–	–	–	–	1 (10%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Clitoral gland	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic active	3 (30%)	–	–	–	–	–
Ovary	(10)	(0)	(1)	(0)	(0)	(10)
Left, cyst	–	–	1 (100%)	–	–	–
Uterus	(10)	(0)	(0)	(0)	(0)	(10)
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mandibular	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Spleen	(10)	(0)	(0)	(0)	(0)	(10)
Lymphoid follicle, depletion cellular	1 (10%)	–	–	–	–	–
Thymus	(10)	(0)	(0)	(0)	(0)	(10)
Integumentary System						
Mammary gland	(10)	(0)	(0)	(0)	(0)	(10)
Skin	(10)	(0)	(0)	(0)	(0)	(10)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(0)	(10)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	2 (20%)	1 (10%)	2 (20%)	2 (20%)	–	–
Inflammation, granulomatous	–	3 (30%)	6 (60%)	6 (60%)	6 (60%)	5 (50%)
Inflammation, acute	1 (10%)	–	–	–	–	–
Inflammation, chronic active	1 (10%)	4 (40%)	3 (30%)	6 (60%)	6 (60%)	8 (80%)
Metaplasia, osseous	–	–	–	1 (10%)	–	–
Bronchiole, metaplasia, squamous	–	–	–	–	–	1 (10%)
Nose	(10)	(0)	(0)	(0)	(0)	(10)
Squamous epithelium, hyperplasia	–	–	–	–	–	1 (10%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Trachea	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	5 (50%)	–	–	–	–	4 (40%)
Special Senses System						
Eye	(10)	(0)	(0)	(0)	(0)	(10)
Harderian gland	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	1 (10%)	–	–	–	–	–
Inflammation, chronic active	2 (20%)	–	–	–	–	3 (30%)
Bilateral, infiltration cellular, mononuclear cell	–	–	–	–	–	1 (10%)
Bilateral, inflammation, chronic active	1 (10%)	–	–	–	–	–
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	–	–	–	–	–	1 (10%)
Mineralization	7 (70%)	–	–	–	–	5 (50%)
Nephropathy	2 (20%)	–	–	–	–	1 (10%)
Urinary bladder	(10)	(0)	(0)	(0)	(0)	(10)

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

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	0	0	0	0	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	–	–	–	–	–	1 (10%)
Liver	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	3 (30%)	–	–	–	–	4 (40%)

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic active	–	–	–	–	–	1 (10%)
Cardiovascular System						
None	–	–	–	–	–	–
Endocrine System						
None	–	–	–	–	–	–
General Body System						
None	–	–	–	–	–	–
Genital System						
Prostate	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	4 (40%)	–	–	–	–	4 (40%)
Epithelium, hyperplasia	1 (10%)	–	–	–	–	–
Hematopoietic System						
None	–	–	–	–	–	–
Integumentary System						
None	–	–	–	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
Lung	(10)	(0)	(0)	(0)	(0)	(10)
Alveolus, infiltration cellular, mononuclear cell	–	–	–	–	–	1 (10%)
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	3 (30%)	–	–	–	–	3 (30%)
Nephropathy	–	–	–	–	–	1 (10%)

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

Table A-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	0	0	0	0	10
Alimentary System						
Liver	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell	9 (90%)	–	–	–	–	10 (100%)
Cardiovascular System						
None	–	–	–	–	–	–
Endocrine System						
None	–	–	–	–	–	–
General Body System						
None	–	–	–	–	–	–
Genital System						
None	–	–	–	–	–	–
Hematopoietic System						
None	–	–	–	–	–	–
Integumentary System						
None	–	–	–	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
Lung	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic	1 (10%)	–	–	–	–	–
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(0)	(10)
Casts protein	1 (10%)	–	–	–	–	–

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Infiltration cellular, mononuclear cell	4 (40%)	–	–	–	–	7 (70%)
Mineralization	–	–	–	–	–	1 (10%)
Nephropathy	1 (10%)	–	–	–	–	–

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

Appendix B. Clinical Pathology Results

Tables

Table B-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Hematology						
n						
Day 23	10	9	10	10	10	8
Week 14	8	7	8	10	10	9
Hematocrit (%)						
Day 23	50.1 ± 0.6	50.5 ± 0.9	50.9 ± 1.0	50.3 ± 0.7	51.1 ± 1.6	50.4 ± 1.6
Week 14	49.0 ± 0.6	47.8 ± 0.4	47.7 ± 0.5	48.1 ± 1.0	47.5 ± 0.5	47.1 ± 0.4
Hemoglobin (g/dL)						
Day 23	15.4 ± 0.2	15.4 ± 0.3	15.6 ± 0.2	15.4 ± 0.2	15.5 ± 0.4	15.3 ± 0.4
Week 14	15.4 ± 0.2	15.1 ± 0.1	14.9 ± 0.1	15.1 ± 0.3	15.0 ± 0.1	14.8 ± 0.1
Erythrocytes (10^6/μL)						
Day 23	8.38 ± 0.08	8.50 ± 0.14	8.62 ± 0.12	8.56 ± 0.12	8.64 ± 0.26	8.58 ± 0.25
Week 14	9.56 ± 0.09	9.37 ± 0.04	9.39 ± 0.09	9.46 ± 0.13	9.41 ± 0.08	9.28 ± 0.09
Reticulocytes (10^3/μL)						
Day 23	370 ± 17	379 ± 13	408 ± 16	382 ± 8	408 ± 10	415 ± 14
Week 14	173 ± 19	193 ± 12	186 ± 10	192 ± 8	172 ± 10	197 ± 3
Mean cell volume (fL)						
Day 23	59.8 ± 0.3	59.4 ± 0.4	59.0 ± 0.4	58.8 ± 0.3	59.1 ± 0.2	58.7 ± 0.4
Week 14	51.3 ± 0.2	51.0 ± 0.4	50.8 ± 0.3	50.8 ± 0.4	50.6 ± 0.3	50.8 ± 0.4
Mean cell hemoglobin (pg)						
Day 23	18.4 ± 0.1	18.2 ± 0.1	18.1 ± 0.1*	18.0 ± 0.1**	18.0 ± 0.1**	17.8 ± 0.1**
Week 14	16.1 ± 0.1	16.2 ± 0.2	15.8 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	16.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 23	30.8 ± 0.2	30.6 ± 0.2	30.6 ± 0.2	30.6 ± 0.1	30.4 ± 0.1	30.3 ± 0.2
Week 14	31.4 ± 0.2	31.7 ± 0.2	31.2 ± 0.1	31.4 ± 0.2	31.6 ± 0.2	31.4 ± 0.1
Platelets (10^3/μL)						
Day 23	914 ± 26	937 ± 41	987 ± 32	960 ± 19	893 ± 67	1,004 ± 27
Week 14	754 ± 21	815 ± 15	838 ± 19*	783 ± 20	781 ± 36	834 ± 9*
Leukocytes (10^3/μL)						
Day 23	7.82 ± 0.24	8.66 ± 0.38	8.42 ± 0.54	8.14 ± 0.19	8.87 ± 0.31	8.64 ± 0.66
Week 14	7.06 ± 0.51	7.15 ± 0.53	6.92 ± 0.34	7.24 ± 0.36	6.77 ± 0.54	6.95 ± 0.44
Segmented neutrophils (10^3/μL)						
Day 23	1.48 ± 0.12	1.98 ± 0.20	1.56 ± 0.11	1.52 ± 0.12	2.13 ± 0.10**	1.95 ± 0.15

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Week 14	1.33 ± 0.11	1.49 ± 0.16	1.68 ± 0.14	1.52 ± 0.13	1.50 ± 0.13	1.36 ± 0.12
Lymphocytes (10^3/μL)						
Day 23	6.01 ± 0.17	6.31 ± 0.28	6.55 ± 0.44	6.32 ± 0.22	6.25 ± 0.30	6.31 ± 0.50
Week 14	5.45 ± 0.52	5.39 ± 0.43	4.98 ± 0.30	5.43 ± 0.27	4.99 ± 0.44	5.35 ± 0.38
Monocytes (10^3/μL)						
Day 23	0.20 ± 0.01	0.23 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.24 ± 0.02	0.25 ± 0.03
Week 14	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	0.14 ± 0.02
Basophils (10^3/μL)						
Day 23	0.080 ± 0.015	0.073 ± 0.011	0.059 ± 0.011	0.044 ± 0.007	0.106 ± 0.020	0.090 ± 0.029
Week 14	0.025 ± 0.004	0.026 ± 0.004	0.030 ± 0.005	0.025 ± 0.003	0.027 ± 0.006	0.024 ± 0.003
Eosinophils (10^3/μL)						
Day 23	0.05 ± 0.02	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.14 ± 0.03*	0.05 ± 0.01
Week 14	0.11 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.08 ± 0.01
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	9	9
Week 14	10	8	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.1 ± 0.6[b]	10.5 ± 0.7	10.1 ± 0.5[b]	10.4 ± 0.6[b]	10.9 ± 0.6[b]	11.0 ± 0.6
Day 23	10.9 ± 0.3	11.3 ± 0.5	9.6 ± 0.5	11.6 ± 0.5	11.6 ± 0.4	10.9 ± 0.8
Week 14	15.1 ± 0.8	15.6 ± 1.0	12.9 ± 0.5	11.5 ± 0.5**	16.1 ± 0.6	14.4 ± 0.7
Creatinine (mg/dL)						
Day 4	0.50 ± 0.00[b]	0.50 ± 0.00	0.47 ± 0.02[b]	0.49 ± 0.01[b]	0.49 ± 0.01[b]	0.45 ± 0.02**
Day 23	0.60 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.57 ± 0.03	0.56 ± 0.02	0.54 ± 0.02
Week 14	0.80 ± 0.01	0.78 ± 0.02	0.75 ± 0.02	0.74 ± 0.02	0.77 ± 0.02	0.74 ± 0.02
Glucose (mg/dL)						
Day 4	123 ± 2[b]	122 ± 2	119 ± 3[b]	123 ± 4[b]	121 ± 2[b]	123 ± 3
Day 23	165 ± 5	157 ± 6	162 ± 7	155 ± 7	153 ± 4	159 ± 4
Week 14	263 ± 8	244 ± 10	255 ± 11	268 ± 13	257 ± 7	249 ± 10
Total protein (g/dL)						
Day 4	5.9 ± 0.1[b]	5.9 ± 0.1	5.9 ± 0.1[b]	5.9 ± 0.1[b]	5.8 ± 0.1[b]	5.9 ± 0.1
Day 23	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Week 14	7.2 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	7.1 ± 0.1	7.0 ± 0.1

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Albumin (g/dL)						
Day 4	4.3 ± 0.0[b]	4.2 ± 0.1	4.3 ± 0.1[b]	4.3 ± 0.1[b]	4.2 ± 0.0[b]	4.3 ± 0.0
Day 23	4.2 ± 0.0	4.4 ± 0.0*	4.3 ± 0.1	4.4 ± 0.1*	4.4 ± 0.1*	4.4 ± 0.1
Week 14	4.6 ± 0.1	4.6 ± 0.1	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Cholesterol (mg/dL)						
Day 4	98 ± 3	89 ± 2	94 ± 3	89 ± 2*	87 ± 3**	85 ± 3**
Day 23	88 ± 2	80 ± 2	79 ± 2	81 ± 2	77 ± 2*	81 ± 2
Week 14	76 ± 3	75 ± 3	69 ± 2	64 ± 2**	70 ± 2	67 ± 4
Alanine aminotransferase (IU/L)						
Day 4	73 ± 2	71 ± 2	69 ± 2	73 ± 3	71 ± 2	71 ± 2
Day 23	56 ± 2	64 ± 1*	60 ± 1	63 ± 2	62 ± 2	63 ± 2
Week 14	116 ± 23	83 ± 8	76 ± 10	84 ± 13	96 ± 6	75 ± 6
Alkaline phosphatase (IU/L)						
Day 4	665 ± 12	640 ± 14	639 ± 16	620 ± 9	629 ± 13	611 ± 17
Day 23	524 ± 10	528 ± 12	544 ± 14	521 ± 15	517 ± 13	511 ± 16
Week 14	275 ± 8	263 ± 6	257 ± 8	258 ± 10	294 ± 7	265 ± 8
Creatine kinase (IU/L)						
Day 4	441 ± 35[b]	646 ± 186	364 ± 34[b]	545 ± 48[b]	623 ± 77[b]	469 ± 40
Day 23	615 ± 89	559 ± 55	458 ± 75	467 ± 64	491 ± 48	725 ± 125
Week 14	360 ± 130	142 ± 17	224 ± 52	248 ± 50	112 ± 4**	140 ± 9
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 1	14 ± 1	15 ± 1	13 ± 1	13 ± 1	14 ± 1
Day 23	18 ± 1	19 ± 2	15 ± 1*	16 ± 1	15 ± 1	16 ± 1
Week 14	44 ± 13	29 ± 3	31 ± 8	46 ± 15	28 ± 1	25 ± 2*
Bile acids (µmol/L)						
Day 4	16.9 ± 2.6	12.9 ± 1.4	17.0 ± 3.6	13.6 ± 2.5	9.6 ± 1.6	14.7 ± 1.4
Day 23	9.6 ± 1.0	13.8 ± 1.9	14.1 ± 1.1	11.5 ± 1.6	9.3 ± 1.3	10.2 ± 1.4
Week 14	19.7 ± 2.3	17.6 ± 3.1	17.7 ± 2.6	20.4 ± 2.5	16.0 ± 1.8	15.6 ± 1.7
Total thyroxine (µg/dL)						
Day 4	7.05 ± 0.26	6.61 ± 0.24	6.58 ± 0.29	6.31 ± 0.29	6.50 ± 0.30	6.05 ± 0.22
Day 23	6.45 ± 0.19	6.88 ± 0.18	6.65 ± 0.27	6.83 ± 0.23	7.14 ± 0.34[c]	6.51 ± 0.14
Week 14	3.16 ± 0.25	3.53 ± 0.29	2.88 ± 0.20	3.24 ± 0.28	3.17 ± 0.20	2.98 ± 0.16
Total triiodothyronine (ng/dL)						
Day 23	158.3 ± 4.3	164.2 ± 8.9	155.1 ± 7.7	175.9 ± 6.8	170.6 ± 8.5[c]	162.6 ± 9.2
Week 14	109.0 ± 9.2[b]	106.1 ± 5.8	99.9 ± 6.2[b]	109.5 ± 5.6	91.2 ± 7.9	97.9 ± 9.2

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Thyroid stimulating hormone (ng/mL)						
Day 4	6.36 ± 0.28	6.47 ± 0.81	6.44 ± 0.17	6.16 ± 0.41	5.79 ± 0.27	6.56 ± 0.61
Day 23	10.00 ± 0.68	10.05 ± 0.60	11.23 ± 1.51	11.32 ± 0.94[b]	9.38 ± 0.30	9.62 ± 0.36
Week 14	9.91 ± 0.53	9.96 ± 0.54	10.94 ± 0.80	11.28 ± 1.31	8.84 ± 0.76[b]	10.85 ± 0.79
Female						
Hematology						
n						
Day 23	10	7	8	7	9	9
Week 14	9	10	10	10	10	10
Hematocrit (%)						
Day 23	49.0 ± 0.7	49.9 ± 2.2	49.3 ± 1.4	49.2 ± 0.9	48.8 ± 0.4	47.5 ± 0.5
Week 14	45.9 ± 0.4	45.9 ± 0.3	45.6 ± 0.4	46.6 ± 0.6	45.3 ± 0.3	44.7 ± 0.4
Hemoglobin (g/dL)						
Day 23	15.6 ± 0.2	15.8 ± 0.6	15.6 ± 0.4	15.7 ± 0.3	15.5 ± 0.1	15.1 ± 0.1
Week 14	15.0 ± 0.1	15.1 ± 0.1	14.7 ± 0.1	15.0 ± 0.2	14.6 ± 0.1*	14.5 ± 0.1**
Erythrocytes (10^6/µL)						
Day 23	8.50 ± 0.14	8.65 ± 0.34	8.53 ± 0.19	8.58 ± 0.15	8.56 ± 0.05	8.36 ± 0.08
Week 14	8.57 ± 0.07	8.47 ± 0.05	8.54 ± 0.08	8.72 ± 0.10	8.55 ± 0.05	8.38 ± 0.09
Reticulocytes (10^3/µL)						
Day 23	194 ± 5	214 ± 16	208 ± 10	183 ± 3	209 ± 7	214 ± 10
Week 14	193 ± 7	194 ± 8	206 ± 9	184 ± 9	194 ± 7	209 ± 9
Mean cell volume (fL)						
Day 23	57.6 ± 0.2	57.6 ± 0.3	57.8 ± 0.4	57.4 ± 0.2	57.0 ± 0.3	56.8 ± 0.3
Week 14	53.6 ± 0.3	54.2 ± 0.2	53.3 ± 0.4	53.5 ± 0.3	53.0 ± 0.2	53.3 ± 0.3
Mean cell hemoglobin (pg)						
Day 23	18.4 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.1 ± 0.1	18.1 ± 0.1
Week 14	17.5 ± 0.1	17.8 ± 0.1	17.2 ± 0.1	17.3 ± 0.1	17.1 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 23	31.9 ± 0.1	31.8 ± 0.2	31.6 ± 0.2	31.9 ± 0.2	31.7 ± 0.2	31.9 ± 0.2
Week 14	32.7 ± 0.2	32.9 ± 0.2	32.3 ± 0.1	32.3 ± 0.2	32.3 ± 0.1	32.5 ± 0.1
Platelets (10^3/µL)						
Day 23	846 ± 37	873 ± 41	949 ± 30	880 ± 21	795 ± 80	941 ± 65
Week 14	787 ± 28	827 ± 43	916 ± 37*	817 ± 57	883 ± 32	918 ± 47
Leukocytes (10^3/µL)						
Day 23	9.57 ± 0.41	9.94 ± 0.45	8.72 ± 0.53	9.52 ± 0.74	9.08 ± 0.48	9.63 ± 0.62

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Week 14	8.33 ± 0.80	7.21 ± 0.26	7.57 ± 0.67	8.50 ± 0.56	7.86 ± 0.41	8.19 ± 0.37
Segmented neutrophils (10^3/μL)						
Day 23	1.65 ± 0.15	1.99 ± 0.15	1.72 ± 0.17	2.04 ± 0.22	2.09 ± 0.18	2.06 ± 0.18
Week 14	1.55 ± 0.12	1.37 ± 0.11	1.52 ± 0.14	1.59 ± 0.11	1.74 ± 0.11	1.77 ± 0.11
Lymphocytes (10^3/μL)						
Day 23	7.49 ± 0.32	7.47 ± 0.43	6.54 ± 0.39	6.99 ± 0.45	6.55 ± 0.41	7.15 ± 0.46
Week 14	6.32 ± 0.59	5.52 ± 0.23	5.75 ± 0.53	6.53 ± 0.49	5.78 ± 0.34	6.07 ± 0.30
Monocytes (10^3/μL)						
Day 23	0.25 ± 0.02	0.26 ± 0.03	0.23 ± 0.02	0.27 ± 0.04	0.25 ± 0.03	0.27 ± 0.02
Week 14	0.32 ± 0.13	0.18 ± 0.01	0.18 ± 0.02	0.22 ± 0.03	0.21 ± 0.02	0.22 ± 0.01
Basophils (10^3/μL)						
Day 23	0.102 ± 0.009	0.094 ± 0.023	0.106 ± 0.029	0.086 ± 0.014	0.084 ± 0.009	0.083 ± 0.010
Week 14	0.049 ± 0.009	0.050 ± 0.006	0.038 ± 0.006	0.066 ± 0.012	0.044 ± 0.004	0.052 ± 0.010
Eosinophils (10^3/μL)						
Day 23	0.09 ± 0.02	0.13 ± 0.03	0.12 ± 0.04	0.14 ± 0.05	0.11 ± 0.02	0.07 ± 0.01
Week 14	0.10 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.07 ± 0.01
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	9
Day 23	10	10	10	9	10	10
Week 14	8	9	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.6 ± 0.6[b]	11.2 ± 0.9	11.6 ± 0.7[b]	10.4 ± 0.5	9.7 ± 0.6[b]	9.4 ± 0.6
Day 23	11.5 ± 0.5	12.0 ± 0.4[b]	12.1 ± 0.5	11.8 ± 0.9	12.0 ± 0.7	10.6 ± 0.5
Week 14	13.4 ± 0.5	14.9 ± 0.4	14.5 ± 0.5	13.1 ± 0.3	13.3 ± 0.5	14.3 ± 0.5
Creatinine (mg/dL)						
Day 4	0.47 ± 0.02[b]	0.46 ± 0.02	0.46 ± 0.02[b]	0.43 ± 0.02	0.42 ± 0.01[b]	0.41 ± 0.01*
Day 23	0.58 ± 0.01	0.58 ± 0.02[b]	0.59 ± 0.01	0.59 ± 0.01	0.55 ± 0.02	0.57 ± 0.02
Week 14	0.70 ± 0.02	0.73 ± 0.03	0.70 ± 0.01	0.69 ± 0.02	0.69 ± 0.02	0.71 ± 0.02
Glucose (mg/dL)						
Day 4	121 ± 3[b]	122 ± 3	121 ± 4[b]	113 ± 2	119 ± 3[b]	111 ± 3*
Day 23	137 ± 5	139 ± 5[b]	151 ± 7	146 ± 9	149 ± 4	131 ± 4
Week 14	215 ± 12	233 ± 14	218 ± 5	218 ± 7	212 ± 7	208 ± 8
Total protein (g/dL)						
Day 4	5.9 ± 0.1[b]	5.9 ± 0.1	6.1 ± 0.1[b]	5.9 ± 0.1	5.8 ± 0.1[b]	5.8 ± 0.1

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Day 23	6.2 ± 0.1	6.3 ± 0.1[b]	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
Week 14	6.7 ± 0.2	6.7 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1
Albumin (g/dL)						
Day 4	4.3 ± 0.1[b]	4.3 ± 0.1	4.4 ± 0.1[b]	4.3 ± 0.0	4.2 ± 0.1[b]	4.3 ± 0.1
Day 23	4.5 ± 0.0	4.5 ± 0.1[b]	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.0
Week 14	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Cholesterol (mg/dL)						
Day 4	98 ± 3	93 ± 2	93 ± 2	92 ± 3	84 ± 2**	89 ± 2**[c]
Day 23	87 ± 2	82 ± 2	83 ± 3	81 ± 2*	74 ± 1**	77 ± 2**
Week 14	74 ± 3	72 ± 2	67 ± 2	68 ± 2	64 ± 1**	69 ± 2
Alanine aminotransferase (IU/L)						
Day 4	63 ± 2	66 ± 2	67 ± 2	65 ± 3	60 ± 3	67 ± 12
Day 23	43 ± 2	47 ± 2	52 ± 2**	53 ± 2**	49 ± 1**	51 ± 2**
Week 14	71 ± 16	87 ± 12*	69 ± 5	72 ± 5	69 ± 8	70 ± 3
Alkaline phosphatase (IU/L)						
Day 4	565 ± 16	552 ± 8	578 ± 12	550 ± 21	526 ± 15	509 ± 27
Day 23	382 ± 11	383 ± 8	425 ± 8**	413 ± 9	388 ± 8	390 ± 9
Week 14	286 ± 9	279 ± 10	267 ± 12	270 ± 5	271 ± 8	246 ± 5**
Creatine kinase (IU/L)						
Day 4	559 ± 37[d]	656 ± 88	545 ± 59[b]	661 ± 71	651 ± 77[b]	546 ± 62
Day 23	407 ± 56	583 ± 74[b]	617 ± 107	517 ± 78	516 ± 63	638 ± 79
Week 14	178 ± 43	318 ± 81	228 ± 76	180 ± 58	196 ± 45	174 ± 56
Sorbitol dehydrogenase (IU/L)						
Day 4	9 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1	11 ± 2
Day 23	14 ± 1	13 ± 0	16 ± 1	14 ± 1	14 ± 1	13 ± 1
Week 14	40 ± 19	37 ± 11	25 ± 1	28 ± 1	26 ± 3	26 ± 2
Bile acids (µmol/L)						
Day 4	9.6 ± 1.7	14.9 ± 3.2	11.2 ± 1.1	12.1 ± 2.3	9.2 ± 0.9	10.6 ± 2.4
Day 23	12.1 ± 1.5	8.9 ± 1.2	12.7 ± 3.4	14.7 ± 1.1	12.0 ± 2.8	13.1 ± 1.8
Week 14	25.5 ± 3.4	29.0 ± 4.7	29.5 ± 4.4	21.4 ± 3.5	25.5 ± 3.3	26.6 ± 4.6
Total thyroxine (µg/dL)						
Day 4	5.52 ± 0.18	5.36 ± 0.26	4.78 ± 0.34	4.87 ± 0.37	5.13 ± 0.31	4.51 ± 0.35[c]
Day 23	5.03 ± 0.26	5.44 ± 0.21	5.18 ± 0.31	5.21 ± 0.24[c]	5.68 ± 0.23	5.94 ± 0.26
Week 14	3.42 ± 0.42[b]	3.28 ± 0.23[c]	3.84 ± 0.22	4.15 ± 0.22	3.56 ± 0.21	3.48 ± 0.22

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Total triiodothyronine (ng/dL)						
Day 23	147.4 ± 7.3	153.9 ± 8.5	152.2 ± 6.5	158.6 ± 8.6[c]	136.7 ± 5.1	147.6 ± 5.7
Week 14	128.4 ± 4.9	107.8 ± 7.1	128.8 ± 6.2[b]	139.6 ± 7.4	115.3 ± 7.7	123.2 ± 9.2
Thyroid stimulating hormone (ng/mL)						
Day 4	7.09 ± 0.25	6.77 ± 0.17	6.68 ± 0.20	6.64 ± 0.12	6.60 ± 0.34	6.91 ± 0.64[c]
Day 23	7.79 ± 0.41	7.59 ± 0.31	8.13 ± 0.27[b]	7.76 ± 0.26[c]	7.93 ± 0.24	7.55 ± 0.20
Week 14	9.46 ± 0.66	7.73 ± 0.43	8.64 ± 0.35	7.41 ± 0.28*	7.95 ± 0.54	7.49 ± 0.21*

*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 = 10.
[d]n = 8.

Table B-2. Hematology Data for Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Male						
n	10	10	10	9	10	9
Hematocrit (%)	51.0 ± 0.6	50.2 ± 0.8	52.0 ± 1.3	50.2 ± 1.0	50.9 ± 0.9	50.7 ± 0.9
Hemoglobin (g/dL)	17.4 ± 0.2	16.9 ± 0.3	17.6 ± 0.5	16.9 ± 0.4	17.2 ± 0.3	17.0 ± 0.3
Erythrocytes (10^6/µL)	11.34 ± 0.15	11.12 ± 0.15	11.59 ± 0.29	11.04 ± 0.23	11.36 ± 0.20	11.26 ± 0.15
Reticulocytes (10^3/µL)	278 ± 9	300 ± 7	304 ± 10	294 ± 11	298 ± 8	286 ± 10
Mean cell volume (fL)	45.0 ± 0.2	45.1 ± 0.2	44.9 ± 0.1	45.4 ± 0.3	44.9 ± 0.2	45.0 ± 0.3
Mean cell hemoglobin (pg)	15.3 ± 0.0	15.2 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.0 ± 0.1	33.7 ± 0.1	33.8 ± 0.1	33.7 ± 0.2	33.8 ± 0.1	33.6 ± 0.2
Platelets (10^3/µL)	747 ± 63	837 ± 40	742 ± 54	888 ± 57	826 ± 57	812 ± 62
Leukocytes (10^3/µL)	3.03 ± 0.22	3.88 ± 0.22	3.33 ± 0.24	4.16 ± 0.47	3.97 ± 0.33	3.84 ± 0.26
Segmented neutrophils (10^3/µL)	0.40 ± 0.04	0.50 ± 0.05	0.43 ± 0.06	0.58 ± 0.10	0.55 ± 0.07	0.59 ± 0.06
Lymphocytes (10^3/µL)	2.50 ± 0.18	3.21 ± 0.17	2.76 ± 0.19	3.36 ± 0.34	3.27 ± 0.28	3.09 ± 0.22
Monocytes (10^3/µL)	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.12 ± 0.03	0.07 ± 0.01	0.07 ± 0.01
Basophils (10^3/µL)	0.005 ± 0.002	0.007 ± 0.002	0.010 ± 0.003	0.009 ± 0.003	0.005 ± 0.002	0.007 ± 0.002
Eosinophils (10^3/µL)	0.07 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
Female						
n	10	10	10	10	10	10
Hematocrit (%)	48.4 ± 0.6	48.5 ± 0.8	48.4 ± 0.4	48.1 ± 1.1	48.8 ± 0.8	48.6 ± 0.8

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Hemoglobin (g/dL)	16.5 ± 0.3	16.5 ± 0.3	16.4 ± 0.2	16.4 ± 0.4	16.6 ± 0.3	16.5 ± 0.3
Erythrocytes (10^6/μL)	10.83 ± 0.13	10.95 ± 0.17	10.88 ± 0.10	10.87 ± 0.24	11.06 ± 0.16	10.96 ± 0.18
Reticulocytes (10^3/μL)	295 ± 10	327 ± 22	303 ± 22	276 ± 16	261 ± 20	262 ± 13
Mean cell volume (fL)	44.7 ± 0.2	44.3 ± 0.1	44.5 ± 0.1	44.2 ± 0.1	44.2 ± 0.2	44.3 ± 0.1
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.2	34.1 ± 0.2	33.9 ± 0.1	34.1 ± 0.1	33.9 ± 0.1	33.9 ± 0.2
Platelets (10^3/μL)	676 ± 61	701 ± 67	729 ± 35	698 ± 55	811 ± 82	773 ± 61
Leukocytes (10^3/μL)	3.24 ± 0.31	3.51 ± 0.20	3.13 ± 0.25	3.27 ± 0.13	3.56 ± 0.23	3.76 ± 0.20
Segmented neutrophils (10^3/μL)	0.28 ± 0.03	0.29 ± 0.04	0.25 ± 0.05	0.34 ± 0.07	0.40 ± 0.03	0.41 ± 0.03*
Lymphocytes (10^3/μL)	2.84 ± 0.28	3.05 ± 0.19	2.71 ± 0.20	2.80 ± 0.09	2.98 ± 0.19	3.20 ± 0.17
Monocytes (10^3/μL)	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Basophils (10^3/μL)	0.005 ± 0.002	0.011 ± 0.003	0.004 ± 0.002	0.002 ± 0.001	0.009 ± 0.002	0.009 ± 0.002
Eosinophils (10^3/μL)	0.05 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.10 ± 0.02	0.06 ± 0.01

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

Appendix C. Organ Weights and Organ-Weight-To-Body-Weight Ratios

Tables

Table C-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	10	8	10	10	10	10
Necropsy body wt	330 ± 9	344 ± 8	329 ± 7	342 ± 6	333 ± 5	320 ± 8
Heart						
Absolute	0.93 ± 0.03	0.99 ± 0.04	0.95 ± 0.03	1.00 ± 0.03	0.92 ± 0.02	0.90 ± 0.02
Relative	2.81 ± 0.03	2.87 ± 0.07	2.89 ± 0.04	2.92 ± 0.08	2.75 ± 0.05	2.82 ± 0.04
R. Kidney						
Absolute	0.83 ± 0.02	0.89 ± 0.02	0.85 ± 0.02	0.92 ± 0.02*	0.83 ± 0.02	0.83 ± 0.03
Relative	2.54 ± 0.05	2.59 ± 0.05	2.58 ± 0.05	2.70 ± 0.05	2.49 ± 0.05	2.58 ± 0.04
Liver						
Absolute	10.36 ± 0.31	11.65 ± 0.25*	10.58 ± 0.27	11.40 ± 0.25	10.72 ± 0.29	10.82 ± 0.37
Relative	31.43 ± 0.53	33.89 ± 0.45**	32.12 ± 0.37	33.37 ± 0.35*	32.18 ± 0.51	33.77 ± 0.44**
Lung						
Absolute	2.13 ± 0.12	1.94 ± 0.11	2.10 ± 0.17	2.38 ± 0.20	2.05 ± 0.08	2.16 ± 0.13
Relative	6.46 ± 0.34	5.63 ± 0.29	6.35 ± 0.46	6.94 ± 0.53	6.19 ± 0.30	6.75 ± 0.37
Spleen						
Absolute	0.605 ± 0.014	0.617 ± 0.011	0.596 ± 0.017	0.631 ± 0.009	0.571 ± 0.014	0.586 ± 0.014
Relative	1.84 ± 0.03	1.80 ± 0.03	1.81 ± 0.04	1.85 ± 0.04	1.72 ± 0.04	1.84 ± 0.05
R. Testis						
Absolute	1.357 ± 0.022	1.356 ± 0.027	1.336 ± 0.011	1.393 ± 0.022	1.321 ± 0.024	1.331 ± 0.041
Relative	4.134 ± 0.086	3.945 ± 0.050	4.069 ± 0.079	4.090 ± 0.102	3.972 ± 0.063	4.162 ± 0.086
Thymus						
Absolute	0.341 ± 0.009	0.354 ± 0.022	0.343 ± 0.010	0.329 ± 0.017	0.296 ± 0.012	0.310 ± 0.013
Relative	1.035 ± 0.020	1.031 ± 0.068	1.044 ± 0.033	0.964 ± 0.051	0.891 ± 0.035	0.972 ± 0.044
Female						
n	9	10	10	10	10	10
Necropsy body wt	191 ± 4	195 ± 4	188 ± 4	194 ± 4	188 ± 3	190 ± 4
Heart						
Absolute	0.65 ± 0.01	0.65 ± 0.02	0.63 ± 0.02	0.65 ± 0.02	0.61 ± 0.01	0.64 ± 0.01
Relative	3.39 ± 0.07	3.33 ± 0.08	3.33 ± 0.06	3.34 ± 0.07	3.25 ± 0.06	3.37 ± 0.07
R. Kidney						
Absolute	0.55 ± 0.01	0.56 ± 0.02	0.55 ± 0.01	0.56 ± 0.01	0.52 ± 0.01	0.55 ± 0.01
Relative	2.87 ± 0.04	2.85 ± 0.06	2.90 ± 0.05	2.89 ± 0.04	2.79 ± 0.05	2.88 ± 0.04

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Liver						
Absolute	5.91 ± 0.14	6.21 ± 0.14	6.09 ± 0.14	6.18 ± 0.12	5.90 ± 0.09	6.25 ± 0.12
Relative	30.93 ± 0.46	31.88 ± 0.52	32.37 ± 0.38	31.92 ± 0.44	31.47 ± 0.53	33.03 ± 0.23**
Lung						
Absolute	1.56 ± 0.18	1.39 ± 0.09	1.40 ± 0.11	1.56 ± 0.10	1.32 ± 0.06	1.43 ± 0.08
Relative	8.14 ± 0.88	7.13 ± 0.44	7.47 ± 0.58	8.05 ± 0.50	7.09 ± 0.37	7.56 ± 0.46
Spleen						
Absolute	0.433 ± 0.009	0.462 ± 0.018	0.459 ± 0.011	0.467 ± 0.013	0.447 ± 0.009	0.476 ± 0.014
Relative	2.27 ± 0.06	2.37 ± 0.09	2.45 ± 0.06	2.41 ± 0.06	2.39 ± 0.07	2.52 ± 0.08
Thymus						
Absolute	0.244 ± 0.009	0.250 ± 0.010	0.258 ± 0.008	0.267 ± 0.021	0.237 ± 0.008	0.264 ± 0.012
Relative	1.277 ± 0.041	1.287 ± 0.052	1.371 ± 0.027	1.369 ± 0.096	1.268 ± 0.050	1.397 ± 0.072

*Significantly different ($P \leq 0.05$) from the vehicle control group by 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 C-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	36.8 ± 1.0	39.0 ± 0.9	38.3 ± 1.0	39.4 ± 1.5	38.2 ± 0.9	39.1 ± 1.1
Heart						
Absolute	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Relative	4.90 ± 0.16	4.58 ± 0.13	5.10 ± 0.16	4.94 ± 0.15	5.09 ± 0.19	4.82 ± 0.16
R. Kidney						
Absolute	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.01
Relative	7.33 ± 0.23	7.15 ± 0.12	7.36 ± 0.18	7.14 ± 0.19	7.23 ± 0.15	6.86 ± 0.14
Liver						
Absolute	1.43 ± 0.07	1.62 ± 0.03	1.58 ± 0.06	1.67 ± 0.13	1.58 ± 0.04	1.62 ± 0.08
Relative	38.91 ± 1.44	41.67 ± 0.93	41.36 ± 1.02	42.05 ± 1.76	41.29 ± 0.56	41.22 ± 1.06
Lung						
Absolute	0.32 ± 0.01	0.33 ± 0.01	0.34 ± 0.02	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
Relative	8.85 ± 0.41	8.44 ± 0.23	8.83 ± 0.42	8.08 ± 0.31	8.49 ± 0.23	8.25 ± 0.22
Spleen						
Absolute	0.063 ± 0.001	0.065 ± 0.002	0.067 ± 0.002	0.067 ± 0.003	0.066 ± 0.001	0.061 ± 0.001

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Relative	1.71 ± 0.03	1.67 ± 0.04	1.75 ± 0.06	1.70 ± 0.04	1.73 ± 0.05	1.58 ± 0.03
R. Testis						
Absolute	0.118 ± 0.002	0.118 ± 0.002	0.116 ± 0.003	0.118 ± 0.002	0.118 ± 0.002	0.116 ± 0.002
Relative	3.244 ± 0.118	3.047 ± 0.072	3.049 ± 0.068	3.014 ± 0.091	3.099 ± 0.082	2.981 ± 0.060
Thymus						
Absolute	0.053 ± 0.005	0.054 ± 0.004	0.051 ± 0.003	0.054 ± 0.004	0.051 ± 0.002	0.054 ± 0.006
Relative	1.427 ± 0.103	1.388 ± 0.085	1.342 ± 0.061	1.368 ± 0.056	1.326 ± 0.040	1.356 ± 0.120
Female						
Necropsy body wt	29.2 ± 1.0	31.6 ± 1.3	32.4 ± 0.7	31.0 ± 0.8	31.0 ± 0.8	30.2 ± 0.9
Heart						
Absolute	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.00	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.01
Relative	4.72 ± 0.22	4.47 ± 0.22	4.49 ± 0.18	4.78 ± 0.18	4.64 ± 0.13	4.77 ± 0.17
R. Kidney						
Absolute	0.17 ± 0.00	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.00	0.16 ± 0.01	0.16 ± 0.01
Relative	5.66 ± 0.13	5.26 ± 0.10	5.26 ± 0.14	5.31 ± 0.13	5.29 ± 0.07	5.33 ± 0.18
Liver						
Absolute	1.10 ± 0.02	1.22 ± 0.05*	1.25 ± 0.03**	1.16 ± 0.03	1.21 ± 0.04	1.22 ± 0.03*
Relative	37.75 ± 1.14	38.79 ± 1.18	38.75 ± 0.85	37.55 ± 0.64	38.88 ± 0.41	40.57 ± 1.16
Lung						
Absolute	0.29 ± 0.02	0.30 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.30 ± 0.01
Relative	9.99 ± 0.42	9.54 ± 0.52	9.64 ± 0.27	10.10 ± 0.17	9.83 ± 0.36	9.76 ± 0.27
Spleen						
Absolute	0.080 ± 0.002	0.081 ± 0.003	0.084 ± 0.003	0.084 ± 0.003	0.083 ± 0.003	0.081 ± 0.002
Relative	2.76 ± 0.10	2.58 ± 0.09	2.61 ± 0.11	2.72 ± 0.10	2.66 ± 0.06	2.70 ± 0.10
Thymus						
Absolute	0.050 ± 0.003	0.058 ± 0.002	0.057 ± 0.002	0.052 ± 0.002	0.054 ± 0.002	0.054 ± 0.002
Relative	1.719 ± 0.088	1.855 ± 0.074	1.774 ± 0.060	1.681 ± 0.067	1.739 ± 0.048	1.819 ± 0.094

*Significantly different ($P \leq 0.05$) from the vehicle control group by 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 D. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Figures

Table D-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	330 ± 9	342 ± 6	333 ± 5	320 ± 8
L. Cauda epididymis	0.1323 ± 0.0044	0.1429 ± 0.0031	0.1320 ± 0.0026	0.1322 ± 0.0055
L. Epididymis	0.3898 ± 0.0077	0.4125 ± 0.0068	0.3925 ± 0.0064	0.3843 ± 0.0103
L. Testis	1.4025 ± 0.0276	1.4796 ± 0.0229	1.4196 ± 0.0222	1.3703 ± 0.0500
Spermatid measurements				
Spermatid heads (10^6/g testis)	164.1 ± 8.2	175.1 ± 5.9	175.1 ± 4.5	166.6 ± 3.3
Spermatid heads (10^6/testis)	192.4 ± 9.3	215.5 ± 5.9	209.5 ± 4.8	189.9 ± 6.3
Epididymal spermatozoal measurements				
Sperm motility (%)	86 ± 0	84 ± 1	84 ± 0	85 ± 1
Sperm (10^6/g cauda epididymis)	809.7 ± 34.3	738.6 ± 49.4	721.1 ± 47.6	732.8 ± 51.2
Sperm (10^6/cauda epididymis)	107.4 ± 6.3	105.8 ± 7.8	94.7 ± 5.4	95.8 ± 5.7

[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 D-2. Estrous Cycle Characterization for Female Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Number weighed at necropsy	9	10	10	10
Necropsy body wt (g)	191 ± 4	194 ± 4	188 ± 3	190 ± 4
Proportion of regular cycling females[b]	9/9	10/10	10/10	10/10
Estrous cycle length (days)	4.9 ± 0.06	4.8 ± 0.13	4.9 ± 0.07	4.9 ± 0.08
Estrous stages (% of cycle)				
Diestrus	50.0	60.8	60.8	56.7
Proestrus	18.3	15.0	18.3	16.7
Estrus	20.8	22.5	20.8	25.0
Metestrus	0.8	1.7	0.0	1.7

[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 weights) or Dunn's test (estrous cycle length). 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 extended estrus or diestrus.
[b]Number of females with a regular cycle/number of females cycling.

Table D-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Stage	Comparison	P Value	Trend[a]
Overall tests	Overall	1	
Overall tests	250 mg/kg vs. vehicle controls	0.971	N
Overall tests	500 mg/kg vs. vehicle controls	0.996	N
Overall tests	1,000 mg/kg vs. vehicle controls	0.996	N
Extended estrus	Overall	0.945	
Extended estrus	250 mg/kg vs. vehicle controls	0.702	N
Extended estrus	500 mg/kg vs. vehicle controls	0.702	N
Extended estrus	1,000 mg/kg vs. vehicle controls	0.864	–
Extended diestrus	Overall	0.999	
Extended diestrus	250 mg/kg vs. vehicle controls	0.933	–
Extended diestrus	500 mg/kg vs. vehicle controls	0.958	–
Extended diestrus	1,000 mg/kg vs. vehicle controls	0.948	N
Extended metestrus	Overall	1	
Extended metestrus	250 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	250 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	250 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	250 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	–

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

Table D-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	500 mg/kg	1,000 mg/kg	2,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	36.8 ± 1.0	39.4 ± 1.5	38.2 ± 0.9	39.1 ± 1.1
L. Cauda epididymis	0.0152 ± 0.0006	0.0167 ± 0.0016	0.0146 ± 0.0005	0.0149 ± 0.0008
L. Epididymis	0.0428 ± 0.0009	0.0449 ± 0.0014	0.0433 ± 0.0011	0.0405 ± 0.0009
L. Testis	0.1102 ± 0.0016	0.1139 ± 0.0023	0.1135 ± 0.0027	0.1096 ± 0.0015
Spermatid measurements				
Spermatid heads (10^6/g testis)	210.0 ± 6.3	230.9 ± 11.8	221.7 ± 13.9	230.2 ± 7.1
Spermatid heads (10^6/testis)	21.4 ± 0.8	23.7 ± 1.1	22.9 ± 1.6	22.3 ± 0.8
Epididymal spermatozoal measurements				
Sperm motility (%)	86 ± 1	85 ± 1	85 ± 1	85 ± 1
Sperm (10^6/g cauda epididymis)	1,477.4 ± 147.4	1,448.0 ± 139.8	1,391.0 ± 132.9	1,672.3 ± 153.7
Sperm (10^6/cauda epididymis)	22.3 ± 2.2	22.9 ± 1.6	20.5 ± 2.2	24.8 ± 2.3

[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 D-5. Estrous Cycle Characterization for Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	29.2 ± 1.0	31.0 ± 0.8	31.0 ± 0.8	30.2 ± 0.9
Proportion of regular cycling females[b]	8/9	9/10	7/9	8/9
Estrous cycle length (days)	4.6 ± 0.18[c]	5.2 ± 0.44	4.5 ± 0.17[c]	4.5 ± 0.17[c]
Estrous stages (% of cycle)				
Diestrus	35.8	39.2	46.7	38.3
Proestrus	0.0	0. 0	0.0	0.0
Estrus	44.2	39.2	35.8	39.2
Metestrus	19.2	21.7	17.5	22.5
Uncertain diagnosis	0.8	0.0	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). Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated that 1,000 mg/kg females spent significantly more time in extended diestrus (P = 0.004) than did the vehicle control group.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle unclear in 1 of 10 animals.

Table D-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Stage	Comparison	P Value	Trend[a]
Overall tests	Overall	0.636	
Overall tests	500 mg/kg vs. Vehicle Controls	0.368	–
Overall tests	1,000 mg/kg vs. Vehicle Controls	0.719	–
Overall tests	2,000 mg/kg vs. Vehicle Controls	0.44	–
Extended estrus	Overall	0.579	
Extended estrus	500 mg/kg vs. Vehicle Controls	0.351	N
Extended estrus	1,000 mg/kg vs. Vehicle Controls	0.351	N
Extended estrus	2,000 mg/kg vs. Vehicle Controls	0.764	N
Extended diestrus	Overall	0.001	
Extended diestrus	500 mg/kg vs. Vehicle Controls	0.06	–
Extended diestrus	1,000 mg/kg vs. Vehicle Controls	0.004	–
Extended diestrus	2,000 mg/kg vs. Vehicle Controls	0.069	–
Extended metestrus	Overall	1	
Extended metestrus	500 mg/kg vs. Vehicle Controls	1	–
Extended metestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Extended metestrus	2,000 mg/kg vs. Vehicle Controls	1	–
Extended proestrus	Overall	1	
Extended proestrus	500 mg/kg vs. Vehicle Controls	1	–
Extended proestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Extended proestrus	2,000 mg/kg vs. Vehicle Controls	1	–
Skipped estrus	Overall	1	
Skipped estrus	500 mg/kg vs. Vehicle Controls	1	–
Skipped estrus	1,000 mg/kg vs. Vehicle Controls	1	–
Skipped estrus	2,000 mg/kg vs. Vehicle Controls	1	–
Skipped diestrus	Overall	1	
Skipped diestrus	500 mg/kg vs. Vehicle Controls	1	–
Skipped diestrus	1,000 mg/kg vs. Vehicle Controls	1	–
Skipped diestrus	2,000 mg/kg vs. Vehicle Controls	1	–
Summary of Significant Groups			
Extended diestrus	1,000 mg/kg vs. Vehicle Controls	0.004	–

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

DOSE (MG/KG)																					
0							D	P	E	D	D	D	P	E	D	D	D	P			
0							D	P	E	D	D	D	P	E	D	D	D	P			
0									E	D	D	D	P	E	D	D	D	P	E	D	
0									P	E	D	D	D	E	E	D	D	D	P	E	
0									P	E	D	D	D	E	D	D	D	E	E	D	
0							D	P	E	D	D	D	P	E	D	D	D	P			
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	P	E	M	D	D	P	E	D	D	D	P			
250									E	D	D	D	P	E	D	D	D	P	E	D	
250									E	D	D	D	P	E	D	D	D	P	E	D	
250									P	E	D	D	D	E	E	D	D	D	D	P	
250						D	D	P	E	D	D	D	P	E	D	D	D				
250									E	D	D	D	P	E	D	D	D	P	E	D	
250							D	D	D	E	D	D	D	E	M	D	D	E			
250									E	D	D	D	P	E	D	D	D	P	E	D	
250						D	D	P	E	D	D	D	P	E	D	D	D				
250						D	D	D	P	E	D	D	D	E	M	D	D				
250								P	E	D	D	D	P	E	D	D	D	P	E		
500						D	D	P	E	D	D	D	P	E	D	D	D				
500							D	P	E	D	D	D	P	E	D	D	D	E			
500					D	D	D	P	E	D	D	D	P	E	D	D					
500								D	P	E	D	D	D	E	E	D	D	D	E		
500								P	E	D	D	D	P	E	D	D	D	P	E		
500							D	P	E	D	D	D	P	E	D	D	D	P			
500							D	P	E	D	D	D	P	E	D	D	D	P			
500					D	D	D	P	E	D	D	D	P	E	D	D					
500					D	D	D	P	E	D	D	D	P	E	D	D					
500									E	D	D	D	P	E	D	D	D	P	E	D	
1,000							D	P	E	D	D	D	P	E	D	D	D	P			
1,000								P	E	D	D	D	P	E	D	D	D	P	E		
1,000						D	D	P	E	D	D	D	P	E	D	D	D				
1,000									E	E	D	D	D	E	E	D	D	D	P	E	
1,000									E	D	D	D	P	E	D	D	D	P	E	D	
1,000										E	D	D	D	E	D	D	D	P	E	D	D
1,000					D	D	D	P	E	D	D	D	P	E	D	D					
1,000								E	E	M	D	D	P	E	D	D	D	P	E		
1,000							D	P	E	M	D	D	P	E	D	D	D	E			
1,000							D	P	E	D	D	D	P	E	D	D	D	E			

Figure D-1. Vaginal Cytology Plots for Female Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Daily vaginal lavage samples were collected from each animal, and estrous stage was determined based on vaginal cytology. Individual females are aligned by their second estrus and color coded based on estrous stage to aid in visual comparisons amongst the groups. D = diestrus, P = proestrus, E = estrus, M = metestrus.

DOSE (MG/KG)																							
0										E	M	D	D	E	E	M	D	D	E	E	M		
0							D	D	E	E	M	D	D	E	E	M	D	D					
0								D	E	E	M	D	D	E	E	M	D	E	E				
0									E	E	M	D	D	E	E	M	D	D	E	E			
0										E	E	M	D	E	E	M	D	E	E	M	D		
0		D	E	E	M	D	D	D	D	D	D	IC	D										
0								D	D	E	E	M	D	E	E	M	D	E	E				
0										E	E	M	D	E	E	E	M	D	E	E	E		
0											E	M	D	E	E	M	D	E	E	M	D	E	
0						M	D	D	E	E	M	D	D	E	E	M	D						
500										E	E	M	D	E	E	M	D	D	D	E	M		
500						M	D	D	E	E	M	D	D	E	E	M	D						
500										E	M	D	D	E	E	M	D	D	E	E	M		
500										E	M	D	D	E	E	M	D	D	E	E	M		
500						E	M	D	D	D	D	D	D	E	E	D	D						
500											E	M	D	E	E	M	D	D	E	E	M	D	
500									E	E	M	D	D	E	E	M	D	D	E	E			
500										E	M	D	D	E	E	M	D	D	E	E	M		
500										E	M	D	D	E	E	M	D	D	E	E	M		
500							D	D	D	E	E	M	D	E	E	M	D	D					
1,000						M	D	D	E	E	M	D	D	E	E	M	D						
1,000						M	D	D	E	E	M	D	D	E	E	D	D						
1,000						M	D	D	E	E	M	D	D	E	E	M	D						
1,000									E	E	M	D	D	E	E	M	D	D	E	E			
1,000									D	E	E	M	D	E	E	M	D	D	D	E			
1,000		D	D	D	D	D	D	D	D	D	E	D	D										
1,000					D	D	D	D	D	D	E	D	D	E	E	M							
1,000										E	E	M	D	E	E	M	D	D	D	D	D		
1,000									D	E	E	M	D	E	E	M	D	E	E	M			
1,000								M	D	E	E	M	D	E	E	M	D	E	E				
2,000											E	M	D	E	E	M	D	D	D	E	E	M	
2,000									D	E	E	M	D	E	E	M	D	E	E	M			
2,000						M	D	D	E	E	M	D	D	E	E	M	D						
2,000							M	D	D	E	E	M	D	E	E	E	M	D					
2,000						M	D	D	E	E	M	D	D	E	E	M	D						
2,000										E	E	M	D	E	E	M	D	D	E	E	M		
2,000									D	E	E	M	D	E	E	M	D	E	E	M			
2,000								D	D	D	E	M	D	E	M	D	D	E	E				
2,000										E	E	M	D	E	E	M	D	E	E	M	D		
2,000		D	D	D	D	D	D	D	D	D	E	M	D										

Figure D-2. Vaginal Cytology Plots for Female Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Daily vaginal lavage samples were collected from each animal, and estrous stage was determined based on vaginal cytology. Individual females are aligned by their second estrus and color coded based on estrous stage to aid in visual comparisons amongst the groups. D = diestrus, P = proestrus, E = estrus, M = metestrus, IC = insufficient number of cells to determine stage.

Appendix E. Genetic Toxicology

Tables

Table E-1. Mutagenicity of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	With 10% Rat S9
TA102	0	332 ± 24	397 ± 20
	100	357 ± 22	380 ± 12
	333	302 ± 19	399 ± 2
	1,000	302 ± 6	345 ± 8
	3,333	264 ± 10[b]	343 ± 14[b]
	10,000	304 ± 9[b]	391 ± 12[b]
Trial summary		Negative	Negative
Positive control[c]		999 ± 16	1,626 ± 24
TA100	0	146 ± 8	138 ± 6
	100	146 ± 11	137 ± 6
	333	145 ± 6	100 ± 11
	1,000	160 ± 8[b]	131 ± 9[b]
	3,333	193 ± 14[d]	158 ± 7[d]
	10,000	186 ± 4[d]	145 ± 8[d]
Trial summary		Negative	Negative
Positive control		534 ± 33	759 ± 27
TA98	0	14 ± 2	23 ± 0
	100	14 ± 0	22 ± 3
	333	14 ± 2	23 ± 1
	1,000	16 ± 2[b]	29 ± 1[b]
	3,333	15 ± 3[d]	23 ± 4[d]
	10,000	15 ± 1[d]	23 ± 5[d]
Trial summary		Negative	Negative
Positive control		78 ± 7	488 ± 34

[a]Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger et al.[38]. Dimethylsulfoxide was the solvent control.
[b]Precipitate on plate.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100), cumene hydroperoxide (TA102), and 4-nitro-*o*-phenylenediamine (TA98). The positive controls for metabolic activation were 2-aminoanthracene (TA98 and TA100) or sterigmatocystin (TA102).
[d]Slight toxicity and precipitate on plate.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) 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	2.20 ± 0.41		2.90 ± 0.21
Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)					
	125	5	2.00 ± 0.42	0.6213	3.10 ± 0.22
	250	5	2.20 ± 0.41	0.5000	3.24 ± 0.13
	500	5	2.20 ± 0.20	0.5000	3.52 ± 0.38
	1,000	5	2.40 ± 0.37	0.3839	3.04 ± 0.18
	2,000	5	2.20 ± 0.12	0.5000	2.72 ± 0.18
			P = 0.412[e]		
Female					
Corn oil	0	5	1.40 ± 0.24		3.40 ± 0.20
Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)					
	125	5	1.50 ± 0.16	0.4263	3.40 ± 0.06
	250	5	1.30 ± 0.30	0.5764	3.74 ± 0.16
	500	5	1.40 ± 0.37	0.5000	2.94 ± 0.41
	1,000	5	2.40 ± 0.37	0.0522	3.32 ± 0.23
	2,000	5	1.90 ± 0.33	0.1918	3.28 ± 0.18
			P = 0.068		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[39]. 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 Cochran-Armitage trend test; significant at $P \leq 0.025$.

Appendix F. Microsomal Protein Concentration and Liver Enzyme Activity Data

Tables

Table F-1. Microsomal Protein Concentration and Liver Enzyme Activities for Rats in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n						
Day 23	10	10	10	10	10	9
Week 14	10	8	10	10	10	10
Microsomal protein (mg/mL)						
Day 23	10.54 ± 0.31	11.70 ± 0.64	17.17 ± 0.77**	18.26 ± 0.54**	17.35 ± 0.22**	18.28 ± 0.52**
Week 14	12.89 ± 0.65	13.47 ± 1.05	15.61 ± 1.13*	17.91 ± 1.26**	17.31 ± 0.71**	17.73 ± 0.94**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 23	0.914 ± 0.038	0.852 ± 0.040	0.579 ± 0.049**	0.534 ± 0.020**	0.504 ± 0.024**	0.479 ± 0.029**
Week 14	0.762 ± 0.062	0.735 ± 0.030	0.629 ± 0.038	0.513 ± 0.014**	0.567 ± 0.027**	0.526 ± 0.018**
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Day 23	60.1 ± 2.2	55.6 ± 2.9	37.4 ± 2.9**	39.5 ± 1.5**	38.2 ± 2.3**	35.1 ± 1.9**
Week 14	48.2 ± 1.9	47.7 ± 2.0	39.1 ± 2.6*	35.4 ± 1.6**	39.2 ± 1.4**	34.7 ± 0.9**
7-Pentoxyresorufin-*O*-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
Day 23	8.4 ± 0.2	7.7 ± 0.3	5.7 ± 0.3**	5.5 ± 0.2**	5.7 ± 0.4**	4.9 ± 0.3**
Week 14	8.8 ± 0.3	8.9 ± 0.6	7.0 ± 0.3**	6.6 ± 0.3**	7.2 ± 0.4**	6.3 ± 0.1**
UDP-Glucuronosyltransferase (UDP-GT) (pmol/minute per mg microsomal protein)						
Day 23	3.24 ± 0.14	3.11 ± 0.12	2.55 ± 0.15**	2.51 ± 0.13**	2.48 ± 0.12**	2.47 ± 0.11**
Week 14	4.29 ± 0.23	3.81 ± 0.17	3.68 ± 0.19	3.46 ± 0.19*	3.71 ± 0.21*	3.47 ± 0.15**
Female						
n						
Day 23	10	10	10	10	10	10
Week 14	9	10	10	10	10	10
Microsomal protein (mg/mL)						
Day 23	10.32 ± 0.28	10.58 ± 0.21	16.84 ± 0.84**	17.74 ± 0.43**	18.01 ± 0.20**	18.60 ± 0.41**
Week 14	14.88 ± 1.03	15.37 ± 0.67	17.79 ± 0.85*	18.96 ± 0.75**	18.69 ± 0.59**	19.46 ± 0.63**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 23	0.787 ± 0.041	0.739 ± 0.033	0.399 ± 0.049**	0.371 ± 0.024**	0.361 ± 0.015**	0.336 ± 0.010**
Week 14	0.763 ± 0.055	0.716 ± 0.035	0.613 ± 0.025*	0.581 ± 0.032**	0.595 ± 0.022**	0.586 ± 0.039**
7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Day 23	59.0 ± 2.0	57.8 ± 1.4	34.1 ± 2.3**	34.9 ± 2.3**	32.4 ± 2.0**	30.7 ± 2.8**
Week 14	63.2 ± 2.1	58.9 ± 2.3	52.3 ± 2.6**	48.6 ± 1.8**	51.3 ± 0.9**	47.8 ± 2.1**
7-Pentoxyresorufin-O-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
Day 23	6.1 ± 0.3	5.6 ± 0.3	3.2 ± 0.3**	3.7 ± 0.2**	3.3 ± 0.2**	3.0 ± 0.2**
Week 14	6.4 ± 0.2	6.3 ± 0.3	4.8 ± 0.3**	5.1 ± 0.2**	5.5 ± 0.3**	5.2 ± 0.2**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
UDP-Glucuronosyltransferase (UDP-GT) (pmol/minute per mg microsomal protein)						
Day 23	3.25 ± 0.16	3.25 ± 0.13	2.50 ± 0.21**	2.72 ± 0.14*	2.66 ± 0.20*	2.73 ± 0.19*
Week 14	4.48 ± 0.16	4.72 ± 0.13	4.44 ± 0.21	4.11 ± 0.11	4.23 ± 0.18	4.18 ± 0.13

*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.

Table F-2. Microsomal Protein Concentration and Liver Enzyme Activities for Mice in the Three-month Gavage Study of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Male						
n	10	10	10	10	10	10
Microsomal protein (mg/mL)						
Week 14	11.65 ± 0.98	13.93 ± 0.92	18.10 ± 0.93**	21.98 ± 1.46**	20.21 ± 1.05**	21.35 ± 1.00**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Week 14	0.961 ± 0.062	0.838 ± 0.076	0.601 ± 0.044**	0.558 ± 0.033**	0.514 ± 0.033**	0.532 ± 0.030**
7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	183.1 ± 6.2	167.7 ± 9.8	132.0 ± 8.6**	116.3 ± 4.5**	103.8 ± 5.4**	108.5 ± 3.8**
7-Pentoxyresorufin-O-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
Week 14	8.7 ± 0.3	7.1 ± 0.4**	5.4 ± 0.2**	5.2 ± 0.2**	4.5 ± 0.2**	4.1 ± 0.2**
UDP-Glucuronosyltransferase (UDP-GT) (pmol/minute per mg microsomal protein)						
Week 14	2.14 ± 0.09	2.08 ± 0.16	1.96 ± 0.13	1.88 ± 0.13	1.77 ± 0.11	1.85 ± 0.12
Female						
n	10	10	10	10	9	10
Microsomal protein (mg/mL)						
Week 14	12.42 ± 0.50	14.91 ± 1.93	18.37 ± 1.16**	21.02 ± 0.67**	23.74 ± 1.63**	22.52 ± 1.12**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Week 14	0.657 ± 0.047	0.544 ± 0.041	0.378 ± 0.027**	0.393 ± 0.015**	0.389 ± 0.028**	0.331 ± 0.015**
7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	149.8 ± 9.7	129.0 ± 10.8	93.8 ± 5.4**	110.4 ± 4.7**	98.5 ± 6.2**	89.4 ± 3.3**
7-Pentoxyresorufin-O-dealkylase (PROD) (pmol/minute per mg microsomal protein)						
Week 14	14.5 ± 0.9	12.0 ± 1.1	8.8 ± 0.6**	9.2 ± 0.4**	8.8 ± 0.5**	7.8 ± 0.3**
UDP-Glucuronosyltransferase (UDP-GT) (pmol/minute per mg microsomal protein)						
Week 14	3.54 ± 0.23	3.20 ± 0.19	2.42 ± 0.18**	2.49 ± 0.17**	2.44 ± 0.17**	2.38 ± 0.13**

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

Appendix G. Chemical Characterization and Dose Formulation Studies

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Tables

Figures

G.1. Procurement and Characterization

G.1.1. Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) was obtained from Great Lakes Chemical Corporation (West Lafayette, IN) in one lot (534106) that was used during the 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO). The study laboratory at Battelle Columbus Operations (Columbus, OH) conducted additional identity analyses and confirmed purity. Reports on analyses performed in support of the tetrabromobisphenol A-bis(2,3-dibromopropyl ether) studies are on file at the National Institute of Environmental Health Sciences.

Lot 534106 of the test chemical, a white powder, was identified as tetrabromobisphenol A-bis(2,3-dibromopropyl ether) by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy. The study laboratory confirmed the identity of the test article using IR spectroscopy. All spectra were consistent with the literature spectra of tetrabromobisphenol A-bis(2,3-dibromopropyl ether)[45; 46]. Representative IR and NMR spectra are presented in Figure G-1 and Figure G-2, respectively.

For lot 534106, the analytical chemistry laboratory determined the melting point using differential scanning calorimetry (DSC) and the purity profile using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection; in addition, the analytical chemistry laboratory determined the tentative identity of a single impurity using liquid chromatography (LC) coupled with mass spectrometry (MS).

DSC was conducted with a Perkin Elmer Diamond Differential Scanning Calorimeter (Perkin Elmer, Waltham, MA), an indium reference, and a heating ramp from 25° to 300°C at 10°C per minute after an initial 1 minute hold. HPLC/UV was conducted using system A, and LC/MS was conducted using system A coupled with an MS detector (Micromass Quattro LC Dual Quadrupole, Waters Corporation, Milford, MA) operated in full scan mode with negative atmospheric pressure chemical ionization.

A) The HPLC system included a liquid chromatograph (Waters Corporation), an Inertsil® ODS-2, 250 mm × 4.6 mm, 5 μm particle size column (Alltech Associates, Inc., Deerfield, IL), an isocratic mobile phase of 90:10 acetonitrile with 0.5% acetic acid:18 mM aqueous ammonium acetate with 0.5% acetic acid, UV detection at 280 nm, and a flow rate of 1 mL/minute.

Melting point analysis by DSC indicated that lot 534106 of the test article contained impurities. Purity profile analysis by HPLC/UV detected 12 reportable impurities with a total relative peak area of approximately 5.8%. One impurity (approximately 2% relative to the total peak area) was tentatively identified as 1,3-dibromo-2-{[(2E)-3-bromoprop-2-en-1-yl]oxy}-5-{1-[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl]-1-methylethyl}benzene by LC/MS. The overall purity of lot 534106 was determined to be approximately 94%.

To ensure stability, the bulk chemical was stored at room temperature (~25°C) in amber glass bottles. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month studies using HPLC/UV by system A, and no degradation of the test article was detected.

G.1.2. Corn Oil

Corn oil was obtained in two lots (UU0854 and UW0493) from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 3-month 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.

G.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared four times by mixing tetrabromobisphenol A-bis(2,3-dibromopropyl ether) with corn oil to give the required concentrations (Table G-1). The dose formulations were stored in sealed clear glass bottles enclosed in amber plastic bags at room temperature and used within 41 days.

Homogeneity studies of 1.0 and 600 mg/mL formulations, and stability studies of the 1.0 mg/mL formulation were performed by the analytical chemistry laboratory using HPLC/UV by system B; the study laboratory used the same analytical system to perform additional homogeneity studies of 12.5, 25, 50, 400, and 800 mg/mL formulations and a gavageability study of a 200 mg/mL dose formulation.

B) The HPLC system included a liquid chromatograph (Waters Corporation or Agilent Inc., Palo Alto, CA), an Inertsil C_{18}, 250 mm × 4.6 mm, 5 μm particle size column (Alltech Associates, Inc.), an isocratic mobile phase of 95:5 acetonitrile:water, with UV detection at 280 nm, and a flow rate of 1.0 mL/minute.

Homogeneity was deemed acceptable for all of the formulations; stability was confirmed for at least 42 days for dose formulations stored in sealed glass containers at temperatures up to 25°C, and for 3 hours under simulated animal room conditions. Gavageability of the 200 mg/mL dose formulation was confirmed by the study laboratory.

Periodic analyses of the dose formulations of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) were conducted by the study laboratory using HPLC/UV by system B. During the 3-month studies, the dose formulations were analyzed three times; 14 of the 15 dose formulations for rats and mice were within 10% of the target concentrations (Table G-2). Animal room samples of these dose formulations were also analyzed; 14 of 18 animal room samples for rats and 12 of 15 for mice were within 10% of the target concentrations.

Table G-1. Preparation and Storage of Dose Formulations in the Three-month Gavage Studies of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Three-month Studies
Preparation
Dose formulations were prepared by adding the appropriate weight of tetrabromobisphenol A-bis(2,3-dibromopropyl ether) directly into an appropriate sized 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, and the beaker contents were diluted to final volume with corn oil and stirred using an overhead stirrer with a vigorous vortex for approximately 15 minutes; during this mixing, the bottom of the beaker was periodically scraped with a spatula to ensure that the test article did not stick to it. Dose formulations were prepared four times.
Chemical Lot Number
534106
Maximum Storage Time
42 days
Storage Conditions
Stored in sealed clear glass bottles in amber plastic bags at 25°C.
Study Laboratory
Battelle Columbus Operations (Columbus, OH)

Table G-2. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Three-month Gavage Studies of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats and Mice				
December 28, 2005[b]	January 4–5, 2006	12.5	12.23	−2
		50	48.29	−3
		100	100.3	0
		200	191.4	−4
January 6, 2006	January 6, 2006	25	25.15	+1
February 20, 2006	February 22–23, 2006	12.5	10.89[c]	−13
		25	23.48	−6
		50	49.61	−1
		100	89.98	−10
		200	184.8	−8
March 16, 2006	March 17–18, 2006	12.5	12.45	0
		25	24.85	−1
		50	46.15	−8
		100	94.33	−6
		200	197.7	−1

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Animal Room Samples				
Rats				
December 28, 2005[b]	February 3–4, 2006	12.5	11.84	−5
		50	45.96	−8
		100	91.16	−9
		200	194.9	−3
January 6, 2006	January 6, 2006	25	24.63	−1
February 20, 2006	March 29–30, 2006	12.5	11.92	−5
		25	24.16	−3
		50	47.96	−4
		100	91.77	−8
		200	182.8	−9
March 16, 2006	April 18–19, 2006	12.5	14.14	+13
		25	25.97	+4
		50	78.64[d]	+57
		50	44.56[e]	−11
		100	92.49	−8
		200	186.7	−7
	May 12, 2006	12.5	11.82[f]	−5
		50	43.85[f]	−12
Mice				
December 28, 2005[b]	February 3–4, 2006	12.5	13.01	+4
		50	50.53	+1
		100	91.09	−9
		200	193.6	−3
January 6, 2006	January 6, 2006	25	25.09	0
February 20, 2006	March 29–30, 2006	12.5	12.09	−3
		25	23.95	−4
		50	48.26	−3
		100	94.36	−6
		200	183.7	−8
March 16, 2006	April 18–19, 2006	12.5	14.87	+19
		25	30.33	+21
		50	56.26	+13

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		100	92.34	−8
		200	183.6	−8

[a]Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 12.5 mg/mL = 62.5 mg/kg, 25 mg/mL = 125 mg/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; 12.5 mg/mL = 125 mg/kg, 25 mg/mL = 250 mg/kg, 50 mg/mL = 500 mg/kg, 100 mg/mL = 1,000 mg/kg, 200 mg/mL = 2,000 mg/kg.
[b]The 25 mg/mL formulation prepared on December 28, 2005, was replaced by one prepared on January 6, 2006, due to a preparation error.
[c]Formulation was outside the acceptable range of ± 10% of target concentration, but used at NTP's direction.
[d]Results indicated that this formulation was not adequately resuspended prior to sampling.
[e]Two additional replicates were prepared from the same 50 mg/mL formulation, analyzed, and had peak responses that were closer to meeting the acceptance criterion.
[f]Because sufficient volume of this dose formulation remained, NTP directed that it be resampled and reanalyzed.

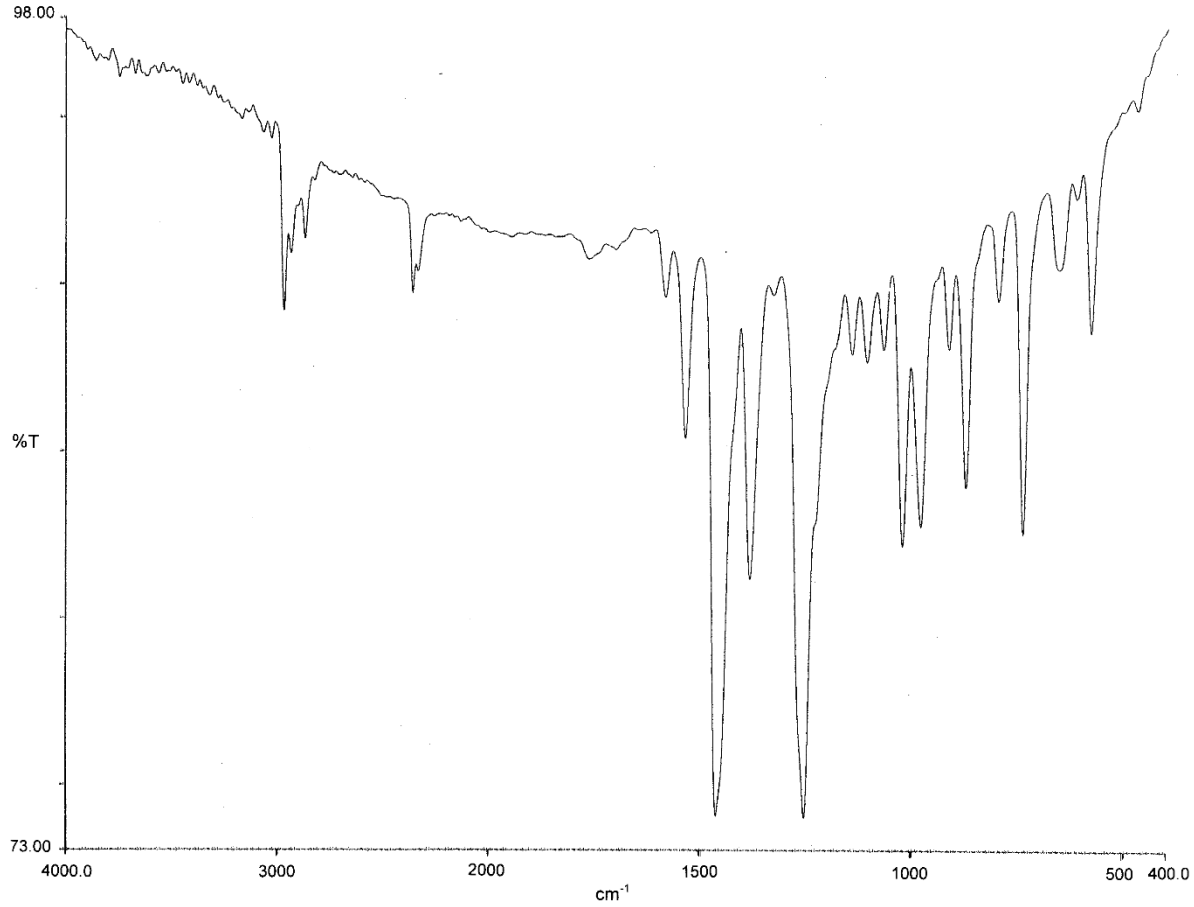

Figure G-1. Infrared Absorption Spectrum of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

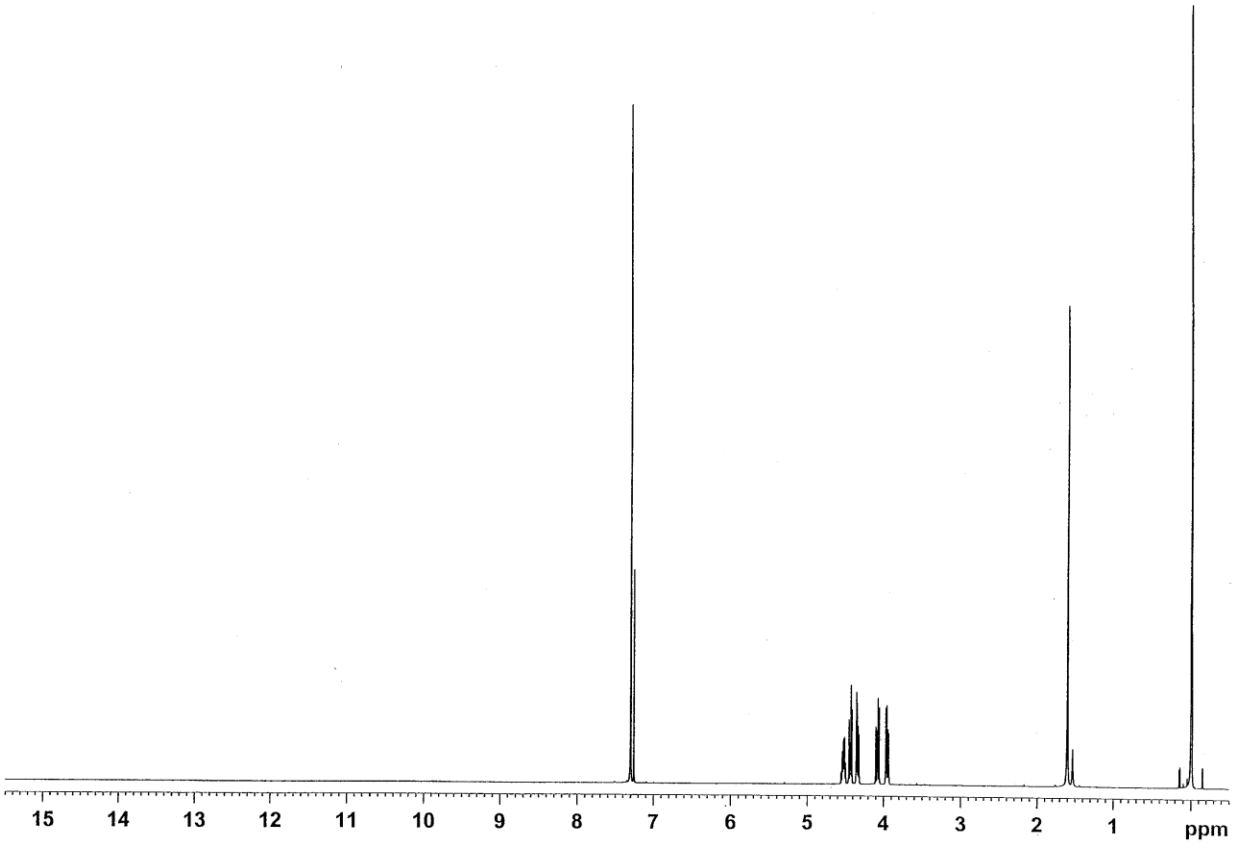

Figure G-2. Proton Nuclear Magnetic Resonance Spectrum of Tetrabromobisphenol A-bis(2,3-dibromopropyl ether)

Appendix H. Ingredients, Nutrient Composition, and Contaminant Levels In NTP-2000 Rat and Mouse Ration

Tables

Table H-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 H-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

	Amount	Source
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 H-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.4 ± 0.71	13.5–5.2	4
Crude fat (% by weight)	8.1 ± 0.14	8.0–8.3	4
Crude fiber (% by weight)	9.3 ± 0.57	8.7–10.0	4
Ash (% by weight)	5.2 ± 0.22	4.9–5.4	4
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.070	0.670–0.970	22
Cystine	0.220 ± 0.024	0.150–0.250	22
Glycine	0.701 ± 0.041	0.620–0.800	22
Histidine	0.352 ± 0.077	0.270–0.680	22
Isoleucine	0.546 ± 0.044	0.430–0.660	22
Leucine	1.095 ± 0.067	0.960–1.240	22
Lysine	0.711 ± 0.114	0.310–0.860	22
Methionine	0.409 ± 0.046	0.260–0.490	22
Phenylalanine	0.628 ± 0.040	0.540–0.720	22
Threonine	0.505 ± 0.043	0.430–0.610	22
Tryptophan	0.150 ± 0.028	0.110–0.200	22
Tyrosine	0.401 ± 0.061	0.280–0.540	22
Valine	0.665 ± 0.043	0.550–0.730	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)	3,490 ± 43	3,150–4,090	4
Vitamin D (IU/kg)	1,000[a]	–	–

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm)[b]	6.5 ± 0.22	6.3–6.8	4
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 B_{12} (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm)[b]	2,846 ± 485	1,820–3,790	22
Minerals			
Calcium (%)	0.991 ± 0.062	0.938–1.080	4
Phosphorus (%)	0.585 ± 0.020	0.566–0.607	4
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	20

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.28 ± 0.073	0.20–0.38	4
Cadmium (ppm)	0.05 ± 0.009	0.04–0.07	4
Lead (ppm)	0.09 ± 0.005	0.09–0.09	4
Mercury (ppm)	<0.02	–	4
Selenium (ppm)	0.41 ± 0.044	0.38–0.48	4

	Mean ± Standard Deviation[b]	Range	Number of Samples
Aflatoxins (ppb)	<5.00	–	4
Nitrate nitrogen (ppm)[c]	21.3 ± 3.08	16.8–23.7	4
Nitrite nitrogen (ppm)[c]	2.50 ± 1.56	0.30–3.86	4
BHA (ppm)[d]	<1.0	–	4
BHT (ppm)[d]	<1.0	–	4
Aerobic plate count (CFU/g)	10 ± 0.0	10	4
Coliform (MPN/g)	3.0 ± 0.0	3.0	4
Escherichia coli (MPN/g)	<10	–	4
Salmonella (MPN/g)	Negative	–	4
Total nitrosoamines (ppb)[e]	6.9 ± 2.07	5.3–9.9	4
N-Nitrosodimethylamine (ppb)[e]	4.1 ± 1.54	2.9–6.3	4
N-Nitrosopyrrolidine (ppb)[e]	2.8 ± 0.85	1.8–3.6	4
Pesticides (ppm)			
α-BHC	<0.01	–	22
β-BHC	<0.02	–	22
γ-BHC	<0.01	–	22
δ-BHC	<0.01	–	22
Heptachlor	<0.01	–	22
Aldrin	<0.01	–	22
Heptachlor epoxide	<0.01	–	22
DDE	<0.01	–	22
DDD	<0.01	–	22
DDT	<0.01	–	22
HCB	<0.01	–	22
Mirex	<0.01	–	22
Methoxychlor	<0.05	–	22
Dieldrin	<0.01	–	22
Endrin	<0.01	–	22
Telodrin	<0.01	–	22
Chlordane	<0.05	–	22
Toxaphene	<0.10	–	22
Estimated PCBs	<0.20	–	22
Ronnel	<0.01	–	22
Ethion	<0.02	–	22
Trithion	<0.05	–	22

	Mean ± Standard Deviation[b]	Range	Number of Samples
Diazinon	<0.10	–	22
Methyl chlorpyrifos	0.096 ± 0.070	0.035–0.186	4
Methyl parathion	<0.02	–	22
Ethyl parathion	<0.02	–	22
Malathion	0.285 ± 0.248	0.020–0.619	4
Endosulfan I	<0.01	–	22
Endosulfan II	<0.01	–	22
Endosulfan sulfate	<0.03	–	22

[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 I. Sentinel Animal Program

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Tables

I.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 toxicological evaluation of compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. 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, allowed to clot, and the serum was separated. All samples were processed appropriately and tested at BioReliance Corporation (Rockville, MD) for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood samples were collected from five male and five female rats and mice at each time point.

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

Method and Test	Time of Collection
Rats	
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
RCV/SDA	End of quarantine, study termination
Mice	
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 encephalomyelitis virus)	End of quarantine, 4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	End of quarantine, 4 weeks, study termination
Mouse adenoma virus-1	End of quarantine, 4 weeks, study termination
MHV (mouse hepatitis virus)	End of quarantine, 4 weeks, study termination
MMV VP2 (mouse minute virus viral protein 2)	End of quarantine, 4 weeks, study termination
MPV VP2 (mouse parvovirus viral protein 2)	End of quarantine, 4 weeks, study termination
PVM	End of quarantine, 4 weeks, study termination
Reovirus 3	End of quarantine, 4 weeks, study termination
Sendai	End of quarantine, 4 weeks, study termination

I.2. Results

All test results were negative.

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