NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 398

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF POLYBROMINATED BIPHENYLS
(FIREMASTER FF-1®)

(CAS NO. 67774-32-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report and individual animal data are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT

ON THE PERINATAL

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P.O. Box 12233
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August 1993

NTP TR 398

NIH Publication No. 93-2853

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

C.J. Alden, Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
R.S. Chhabra, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.K. Haseman, Ph.D.
H.B. Matthews, Ph.D.
G.N. Rao, D.V.M., Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Battelle Columbus Laboratories
Conducted studies, evaluated pathology findings

B.D. Carlton, Ph.D., Principal Investigator
P.J. Kurtz, Ph.D., Principal Investigator

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
H.R. Brown, D.V.M., M.S.
B.F. Hamilton, D.V.M., Ph.D.

Integrated Laboratory Systems
Prepared quality assurance audits

S.L. Smith, J.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (7 July 1989)

J.C. Seely, D.V.M., Chair
Pathco, Inc.
H.R. Brown, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
W.R. Brown, D.V.M., Ph.D.
Research Pathology Services, Inc.
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
M.P. Jokinen, D.V.M.
National Toxicology Program
C. Keenan, V.M.D.
Merck, Sharp & Dohme Research Laboratories
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program

Evaluated slides, prepared pathology report on mice (20 June 1989)

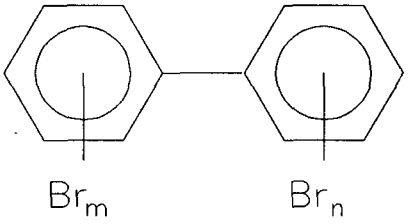
M.A. Stedham, D.V.M., M.S., Chair
Pathology Associates, Inc.
K.M. Ayers, D.V.M.
Burroughs Wellcome
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
M.R. Elwell, D.V.M., Ph.D.
National Toxicology Program
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
Z. Ruben
G.D. Searle & Company

Biotechnical Services, Inc.
Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
G.F. Corley, D.V.M.
P.R. Dennis, M.C.M.
T.A. King-Hunter, B.S.
K.D. Mencer, B.A.
W.D. Sharp, B.A., B.S.

CONTENTS

ABSTRACT



[Sum of m and n ranges from 2 to 7]

POLYBROMINATED BIPHENYLS (FIREMASTER FF-1®)

CAS No. 67774-32-7

Chemical Formula: $C_{12}H_{(10-m-n)}Br_{(m+n)}$ Molecular Weight: 144.21+78.90(m+n)

Synonyms: PBBs; polybrominated biphenyl mixture; hexabromobiphenyl (technical grade); brominated biphenyls; polybromobiphenyls

Polybrominated biphenyls are synthetic chemicals used as flame retardants. The technical product used in these studies, Firemaster FF-1®, is a mixture of brominated biphenyls. Firemaster FF-1® is a known liver carcinogen in rats and mice and is one of three compounds chosen by the National Toxicology Program to investigate the potential value of perinatal exposures in assessing chemical carcinogenicity.

Chronic toxicity and carcinogenicity studies of polybrominated biphenyls (Firemaster FF-1®) were conducted in F344/N rats and B6C3F$_1$ mice of each sex. The studies were designed to determine: a) the effects of polybrominated biphenyls in rats and mice receiving adult (F$_1$) exposure only (a typical carcinogenicity study), b) the toxic and carcinogenic effects of polybrominated biphenyls in rats and mice receiving perinatal (F$_0$) exposure only (dietary exposure of dams prior to breeding and throughout gestation and lactation), and c) the effects of combined perinatal and adult exposure to polybrominated biphenyls.

STUDIES IN F344/N RATS

The exposure levels selected for F$_1$ exposure, based on studies of polybrominated biphenyls in the literature, were 3, 10, and 30 ppm. In a preliminary study to determine the perinatal dietary concentrations for the 2-year study, female rats were administered 1 to 30 ppm polybrominated biphenyls in the feed beginning 60 days prior to breeding and continuing throughout gestation, lactation, and up to 4 weeks postweaning. The mean preweaning litter weight of the 30 ppm group was less than 80% of the mean litter weight of the control group at days 0, 4, and 12. At weaning, the mean weight of litters in this group was 80% of the control group mean. The final mean body weights (28 days after weaning) of males and females receiving 30 ppm were 13% to 19% lower than the final mean body weights of the controls. Therefore, dietary concentrations of 0, 1, 3, and 10 ppm were selected for the F$_0$ exposure levels in the 2-year study. The eight F$_0$:F$_1$ exposure combinations selected for the 2-year study are shown in the following table.

Exposure Groups and Numbers of Rats[a]

F_0 Concentration[b] (ppm)	F_1 Concentration[c] (ppm)			
	0	3	10	30
0	60	–	60	60
1	–	60	–	–
3	–	–	60	–
10	60	–	60	60

[a] Ten rats from each group were evaluated at 9 months.
[b] Concentration of polybrominated biphenyls in feed through breeding, gestation, and lactation until pups were 8 weeks of age
[c] Concentration of polybrominated biphenyls in feed given to rats beginning at 8 weeks of age for 2 years

Adult-Only Exposure

The major organ affected by toxicity of polybrominated biphenyls was the liver. Rats evaluated at 9 months had decreased body weights, hepatomegaly, nonneoplastic histopathologic changes in the liver, mild anemia, increases in serum cholesterol concentrations, and decreases in serum triglyceride concentrations (males only). In rats receiving adult-only exposure (F_0:F_1 concentrations of 0:10 or 0:30 ppm), there were no significant effects on survival. Mean body weights were significantly reduced in 0:10 and 0:30 ppm male rats and in 0:30 ppm female rats. Males and females exposed to 0:10 or 0:30 ppm had increased incidences of hepatocellular neoplasms (males: 0:0 ppm, 1/50; 0:10 ppm, 12/49; 0:30 ppm, 41/50; females: 0/50, 12/50, 39/50). Increased incidences of the following nonneoplastic lesions were associated with the administration of polybrominated biphenyls: eosinophilic foci, cytoplasmic vacuolization, oval cell hyperplasia, and hypertrophy in the liver of males and females; acanthosis, inflammation, and ulceration of the forestomach in exposed males; and cystic endometrial hyperplasia of the uterus in 0:30 ppm females.

Perinatal-Only Exposure

For rats receiving only perinatal exposure (10:0 ppm), there were no changes in survival or body weights compared to the 0:0 ppm control groups. In female rats, there were no effects on neoplasm incidences, but perinatal exposure was associated with a marginally increased incidence of hepatocellular adenoma in male rats (0:0 ppm, 1/50; 10:0 ppm, 5/50). The incidences of nonneoplastic lesions in the liver were increased in exposed males (eosinophilic foci and cytoplasmic vacuolization) and females (eosinophilic foci).

Combined Perinatal and Adult Exposure

Combined perinatal and adult exposure resulted in marginally reduced survival compared to the 0:0 ppm control group for male rats in the 3:10, 10:10, and 10:30 ppm groups. No significant survival differences were observed in female rats. The final mean body weights of male and female rats receiving 3:10, 10:10, or 10:30 ppm were lower than those of the 0:0 ppm controls.

In male rats, there were no enhancing effects of combined perinatal and adult exposure on the incidence of hepatocellular neoplasms. However, perinatal exposure enhanced the development of liver neoplasms in female rats receiving 10 or 30 ppm adult exposure. A combined analysis of all male and female exposure groups also revealed increased incidences of mononuclear cell leukemia that were considered related to polybrominated biphenyls exposure.

STUDIES IN B6C3F_1 MICE

The exposure levels selected for the F_1 exposure, based on studies of polybrominated biphenyls in the literature, were 3, 10, and 30 ppm. In a preliminary study to determine the perinatal dietary concentrations for the 2-year study, female C57BL/6N mice

were exposed to 1 to 30 ppm polybrominated biphenyls in the feed beginning 60 days before breeding to C3H/HeN males, continuing throughout gestation and lactation and up to 4 weeks postweaning. There were no clear chemical-related effects on survival or growth at any phase of the study; therefore, 0, 3, 10, and 30 ppm dietary concentrations were selected for the F_0 exposure levels in the 2-year study. The eight F_0:F_1 exposure combinations selected for the 2-year study are shown in the table below.

Adult-Only Exposure

The major organ affected by toxicity of polybrominated biphenyls was the liver. Animals evaluated at 9 months had lower body weights than the controls, hepatomegaly, and histopathologic changes in the liver. In mice receiving adult-only exposure, no males or females in the 0:30 ppm group survived to the end of the study. Neither survival nor body weights were affected in the 0:10 ppm groups. Males and females receiving 0:10 or 0:30 ppm had markedly increased incidences of hepatocellular neoplasms (males: 0:0 ppm, 16/50; 0:10 ppm, 48/49; 0:30 ppm, 48/50; females: 5/50, 42/50, 47/48). Increased incidences of nonneoplastic liver lesions including cytomegaly (hypertrophy), fatty change (cytoplasmic vacuolization), bile duct hyperplasia, eosinophilic and clear cell foci, and necrosis of individual hepatocytes were related to treatment with polybrominated biphenyls. Increased incidences and severity of chronic nephropathy in the kidney and excessive hematopoiesis in the spleen of 0:30 ppm males and females were also considered to be related to exposure to polybrominated biphenyls.

Perinatal-Only Exposure

There were no survival or body weight differences in mice receiving only perinatal exposure (30:0 ppm). Perinatal exposure resulted in significantly increased incidences of hepatocellular neoplasms in males and females. The incidences of nonneoplastic lesions (cytomegaly, eosinophilic foci, clear cell foci) were increased in males and females.

Combined Perinatal and Adult Exposure

Combined perinatal and adult exposure resulted in markedly reduced survival for females in the 30:10 ppm group; no mice receiving 30:30 ppm survived to the end of the study. In those groups receiving adult exposure of 30 ppm, mean body weights were not affected.

The incidence of hepatocellular neoplasms in male and female mice was significantly increased. At the 9-month interim evaluation the incidence of hepatocellular adenomas was significantly increased in males (0:30 ppm, 1/10; 30:30 ppm, 7/10). The incidence of hepatocellular adenomas in 30:30 ppm females was similar to that of 0:30 ppm females (0:30 ppm, 0/10; 30:30 ppm, 3/10). At the end of the study the incidence of hepatocellular adenomas in males was statistically increased (0:30 ppm, 42/50; 30:30 ppm, 48/50). The incidence of hepatocellular adenomas in 30:30 ppm females was statistically decreased compared to that of 0:30 ppm females (0:30 ppm, 46/48; 30:30 ppm, 41/47). It was not possible to assess the potential enhancing effect of combined perinatal and adult exposure on hepatocellular neoplasms because adult-only exposure resulted in such high (84% to 98%) liver neoplasm incidences.

Exposure Groups and Numbers of Mice[a]

F_0 Concentration[b] (ppm)	F_1 Concentration[c] (ppm)			
	0	3	10	30
0	60	–	60	60
3	–	60	–	–
10	–	–	60	–
30	60	–	60	60

[a] Ten mice from each group were evaluated at 9 months.
[b] Concentration of polybrominated biphenyls in feed through breeding, gestation, and lactation until pups were 8 weeks of age
[c] Concentration of polybrominated biphenyls in feed given to mice beginning at 8 weeks of age for 24 months

CONCLUSIONS

Adult-Only Exposure

Under the conditions of these 2-year, adult-only, dietary exposure studies, there was *clear evidence of carcinogenic activity** for polybrominated biphenyls in male and female F344/N rats and male and female B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms.

Perinatal-Only Exposure

Perinatal exposure alone (through dietary administration of 10:0 ppm polybrominated biphenyls to the dams) had no effect on the incidences of neoplasms in female F344/N rats, but in male F344/N rats, perinatal exposure was associated with a marginally increased incidence of hepatocellular adenomas that may have been related to chemical administration. In male and female B6C3F$_1$ mice, perinatal exposure to 30:0 ppm polybrominated biphenyls resulted in significantly increased incidences of hepatocellular neoplasms. The incidences of a number of nonneoplastic lesions in the liver (cytomegaly, eosinophilic focus, and clear cell focus) were increased in male and female B6C3F$_1$ mice.

Combined Perinatal and Adult Exposure

Combined perinatal and adult dietary exposure to polybrominated biphenyls confirmed the findings of the adult-only exposures for the increased incidences of hepatocellular neoplasms in F344/N rats and B6C3F$_1$ mice. In male F344/N rats, there were no enhancing effects of combined perinatal and adult exposure. However, perinatal exposure enhanced the susceptibility of female F344/N rats receiving adult exposure of 10 or 30 ppm to the induction of liver neoplasms.

For male and female F344/N rats, a combined analysis of the incidences of leukemia in the adult-only, perinatal-only, and combined perinatal and adult exposure groups revealed an apparent association between increasing incidences of mononuclear cell leukemia and exposure to polybrominated biphenyls.

In male and female B6C3F$_1$ mice, it was not possible to adequately assess the enhancing effects of combined perinatal and adult exposure on hepatocellular neoplasms, because adult-only exposure to 10 or 30 ppm polybrominated biphenyls resulted in high incidences (84% to 98%) of liver neoplasms. However, with increased perinatal exposure, there were increases in the numbers of B6C3F$_1$ mice with hepatocellular carcinomas and in the numbers of B6C3F$_1$ mice with multiple hepatocellular adenomas, which suggests an enhancement of polybrominated biphenyls-related hepatocellular carcinogenicity associated with perinatal exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 11.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on polybrominated biphenyls (Firemaster FF-1®) on June 23, 1992, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

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

Gary P. Carlson, Ph.D., Chair
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Paul T. Bailey, Ph.D.
Toxicology Division
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H.*
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

Kowetha A. Davidson, Ph.D.
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Harold Davis, D.V.M., Ph.D.
School of Aerospace Medicine
Brooks Air Force Base, TX

Jay I. Goodman, Ph.D., Principal Reviewer
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

David W. Hayden, D.V.M., Ph.D.
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Curtis D. Klaassen, Ph.D.*
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Daniel S. Longnecker, M.D.*
Department of Pathology
Dartmouth Medical School
Lebanon, NH

Barbara McKnight, Ph.D., Principal Reviewer*
Department of Biostatistics
University of Washington
Seattle, WA

Ellen K. Silbergeld, Ph.D.
University of Maryland Medical School
Baltimore, MD

Matthew J. van Zwieten, D.V.M., Ph.D.
Department of Safety Assessment
Merck, Sharp & Dohme Research Laboratories
West Point, PA

Lauren Zeise, Ph.D., Principal Reviewer
California Department of Health Services/RCHAS
Berkeley, CA

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 23, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of polybrominated biphenyls (Firemaster FF-1®) received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis studies of polybrominated biphenyls (PBBs) by discussing the rationale for incorporating perinatal exposure into the study design. The study designs included conventional 2-year exposure of adult animals, perinatal exposure only, and perinatal plus adult exposure. The studies were intended to compare and evaluate the potential values of perinatal exposures in assessing chemical carcinogenicity. Dr. Chhabra described the experimental design, reported on survival and body weight effects, and commented on neoplasms and nonneoplastic lesions in rats and mice. The proposed conclusions were:

Adult-Only Exposure

Under the conditions of these 2-year, adult-only, dietary exposure studies, there was *clear evidence of carcinogenic activity* for polybrominated biphenyls (PBBs) in male and female F344/N rats and male and female B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms.

Perinatal-Only Exposure

Perinatal exposure alone (through dietary administration of 10 ppm PBBs to the dams) had no effect on the incidences of neoplasms in female F344/N rats, but in male rats, perinatal exposure was associated with a marginally increased incidence of hepatocellular adenomas that may have been related to chemical administration. In male and female B6C3F$_1$ mice, perinatal exposure to 30 ppm PBBs resulted in significantly increased incidences of hepatocellular neoplasms.

Combined Perinatal and Adult Exposure

Combined perinatal and adult dietary exposure to PBBs confirmed the findings of the adult-only exposures for the increased incidences of hepatocellular neoplasms in rats and mice. In male rats, there were no enhancing effects of combined perinatal and adult exposure. However, perinatal exposure enhanced the susceptibility of female rats receiving adult exposure of 10 or 30 ppm to the induction of liver neoplasms. For male and female rats, a combined analysis of the incidences of leukemia in the adult-only, perinatal-only, and combined perinatal and adult exposure groups revealed an apparent association between increasing incidences of mononuclear cell leukemia and exposure to PBBs. In male and female mice, it was not possible to adequately assess the enhancing effects of combined perinatal and adult exposure on hepatocellular neoplasms, because adult-only exposure to 10 or 30 ppm PBBs resulted in high incidences (84% to 98%) of liver neoplasms. However, with increased perinatal exposure, there were increases in the numbers of mice with hepatocellular carcinomas and in the numbers of mice with multiple hepatocellular adenomas, which suggests an enhancement of PBB-related hepatocellular carcinogenicity associated with perinatal exposure.

Dr. Goodman, a principal reviewer, agreed in principle with the proposed conclusions. However, he thought that in the conclusions for male rats receiving perinatal-only exposure, the phrase "a marginally increased incidence of" should be changed to "an equivocal increase in the incidence of" to better characterize the questionable nature of the increase. Also, under the heading "Combined Perinatal and Adult Exposure," he suggested that all reference to mononuclear cell leukemia be omitted. Dr. Chhabra noted that life table analyses of data from all eight experimental groups indicated that significant increases in incidences of leukemia were associated with increasing concentration levels of PBBs in adult rats. Dr. Goodman proposed omitting from consideration of possible carcinogenicity of PBBs certain exposure groups in which the maximum tolerated dose (MTD) appears to have been exceeded, noting that this would not change the overall level of evidence. Dr. J.K. Haseman, NIEHS, commented that the reduced weight gain in these groups may have resulted from the hepatocellular carcinogenicity. Dr. M.R. Elwell, NIEHS, thought the high incidence of leukemia also could have been a contributing factor. Dr. Goodman said the argument that PBBs

were genotoxic was weak and should be deleted. Dr. Chhabra agreed.

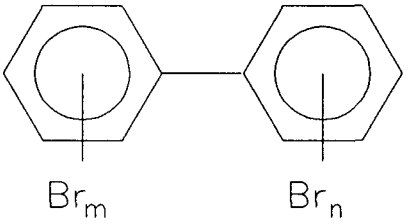
Dr. Zeise, the second principal reviewer, also agreed in principle with the proposed conclusions, but said they should note that the power of the study in rats to distinguish the impact of perinatal exposure may have been compromised by an inadvertent exposure of control rats to PBBs. Dr. Chhabra said there was certainty that control animals did not receive PBBs in the diet and stated that it is common to find PBBs in control animal tissues because PBBs are a ubiquitous environmental contaminant. Dr. Zeise said the decreases in neoplasms and nonneoplastic lesions noted for mice were difficult to interpret without results of statistical comparisons which account for the poor survival of treated mice.

Because Dr. McKnight, the third principal reviewer, was unable to attend the meeting, Dr. L. Hart, NIEHS, read her review into the record. Dr. McKnight agreed in principle with the conclusions but asked whether increases in thyroid follicular cell adenomas should be mentioned as part of the effects for male mice under combined perinatal and adult exposure. Dr. Haseman said the presence of an adenoma in an untreated control male mouse resulted in lack of statistical significance. Dr. McKnight questioned the statement in the results that for female rats there was significantly increased incidence of liver neoplasms from perinatal exposure alone, and suggested that it would be better to say that there was an enhancing effect of perinatal exposure on the incidence of liver neoplasms in animals exposed as adults. Dr. McKnight said the statistical analyses for the combined perinatal and adult exposures should be presented in the appendixes. Dr. Haseman said that this would be done.

Dr. Silbergeld asked whether there had been contamination of the test mixture with brominated dibenzofurans, compounds for which there had been recent toxicity studies reported. Dr. T.J. Goehl, NIEHS, said a mass spectral analysis had been done for dibenzofurans. The fragmentation patterns would indicate the number of bromines but not their position on the furan ring. Dr. Silbergeld commented that some of the brominated biphenyls were known to induce anorexia, a property which might have contributed to the reduced weight gain noted by Dr. Goodman.

Dr. Silbergeld moved that the Technical Report on polybrominated biphenyls be accepted with the conclusions as written for male and female rats and mice under the three combinations of Adult-Only Exposure, Perinatal-Only Exposure, and Combined Perinatal and Adult Exposure. Dr. Zeise seconded the motion. Dr. Goodman offered an amendment: "The MTD was deemed to have been exceeded in male and female rats in the 0:30 ppm and 10:30 ppm dose groups, based on an excessive (i.e., 20% to 29%) decrease in body weight gain. Therefore, the carcinogenicity data obtained from the exposure groups in question were not considered in this assessment of the carcinogenicity of the PBBs." The amendment was tabled for lack of a second. Dr. Silbergeld's motion was then accepted unanimously with eight votes.

INTRODUCTION



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POLYBROMINATED BIPHENYLS (FIREMASTER FF-1®)

CAS No. 67774-32-7

Chemical Formula: $C_{12}H_{(10-m-n)}Br_{(m+n)}$ Molecular Weight: 144.21+78.90(m+n)

Synonyms: PBBs; polybrominated biphenyl mixture; hexabromobiphenyl (technical grade); brominated biphenyls; polybromobiphenyls

A series of mishaps with certain therapeutic agents and environmental toxicants has focused attention on the responses of developing organisms to diverse types of biologically active molecules. The occurrence of congenital defects in children resulting from the use of thalidomide by pregnant women, cancer in the daughters of women exposed to diethylstilbestrol during pregnancy, and episodes of congenital methylmercury poisoning have stimulated research in perinatal toxicology (Herbst *et al.*, 1971, 1975; Amin-Zaki *et al.*, 1974). During the perinatal period from conception to birth or weaning, some physiologic barriers such as the blood-brain barrier and certain aspects of the excretory, metabolizing, and gastrointestinal systems are not fully developed. Therefore, developing organisms can be more susceptible to the toxic effects of environmental or therapeutic agents (Lewerenz, 1982; Miller, 1983).

Recognition of the heightened sensitivity of developing organisms to chemical toxicity has led to a number of human and laboratory animal studies. Examples of epidemiological studies include evaluations of the relationships between brain neoplasms in children and the occupational exposure of parents to carcinogens (Peters *et al.*, 1981), childhood cancer and parental cigarette smoking (Grufferman *et al.*, 1983; Stjernfeldt *et al.*, 1986; Pershagen, 1989), and childhood leukemia and occupational and home exposure of parents to carcinogens (Lowengart *et al.*, 1987). Arundel and Kinnier-Wilson (1986) have reviewed 14 epidemiology studies that investigate a possible association between childhood cancer and parental occupational exposure to carcinogens. The contradictory observations suggest that more investigations are needed in this field.

Although human data are limited, information on perinatal toxicology and carcinogenesis in laboratory animals began accumulating when Larsen *et al.* (1947) reported a high incidence of lung neoplasms in offspring when pregnant strain A mice were administered urethane 1 day before delivery. This finding of an increased susceptibility of the fetal lung to urethane carcinogenesis was confirmed by Klein (1952). Pietra *et al.* (1959) reported that 12-hour-old mice given a single injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) had a 32% incidence of lymphomas at 15.3 weeks of age, a relatively short period for expression of a neoplasmigenic effect. Similar decreases in the latency period for expression of neoplasmigenic effects were obtained with benzo(a)pyrene, 3-methylcholanthrene, and urethane (Pietra *et al.*, 1961). Druckery *et al.* (1966) reported

that the teratogen ethylnitrosourea, administered by a single injection to pregnant rats, produced brain neoplasms in offspring at an average age of 160 days, compared to an average age of 360 days for animals exposed to ethylnitrosourea as young adults. The increased sensitivity of fetal nervous tissue to ethylnitrosourea was further studied in Fischer and Sprague-Dawley rats by Swenberg *et al.* (1972), who evaluated the dose-relationship of transplacental brain neoplasm development and concluded that the age at which an animal develops neoplasia following exposure is a function of the dose levels used. Spontaneous neoplasms of the brain and nerves are rare in mice. However, perinatal exposure of several mouse strains to ethylnitrosourea caused a 6% incidence of neurogenic neoplasms, whereas postnatal ethylnitrosourea exposure resulted in an incidence of only 0.33% (Wechsler *et al.*, 1979). Furthermore, certain types of neoplasms, such as medulloblastomas, astrocytomas, and meningeal neoplasms, were observed only in mice exposed to ethylnitrosourea perinatally.

The carcinogenic response of various tissues following transplacental, neonatal-infant, or adult exposure of mice to a single administration of ethylnitrosourea was studied by Vesselinovitch *et al.* (1979). These studies showed that the age of the animals at the time of exposure to a carcinogen is the most effective modulator of carcinogenesis in the liver, lung, stomach, ovary, and lymphoreticular tissues. Tomatis (1979) reported that exposure of mice to DMBA and of rats to ethylnitrosourea or methylnitrosourea during pregnancy resulted in a high incidence of neoplasms in animals of the first generation and in an increased incidence of neoplasms at specific sites in untreated animals of the second and third generations. Germ cell mutation caused by perinatal exposure to a carcinogen was reported by Nomura (1982). The exposure of parent ICR mice to X-rays or urethane resulted in a 90% incidence of lung neoplasms in the offspring; the inheritability of carcinogenic effects in F_1 and F_2 generations was shown. Yamasaki *et al.* (1987) reported that fetal c-Ha-*ras* can be transplacentally activated through a specific point mutation by a carcinogen. Also, when administered to pregnant ICR mice on day 18 of gestation, safrole, 4-aminobiphenyl, and benzo(a)pyrene bind to the DNA of the maternal uterus and placenta and the maternal and fetal liver, lung, kidney, heart, brain, intestine, and skin (Lu *et al.*, 1986).

Toxicology endpoints other than carcinogenicity have also been studied in laboratory animals after perinatal exposure. The toxicity of chemicals to the nervous (Adams and Buelke-Sam, 1981), reproductive (McLachlan *et al.*, 1981), and immune systems (Roberts and Chapman, 1981) is a subject of continuing public health and scientific interest. The field of perinatal toxicology and carcinogenesis has been extensively reviewed (IARC, 1973; NCI, 1979; Alexandrov, 1983; Miller, 1983; Tomatis, 1988). A review of environmental, occupational, and therapeutic exposure data by Schardein and Keller (1989) has identified 54 chemicals as potential developmental toxicants in humans.

STUDY RATIONALE

The evaluation of chemicals for carcinogenicity in rodents is usually accomplished by exposing animals to a chemical for 2 years, beginning when the animals are approximately 6 to 8 weeks old (Chhabra *et al.*, 1990). In 1976, a symposium was organized by the National Cancer Institute on perinatal carcinogenesis (NCI, 1979); this group recommended that the perinatal period be incorporated into the period of exposure for conventional carcinogenicity studies (Swenberg, 1979; Vesselinovitch *et al.*, 1979). Therefore, the National Institute of Environmental Health Sciences designed the present studies to incorporate the perinatal period, including exposure of maternal animals prior to breeding, through gestation, lactation, and weaning, followed by conventional exposure of the offspring for 2 years, to compare the sensitivity of the combined perinatal and adult exposure bioassay with the conventional bioassay for detecting carcinogenicity. Three chemicals, ethylene thiourea (ETU), 5,5′-diphenylhydantoin (phenytoin), and polybrominated biphenyls (Firemaster FF-1®), were selected for these combined perinatal and adult exposure studies. These chemicals can cross the placenta and be secreted in the milk so that developing fetuses and neonates are exposed during the gestation and lactation periods. This report describes the results of the carcinogenicity studies of polybrominated biphenyls. The studies on ETU have been reported (NTP, 1992).

CHEMICAL AND PHYSICAL PROPERTIES

Polybrominated biphenyls are synthetic flame retardants. The technical products are white powders

containing mixtures of brominated biphenyls, with two to seven bromines per biphenyl, and 2% calcium silicate, an anticaking agent. The primary brominated biphenyl in polybrominated biphenyl mixtures, hexabromobiphenyl, constitutes 60% to 90% of the mixture, and the principal hexabromobiphenyl is the 2,2′,4,4′,5,5′-isomer. Polybrominated biphenyls are insoluble in water, soluble in fat, and slightly to highly soluble in various organic solvents (IARC, 1986).

Use and Human Exposure

Polybrominated biphenyls were used as flame retardants for synthetic fibers and molded thermoplastic parts in the early 1970's. After a total production of 5.1 million kg of hexabromobiphenyl from 1970 to 1974, the sole United States manufacturer of hexabromobiphenyl ceased production in November 1974. This action was taken following an incident in 1973 in which approximately 295 kg of polybrominated biphenyls was inadvertently substituted for magnesium oxide during the formulation of animal feed at a chemical plant in Michigan. As a result, millions of Michigan residents were exposed to polybrominated biphenyls through the ingestion of contaminated dairy products (Sleight, 1979; IARC, 1986). Random sampling from nursing mothers in Michigan indicated that 96% of the population in the lower peninsula had detectable body burdens (Brilliant *et al.*, 1978).

Absorption, Distribution, Metabolism, and Excretion

Studies conducted with various species indicate that polybrominated biphenyls are rapidly and almost completely absorbed from the gastrointestinal tract. Highly brominated biphenyls are absorbed less efficiently than less brominated biphenyls. The tissue distribution of polybrominated biphenyls follows a similar pattern in all species studied. The highest concentrations are found in adipose tissue and liver. Changes in physiological status that cause mobilization of fat from adipose tissue can also cause mobilization of polybrominated biphenyls, causing a new tissue equilibrium to be established (Fries, 1985).

Rickert *et al.* (1978) studied tissue distribution of polybrominated biphenyls in pregnant and lactating rats and in their offspring following dietary exposure. The studies concluded that: a) lactation did not significantly alter the concentration of polybrominated biphenyls found in tissues other than mammary tissue, b) polybrominated biphenyl concentrations were higher in neonatal livers than in the livers of nursing dams, and c) transfer of polybrominated biphenyls via the milk appeared to contribute more to the accumulation of polybrominated biphenyls in the newborn tissues than did placental transfer.

The rate and pathways for metabolism of several tetra-, penta-, or hexabromobiphenyls were studied in *in vitro* rat liver microsomal preparations by Mills *et al.* (1985). Ring hydroxylation of some di-, tri-, and tetrabromobiphenyls was demonstrated in preparations of liver microsomes from animals pretreated with phenobarbital or methylcholanthrene. Dibromobiphenyl was the most rapidly metabolized bromobiphenyl, followed by tri-and tetrabromobiphenyl, in decreasing order. Apparently, further bromination prevented metabolism, because penta- and hexabromobiphenyls were not metabolized. Hexa isomers are reported to be more toxic than tetra isomers (Millis *et al.*, 1985; Mills *et al.*, 1985). The reasons for decreased metabolism of the more highly brominated biphenyls are not clear. However, Matthews and Kato (1979) have proposed that the large bromine atoms can effectively hinder the approach of the enzyme to the carbon skeleton, and the electrochemical properties cause the bromine atoms to inhibit the formation of an arene oxide intermediate.

Elimination of highly brominated biphenyls is a slow process. Because there is little or no metabolism of the polybrominated biphenyls *in vivo*, they are excreted as parent compound through biliary or intestinal excretion with elimination in feces, elimination via fat-containing secretions such as milk, or elimination through egg production and transfer to the fetus and associated products of conception (Matthews *et al.*, 1977; Fries, 1985). The major component of polybrominated biphenyls, 2,2′,4,4′,5,5′-hexabromobiphenyl, appears to be the most persistent in various species studied (Wolff and Selikoff, 1979).

Toxicity

Humans

There have been numerous studies of the health effects of polybrominated biphenyls in humans.

Symptoms including fatigue, depression, headache, blurred vision, and muscular weakness were the earliest effects reported following the accidental exposures in Michigan, and these symptoms occurred more frequently in exposed Michigan residents than in unexposed Wisconsin residents (Valciukas *et al.*, 1979). In a study by Stross (1979), physical examination of polybrominated biphenyl-exposed individuals revealed hepatomegaly; however, few results of liver function tests were abnormal. Psychological tests identified no consistent findings other than reactive depression. No organic central nervous system changes were found, and it was concluded that the people of Michigan were not at great risk of suffering ill health effects. Workers who had manufactured polybrominated biphenyls and had the highest concentrations of polybrominated biphenyls in their adipose tissue had normal mean scores in all memory tests (Brown *et al.*, 1981). A study of immunologic dysfunction among polybrominated biphenyl-exposed Michigan dairy farmers showed a significant decrease in the absolute numbers and percentages of T- and B-lymphocytes and an increase in the number of lymphocytes with no detectable surface markers. In addition, there was a significant reduction in *in vitro* immune function. Both the T- and B-lymphocyte subpopulations showed evidence of functional defects (Bekesi *et al.*, 1979, 1987).

In one study, the distribution of polybrominated biphenyls in body tissues and the partitioning ratio between types of tissues were determined for adult male and female Michigan chemical manufacture workers (Eyster *et al.*, 1983). The men and women had similar ratios of the various polybrominated biphenyls in their adipose tissue. Cord blood contained about 10% of the concentration of polybrominated biphenyls found in maternal serum, indicating a partial placental transfer from mother to fetus. The concentration of polybrominated biphenyls in milk was over 100 times greater than the concentration in maternal serum. The polybrominated biphenyl concentration in the feces represented only a minor proportion of the total body burden, suggesting a slow rate of excretion. Tuey and Matthews (1980) estimated that the half-life for elimination of the polybrominated biphenyl body burden in humans was 6.5 years.

Animals

The acute oral LD_{50} of polybrominated biphenyls in rats is 21.5 g/kg, indicating a compound of extremely low acute toxicity (Sleight and Sanger, 1976). Gupta and Moore (1979) estimated the 1-month oral LD_{50} (90-day observation) in rats to be 65 mg/kg per day in females and 149 mg/kg per day in males. Pathologic changes included liver enlargement and atrophy of the spleen and thymus. Dermal application of polybrominated biphenyls to rabbit ears resulted in hyperkeratosis (Needham *et al.*, 1982).

In subchronic studies, polybrominated biphenyl administration has been associated with gross and histopathologic changes in the liver and, to a lesser extent, the kidney and thyroid. Administration of polybrominated biphenyls to rats for 30 days at doses ranging from 1 to 500 ppm in the diet resulted in increased liver-weight-to-body-weight ratios in all groups. Histopathology of the livers revealed swelling and vacuolization of the hepatocytes and increased numbers of mitochondria, proliferation of smooth endoplasmic reticulum, and myeloid bodies at the highest doses (Sleight and Sanger, 1976). Akoso *et al.* (1982) reported increased thyroid weights and decreased triiodothyronine concentrations in rats fed 100 ppm polybrominated biphenyls for 30 days. Extensive thyroid hyperplasia, follicular cell hypertrophy, and a lack of colloid were reported.

In a 6-month toxicity study of polybrominated biphenyls at dose levels of 0.1 to 10 mg/kg by gavage with mice and rats, body weights were decreased and feed consumption was increased in both species, indicating poor feed utilization (Gupta *et al.*, 1983). Decreased thymus weights in rats and increased liver weights were observed in both species. Hepatocytomegaly, proliferation of endoplasmic reticulum, and excess lipid accumulation were noted in the livers. Hepatic porphyria was observed in both species. Histopathologic changes in the thyroid and decreased serum triiodothyronine concentrations were also noted. Cook *et al.* (1978) found chronic inflammatory lymphocytic infiltrates in the liver, kidney, lung, and small intestine of Michigan dairy cows exposed to polybrominated biphenyls. The histopathologic changes in the spleen and lymph node and the prevention of the typical thymic involution indicated a

high degree of immunological activity; however, the high susceptibility of the animals to infections suggested impairment of the immune system. The testes of some exposed bulls were devoid of spermatozoa, and hyperkeratosis was noted in some animals.

Polybrominated biphenyls can alter the immune response in animals. Depressed cell-mediated and humoral immunity has been reported in rats and mice (Luster *et al.*, 1978; Fraker, 1980). Fraker reported depressed IgM and IgG levels in mice exposed to polybrominated biphenyls in the diet; both B-cells and helper T-cells were apparently affected. At the highest exposure level, 1,000 ppm, 40% of the mice were athymic and incapable of producing an immune response after 14 days of exposure (Fraker, 1980).

Polybrominated biphenyls are neurotoxic in rats. Oral exposure to polybrominated biphenyls at a cumulative dose of 390 or 1,300 mg/kg over 6 months or 660 mg/kg over 1 month produced a decrement in muscular functioning, including a depression in locomotor activity, impaired forelimb grip strength, and decreased hindlimb extensor responses (Tilson and Cabe, 1979). Gause *et al.* (1979) demonstrated that Ca^{++} binding to synaptic plasma membranes is significantly reduced in polybrominated biphenyl-treated rats due to a loss of Ca^{++} binding sites on the membrane. Because Ca^{++} uptake is necessary for neurotransmitter release, this reduced binding may be involved in behavioral changes.

Polybrominated biphenyls appear to be weakly teratogenic, causing exencephaly and cleft palate in mice (Corbett *et al.*, 1975). Rats, mice, and nonhuman primates exposed prenatally to polybrominated biphenyls have low birth weights and decreased growth rates (Corbett *et al.*, 1975; Lambrecht *et al.*, 1978). McCormack *et al.* (1981) demonstrated the biological stability of polybrominated biphenyls in a multigeneration rat study. Polybrominated biphenyls fed to pregnant and lactating dams induced liver changes including enlargement, histopathologic changes, and decreased vitamin A content in the F_1 and F_2 generations. Liver changes in the F_2 generation included cytoplasmic vacuolization and proliferation of smooth endoplasmic reticulum, indicative of microsomal enzyme induction.

Polybrominated biphenyls are potent inducers of hepatic microsomal enzymes and produce a "mixed" type induction; that is, they induce enzymes typical of both phenobarbital and 3-methylcholanthrene (Dent *et al.*, 1976). Microsomal enzyme induction was seen in mice, dogs, pigs, cows, hamsters, and guinea pigs (Fries, 1985).

GENETIC TOXICITY

Polybrominated biphenyls have been assayed for mutagenic and clastogenic activity in a variety of bacterial and mammalian test systems. Hexabromobiphenyl was negative for induction of gene mutations in four strains of *Salmonella typhimurium* (Haworth *et al.*, 1983), and polybrominated biphenyls were negative for mutation induction at the HGPRT locus in rat liver epithelial cells and in human fibroblasts cultured with rat hepatocytes to provide exogenous metabolic activation (Williams *et al.*, 1984). In addition, no increase in the frequency of chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with hexabromobiphenyl (Galloway *et al.*, 1987) or in bone marrow cells of male Holtzman rats administered polybrominated biphenyls in feed or Swiss albino mice administered polybrominated biphenyls by gavage (Garthoff *et al.*, 1977; Wertz and Ficsor, 1978). A slight increase in the frequency of sister chromatid exchanges was noted in Chinese hamster ovary cells treated with concentrations of hexabromobiphenyl which produced severe cell cycle delay (Galloway *et al.*, 1987). Finally, results were uniformly negative in tests for induction of unscheduled DNA synthesis in rat hepatocytes assayed *in vitro* as primary cultures (Williams *et al.*, 1984; Tennant *et al.*, 1986) or *in vivo* (Mirsalis *et al.*, 1985). Thus, the available data indicate that polybrominated biphenyls are not genotoxic.

Two structural analogues, decabromobiphenyl and Firemaster 680®, the specific polybrominated biphenyl mixture involved in the Michigan incident, were negative for mutation induction in *S. typhimurium* (Millischer *et al.*, 1979; Zeiger *et al.*, 1987). Doses of 5,000 to 20,000 mg/kg decabromobiphenyl, administered twice by gavage at 24-hour intervals, were also reported to be negative in a test for micronucleus induction in bone marrow polychromatic erythrocytes of CFLP mice (Millischer *et al.*, 1979).

CARCINOGENICITY

Polybrominated biphenyls are carcinogenic in rats and mice. Male and female Sherman rats were given a single 1,000 mg/kg dose of polybrominated

biphenyls by gavage and were examined at 2, 6, 10, or 14 months (Kimbrough *et al.*, 1978). An increased incidence of neoplastic liver nodules was observed in treated animals at 14 months. In a follow-up study, Kimbrough *et al.* (1981) administered a single 1,000 mg/kg dose, 12 doses of 100 mg/kg, or a single dose of 200 mg/kg polybrominated biphenyls by gavage to female Sherman rats. The animals were killed 22 to 26 months after the last dose. The incidence of hepatocellular carcinomas was 24 of 58 in the 1,000 mg/kg group and 17 of 28 in the group administered 12 doses of 100 mg/kg. In the group receiving a single dose of 200 mg/kg, the liver of 5 of 16 animals had neoplastic nodules. The liver of control rats had no neoplastic nodules.

Studies conducted by the NTP showed that polybrominated biphenyls are carcinogenic in Fischer 344/N rats and B6C3F$_1$ mice (Gupta *et al.*, 1983; NTP, 1983). Rats and mice of each sex were given 125 gavage doses of polybrominated biphenyls over a 6-month period at concentrations of 0, 0.1, 0.3, 1.0, 3.0, and 10 mg/kg body weight and were observed for an additional period of 23 months for rats and 24 months for mice. In rats, there were significant increases of hepatocellular carcinomas in the 3 and 10 mg/kg dose groups (3 mg/kg: males, 7/33; females, 3/19; 10 mg/kg: males, 7/31; females, 7/20). No hepatocellular carcinomas were seen in control rats. The incidence of hepatocellular carcinomas in mice was increased only in the 10 mg/kg group (males: control, 12/25; 10 mg/kg, 21/22; females: control, 0/13; 10 mg/kg, 7/8).

The International Agency for Research on Cancer (IARC, 1987) has determined that there is sufficient evidence to consider polybrominated biphenyls carcinogenic in animals. A study of the mortality of workers potentially exposed to a number of chemicals did not show an increased incidence of neoplasms in populations exposed to polybrominated biphenyls (Wong *et al.*, 1984).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF POLYBROMINATED BIPHENYLS

Polybrominated biphenyls were obtained from Monsanto Corporation (St. Louis, MO) in one lot (FF1312-FT), which was used throughout the studies. Purity and identity analyses were conducted by the study laboratory, Battelle Columbus Laboratories (Columbus, OH), and are discussed in Appendix F. The bulk chemical was identified as a mixture of polybrominated biphenyls by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy.

The purity of the material was determined by elemental analyses, Karl Fischer water analysis, ashing to determine inorganic content, gas chromatography, high-performance liquid chromatography, and gas chromatography/mass spectroscopy. Elemental analyses for carbon and hydrogen were slightly higher and analysis for bromine was slightly lower than the theoretical values for hexabromobiphenyl. Karl Fischer water analysis indicated 0.15% water. Ashing indicated 1.4% inorganic material. Gas chromatography indicated nine components by electron capture detection and 10 components by flame ionization detection. High-performance liquid chromatography indicated eight components. Gas chromatography/mass spectroscopy indicated nine components, with the major component identified as a hexa isomer (75%-80%), and also detected biphenyl.

Stability studies performed by gas chromatography indicated that polybrominated biphenyls were stable as a bulk chemical for 2 weeks at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at 4° C at the testing laboratory throughout the studies. The stability of the bulk chemical was monitored periodically by spectroscopy and gas chromatography during the 2-year studies. No significant deterioration of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared weekly by mixing appropriate amounts of polybrominated biphenyls with feed (Table F2). Stability studies were conducted by the study laboratory using gas chromatography. Stability of the dose formulations was verified for at least 7 days when stored at or below room temperature. During the gestational and 2-year studies, the dose formulations were stored in plastic-lined tin cans at or below room temperature.

During the 2-year studies, the study laboratory conducted periodic analyses of the dose formulations using gas chromatography as described in Appendix F. Approximately 77% (43/56) of the dose formulations were prepared within 10% of the target concentrations (Table F3).

GESTATIONAL STUDIES AND DETERMINATION OF MAXIMUM PERINATAL DOSE

Female F344/N rats and C57BL/6N mice were exposed to 0, 1, 3, 10, or 30 ppm polybrominated biphenyls in feed for 60 days before breeding and throughout gestation and lactation. Females were bred to previously unexposed male F344/N rats or C3H/HeN mice. Four pregnant animals from each group were evaluated on prenatal day 18 (rats) or 17 (mice) for numbers of implantations, live fetuses, fetuses per litter, fetal weights, placental weights, and absolute and relative liver weights. Litter weights of rats were recorded on day 0. Pups were culled to eight per litter on day 4 (rats) or day 7 (mice) and weighed. Studies were performed on day 12 postpartum on four dams and litters (culled to five pups per litter) from each exposure group to determine absolute and relative liver weights.

After being weaned on day 28 postpartum, selected weanlings (10 per group) were continued at the same exposure level for 4 weeks. No more than one male and one female rat or mouse from the same litter were placed in the postweaning exposure groups. Following the 28-day period of exposure, all animals were killed with carbon dioxide and a complete necropsy was performed. Livers were weighed for all animals. A histopathologic examination was performed on all major organs from control and

30 ppm animals. In addition to gross lesions, the liver was examined from all exposed animals. Tissues examined are listed in Table 1.

2-YEAR STUDIES

Study Design

Groups of 60 rats and 60 mice received perinatal exposure (F_0), adult exposure (F_1), or both to various concentrations of polybrominated biphenyls (Table 1).

Female F344/N rats were exposed to 0, 1, 3, or 10 ppm in feed for 60 days before breeding. Female C57BL/6N mice were exposed to 0, 3, 10, or 30 ppm in feed for 60 days before breeding. After breeding to previously unexposed males (F344/N rats, C3H/HeN mice), all females were housed singly and were continued on their previous diet. Exposure continued throughout pregnancy and lactation. Weaning occurred on day 28 postpartum, and dietary exposure at these same concentrations continued until the pups were approximately 8 weeks of age. See Figure 1 for the outline of the study design.

On postpartum day 4 (rats) or day 7 (mice), litters were culled to a maximum of eight pups, and the number, sex, and body weight of pups were recorded. After weaning, pups were weighed and separated by sex, and litter mates were cohoused. At approximately 8 weeks of age, groups of 60 male and 60 female pups began receiving the adult (F_1) dietary concentrations (0, 3, 10, or 30 ppm), and were continued on these diets for up to 2 years. After 9 months of polybrominated biphenyls administration, 10 animals from each group were evaluated.

Source and Specification of Breeder Animals

Male and female F344/N rats and male C3H/HeN and female C57BL/6N mice were obtained from Charles River Breeding Laboratories (Kingston, NY). Rats were observed for 17 to 24 days and mice for 9 to 23 days. Rats were 7 to 9 weeks old and mice were 5 to 9 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Animals were housed five per cage. Cages were rotated within racks and racks were rotated within rooms every week. Feed and water were available *ad libitum*. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

Clinical observations were made twice daily, and findings were recorded. F_1 animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter. Necropsies were performed on all animals.

At the 9-month interim evaluation, blood was drawn from the orbital sinus plexus of rats for hematology and clinical chemistry analyses. The parameters measured are listed in Table 1. The adrenal gland, brain, heart, right kidney, liver, lung, ovary, pituitary gland, prostate gland, right testis, thymus, thyroid gland, and uterus of each animal were weighed at necropsy. Polybrominated biphenyl levels in pooled livers were determined at 9 months from groups of three male or female rats receiving 0:0, 0:30, 10:0, or 10:30 ppm and three male or female mice receiving 0:0, 0:30, 30:0, or 30:30 ppm. Further details of the interim evaluations are presented in Table 1.

Animals found in a moribund state, selected for the 9-month interim evaluations, or surviving to the end of the 2-year studies were killed with CO_2. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on all animals evaluated at 9 months, all animals dying or killed moribund prior to the end of the studies, all control animals, all rats receiving 0:30 (F_0:F_1) or 10:30 ppm, and all mice receiving 0:30 or 30:30 ppm. Tissues examined are listed in Table 1.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Toxicology Data Management System. The microscope slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet-tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated by the quality assessment laboratory. The liver, spleen, and forestomach of

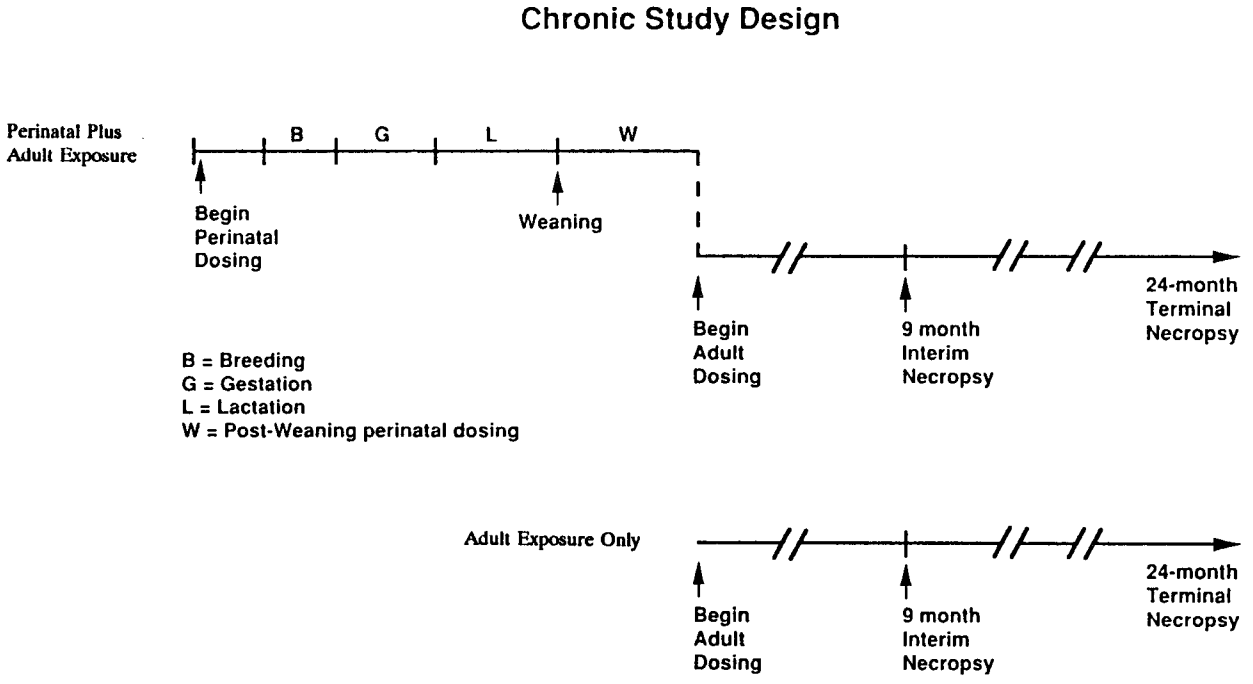

FIGURE 1
Chronic Study Design

male and female rats and the liver, kidney, spleen, mesenteric lymph nodes, and pancreas from male and female mice were reviewed microscopically by the quality assessment pathologist for both neoplastic and nonneoplastic lesions. All neoplastic diagnoses in all tissues from all rats and mice and all tissues from a randomly selected 10% of the control and high-dose rats and mice were reevaluated microscopically by a quality assessment pathologist.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnosis between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chair to the PWG for review. Tissues examined included the liver and forestomach of male rats, the liver of female rats, the kidney, liver, skin, and thyroid gland of male mice, and the bone marrow, kidney, liver, pituitary gland, skin, thyroid gland, and uterus of female mice. The PWG members included the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of exposure groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the PWG consensus. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

The experimental design of these studies was complex (a 4 × 4 matrix with missing cells), and both perinatal and postnatal effects were evaluated. The effect of adult-only exposure to polybrominated biphenyls (i.e., the standard 2-year study design) was analyzed by comparison of F_0:F_1 groups 0:0, 0:10, and 0:30. To determine perinatal effects, supplemental analyses were carried out in addition to the usual comparison of exposed groups to controls. Specifically, for a fixed adult (F_1) exposure concentration, the effect of varying perinatal (F_0) exposure was evaluated. For example, in rats, comparisons were made between groups 0:0 and 10:0, among groups 0:10, 3:10, and 10:10, and between groups 0:30 and 10:30. Comparisons were also made between groups with varying perinatal and adult exposure concentrations and the 0:0 ppm control group. It is recognized that these multiple comparisons are not all strictly independent, but taken collectively, they should provide a reasonable evaluation of the overall effects of perinatal and adult exposure to polybrominated biphenyls.

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Statistical analyses for a possible dose-related effect on survival were performed using the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of all nonneoplastic lesions and most neoplasms (Tables A2, B2, C2, and D2) are also given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not

significantly enhance the fit of the model. The exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms (e.g., leukemia), and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of neoplasm incidence and reported P values are one sided. The procedures described above were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in these studies were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluations, the Fisher exact test, a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the multiple comparison procedures of Dunnett (1955). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the multiple comparison methods of Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's or Dunn's test).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of neoplasm incidence. Consequently, control neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Quality Assurance Methods

As study records for the 2-year studies were submitted to the NTP archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of the NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all had been resolved or were otherwise addressed during the preparation of this Technical Report.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polybrominated Biphenyls

Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species F_0 and F_1 rats: F344/N F_0 mice: C3H/HeN males and C57BL/6N females F_1 mice: B6C3F_1	F_0 and F_1 rats: F344/N F_0 mice: C3H/HeN males and C57BL/6N females F_1 mice: B6C3F_1
Animal Source F_0: Charles River Breeding Laboratories (Kingston, NY) F_1: bred at the study laboratory from F_0 animals	F_0: Charles River Breeding Laboratories (Kingston, NY) F_1: bred at the study laboratory from F_0 animals
Size of Study Groups 10 males and 10 females	60 males and 60 females
Doses Rats and mice: 0, 1, 3, 10, or 30 ppm polybrominated biphenyls in feed	F_0 females administered perinatal (F_0) doses in feed from 60 days before breeding through the weaning of the F_1 generation; pups administered same diet as dams from weaning at week 4 until 8 weeks of age, then administered adult (F_1) doses. The following concentrations (ppm) of polybrominated biphenyls were administered in feed:
Time Held Before Study F_0 females: 10 days (rats); 24-31 days (mice)	F_0 females: 17-24 days (rats); 9-23 days (mice)
Average Age When Placed on Study F_0 females: 6-7 weeks (rats); 8-10 weeks (mice)	F_0 females: 7-9 weeks (rats); 5-9 weeks (mice) F_1: 8 weeks (age when adult dosing began)
Date of First Dose F_0 females: 11 September 1981 (rats); 20 November 1981 (mice)	F_0 females: 18 March 1983 (rats); 16 December 1982 (mice) F_1: 17 August 1983 (male rats) 15 August 1983 (female rats) 18 May 1983 (male mice) 16 May 1983 (female mice)

Rats		Mice	
F_0	F_1	F_0	F_1
0	0	0	0
0	10	0	10
0	30	0	30
1	3	3	3
3	10	10	10
10	0	30	0
10	10	30	10
10	30	30	30

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polybrominated Biphenyls
(continued)

Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Duration of Dosing	
F_0 females: from 60 days before breeding through weaning F_1: 7 days a week for up to 8 weeks (4 weeks post weaning)	F_0 females: from 60 days before breeding through weaning F_1: F_0 doses through gestation, lactation, and 4 weeks post weaning; F_1 doses 7 days/week for 104 weeks (rats) or 105 weeks (mice)
Necropsy Dates	
Rats: 30 January 1982 - 8 February 1982 Mice: 10-19 April 1982	Interim: 15-18 May 1984 (rats); 13-16 February 1984 (mice) Terminal: 12-21 August 1985 (rats); 13-20 May 1985 (mice)
Average Age at Necropsy	
F_0: 24-25 weeks F_1: 8 weeks	F_0: 24-25 weeks F_1: 11 or 26 months
Method of Sacrifice	
CO_2	CO_2
Animals per Cage	
F_0 rats: 1 female and 1 male at night during breeding F_0 mice: 3 females and 1 male during breeding Females housed singly after becoming pregnant F_1: 5 after weaning	F_0 rats: 2 females and 1 male during breeding F_0 mice: 3 females and 1 male during breeding Females housed singly after becoming pregnant F_1: 5 after weaning
Method of Animal Distribution	
F_0 females: Randomized by weight with a computer randomization program F_1: Random among littermates of same sex; groups included no more than one male and one female from a single litter	F_0 females: Randomized by weight with a computer randomization program F_1: Random among littermates of same sex; groups included no more than two males and two females from a single litter
Method of Animal Identification	
Ear tag	Ear tag
Diet	
Purina Certified Rodent Chow meal (No. 5002); available *ad libitum* except to rats during breeding, when available only during the day	Purina Certified Rodent Chow meal (No. 5002); available *ad libitum*
Water	
Tap water (City of Columbus) via plastic disposable water bottles, available *ad libitum*	Tap water (City of Columbus) via plastic disposable water bottles, available *ad libitum*
Cages	
Polycarbonate (Lab Products, Inc., Garfield, NJ), changed once or twice weekly, except during week 1 postpartum	Polycarbonate (Lab Products, Inc., Garfield, NJ), changed three times weekly, except during week 1 postpartum

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polybrominated Biphenyls
(continued)

Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Bedding	
Absorb-Dri hardwood chips (Absorb-Dri, Inc., Maywood, NJ), changed once or twice weekly, except during week 1 postpartum	Absorb-Dri hardwood chips (Absorb-Dri, Inc., Maywood, NJ) through 10 September 1984, then BetaChips (Northeastern Products Corp., Warrensburg, NY); changed three times weekly, except during week 1 postpartum
Racks	
Stainless steel (Lab Products, Inc., Garfield, NY), changed once weekly	Stainless steel (Lab Products, Inc., Garfield, NY), changed once weekly
Nesting Material	
None	Nestlets (Ancare Corp., Manhasset, Long Island, NY)
Animal Room Environment	
Average temperature: 22° ± 2.5° C Relative humidity: 43% ± 7.0% Fluorescent light: 12 hours/day Room air changes: 16-20 changes/hour	Average temperature: 18°-27° C (rats); 19°-27° C (mice) Relative humidity: 20%-86% (rats); 20%-69% (mice) Fluorescent light: 12 hours/day Room air changes: 15 changes/hour
Type and Frequency of Observation	
F_0: Observed twice/day; weighed once/week except during immediate postnatal period; clinical observations recorded twice/day F_1: Observed twice/day; litter weights recorded on day 0 (rats); weighed on day 4 (rats) or 7 (mice), on day 28, and once/week thereafter; clinical observations recorded twice/day	F_0: Observed twice/day; weighed once/week except during immediate postnatal period; clinical observations recorded daily F_1: Observed twice/day; weighed on day 4 (rats) or 7 (mice), on day 28, once/week for 17 weeks (through week 13 of adult dosing), once/month thereafter; clinical observations recorded once/week for 13 weeks, once/month thereafter
Necropsy	
F_0: None F_1: Necropsy performed on all animals. Livers were weighed.	F_0: None F_1: Necropsy performed on all animals. The following organs were weighed at 9 months: adrenal gland, brain, heart, right kidney, liver, lung, ovary, pituitary gland, prostate gland, right testis, thymus, thyroid gland, uterus

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Polybrominated Biphenyls
(continued)

Gestational and Maximum Perinatal Dose Determination Studies	2-Year Studies
Histopathology	
Complete histopathology performed on all control and 30 ppm F_1 rats and mice. Tissues examined included: adrenal gland, bone and marrow, brain, clitoral or preputial gland, epididymis, esophagus, gross lesions, heart, kidney, large intestine, liver, lung, lymph node, mammary gland, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, skin, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues examined for lower exposure groups included the liver, spleen, and stomach.	F_0: None F_1: Complete histopathology performed on all animals that died or were killed moribund prior to study termination, all animals from the 9-month interim evaluation, and all animals in the control and high-dose groups. Tissues examined included: adrenal glands (cortex and medulla), bone (femur) and marrow, brain, cecum, clitoral or preputial gland (rats), colon, duodenum, epididymis, esophagus, gallbladder (mice), gross lesions, heart, ileum, jejunum, kidney, liver, lung, mammary gland (females), mandibular or mesenteric lymph nodes, nasal turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, skin, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. At the 9-month interim evaluations, the liver was examined from all other exposure groups of rats and mice. At study termination, gross lesions and liver in mice and gross lesions, liver, spleen, and stomach in rats were examined from all other exposure groups.
Clinical Pathology	
None	Clinical pathology studies on rats of each sex from each exposure group at 9 months. ***Hematology:*** hematocrit, hemoglobin, erythrocyte count, mean erythrocyte volume, platelets, reticulocytes, and total leukocyte count ***Clinical chemistry:*** cholesterol and triglycerides
Tissue Concentration Studies	
None	Polybrominated biphenyl levels in pooled livers were determined at 9 months from groups of three male or female rats receiving 0:0, 0:30, 10:0, or 10:30 ppm and mice receiving 0:0, 0:30, 30:0, or 30:30 ppm.

RESULTS

RATS

GESTATIONAL STUDY: DETERMINATION OF MAXIMUM PERINATAL DOSE

The gestational study was conducted to determine the dietary concentrations for perinatal exposure to be used in the 2-year study. Selected dams from each group were evaluated at gestation day 18 for reproductive effects. The numbers of implantations, live fetuses, and fetuses per litter were slightly decreased for dams that received 3 or 30 ppm (Table 2). All rat dams not designated for evaluation at gestation day 18 survived to the end of the study.

The mean litter weight on day 0 was decreased in the 30 ppm group (Table 3). The number of pups surviving to day 28 was similar among exposed and control groups. Mean pup weight was decreased by approximately 20% in the 30 ppm exposure group. The absolute and relative liver weights of pups in the 10 and 30 ppm groups and relative liver weights of the 1 and 3 ppm groups examined at day 12 were increased compared to those of the controls (Table 4). The body weights and absolute liver weights of dams exposed to 30 ppm were lower than those of the controls at day 12.

Selected pups from the various groups were weaned onto feed containing polybrominated biphenyls. All weanling rats receiving polybrominated biphenyls in feed survived until the end of the study (Table 5). The mean body weight gains and final mean body weights of males and females that received 30 ppm were decreased more than 10% relative to those of the controls.

TABLE 2
Prenatal Day 18 Litter Data for Rats in the Maximum Perinatal Dose Determination Feed Study of Polybrominated Biphenyls

	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm
Litters	4	4	4	4	4
Implantations	39	35	28	40	31
Live fetuses	39	35	24	40	31
Fetuses/litter[a]	9.8 ± 1.3	8.8 ± 1.0	6.0 ± 3.4*	10.0 ± 1.8	7.8 ± 0.5
Fetal weight[a]	1.29 ± 0.15	1.13 ± 0.33**	1.30 ± 0.15	1.31 ± 0.16	1.37 ± 0.08
Fetal liver weight[a]					
Absolute	0.09 ± 0.01	0.09 ± 0.01[b]	0.09 ± 0.01	0.10 ± 0.02	0.10 ± 0.01
Relative	6.98 ± 0.60	6.72 ± 0.53	6.98 ± 0.39	7.47 ± 0.78	6.45 ± 0.36
Placental weight[a]	0.35 ± 0.03	0.35 ± 0.07	0.39 ± 0.06	0.36 ± 0.04	0.38 ± 0.03

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Mean ± standard deviation. Fetal body, liver, and placental weights are given in grams; relative liver weights are given as percent of body weight.
[b] Liver weights from one group were excluded because fetuses appeared to be on day 16 of gestation.

TABLE 3
Survival, Sex Ratios, and Mean Body Weights of Rat Pups in the Maximum Perinatal Dose Determination Feed Study of Polybrominated Biphenyls

	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm
Precull					
Litters on day 0[a]	19	22	17	21	20
Pups on day 0	162	159	134	176	145
Litter weight on day 0[b]	49.5 ± 5.8	41.9 ± 12.8	46.5 ± 9.8	46.3 ± 8.6	38.8 ± 13.6**
Pups dead days 0-4	0	0	0	1	2
Postcull					
Litters on day 4	19	22	17	21	19
Pups on day 4	162	159	134	175	143
Pup weight on day 4[b]	8.8 ± 0.9	8.3 ± 0.9	8.6 ± 0.9	7.8 ± 1.2**	6.9 ± 0.8**
Male/female ratio on day 4	0.97	1.30	1.09	1.08	1.01
Pups dead days 4-28	43	39	40	52	47
Litters on day 28[c]	15	18	13	17	14
Pups on day 28	119	120	94	123	96
Pup weight on day 28[b]	58.3 ± 5.8	58.4 ± 4.9	59.3 ± 5.4	54.3 ± 6.3	46.7 ± 4.1**

** Significantly different (P≤0.01) from the control group by Dunnett's test
[a] Does not include four litters per group evaluated on gestation day 18
[b] Body weights are given in grams (mean ± standard deviation).
[c] Does not include litters evaluated on day 12 postpartum

TABLE 4
Day 12 Postpartum Relative Liver Weights for Rat Pups Exposed Perinatally to Polybrominated Biphenyls and Rat Dams Exposed to Polybrominated Biphenyls in the Diet[a]

	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm
Pups					
n	20	20	20	20	20
Body weight	22.0 ± 1.0	21.0 ± 2.4	19.2 ± 0.8**	19.8 ± 1.5**	15.7 ± 1.1**
Liver weight					
Absolute	0.60 ± 0.04	0.62 ± 0.08	0.57 ± 0.04	0.71 ± 0.05**	0.72 ± 0.06**
Relative	2.73 ± 0.14	2.97 ± 0.19**	2.97 ± 0.18**	3.59 ± 0.26**	4.59 ± 0.21**
Dams					
n	4	4	4	4	4
Body weight	237 ± 10	222 ± 16	213 ± 13	215 ± 10	200 ± 17**
Liver weight					
Absolute	11.57 ± 1.21	9.83 ± 0.67	10.66 ± 1.01	10.63 ± 1.06	9.78 ± 0.44*
Relative	4.89 ± 0.50	4.44 ± 0.08	5.01 ± 0.29	4.94 ± 0.37	4.90 ± 0.43

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Liver and body weights are given in grams; relative liver weights are given as percent of body weight (mean ± standard deviation).

TABLE 5
Survival and Mean Body Weights of Weanling Rats in the Maximum Perinatal Dose Determination Feed Study of Polybrominated Biphenyls

Concentration (ppm)	Survival[a]	Mean Body Weight (g)[b]			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	61 ± 2	203 ± 4	142	
1	10/10	62 ± 2	209 ± 3	147	103
3	10/10	64 ± 1	199 ± 3	135	98
10	10/10	56 ± 3	183 ± 5**	127	90
30	10/10	49 ± 1**	177 ± 4**	128	87
Female					
0	10/10	60 ± 2	150 ± 2	90	
1	10/10	58 ± 2	144 ± 2	86	96
3	10/10	55 ± 1	137 ± 2**	82	91
10	10/10	53 ± 2*	137 ± 3**	84	91
30	10/10	46 ± 1**	122 ± 2**	76	81

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving/number initially in group
[b] Weights are given as mean ± standard error.

The only clearly treatment-related lesion was liver enlargement of male and female rats from the 10 and 30 ppm groups. Microscopic examination showed hypertrophy, which consisted of enlarged hepatocytes with foamy-appearing cytoplasm; there was marked variation in the size of hepatocyte nuclei in affected livers. Hepatocyte hypertrophy was present in all male rats in the 10 and 30 ppm groups and in 8 of 10 males in the 3 ppm group. Severity increased in an exposure-related manner; it was minimal to mild in the 3 ppm group and moderate in most male rats in the 30 ppm group. In female rats, the incidence and severity of hepatocyte hypertrophy were decreased compared to male rats; lesions were limited to the 10 ppm group (3 of 10 females) and 30 ppm group (9 of 10 females). The severity of this lesion in females receiving 30 ppm was minimal to mild except in two females (one with moderate severity and one with marked severity).

Dose Selection Rationale

No chemical-related effects on fertility or litter size were observed. Also, there were no chemical-related effects on preweaning or postweaning survival in the F_1 generation. However, the mean preweaning litter weight of the 30 ppm group was less than 80% of the mean litter weight of the control group on days 0, 4, and 12. At weaning, the mean pup weight in the 30 ppm group was 80% of the control group mean pup weight. The final mean body weights of males and females receiving 30 ppm were decreased more than 10% compared to those of the controls. Therefore, 10 ppm was selected as the highest exposure level for the perinatal exposure portion of the carcinogenicity study.

2-YEAR STUDY

9-Month Interim Evaluation

The absolute and relative liver weights of males and the relative liver weights of females receiving F_0:F_1 exposures of 0:10, 0:30, 3:10, 10:10, or 10:30 ppm were significantly greater than those of the controls (Table 6). The relative liver weight of males in the 1:3 ppm group and the absolute liver weight of females in the 0:30 ppm group were also significantly increased.

Regardless of the concentration of polybrominated biphenyls given to the F_0 generation (0, 1, 3, or 10 ppm), exposure of male rats to 10 or 30 ppm polybrominated biphenyls for 9 months produced a mild, normochromic, normocytic anemia, indicated by decreased hematocrit and hemoglobin values and erythrocyte counts in males exposed to 30 ppm and decreased hemoglobin and variable hematocrit values in males exposed to 10 ppm (Table E1). Although the change was not statistically significant, animals exposed to 30 ppm polybrominated biphenyls tended to have slightly smaller red cells (decreased mean cell volumes) with appropriate amounts of hemoglobin for erythrocytes of that size, as indicated by the unchanged mean cell hemoglobin concentrations.

In female rats, the anemia in animals exposed only as adults was very mild; females exposed to 30 ppm had decreased hemoglobin values (Table E1). However, in rats exposed perinatally and for 9 months, the anemia, although mild, was more pronounced (decreased hemoglobin and hematocrit values and erythrocyte counts). Additionally, mild to moderate decreases in platelet counts occurred in female rats exposed to 30 ppm polybrominated biphenyls for 9 months, regardless of perinatal treatment.

TABLE 6
Liver Weights and Liver-Weight-to-Body-Weight Ratios for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0:F_1 Concentration (ppm)	Male			Female		
	Body Weight	Absolute Liver Weight	Relative Liver Weight	Body Weight	Absolute Liver Weight	Relative Liver Weight
0:0	413 ± 8	13.56 ± 0.34	32.68 ± 0.56	219 ± 5	6.46 ± 0.17	29.53 ± 0.31
0:10	397 ± 7	17.66 ± 0.37**	44.59 ± 0.79**	200 ± 3**	6.92 ± 0.10	34.66 ± 0.65**
0:30	351 ± 9**	17.55 ± 0.59**	49.93 ± 0.83**	192 ± 5**[b]	7.57 ± 0.29**	39.35 ± 0.73**
1:3	405 ± 7	14.71 ± 0.38	36.31 ± 0.76**	219 ± 5	6.77 ± 0.15	30.96 ± 0.65
3:10	389 ± 8	17.34 ± 0.49**	44.59 ± 0.68**	202 ± 4*	7.04 ± 0.24	34.94 ± 0.71**
10:0	414 ± 7	14.50 ± 0.46	34.94 ± 0.64	215 ± 3	6.81 ± 0.24	31.82 ± 1.15
10:10	398 ± 9	17.81 ± 0.58**	44.70 ± 0.66**	190 ± 4**	6.77 ± 0.18	35.47 ± 0.78**
10:30	356 ± 7**	17.38 ± 0.57**	48.77 ± 0.98**	182 ± 3**	7.17 ± 0.17	39.48 ± 0.34**

* Significantly different (P≤0.05) from the 0:0 ppm group by Dunnett's test
** P≤0.01
[a] Liver weights and body weights are given in grams; liver-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error). Absolute and relative weights are given for groups of 10 animals unless otherwise specified.
[b] n=9

Serum cholesterol concentrations were increased in male groups receiving adult exposure to 10 or 30 ppm polybrominated biphenyls, with the exception of the 3:10 and 10:10 ppm groups, and triglyceride concentrations were decreased in all male groups receiving adult exposure to 10 or 30 ppm. Treatment of the F_0 generation had no effect on serum cholesterol or triglycerides. Similarly, in female rats, cholesterol concentrations were increased by treatment of the F_1 generation, particularly at the 30 ppm exposure level. Serum triglyceride concentrations in females were not affected by exposure.

Pooled liver tissues from groups of three males or three females in the 0:0, 0:30, 10:0, and 10:30 ppm groups were analyzed in duplicate samples for polybrominated biphenyls concentrations. Tissue concentrations in the 0:0 ppm group were 1.23 ppm for males and 1.24 ppm for females (Table G1). The background levels of polybrominated biphenyls in control animals were within the range reported in earlier studies (Corbett *et al.*, 1975; Kimbrough *et al.*, 1978, 1981; Jensen *et al.*, 1984). Polybrominated biphenyls concentrations in the 10:0 ppm group were 0.89 ppm for males and 2.93 ppm for females. The concentrations in the 0:30 and 10:30 ppm groups were 111.65 and 138.39 ppm for males and 78.58 and 79.03 ppm for females.

At necropsy, there were no gross lesions attributed to polybrominated biphenyls exposure. Microscopic effects clearly related to polybrominated biphenyls exposure consisted of a variety of nonneoplastic liver lesions in male and female rats in the 0:10, 0:30, 3:10, 10:0, 10:10, and 10:30 ppm groups. Liver lesions included hypertrophy and cytoplasmic vacuolization of hepatocytes and foci of cellular alteration (eosinophilic foci). Hepatocellular hypertrophy and cytoplasmic vacuolization were generally centrilobular in location and were of minimal to mild severity, except in the 0:30 and 10:30 ppm groups, where these effects were of mild to moderate severity. Although single occurrences of focal cellular alteration were present in several exposure groups, this histopathologic change was seen primarily in female rats in the 10:10 and 10:30 ppm groups. Foci were identified in the livers of 7 of 10 females that received 10:10 ppm and 5 of 10 females that received 10:30 ppm. No hepatocellular neoplasms were present in rats at the 9-month interim evaluation.

Other microscopic lesions possibly related to polybrominated biphenyls exposure were identified in the thyroid gland of male and female rats in the 0:30 and 10:30 ppm groups. Minimal hypertrophy or hyperplasia of the thyroid follicular epithelium occurred in 5 of 10 males and 5 of 10 females in the 0:30 ppm groups; minimal to mild hypertrophy or hyperplasia was present in 6 of 10 males and 5 of 10 females in the 10:30 ppm groups. Two male and two female rats from the 10:30 ppm groups had minimal to mild focal accumulation of macrophages (histiocytic hyperplasia) in the red pulp of the spleen.

Survival

The survival of male rats in the 0:10, 3:10, 10:10, 0:30, and 10:30 ppm groups was less than that of the controls (Table 7 and Figure 2). There were no significant differences in the survival of female rats. Decreases in survival appeared to be related to the increased incidences of mononuclear cell leukemia in exposed males.

Body Weights, Feed Consumption, and Clinical Findings

Final mean body weights of males exposed to 0:10, 0:30, 3:10, 10:10, or 10:30 ppm and females exposed to 0:30, 10:10, or 10:30 ppm were more than 10% lower than those of the controls (Tables 8 and 9 and Figures 3a,b,c, and d). Feed consumption was similar among exposed and control groups (Tables H1 and H2). Adult consumption of 3, 10, and 30 ppm polybrominated biphenyls provided approximately 0.10, 0.40, 0.45, 0.45, 1.35, and 1.40 mg/kg for males and 0.15, 0.45, 0.45, 0.50, 1.20, and 1.55 mg/kg for females. There were no clinical findings that were clearly related to polybrominated biphenyls exposure.

TABLE 7
Survival of Rats in the 2-Year Feed Study of Polybrominated Biphenyls

	F_0:F_1 Concentration (ppm)							
	0:0	10:0	1:3	0:10	3:10	10:10	0:30	10:30
Male								
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	16	16	15	21	25	26	23	24
Natural deaths	5	4	1	6	5	4	3	7
Animals surviving to study termination	29	30[b]	34	23[b]	20[b]	20[b]	24[b]	19[b]
Percent probability of survival at end of study[c]	58	60	68	46	40	40	48	38
Mean survival (days)[d]	696	684	696	689	663	669	685	663
Survival analysis[e]		P=0.882	P=0.424	P=0.333	P=0.049	P=0.055	P=0.329	P=0.033
Female								
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	10	10	9	12	8	10	10	15
Natural deaths	0	1	1	2	3	2	5	2
Animals surviving to study termination	40	39	40[b]	36	39	38	35[b]	33[b]
Percent probability of survival at end of study[c]	80	78	80	72	78	76	70	66
Mean survival (days)[d]	704	709	705	704	706	698	698	699
Survival analysis[e]		P=0.999	P=1.000N	P=0.524	P=0.999	P=0.772	P=0.356	P=0.224

[a] Censored from survival analyses
[b] Includes one animal that died or was killed moribund during the last week of the study
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice)
[e] The results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A lower mortality in an exposure group is indicated by N.

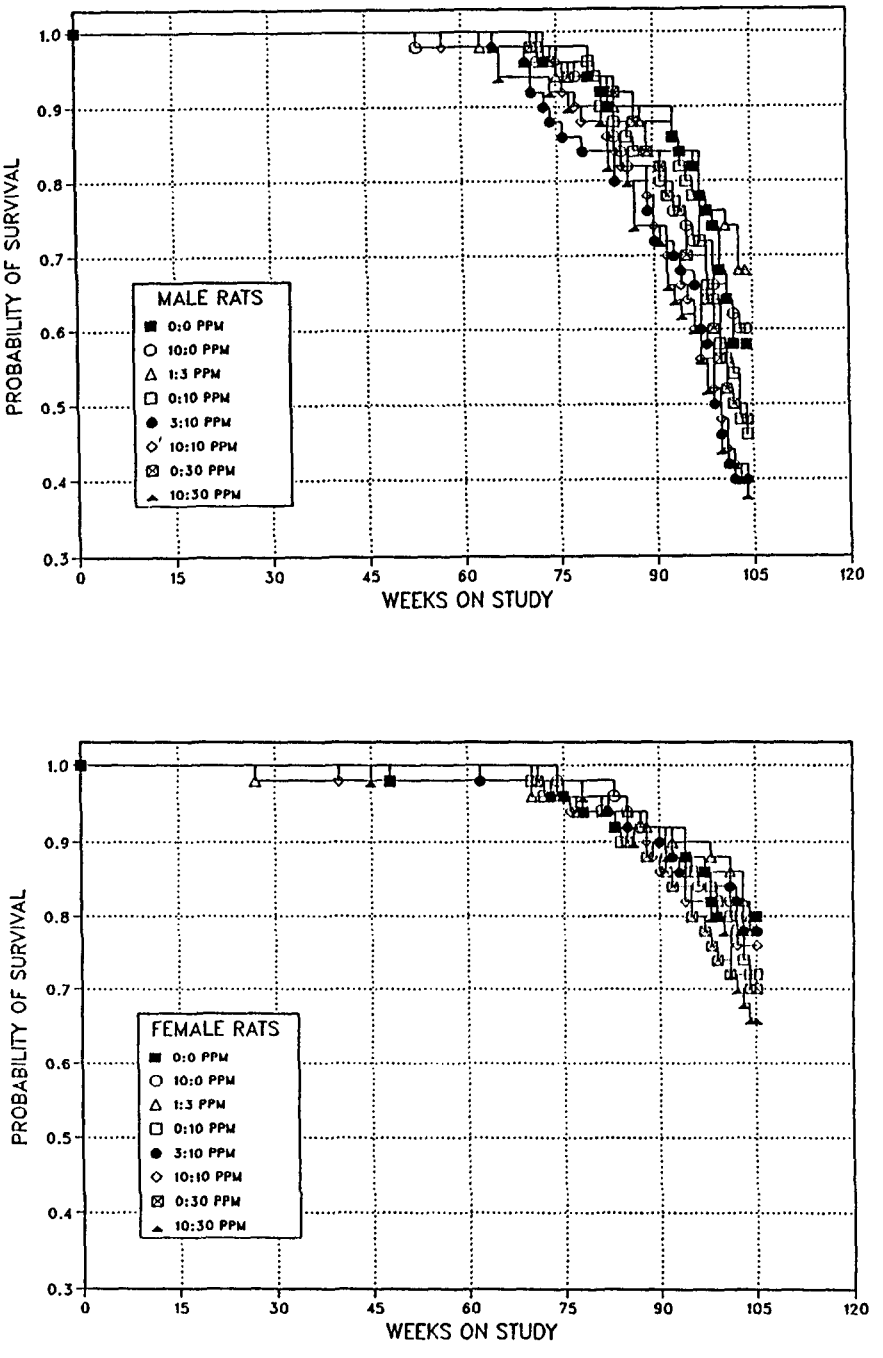

FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered Polybrominated Biphenyls in Feed for 2 Years

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls

Week on Study	0:0 ppm		10:0 ppm			1:3 ppm			0:10 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
-1	194	50	191	98	50	201	104	50	197	102	50
1	222	50	217	98	50	227	102	50	225	101	50
2	244	50	239	98	50	245	100	50	247	101	50
3	257	50	251	98	50	256	100	50	257	100	50
4	269	50	264	98	50	270	100	50	271	101	50
5	276	50	270	98	50	277	100	50	275	100	50
6	285	50	280	98	50	288	101	50	286	100	50
7	294	50	287	98	50	297	101	50	295	100	50
8	302	50	297	98	50	306	101	50	303	100	50
9	311	50	306	98	50	313	101	50	306	98	50
10	320	50	314	98	50	324	101	50	322	101	50
11	327	50	322	98	50	326	100	50	326	100	50
12	332	50	328	99	50	332	100	50	332	100	50
17	360	50	355	99	50	354	98	50	355	99	50
22	375	50	373	99	50	374	100	50	374	100	50
26	386	50	385	100	50	385	100	50	380	98	50
30	405	50	401	99	50	401	99	50	395	98	50
35	415	50	414	100	50	414	100	50	404	97	50
39	414	50	412	100	50	410	99	50	401	97	50
43	431	50	431	100	50	424	98	50	415	96	50
48	434	50	429	99	50	426	98	50	409	94	50
52	442	50	438	99	50	435	98	50	416	94	50
56	448	50	443	99	49	437	98	50	416	93	50
61	451	50	447	99	49	443	98	50	416	92	50
65	451	50	450	100	49	443	98	49	417	92	50
70	459	50	452	98	49	448	98	49	421	92	50
74	459	48	455	99	48	445	97	48	417	91	49
79	459	48	468	102	47	446	97	47	417	91	49
83	459	46	451	98	47	441	96	47	412	90	45
87	461	45	459	100	41	445	97	45	408	89	43
93	461	45	455	99	40	440	95	44	408	89	42
98	451	41	451	100	36	428	95	42	391	87	39
101	437	37	442	101	33	426	97	38	382	87	32
105	436	29	435	100	30	409	94	34	376	86	23
Mean for weeks											
1-13	287		281	98		288	100		287	100	
14-52	407		404	99		403	99		394	97	
53-105	453		451	100		438	97		407	90	

(continued)

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

Week on Study	3:10 ppm			10:10 ppm			0:30 ppm			10:30 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
-1	192	99	50	192	99	50	201	104	50	192	99	50
1	217	98	50	213	96	50	227	102	50	214	96	50
2	235	96	50	235	96	50	248	102	50	233	95	50
3	248	96	50	248	96	50	257	100	50	248	96	50
4	264	98	50	263	98	50	271	101	50	265	99	50
5	269	97	50	270	98	50	277	100	50	270	98	50
6	282	99	50	280	98	50	286	100	50	279	98	50
7	292	99	50	290	99	50	294	100	50	286	97	50
8	301	100	50	298	99	50	302	100	50	292	97	50
9	311	100	50	307	99	50	311	100	50	300	96	50
10	321	100	50	315	98	50	316	99	50	304	95	50
11	325	99	50	320	98	50	323	99	50	310	95	50
12	333	100	50	325	98	50	323	97	50	311	94	50
17	359	100	50	348	97	50	343	95	50	327	91	50
22	375	100	50	368	98	50	353	94	50	336	90	50
26	383	99	50	379	98	50	360	93	50	341	88	50
30	399	99	50	392	97	50	365	90	50	347	86	50
35	406	98	50	401	97	50	367	88	50	352	85	50
39	404	98	50	401	97	50	365	88	50	352	85	50
43	416	97	50	413	96	50	373	87	50	364	84	50
48	409	94	50	411	95	50	368	85	50	359	83	50
52	415	94	50	413	93	50	370	84	50	362	82	50
56	415	93	50	414	92	50	369	82	50	364	81	50
61	417	92	50	417	92	49	371	82	50	364	81	50
65	415	92	50	416	92	49	370	82	50	363	80	50
70	418	91	49	424	92	49	371	81	50	366	80	47
74	413	90	46	409	89	49	368	80	49	366	80	47
79	427	93	43	421	92	44	368	80	47	383	83	45
83	406	88	42	411	90	44	361	79	47	351	76	43
87	407	88	40	409	89	41	358	78	46	354	77	40
93	399	87	36	404	88	37	353	77	41	338	73	37
98	387	86	33	386	86	30	348	77	35	336	75	30
101	377	86	25	375	86	26	341	78	30	321	73	26
105	371	85	20	359	82	21	340	78	25	316	72	20
Mean for weeks												
1-13	283	99		280	98		286	100		276	96	
14-52	396	97		392	96		363	89		349	86	
53-105	404	89		404	89		360	79		352	78	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	10:0 ppm Av. Wt. (g)	10:0 ppm Wt. (% of controls)	10:0 ppm No. of Survivors	1:3 ppm Av. Wt. (g)	1:3 ppm Wt. (% of controls)	1:3 ppm No. of Survivors	0:10 ppm Av. Wt. (g)	0:10 ppm Wt. (% of controls)	0:10 ppm No. of Survivors
-1	137	50	131	96	50	142	104	50	137	100	50
1	155	50	150	97	50	154	99	50	155	100	50
2	161	50	157	98	50	160	99	50	165	102	50
3	167	50	162	97	50	166	99	50	171	102	50
4	173	50	166	96	50	171	99	50	175	101	50
5	172	50	166	97	50	171	99	50	174	101	50
6	175	50	168	96	50	175	100	50	177	101	50
7	171	50	170	99	50	177	104	50	179	105	50
8	179	50	172	96	50	179	100	50	181	101	50
9	183	50	175	96	50	183	100	50	182	99	50
10	182	50	178	98	50	186	102	50	185	102	50
11	184	50	179	97	50	188	102	50	186	101	50
12	186	50	181	97	50	189	102	50	187	101	50
17	193	50	188	97	50	194	101	50	191	99	50
22	201	50	196	98	50	199	99	50	196	98	50
26	203	50	202	100	50	201	99	50	197	97	50
30	213	50	213	100	50	212	100	49	207	97	50
35	217	50	213	98	50	212	98	49	205	94	50
39	215	50	213	99	50	209	97	49	205	95	50
43	226	50	226	100	50	227	100	49	212	94	50
48	224	50	225	100	50	221	99	49	211	94	50
52	238	49	240	101	50	227	95	49	219	92	50
56	239	49	240	100	50	234	98	49	223	93	50
61	246	49	250	102	50	246	100	49	242	98	50
65	250	49	256	102	50	248	99	49	251	100	50
70	267	49	263	99	50	243	91	49	266	100	50
74	267	49	269	101	49	264	99	48	242	91	48
79	281	47	270	96	49	258	92	48	243	86	48
83	279	47	287	103	49	272	97	48	258	92	46
87	280	46	293	105	47	271	97	47	254	91	45
93	291	46	297	102	44	283	97	45	265	91	44
98	295	44	304	103	42	287	97	45	265	90	43
101	292	40	301	103	42	284	97	44	262	90	41
105	293	40	303	103	39	277	95	41	260	89	37
Mean for weeks											
1-13	174		169	97		175	101		176	101	
14-52	214		213	100		211	99		205	96	
53-105	273		278	102		264	97		253	93	

(continued)

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

Week on Study	3:10 ppm Av. Wt. (g)	3:10 ppm Wt. (% of controls)	3:10 ppm No. of Survivors	10:10 ppm Av. Wt. (g)	10:10 ppm Wt. (% of controls)	10:10 ppm No. of Survivors	0:30 ppm Av. Wt. (g)	0:30 ppm Wt. (% of controls)	0:30 ppm No. of Survivors	10:30 ppm Av. Wt. (g)	10:30 ppm Wt. (% of controls)	10:30 ppm No. of Survivors
-1	135	99	50	137	100	50	134	98	50	138	101	50
1	152	102	50	147	95	50	155	100	50	146	94	50
2	161	100	50	152	94	50	162	101	50	153	95	50
3	167	100	50	159	95	50	166	99	50	157	94	50
4	172	99	50	163	94	50	169	98	50	161	93	50
5	170	99	50	164	95	50	168	98	50	161	94	50
6	174	99	50	168	96	50	171	98	50	164	94	50
7	173	101	50	168	98	50	172	101	50	166	97	50
8	173	97	50	170	95	50	174	97	50	168	94	50
9	177	97	50	171	93	50	195	107	50	169	92	50
10	179	98	50	174	96	50	178	98	50	171	94	50
11	180	98	50	174	95	50	177	96	50	170	92	50
12	180	97	50	175	94	50	178	96	50	170	91	50
17	187	97	50	180	93	50	182	94	50	175	91	50
22	192	96	50	183	91	50	185	92	50	176	88	50
26	195	96	50	185	91	50	186	92	50	177	87	50
30	204	96	50	193	91	50	191	90	50	184	86	50
35	202	93	50	193	89	50	189	87	50	183	84	50
39	203	94	50	197	92	50	188	87	50	183	85	50
43	213	94	50	206	91	49	197	87	50	196	87	50
48	214	96	50	207	92	49	195	87	50	190	85	49
52	223	94	50	207	87	49	201	84	50	194	82	49
56	227	95	50	216	90	49	204	85	50	198	83	49
61	237	96	50	233	95	49	208	85	50	203	83	49
65	247	99	49	240	96	49	211	84	50	209	84	49
70	248	93	49	231	87	49	221	83	50	208	78	49
74	252	94	48	229	86	48	217	81	49	208	78	49
79	249	89	48	238	85	47	217	77	48	210	75	48
83	267	96	47	251	90	47	215	77	47	211	76	46
87	270	96	46	254	91	47	214	76	45	208	74	45
93	274	94	44	264	91	43	212	73	43	216	74	44
98	278	94	43	268	91	41	202	68	40	218	74	43
101	273	93	43	268	92	40	210	72	37	211	72	39
105	274	94	39	262	89	38	208	71	36	213	73	33
Mean for weeks												
1-13	172	99		165	95		172	99		163	94	
14-52	204	95		195	91		190	89		184	86	
53-105	258	95		246	90		212	78		209	77	

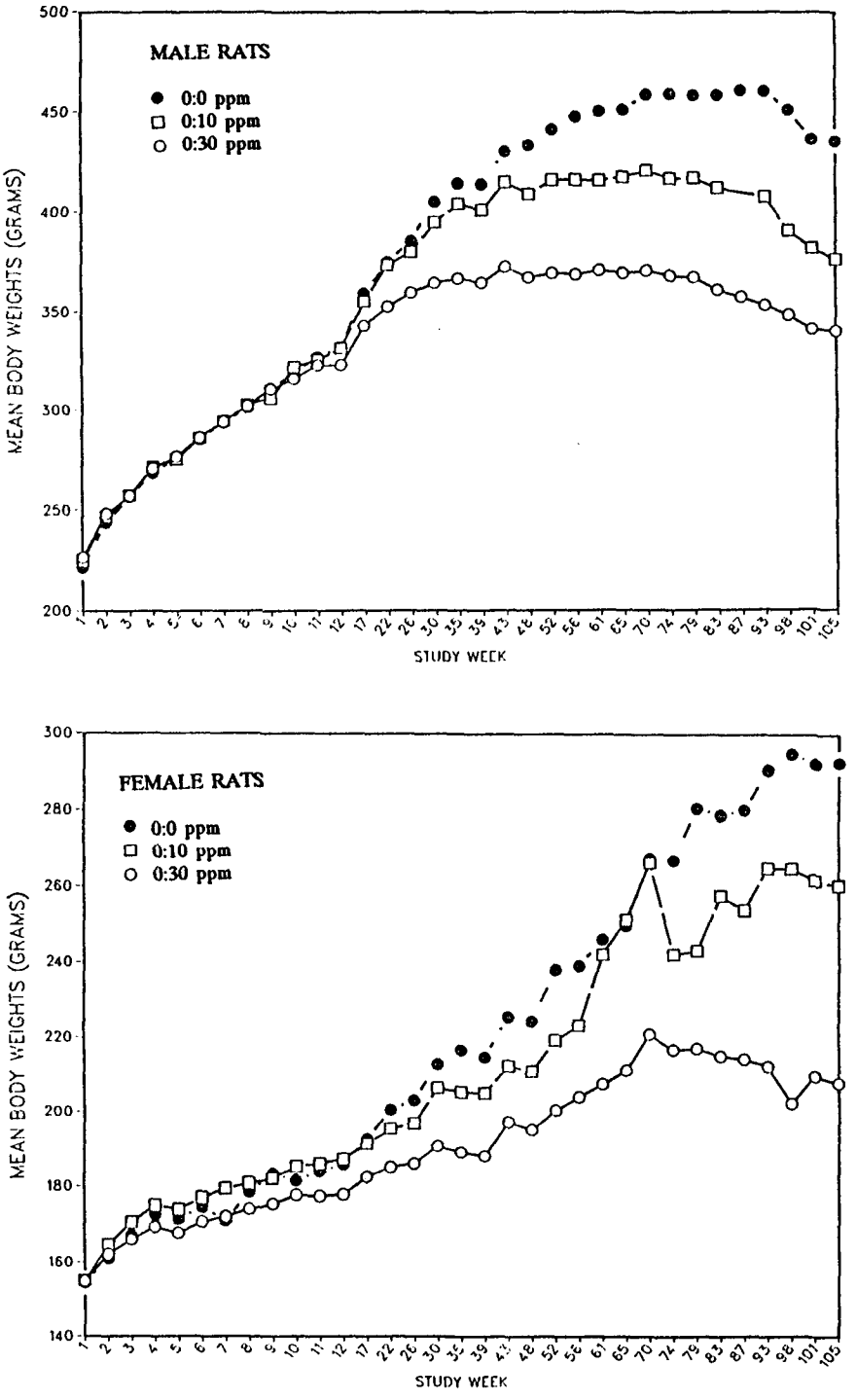

FIGURE 3a
Growth Curves for Rats Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 0:10, and 0:30 ppm Groups

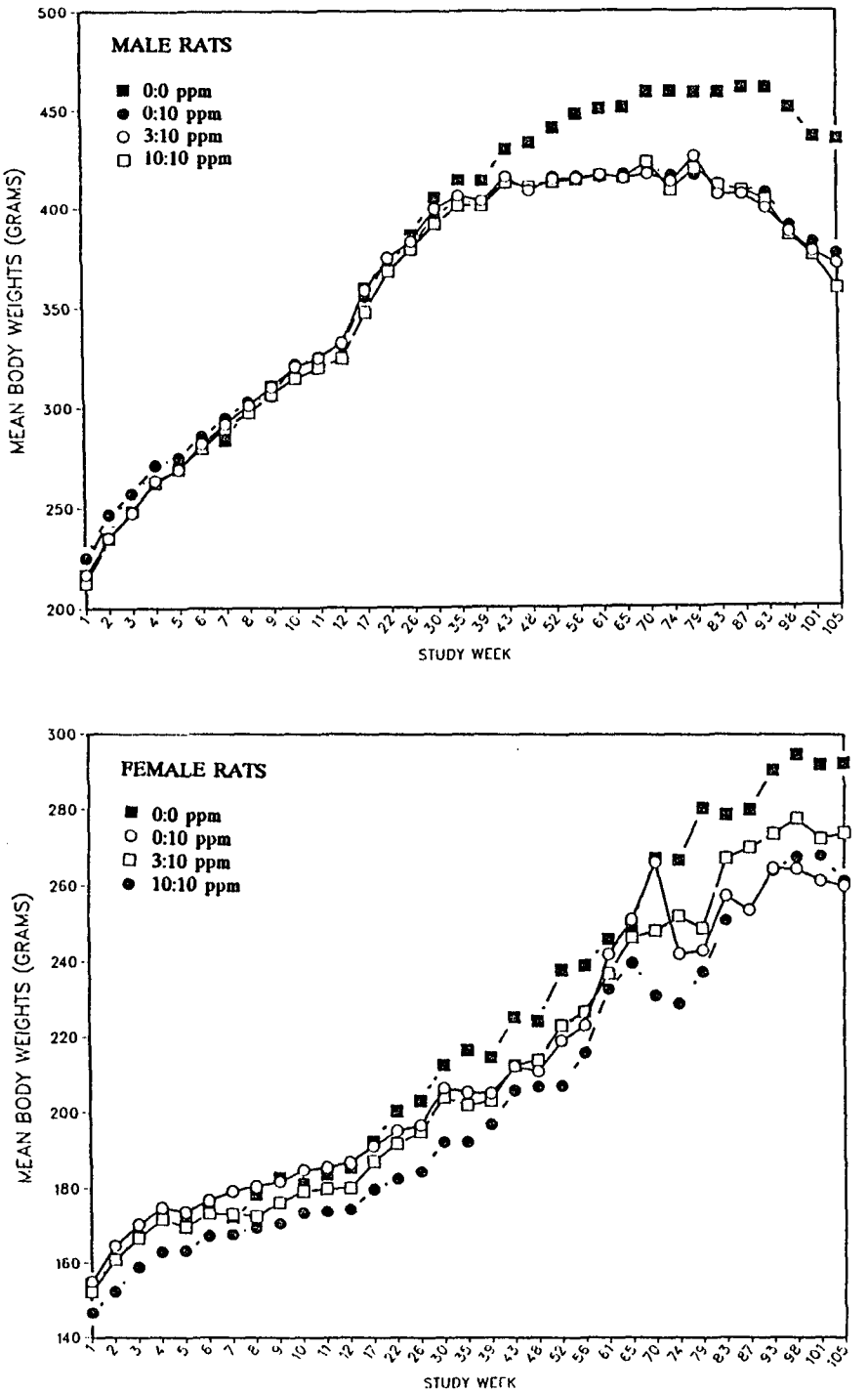

FIGURE 3b
Growth Curves for Rats Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 0:10, 3:10, and 10:10 ppm Groups

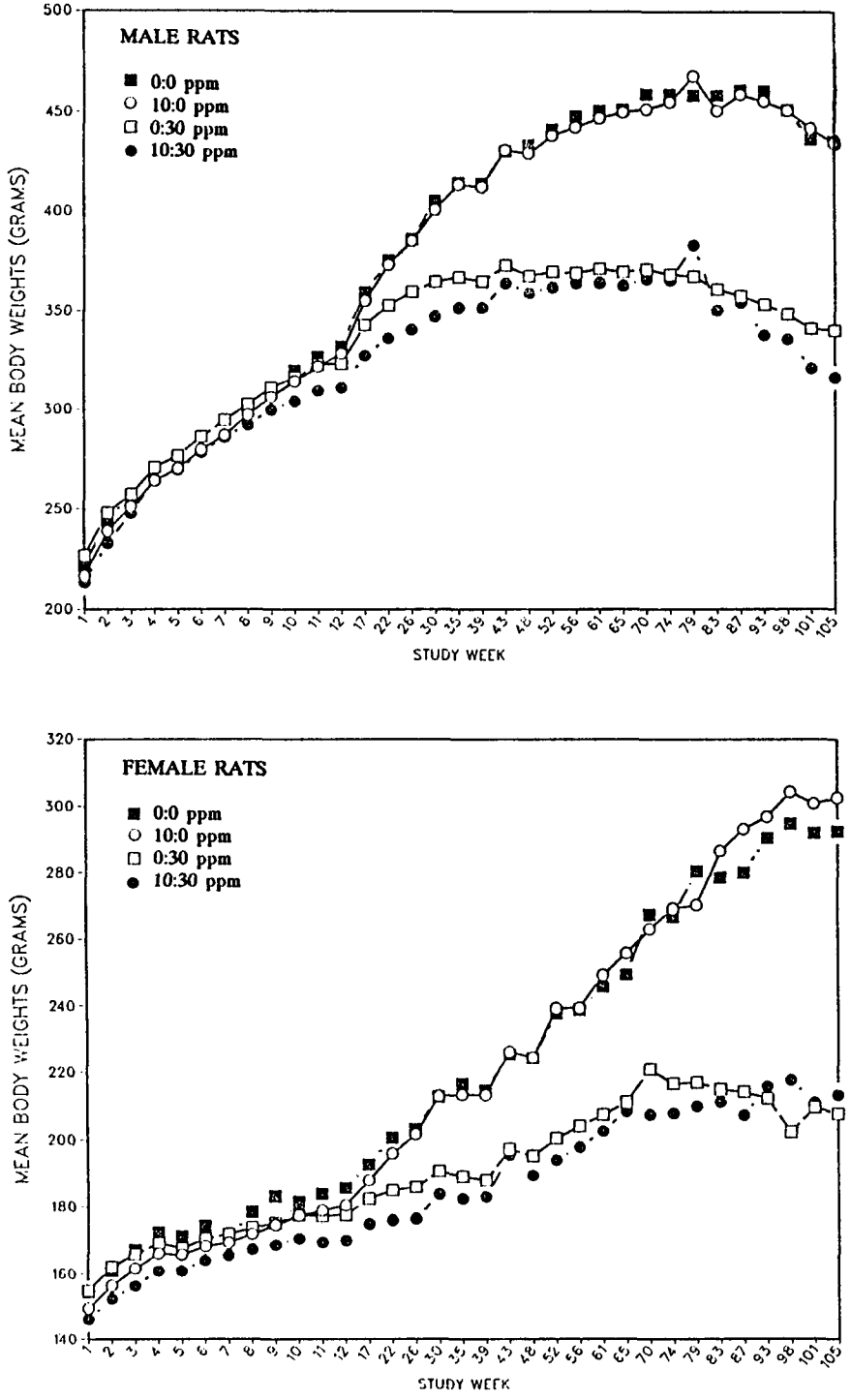

FIGURE 3c
Growth Curves for Rats Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 10:0, 0:30, and 10:30 ppm Groups

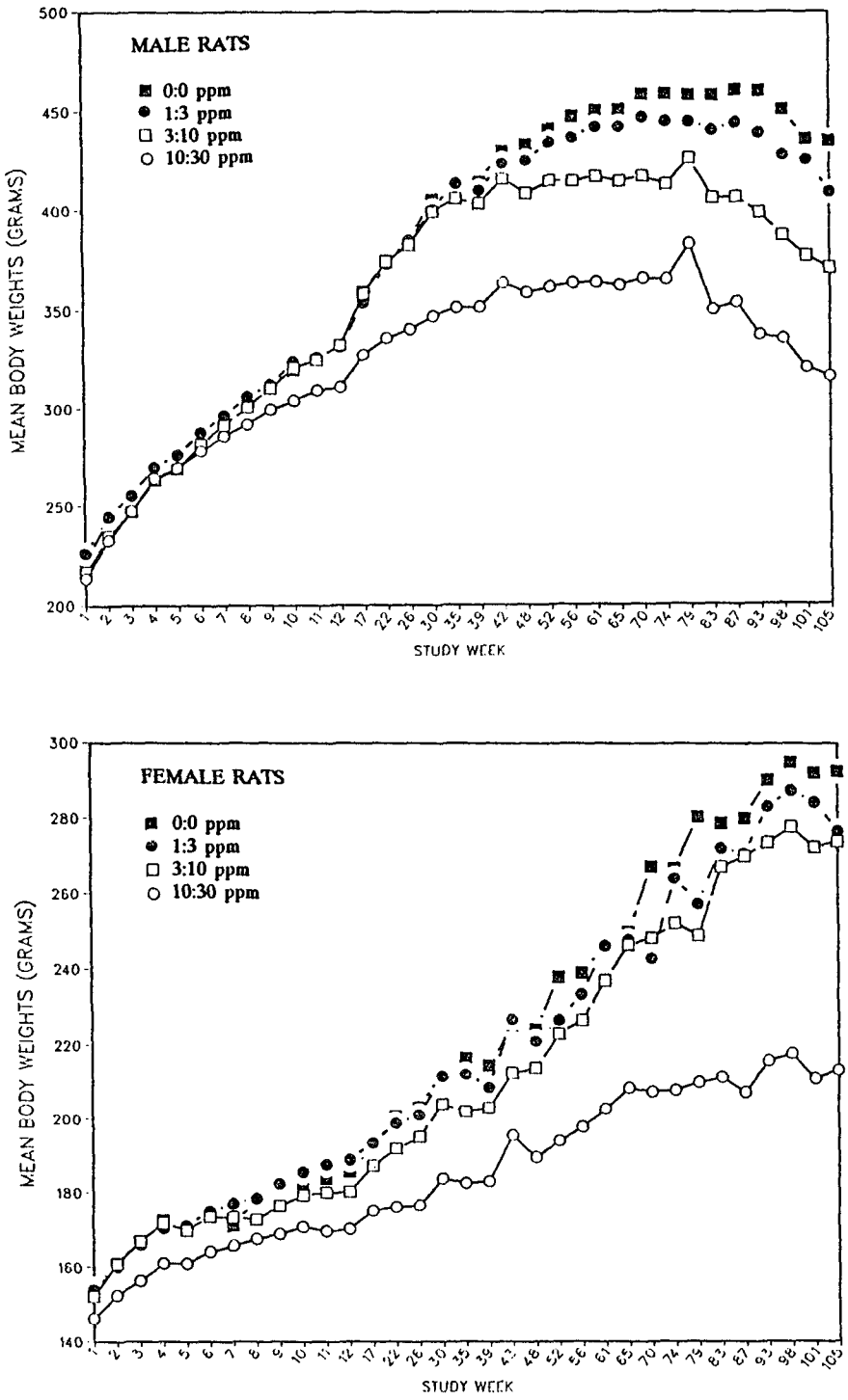

FIGURE 3d
Growth Curves for Rats Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 1:3, 3:10, and 10:30 ppm Groups

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms or nonneoplastic lesions of the liver, forestomach, uterus, adrenal gland, pituitary gland, preputial gland, thyroid gland, and mammary gland in rats.

Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendixes A for male rats and B for female rats. Historical incidences of neoplasms in control rats are given in Tables A3a and A3b for males and B3a and B3b for females.

Effects of Adult-Only Exposure of Rats to Polybrominated Biphenyls

The neoplastic and nonneoplastic effects of adult-only exposure were determined by comparison of the incidences of lesions in the 0:0, 0:10, and 0:30 ppm groups, which correspond to a standard carcinogenicity study. The liver was the only site showing clearly chemical-related increases in the incidences of neoplasms.

Liver: The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in 0:10 and 0:30 ppm rats (Table 10). The majority of male and female rats with hepatocellular adenoma had multiple occurrences of this neoplasm (Tables A1 and B1). The incidence of hepatocellular carcinoma was significantly increased in 0:30 ppm males. Although the combined incidences of adenoma and carcinoma were similar for males and females, there were more carcinomas in 0:30 ppm males (19 carcinomas) than in 0:30 ppm females (four carcinomas). Multiple hepatocellular carcinomas occurred in seven 0:30 ppm males.

The incidences of various nonneoplastic lesions (hypertrophy, eosinophilic focus, cytoplasmic vacuolization, and oval cell hyperplasia) of the liver were increased in exposed males and females (Table 10). Hypertrophy was similar to that seen in the gestational study and at the 9-month interim evaluation and consisted of an enlargement of hepatocytes in the centrilobular region of the liver lobules. Eosinophilic foci of alteration were diagnosed when the cytoplasm of enlarged hepatocytes had a vacuolated or granular eosinophilic appearance; the nuclei of these cells were often enlarged. Eosinophilic foci ranged in size from less than one lobule to a diameter greater than three or four lobules. Cytoplasmic vacuolization was characterized by hepatocytes with foamy, vacuolated cytoplasm that resembled a fatty change. Oval cell hyperplasia was seen only in exposed rats and occurred throughout the liver lobules and in foci of alteration and neoplasms. These small cells with faintly staining cytoplasm and round- to oval-shaped nuclei formed into single or double rows and sometimes produced small, ductular structures. Oval cell hyperplasia was distinctly different from the spontaneously occurring bile duct hyperplasia that also occurred in nearly all control and exposed rats in this study.

Other Lesions: The only other sites of nonneoplastic effects possibly related to treatment were the forestomach in male rats and the uterus in female rats. The incidences of acanthosis (hyperplasia), inflammation, and ulceration of the forestomach were increased in exposed males (acanthosis: 0:0 ppm, 0/50; 0:10 ppm, 7/49; 0:30 ppm, 11/50; inflammation: 0/50, 6/49, 9/50; ulceration: 0/50, 5/49, 6/50). However, the combined incidences of squamous cell papilloma or carcinoma of the forestomach were not increased in exposed males (0/50, 2/49, 1/50). The incidence of splenic fibrosis was significantly increased in 0:30 ppm males (1/50, 4/49, 10/50); this was generally a mild, focal lesion near the hilus and was often secondary to splenic infarction associated with advanced mononuclear cell leukemia. In 0:30 ppm females, the incidence of cystic endometrial hyperplasia of the uterus was increased (0/50, 1/18, 12/49).

Decreasing Incidences of Neoplasms: Male rats in the 0:30 ppm group had significantly decreased incidences of adrenal gland pheochromocytomas (11/49, 1/29, 2/50), pituitary gland adenomas (13/50, 12/32, 4/48), and preputial gland adenomas and carcinomas, combined (7/47, 4/32, 1/44). Females in the 0:30 ppm group had decreased incidences of pituitary gland adenomas (21/50, 18/29, 4/50) and thyroid gland C-cell adenomas (11/50, 3/14, 2/48).

TABLE 10
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups

	0:0 ppm	0:10 ppm	0:30 ppm
Male			
Hypertrophy			
Overall rate[a]	0/50 (0%)	27/49 (55%)**	17/50 (34%)**
Eosinophilic Focus			
Overall rate	18/50 (36%)	44/49 (90%)**	46/50 (92%)**
Cytoplasmic Vacuolization			
Overall rate	5/50 (10%)	28/49 (57%)**	34/50 (68%)**
Oval Cell Hyperplasia			
Overall rate	0/50 (0%)	11/49 (22%)**	37/50 (74%)**
Hepatocellular Adenoma			
Overall rate	1/50 (2%)	10/49 (20%)	38/50 (76%)
Adjusted rate[b]	3.4%	38.3%	92.5%
Terminal rate[c]	1/29 (3%)	7/23 (30%)	21/24 (88%)
First incidence (days)	727 (T)	716	588
Logistic regression test[d]	P<0.001	P=0.002	P<0.001
Hepatocellular Adenoma, Multiple			
Overall rate	0/50 (0%)	6/49 (12%)*	36/50 (72%)**
Hepatocellular Carcinoma			
Overall rate	0/50 (0%)	2/49 (4%)	19/50 (38%)
Adjusted rate	0.0%	5.3%	61.6%
Terminal rate	0/29 (0%)	0/23 (0%)	13/24 (54%)
First incidence (days)	–[e]	678	604
Logistic regression test	P<0.001	P=0.237	P<0.001
Hepatocellular Carcinoma, Multiple			
Overall rate	0/50 (0%)	0/49 (0%)	7/50 (14%)*
Hepatocellular Adenoma or Carcinoma[f]			
Overall rate	1/50 (2%)	12/49 (24%)	41/50 (82%)
Adjusted rate	3.4%	41.6%	97.6%
Terminal rate	1/29 (3%)	7/23 (30%)	23/24 (96%)
First incidence (days)	727 (T)	678	588
Logistic regression test	P<0.001	P<0.001	P<0.001

(continued)

TABLE 10
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

	0:0 ppm	0:10 ppm	0:30 ppm
Female			
Hypertrophy			
Overall rate	0/50 (0%)	32/50 (64%)**	17/50 (34%)**
Eosinophilic Focus			
Overall rate	3/50 (6%)	47/50 (94%)**	48/50 (96%)**
Cytoplasmic Vacuolization			
Overall rate	3/50 (6%)	47/50 (94%)**	50/50 (100%)**
Oval Cell Hyperplasia			
Overall rate	0/50 (0%)	12/50 (24%)**	42/50 (84%)**
Hepatocellular Adenoma			
Overall rate	0/50 (0%)	10/50 (20%)	38/50 (76%)
Adjusted rate	0.0%	26.6%	90.4%
Terminal rate	0/40 (0%)	9/36 (25%)	31/35 (89%)
First incidence (days)	–	565	593
Logistic regression test	P<0.001	P=0.001	P<0.001
Hepatocellular Adenoma, Multiple			
Overall rate	0/50 (0%)	4/50 (8%)	37/50 (74%)**
Hepatocellular Carcinoma			
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	5.6%	10.6%
Terminal rate	0/40 (0%)	2/36 (6%)	3/35 (9%)
First incidence (days)	–	729 (T)	614
Logistic regression test	P=0.045	P=0.215	P=0.063
Hepatocellular Carcinoma, Multiple			
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)
Hepatocellular Adenoma or Carcinoma[g]			
Overall rate	0/50 (0%)	12/50 (24%)	39/50 (78%)
Adjusted rate	0.0%	32.0%	90.6%
Terminal rate	0/40 (0%)	11/36 (31%)	31/35 (89%)
First incidence (days)	–	565	593
Logistic regression test	P<0.001	P<0.001	P<0.001

* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by the logistic regression test
** ($P \leq 0.01$)
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.
[e] Not applicable; no neoplasms in animal group
[f] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 32/1,001 (3.2% ± 3.6%), range 0%–10%
[g] Historical incidence: 6/1,000 (0.6% ± 1.5%), range 0%–6%

Effects of Perinatal-Only Exposure of Rats to Polybrominated Biphenyls

The neoplastic and nonneoplastic effects of perinatal-only exposure were determined by comparison of the incidences of lesions in the 0:0 and 10:0 ppm groups. With the possible exception of the liver, perinatal-only exposure did not increase neoplasm incidences at any sites.

Liver: The incidence of hepatocellular adenomas was marginally (P=0.108) increased in males, but not in females, exposed perinatally to polybrominated biphenyls (Table 11). The incidences of nonneoplastic lesions in the liver were increased in exposed males (eosinophilic focus and cytoplasmic vacuolization) and females (eosinophilic focus).

TABLE 11
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0 and 10:0 ppm Groups

	0:0 ppm	10:0 ppm
Male		
Eosinophilic Focus		
Overall rate[a]	18/50 (36%)	29/50 (58%)**
Cytoplasmic Vacuolization		
Overall rate	5/50 (10%)	18/50 (36%)**
Hepatocellular Adenoma		
Overall rate	1/50 (2%)	5/50 (10%)
Adjusted rate[b]	3.4%	16.7%
Terminal rate[c]	1/29 (3%)	5/30 (17%)
First incidence (days)	727 (T)	727 (T)
Logistic regression test[d]		P=0.108
Female		
Eosinophilic Focus		
Overall rate	3/50 (6%)	19/50 (38%)**
Cytoplasmic Vacuolization		
Overall rate	3/50 (6%)	6/50 (12%)
Hepatocellular Adenoma		
Overall rate	0/50 (0%)	0/50 (0%)

** Significantly different (P≤0.01) from the 0:0 ppm group by the logistic regression test
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.

Effects of Combined Perinatal and Adult Exposure of Rats to Polybrominated Biphenyls

The effects of combined perinatal and adult exposure were determined by comparison of the incidences of lesions in rats in the 0:10, 3:10, and 10:10 ppm groups and in the 0:30 and 10:30 ppm groups.

Liver: In females receiving varying F_0 concentrations and a constant F_1 concentration of 10 or 30 ppm, the incidences of hepatocellular adenoma, hepatocellular adenoma or carcinoma (combined), and multiple hepatocellular neoplasms increased with the F_0 concentration (Tables 12 and 13). The incidence of these neoplasms did not increase with increased F_0 concentration in male rats. The incidence of oval cell hyperplasia was significantly increased in 0:30 ppm males and females. In male and female rats exposed to 30 ppm polybrominated biphenyls for 2 years, perinatal exposure to 10 ppm had little or no effect on the incidences of nonneoplastic lesions in the liver. The incidence and severity of oval cell hyperplasia were similar in the 0:30 and 10:30 ppm groups (Table 13). In female rats receiving F_0:F_1 concentrations of 10:30 ppm, there was an increased incidence of bile duct fibrosis (cholangiofibrosis) (Table 13). Bile duct fibrosis in female rats was characterized by the presence of cysts or large ducts with a hyperplastic, pleomorphic biliary epithelial lining. These structures were surrounded by a chronic inflammatory cell infiltrate and fibrosis. An additional finding in one male rat in each of the 10:30 and 0:30 ppm groups was the focal metaplasia of hepatocytes to a cell type which resembled pancreatic acinar cells (Table A4).

TABLE 12
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 3:10, and 10:10 ppm Groups

	0:10 ppm	3:10 ppm	10:10 ppm
Male			
Oval Cell Hyperplasia			
Overall rate[a]	11/49 (22%)	13/50 (26%)	23/50 (46%)**
Hepatocellular Adenoma			
Overall rate	10/49 (20%)	13/50 (26%)	16/50 (32%)
Adjusted rate[b]	38.3%	51.5%	50.4%
Terminal rate[c]	7/23 (30%)	9/20 (45%)	7/20 (35%)
First incidence (days)	716	484	531
Logistic regression test[d]	P=0.087	P=0.187	P=0.092
Hepatocellular Adenoma, Multiple			
Overall rate	6/49 (12%)	7/50 (14%)	6/50 (12%)
Hepatocellular Carcinoma			
Overall rate	2/49 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	5.3%	5.0%	5.0%
Terminal rate	0/23 (0%)	1/20 (5%)	1/20 (5%)
First incidence (days)	678	727 (T)	727 (T)
Logistic regression test	P=0.489N	P=0.528N	P=0.522N
Hepatocellular Carcinoma, Multiple			
Overall rate	0/49 (0%)	0/50 (0%)	0/50 (0%)
Hepatocellular Adenoma or Carcinoma			
Overall rate	12/49 (24%)	14/50 (28%)	16/50 (32%)
Adjusted rate	41.6%	55.9%	50.4%
Terminal rate	7/23 (30%)	10/20 (50%)	7/20 (35%)
First incidence (days)	678	484	531
Logistic regression test	P=0.184	P=0.263	P=0.200

(continued)

TABLE 12
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 3:10, and 10:10 ppm Groups (continued)

	0:10 ppm	3:10 ppm	10:10 ppm
Female			
Oval Cell Hyperplasia			
Overall rate	12/50 (24%)	19/50 (38%)	34/50 (68%)**
Hepatocellular Adenoma			
Overall rate	10/50 (20%)	22/50 (44%)	35/50 (70%)
Adjusted rate	26.6%	54.8%	83.2%
Terminal rate	9/36 (25%)	21/39 (54%)	31/38 (82%)
First incidence (days)	565	625	608
Logistic regression test	P<0.001	P=0.010	P<0.001
Hepatocellular Adenoma, Multiple			
Overall rate	4/50 (8%)	10/50 (20%)	20/50 (40%)**
Hepatocellular Carcinoma			
Overall rate	2/50 (4%)	1/50 (2%)	8/50 (16%)
Adjusted rate	5.6%	2.2%	19.0%
Terminal rate	2/36 (6%)	0/39 (0%)	5/38 (13%)
First incidence (days)	729 (T)	625	529
Logistic regression test	P=0.009	P=0.502N	P=0.048
Hepatocellular Carcinoma, Multiple			
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)
Hepatocellular Adenoma or Carcinoma			
Overall rate	12/50 (24%)	22/50 (44%)	39/50 (78%)
Adjusted rate	32.0%	54.8%	88.5%
Terminal rate	11/36 (31%)	21/39 (54%)	33/38 (87%)
First incidence (days)	565	625	529
Logistic regression test	P<0.001	P=0.030	P<0.001

** Significantly different (P≤0.01) from the 0:10 ppm group by the logistic regression test
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0:10 ppm group are the P values associated with the trend test. Beneath the 3:10 and 10:10 ppm groups are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.

TABLE 13
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 10:30 ppm Groups

	0:30 ppm	10:30 ppm
Male		
Hypertrophy		
Overall rate[a]	17/50 (34%)	15/50 (30%)
Cytoplasmic Vacuolization		
Overall rate	34/50 (68%)	37/50 (74%)
Oval Cell Hyperplasia		
Overall rate	37/50 (74%)	41/50 (82%)
Hepatocellular Adenoma		
Overall rate	38/50 (76%)	38/50 (76%)
Adjusted rate[b]	92.5%	100%
Terminal rate[c]	21/24 (88%)	19/19 (100%)
First incidence (days)	588	515
Logistic regression test[d]		P=0.248
Hepatocellular Adenoma, Multiple		
Overall rate	36/50 (72%)	35/50 (70%)
Hepatocellular Carcinoma		
Overall rate	19/50 (38%)	23/50 (46%)
Adjusted rate	61.6%	73.4%
Terminal rate	13/24 (54%)	11/19 (58%)
First incidence (days)	604	575
Logistic regression test		P=0.108
Hepatocellular Carcinoma, Multiple		
Overall rate	7/50 (14%)	10/50 (20%)
Hepatocellular Adenoma or Carcinoma		
Overall rate	41/50 (82%)	41/50 (82%)
Adjusted rate	97.6%	100.0%
Terminal rate	23/24 (96%)	19/19 (100%)
First incidence (days)	588	515
Logistic regression test		P=0.204

(continued)

TABLE 13
Liver Lesions in Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 10:30 ppm Groups (continued)

	0:30 ppm	10:30 ppm
Female		
Hypertrophy		
Overall rate	17/50 (34%)	6/50 (12%)**
Cytoplasmic Vacuolization		
Overall rate	50/50 (100%)	40/50 (80%)**
Oval Cell Hyperplasia		
Overall rate	42/50 (84%)	44/50 (88%)
Bile Duct Fibrosis		
Overall rate	3/50 (6%)	19/50 (38%)**
Hepatocellular Adenoma		
Overall rate	38/50 (76%)	45/50 (90%)
Adjusted rate	90.4%	97.8%
Terminal rate	31/35 (89%)	32/33 (97%)
First incidence (days)	593	312
Logistic regression test		P=0.049
Hepatocellular Adenoma, Multiple		
Overall rate	37/50 (74%)	44/50 (88%)*
Hepatocellular Carcinoma		
Overall rate	4/50 (8%)	22/50 (44%)
Adjusted rate	10.6%	53.0%
Terminal rate	3/35 (9%)	14/33 (42%)
First incidence (days)	614	599
Logistic regression test		P<0.001
Hepatocellular Carcinoma, Multiple		
Overall rate	0/50 (0%)	6/50 (12%)*
Hepatocellular Adenoma or Carcinoma		
Overall rate	39/50 (78%)	47/50 (94%)
Adjusted rate	90.6%	100.0%
Terminal rate	31/35 (89%)	33/33 (100%)
First incidence (days)	593	312
Logistic regression test		P=0.016

* Significantly different (P≤0.05) from the 0:30 ppm group by the logistic regression test
** (P≤0.01)
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.

The combined incidences of hepatocellular adenomas and carcinomas for all exposure groups are shown in Table 14. A single logistic regression analysis applied to all eight experimental groups indicates that significant ($P \leq 0.001$) increases in the incidence of hepatocellular neoplasms are associated with increasing F_0 and F_1 concentration levels of polybrominated biphenyls for both males and females. For females, there was also an increased liver neoplasm incidence ($P \leq 0.01$) associated with F_0 exposure as well as a significant ($P \leq 0.01$) $F_0 \times F_1$ interaction (Piegorsch *et al.*, 1986). This interaction implies that an F_0 exposure significantly enhances the effect of an F_1 exposure, as is evident from Table 14.

TABLE 14
Hepatocellular Adenomas and Carcinomas in Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration (ppm)	F_1 Concentration (ppm) 0	3	10	30
Male				
0	1/50	–[b]	12/49**	41/50**
1	–	6/50	–	–
3	–	–	14/50**	–
10	5/50	–	16/50**▲▲	41/50**▲▲
Female				
0	0/50	–	12/50**	39/50**
1	–	3/50	–	–
3	–	–	22/50**	–
10	0/50	–	39/50**▲▲	47/50**▲▲

** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by the logistic regression test
▲▲ Significantly different ($P \leq 0.01$) from the 10:0 ppm group by the logistic regression test
[a] Incidences are given as the number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Animals were not exposed at these concentrations.

Mononuclear Cell Leukemia: Although the incidences of mononuclear cell leukemia were only marginally increased in the adult-only (0:10 and 0:30 ppm) exposure groups, an evaluation of all exposure groups indicated consistent increases in the incidence of this neoplasm in the higher F_1 exposure groups (Table 15). Moreover, mononuclear cell leukemia incidences in some groups fall outside the NTP historical control range (Tables A3b and B3b). Life table analyses of data from all eight experimental groups indicate that significant increases in the incidence of mononuclear cell leukemia are associated with increasing F_1 concentration levels of polybrominated biphenyls ($P \leq 0.05$ for males; $P \leq 0.01$ for females). For males, there was also a marginally significant ($P \leq 0.05$) increase associated with F_0 exposure. There was no significant $F_0 \times F_1$ interaction for males or females.

Decreasing Incidences of Neoplasms: For groups receiving varying F_0 concentrations and an F_1 concentration of 10 ppm, the incidences of mammary gland fibroadenomas were significantly decreased in females (11/21, 7/16, 2/11) and slightly decreased in males (4/23, 0/20, 0/18). No significant decreases occurred in the incidences of neoplasms in rats receiving varying F_0 concentrations and an F_1 concentration of 30 ppm.

TABLE 15
Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration (ppm)	F_1 Concentration (ppm)			
	0	3	10	30
Male				
0	25/50	–[b]	33/50*	31/50
1	–	32/50	–	–
3	–	–	41/50**	–
10	31/50	–	37/50**▲	37/50**▲
Female				
0	14/50	–	22/50	23/50*
1	–	20/50	–	–
3	–	–	17/50	–
10	13/50	–	27/50**▲▲	25/50*▲▲

* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by the life table test
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by the life table test
▲ Significantly different ($P \leq 0.05$) from the 10:0 ppm group by the life table test
▲▲ Significantly different ($P \leq 0.01$) from the 10:0 ppm group by the life table test
[a] Incidences are given as the number of neoplasm-bearing animals/number of animals necropsied.
[b] Animals were not exposed at these concentrations.

MICE

GESTATIONAL STUDY: DETERMINATION OF MAXIMUM PERINATAL DOSE

The gestational study was conducted to determine the dietary concentrations for perinatal exposure to be used in the 2-year study. Selected dams from each exposure group were evaluated at gestation day 17 for reproductive effects. There were no differences in the numbers of implantations, live fetuses, or fetuses per litter for exposed dams, nor were there any significant differences in fetal liver weights (Table 16). All mouse dams not designated for evaluation at gestation day 18 survived to the end of the study.

The number of pup deaths during day 0 through day 7 was increased among exposed mice (Table 17). The number of pups surviving to day 28 was similar among exposed and control groups. Mean pup weight at day 28 was decreased in the 30 ppm group. The absolute and relative liver weights of pups in the 10 and 30 ppm exposure groups examined at day 12 were increased relative to those of the controls (Table 18). The absolute and relative liver weights of dams in the 30 ppm group were higher than those of the controls.

All weanling mice receiving polybrominated biphenyls in feed survived until the end of the study (Table 19). The final mean body weights of control and exposed mice were similar.

No treatment-related gross lesions were observed at necropsy. Treatment-related microscopic lesions were limited to the liver of male and female mice and consisted of enlarged (hypertrophic) hepatocytes with foamy-appearing cytoplasm; there was a marked variation in the size of hepatocyte nuclei in affected livers. In the 30 ppm groups, hepatocellular hypertrophy was of mild to moderate severity in all male mice and of mild severity in one female mouse. Hypertrophy (mild) was present in 8 of 10 male mice receiving 10 ppm. There were no treatment-related microscopic lesions in the liver of male or female mice receiving 1 or 3 ppm or female mice receiving 10 ppm.

Dose Selection Rationale

There were no clearly chemical-related effects on survival or growth at any phase of the study; therefore, 30 ppm was selected as the highest exposure level for the perinatal portion of the carcinogenicity study.

TABLE 16
Prenatal Day 17 Litter Data for Mice in the Maximum Perinatal Dose Determination Feed Study of Polybrominated Biphenyls

	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm
Litters	4	4	4	4	4
Implantations	32	36	33	38	34
Live fetuses	30	35	28	31	32
Fetuses/litter[a]	7.5 ± 1.9	8.8 ± 2.1	7.0 ± 1.4	7.8 ± 2.5	8.0 ± 0.0
Fetal weight[a]	0.91 ± 0.09	0.83 ± 0.14*	0.83 ± 0.08*	0.87 ± 0.06	0.91 ± 0.20
Fetal liver weight[a]					
Absolute	0.061 ± 0.007	0.055 ± 0.011	0.060 ± 0.007	0.062 ± 0.006	0.062 ± 0.011
Relative	6.65 ± 0.67	6.76 ± 1.37	7.34 ± 0.95	7.20 ± 0.74	6.88 ± 0.72
Placental weight[a]	0.11 ± 0.06	0.13 ± 0.08	0.09 ± 0.07	0.11 ± 0.07	0.09 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Mean ± standard deviation. Fetal body, liver, and placental weights are given in grams; relative liver weights are given as percent of body weight.

TABLE 17
Survival, Sex Ratios, and Mean Body Weights of Mouse Pups in the Maximum Perinatal Dose Determination Feed Study of Polybrominated Biphenyls

	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm
Precull					
Litters on day 0[a]	39	38	45	40	33
Pups on day 0	255	285	326	300	242
Pups dead days 0-7	67	128	122	136	98
Postcull					
Litters on day 7	28	28	34	29	22
Pups on day 7	188	157	204	164	144
Pup weight on day 7[b]	3.6 ± 0.5	3.7 ± 0.6	3.7 ± 0.7	3.7 ± 0.7	3.6 ± 0.6
Male/female ratio on day 7	1.01	1.71	0.87	1.18	0.90
Pups dead days 7-28	29	21	9	15	19
Litters on day 28[c]	21	20	29	23	15
Pups on day 28	133	108	167	124	98
Pup weight on day 28	12.5 ± 1.8	12.8 ± 2.2	12.4 ± 2.0	12.7 ± 1.7	11.6 ± 1.4

[a] Does not include four litters per group evaluated for gestation day 18 studies. Differences from the control group were not significant by Dunnett's test.
[b] Body weights are given in grams (mean ± standard deviation).
[c] Does not include litters used for day 12 studies

TABLE 18
Day 12 Postpartum Relative Liver Weights for Mouse Pups Exposed Perinatally to Polybrominated Biphenyls and Mouse Dams Exposed to Polybrominated Biphenyls in the Diet[a]

	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm
Pups					
n	20	20	20	20	20
Body weight	4.9 ± 0.5	5.7 ± 0.5**	5.2 ± 0.8	5.8 ± 0.5**	5.8 ± 0.9**
Liver weight					
Absolute	0.135 ± 0.010	0.176 ± 0.02*	0.156 ± 0.027	0.215 ± 0.025**	0.266 ± 0.052**
Relative	2.78 ± 0.19	3.11 ± 0.13**	2.98 ± 0.21	3.71 ± 0.26**	4.63 ± 0.58**
Dams					
n	4	4	4	4	4
Body weight	29.6 ± 1.8	28.5 ± 2.5	30.1 ± 2.7	29.1 ± 0.8	29.8 ± 3.7
Liver weight					
Absolute	2.16 ± 0.29	2.04 ± 0.32	2.06 ± 0.13	2.31 ± 0.10	3.02 ± 0.44**
Relative	7.30 ± 0.57	7.14 ± 0.58	6.88 ± 0.64	7.94 ± 0.52	10.12 ± 0.44**

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Liver and body weights are given in grams; relative liver weights are given as percent of body weight (mean ± standard deviation).

TABLE 19
Survival and Mean Body Weights of Weanling Mice in the Maximum Perinatal Dose Determination Feed Study of Polybrominated Biphenyls

Concentration (ppm)	Survival[a]	Mean Body Weight (g)[b]			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	13.9 ± 0.6	22.7 ± 0.4	8.8	
1	10/10	13.7 ± 0.6	23.0 ± 0.4	9.3	101
3	10/10	13.1 ± 0.6	22.6 ± 0.5	9.5	100
10	10/10	13.5 ± 0.5	23.4 ± 0.4	9.9	103
30	10/10	12.4 ± 0.6	22.3 ± 0.5	9.9	98
Female					
0	10/10	11.9 ± 0.4	18.4 ± 0.3	6.5	
1	10/10	12.0 ± 0.4	18.5 ± 0.3	6.5	101
3	10/10	12.4 ± 0.6	19.2 ± 0.4	6.8	104
10	10/10	12.0 ± 0.6	18.2 ± 0.4	6.2	99
30	10/10	12.3 ± 0.6	19.1 ± 0.6	6.8	104

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

2-YEAR STUDY

9-Month Interim Evaluation

The absolute and relative liver weights of males in the 0:30 and 30:30 ppm groups and the relative liver weight of males in the 30:10 ppm group were significantly increased (Table 20). The absolute and relative liver weights of females in the 0:10, 0:30, 10:10, 30:10, and 30:30 ppm groups were significantly increased.

Pooled liver tissues from groups of three males or three females in the 0:0, 30:0, 0:30, and 30:30 ppm groups were analyzed for polybrominated biphenyls concentrations. The concentration of polybrominated biphenyls in the 0:0 ppm group was 0 ppm for males and females (Table G2). Polybrominated biphenyls concentrations in the 30:0 ppm group were 24.57 ppm for males and 22.33 ppm for females. The concentrations in the 0:30 ppm group were 248.27 ppm for males and 290.66 ppm for females. The concentrations in the 30:30 ppm group were 246.89 ppm for males and 246.47 ppm for females.

At necropsy, gross lesions attributed to polybrominated biphenyls exposure were observed in the liver of male and female mice. The most consistent finding was the presence of an enlarged liver in male and female mice (37 of 40) from the 0:30 and 30:30 ppm groups. Other gross lesions (discoloration, nodules, masses) were also present in the liver of a majority of mice from these two exposure groups; liver enlargement and focal lesions, as previously described, were also present in the 30:10 ppm groups (primarily in males).

Microscopic lesions clearly related to chemical exposure consisted of neoplasms and a variety of nonneoplastic lesions in the liver of male and female mice. Hepatocellular adenomas occurred in one or more of the 10 male mice from each exposure group (Table 21). In the 30:30 ppm group, 7 of 10 male mice had one or more hepatocellular adenomas; hepatocellular carcinomas did not occur in any male mice at the 9-month interim evaluation. Hepatocellular adenomas were seen less frequently in female mice, occurring in only one female in each of the 0:30, 30:0, and 30:10 ppm groups and three females in the 30:30 ppm group. Multiple adenomas did not occur in female mice at the 9-month interim evaluation. Nonneoplastic lesions related to treatment in mice included hepatocellular cytomegaly (hypertrophy) and fatty change (cytoplasmic vacuolization).

TABLE 20
Liver Weights and Liver-Weight-to-Body-Weight Ratios for Mice at the 9-Month Interim Evaluations in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0:F_1 Concentration	Male Mice			Female Mice		
	Body Weight	Absolute Liver Weight	Relative Liver Weight	Body Weight	Absolute Liver Weight	Relative Liver Weight
0:0	37.1 ± 1.0	1.83 ± 0.04	49.39 ± 1.19	28.3 ± 0.6	1.46 ± 0.03	51.63 ± 0.55
0:10	37.3 ± 1.2	2.51 ± 0.09	67.52 ± 2.22	27.4 ± 0.8	2.16 ± 0.07*	78.65 ± 1.34**
0:30	31.0 ± 0.8**	3.62 ± 0.19**	117.60 ± 8.15**	25.1 ± 0.5**	3.26 ± 0.10**	130.12 ± 5.03**
3:3	39.2 ± 1.5	2.20 ± 0.16	55.61 ± 1.98	27.1 ± 0.7	1.68 ± 0.06	61.92 ± 1.25
10:10	36.7 ± 1.1	2.52 ± 0.07	68.95 ± 1.73	27.0 ± 0.4	2.24 ± 0.05**	83.17 ± 1.58**
30:0	37.4 ± 0.9	2.03 ± 0.05	54.26 ± 1.06	28.3 ± 0.8	1.65 ± 0.05	58.12 ± 1.07
30:10	35.1 ± 1.0	2.66 ± 0.10	76.55 ± 4.08*	28.4 ± 0.5	2.58 ± 0.06**	90.92 ± 2.51**
30:30	31.6 ± 0.8**	5.20 ± 0.62**	163.64 ± 17.72**	26.5 ± 0.6	4.38 ± 0.39**	164.39 ± 13.12**

* Significantly different (P≤0.05) from the 0:0 ppm group by Dunnett's test
** P≤0.01
[a] Liver weights and body weights are given in grams; liver-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error). Absolute and relative weights are given for groups of 10 animals.

TABLE 21
Liver Lesions in Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0:F_1 Concentrations (ppm)	Male Mice		Female Mice	
	Adenoma	Carcinoma	Adenoma	Carcinoma
Constant Adult Exposure/ Varying Perinatal Exposure				
0:10	3/10	0/10	0/10	0/10
10:10	1/10	0/10	0/10	0/10
30:10	4/10	0/10	1/10	0/10
0:30	1/10	0/10	1/10	0/10
30:30	7/10**	0/10	3/10	1/10
Perinatal-Only Exposure				
0:0	1/10	0/10	0/10	0/10
30:0	2/10	0/10	1/10	0/10
Adult-Only Exposure				
0:0	0/10	0/10	0/10	0/10
0:10	3/10	0/10	0/10	0/10
0:30	1/10	0/10	1/10	0/10

** Significantly different (P≤0.01) from the 0:0 and 0:30 ppm groups by the Fisher exact test
[a] Incidences are given as the number of neoplasm-bearing animals/number of animals examined microscopically

Focal cellular alteration (eosinophilic, clear, and basophilic foci) occurred in the liver of mice from most exposure groups; these foci occurred in most 0:30, 30:10, and 30:30 ppm mice. Necrosis of individual hepatocytes occurred in 0:10, 0:30, 30:10, and 30:30 ppm mice, but perinatal exposure alone did not result in hepatocellular necrosis.

Survival

All mice exposed to 0:30 or 30:30 ppm died before the end of the study (Table 22). The survival of 30:10 ppm females was less than that of the controls; survival of males exposed to 3:3 ppm was greater than that of the controls (Table 22 and Figure 4).

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights at week 101 were similar for all groups except the 0:30 ppm males, which did not have sufficient survival to determine representative body weights (Tables 23 and 24 and Figures 5a,b,c, and d). Feed consumption was similar among exposed and control groups (Tables H3 and H4). Adult consumption of 3, 10, and 30 ppm polybrominated biphenyls provided approximately 0.40, 1.35, 1.35, 1.40, 4.85, and 5.10 mg/kg for males and 0.50, 1.70, 1.70, 1.70, 5.50, and 5.60 mg/kg for females. There were no clinical findings attributed to polybrominated biphenyls administration.

TABLE 22
Survival of Mice in the 2-Year Feed Study of Polybrominated Biphenyls

	F_0:F_1 Concentration (ppm)							
	0:0	30:0	3:3	0:10	10:10	30:10	0:30	30:30
Male								
Animals initially in study	60	60	59	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	9	5	2	5	2	3	16	21
Natural deaths	10	13	5	11	14	18	34	29
Animals surviving to study termination	31	32	42[b]	34	34[b]	29[c]	0	0
Percent probability of survival at end of study[d]	62	64	86	68	68	58	0	0
Mean survival (days)[e]	677	689	693	691	679	670	598	502
Survival analysis[f]		P=0.894N	P=0.017N	P=0.538N	P=0.585N	P=0.853	P<0.001	P<0.001
Female								
Animals initially in study	60	60	60	60	60	60	60	60
9-Month interim evaluation[a]	10	10	10	10	10	10	10	10
Moribund	7	3	5	2	4	8	16	15
Natural deaths	4	2	13	10	13	25	34	35
Animals surviving to study termination	39	45	32	38	33	17	0	0
Percent probability of survival at end of study[d]	78	90	64	76	66	34	0	0
Mean survival (days)[e]	694	717	683	710	696	674	560	482
Survival analysis[f]		P=0.161N	P=0.219	P=0.891	P=0.354	P<0.001	P<0.001	P<0.001

[a] Censored from survival analyses
[b] Includes one animal that died during the last week of the studies
[c] Includes two animals that died during the last week of the studies
[d] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations.
[e] Mean of all deaths (uncensored, censored, terminal sacrifice)
[f] The results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A lower mortality in an exposure group is indicated by N.

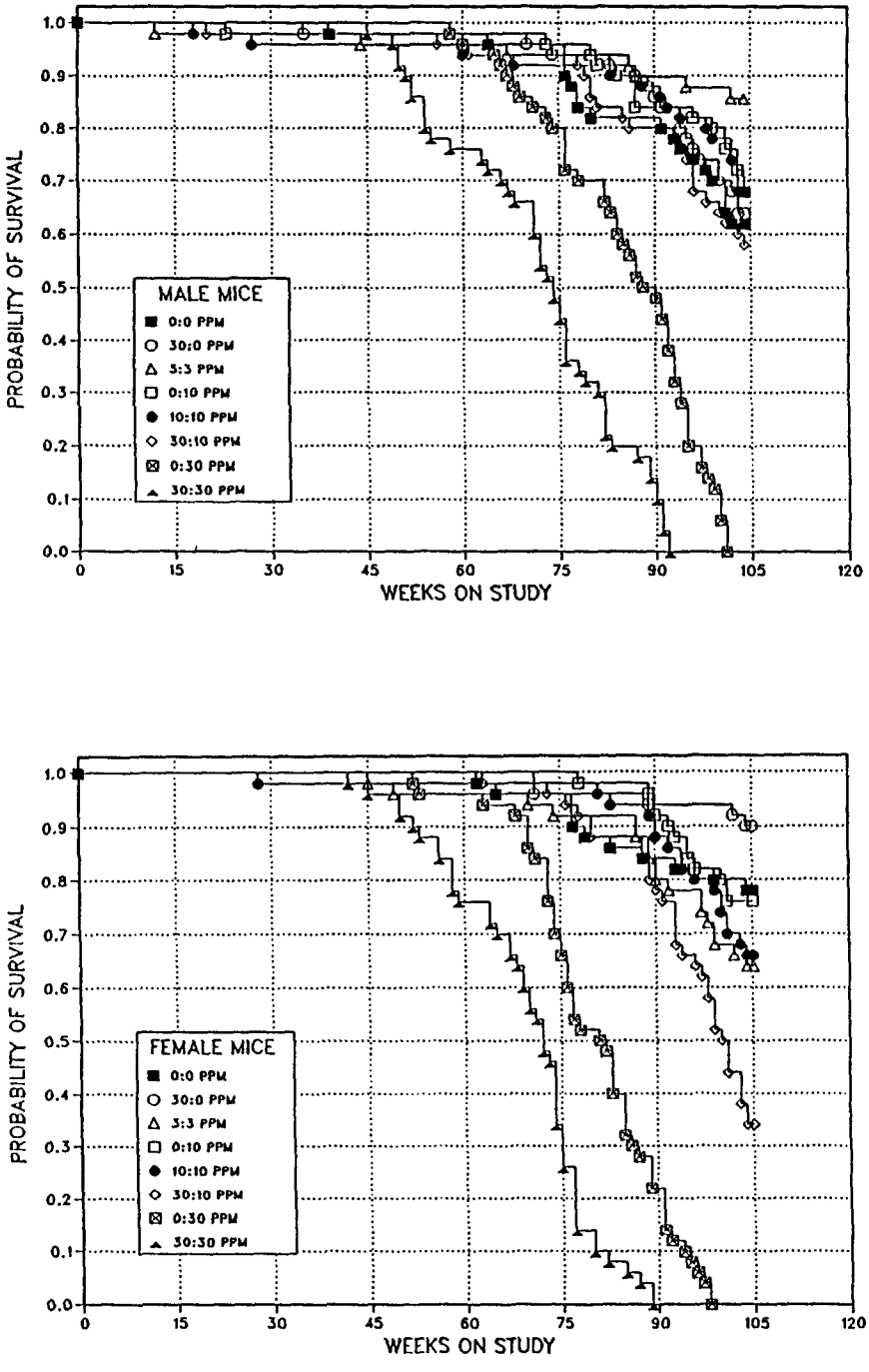

FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered Polybrominated Biphenyls in Feed for 2 Years

TABLE 23
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	30:0 ppm Av. Wt. (g)	30:0 ppm Wt. (% of controls)	30:0 ppm No. of Survivors	3:3 ppm Av. Wt. (g)	3:3 ppm Wt. (% of controls)	3:3 ppm No. of Survivors	0:10 ppm Av. Wt. (g)	0:10 ppm Wt. (% of controls)	0:10 ppm No. of Survivors
-1	25.0	50	24.2	97	50	25.3	101	49	25.1	100	50
1	26.8	50	26.4	99	50	26.8	100	49	27.1	101	50
2	27.4	50	27.4	100	50	27.2	99	49	27.8	101	50
3	27.8	50	27.8	100	50	27.8	100	49	28.4	102	50
4	28.1	50	28.1	100	50	27.6	98	49	28.6	102	50
5	28.5	50	28.5	100	50	28.3	99	49	29.1	102	50
6	29.0	50	28.9	100	50	28.9	100	49	29.6	102	50
7	29.5	50	29.7	101	50	29.4	100	49	30.2	102	50
8	30.0	50	30.0	100	50	29.6	99	49	30.4	101	50
9	30.5	50	30.7	101	50	30.2	99	49	30.8	101	50
10	30.7	50	30.7	100	50	30.1	98	49	31.1	101	50
11	31.0	50	31.1	100	50	30.7	99	48	31.5	102	50
12	31.0	50	31.1	100	50	30.7	99	48	31.7	102	50
13	31.1	50	31.2	100	50	31.0	100	48	31.5	101	50
17	31.7	50	32.6	103	50	32.4	102	48	33.8	107	50
21	33.8	50	33.9	100	50	32.9	97	48	34.1	101	50
26	36.4	50	36.6	101	50	35.4	97	48	35.6	98	49
30	37.0	50	37.5	101	50	35.6	96	48	36.4	98	49
35	37.0	50	37.8	102	49	36.3	98	48	37.0	100	49
40	38.6	49	39.3	102	49	37.5	97	48	38.0	98	49
44	39.0	49	39.0	100	49	36.3	93	48	37.5	96	49
49	40.0	49	40.9	102	49	37.2	93	47	38.4	96	49
53	41.1	49	42.1	102	49	38.6	94	47	39.8	97	49
57	41.3	49	42.3	102	49	40.2	97	47	40.8	99	49
62	41.3	49	42.4	103	49	39.5	96	47	39.8	96	49
66	41.7	48	42.6	102	49	40.5	97	47	40.3	97	49
70	41.5	48	42.6	103	48	40.0	96	46	40.1	97	49
75	41.5	48	43.3	104	47	40.5	98	46	40.0	96	48
79	43.5	42	44.5	102	47	41.7	96	46	40.8	94	48
83	41.2	41	43.6	106	46	41.2	100	46	39.6	96	46
88	40.9	41	41.6	102	45	40.6	99	44	43.1	105	42
93	40.0	40	39.4	99	42	40.0	100	44	38.1	95	42
97	39.0	37	38.9	100	38	39.1	100	43	38.2	98	41
101	37.7	35	38.8	103	35	38.8	103	43	38.3	102	39
Mean for weeks											
1-13	29.3		29.4	100		29.1	99		29.8	102	
14-52	36.7		37.2	101		35.5	97		36.4	99	
53-101	40.9		41.8	102		40.1	98		39.9	98	

(continued)

TABLE 23
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

Week on Study	10:10 ppm			30:10 ppm			0:30 ppm			30:30 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
-1	25.9	104	50	24.4	98	50	24.6	98	50	23.8	95	50
1	27.2	101	50	25.8	96	50	26.7	100	50	26.0	97	50
2	27.9	102	50	26.7	97	50	27.3	100	50	26.7	97	50
3	28.4	102	50	27.5	99	50	28.2	101	50	27.5	99	50
4	28.5	101	50	27.7	99	50	28.6	102	50	28.0	100	50
5	28.9	101	50	28.4	100	50	29.1	102	50	28.2	99	50
6	29.4	101	50	28.7	99	50	29.6	102	50	28.8	99	50
7	30.0	102	50	29.3	99	50	30.5	103	50	29.5	100	50
8	30.2	101	50	29.4	98	50	30.4	101	50	29.6	99	50
9	31.4	103	50	29.9	98	50	30.9	101	50	29.8	98	50
10	31.3	102	50	30.0	98	50	30.9	101	50	30.0	98	50
11	31.5	102	50	30.5	98	50	31.4	101	50	30.3	98	50
12	31.7	102	50	30.7	99	50	31.4	101	50	30.5	98	50
13	31.9	103	50	30.9	99	50	31.5	101	50	30.6	98	50
17	33.6	106	49	32.6	103	50	32.6	103	50	31.6	100	50
21	34.0	101	49	33.3	99	49	32.6	96	50	31.7	94	50
26	36.1	99	48	35.4	97	49	33.8	93	50	32.9	90	50
30	36.7	99	48	36.0	97	49	34.2	92	50	33.6	91	50
35	37.2	101	48	36.8	99	49	34.6	94	50	33.9	92	50
40	37.9	98	48	36.9	96	49	35.3	91	50	34.7	90	50
44	37.2	95	48	36.4	93	49	34.2	88	50	34.0	87	50
49	39.2	98	48	38.0	95	49	34.8	87	50	34.5	86	49
53	40.2	98	48	39.7	97	49	36.1	88	50	35.5	86	43
57	40.9	99	48	40.4	98	48	35.4	86	50	35.5	86	39
62	41.1	100	47	40.6	98	47	35.6	86	48	36.2	87	38
66	41.6	100	47	40.5	97	47	32.7	78	48	35.3	85	36
70	40.7	98	46	40.0	96	47	35.5	86	43	36.6	88	33
75	40.3	97	46	39.5	95	47	36.1	87	40	37.9	91	23
79	40.9	94	46	42.3	97	45	36.9	85	35	37.9	87	16
83	40.2	98	45	38.4	93	42	36.6	89	33	39.4	96	11
88	40.7	100	44	39.5	97	40	36.9	90	26	40.6	99	9
93	38.5	96	42	38.3	96	40	39.0	98	19			
97	38.6	99	41	39.0	100	34	38.5	99	10			
101	38.4	102	39	39.5	105	31	31.0	82	2			
Mean for weeks												
1-13	29.9	102		28.9	99		29.7	101		28.9	99	
14-52	36.5	99		35.7	97		34.0	93		33.4	91	
53-101	40.2	98		39.8	97		35.9	88		37.2	91	

TABLE 24
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls

Week on Study	0:0 ppm Av. Wt. (g)	0:0 ppm No. of Survivors	30:0 ppm Av. Wt. (g)	30:0 ppm Wt. (% of controls)	30:0 ppm No. of Survivors	3:3 ppm Av. Wt. (g)	3:3 ppm Wt. (% of controls)	3:3 ppm No. of Survivors	0:10 ppm Av. Wt. (g)	0:10 ppm Wt. (% of controls)	0:10 ppm No. of Survivors
-1	18.9	50	18.7	99	50	19.2	102	50	19.1	101	50
1	21.5	50	21.2	99	50	21.6	100	50	21.0	98	50
2	22.0	50	22.0	100	50	22.4	102	50	21.7	99	50
3	22.3	50	22.2	100	50	23.1	104	50	22.4	100	50
4	22.6	50	22.3	99	50	23.0	102	50	22.7	100	50
5	23.1	50	22.8	99	50	23.6	102	50	23.1	100	50
6	23.4	50	23.6	101	50	24.1	103	50	23.4	100	50
7	24.4	50	24.2	99	50	24.7	101	50	24.1	99	50
8	24.4	50	24.3	100	50	24.8	102	50	24.3	100	50
9	24.4	50	24.7	101	50	24.9	102	50	24.6	101	50
10	24.2	50	24.4	101	50	25.0	103	50	24.4	101	50
11	24.9	50	24.7	99	50	25.4	102	50	24.8	100	50
12	24.8	50	24.9	100	50	25.6	103	50	24.9	100	50
13	25.0	50	24.9	100	50	25.7	103	50	25.0	100	50
17	25.9	50	26.0	100	50	26.6	103	50	26.2	101	50
21	26.2	50	26.7	102	50	27.1	103	50	25.9	99	50
26	27.4	50	27.7	101	50	27.9	102	50	27.3	100	50
30	27.7	50	28.5	103	50	28.7	104	50	27.9	101	50
35	28.4	50	29.4	104	50	29.2	103	50	28.3	100	50
40	29.2	50	30.1	103	50	30.1	103	50	29.1	100	50
44	29.7	50	30.6	103	50	30.1	101	50	28.7	97	50
49	30.2	50	30.9	102	50	30.8	102	48	30.1	100	50
53	31.8	50	32.5	102	50	31.3	98	48	32.6	103	50
57	32.3	50	33.2	103	50	33.5	104	48	30.8	95	50
62	32.9	49	33.4	102	50	33.5	102	48	31.4	95	50
66	33.3	48	34.0	102	50	34.5	104	48	32.4	97	50
70	33.2	48	34.1	103	50	33.7	102	48	32.4	98	50
75	34.4	48	35.3	103	48	34.6	101	46	32.9	96	50
79	35.9	44	36.2	101	48	35.8	100	46	34.0	95	49
83	37.2	43	37.7	101	48	36.8	99	46	34.1	92	49
88	38.0	42	39.3	103	48	37.8	99	44	33.6	88	49
93	37.8	41	39.4	104	47	37.4	99	39	35.4	94	45
97	39.3	41	39.2	100	47	38.0	97	37	34.8	89	41
101	36.9	40	38.8	105	47	35.5	96	34	35.2	95	39
Mean for weeks											
1-13	23.6		23.6	100		24.1	102		23.6	100	
14-52	28.1		28.7	102		28.8	102		27.9	99	
53-101	35.3		36.1	102		35.2	100		33.3	94	

(continued)

TABLE 24
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

Week on Study	10:10 ppm			30:10 ppm			0:30 ppm			30:30 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
-1	19.8	105	50	20.0	106	50	20.2	107	50	19.9	105	50
1	21.4	100	50	21.4	100	50	21.4	100	50	21.4	100	50
2	22.2	101	50	21.9	100	50	22.1	100	50	21.6	98	50
3	22.8	102	50	22.5	101	50	22.7	102	50	22.4	100	50
4	23.0	102	50	22.6	100	50	22.9	101	50	22.4	99	50
5	23.4	101	50	23.0	100	50	23.3	101	50	22.9	99	50
6	23.9	102	50	23.5	100	50	23.7	101	50	23.0	98	50
7	24.6	101	50	24.0	98	50	24.6	101	50	24.1	99	50
8	24.6	101	50	24.2	99	50	24.5	100	50	24.1	99	50
9	24.8	102	50	24.8	102	50	24.8	102	50	24.7	101	50
10	24.9	103	50	24.7	102	50	24.8	102	50	24.7	102	50
11	25.2	101	50	25.1	101	50	25.3	102	50	25.2	101	50
12	25.6	103	50	25.1	101	50	25.2	102	50	25.3	102	50
13	25.8	103	50	25.2	101	50	25.3	101	50	25.3	101	50
17	26.8	103	50	26.3	102	50	26.3	102	50	26.7	103	50
21	26.8	102	50	26.3	100	50	26.9	103	50	27.1	103	50
26	28.5	104	50	27.7	101	50	27.7	101	50	27.8	101	50
30	28.8	104	49	27.8	100	50	27.7	100	50	28.3	102	50
35	29.4	104	49	28.5	100	50	28.8	101	50	29.1	102	50
40	30.0	103	49	29.0	99	50	29.4	101	50	29.9	102	50
44	30.0	101	49	29.2	98	50	29.7	100	50	30.6	103	49
49	30.4	101	49	29.9	99	50	30.9	102	50	32.8	109	48
53	32.1	101	49	31.8	100	50	32.6	103	48	34.6	109	45
57	32.6	101	49	33.0	102	50	32.3	100	48	35.1	109	42
62	33.0	100	49	33.6	102	50	33.4	102	48	34.7	105	38
66	34.1	102	49	33.9	102	49	32.7	98	48	36.1	108	35
70	33.4	101	49	34.0	102	49	33.9	102	43	37.1	112	29
75	34.6	101	49	35.3	103	48	37.6	109	33	38.8	113	13
79	35.6	99	49	35.8	100	46	37.9	106	26	38.0	106	7
83	35.9	97	47	36.3	98	44	37.9	102	21	39.5	106	4
88	36.3	96	47	38.8	102	42	40.3	106	13	44.5	117	2
93	36.0	95	43	36.9	98	36	37.0	98	6			
97	36.3	92	40	39.2	100	31	35.8	91	3			
101	36.8	100	35	40.5	110	23						
Mean for weeks												
1-13	24.0	102		23.7	100		23.9	101		23.6	100	
14-52	28.8	102		28.1	100		28.4	101		29.0	103	
53-101	34.7	98		35.8	101		36.6	104		37.6	107	

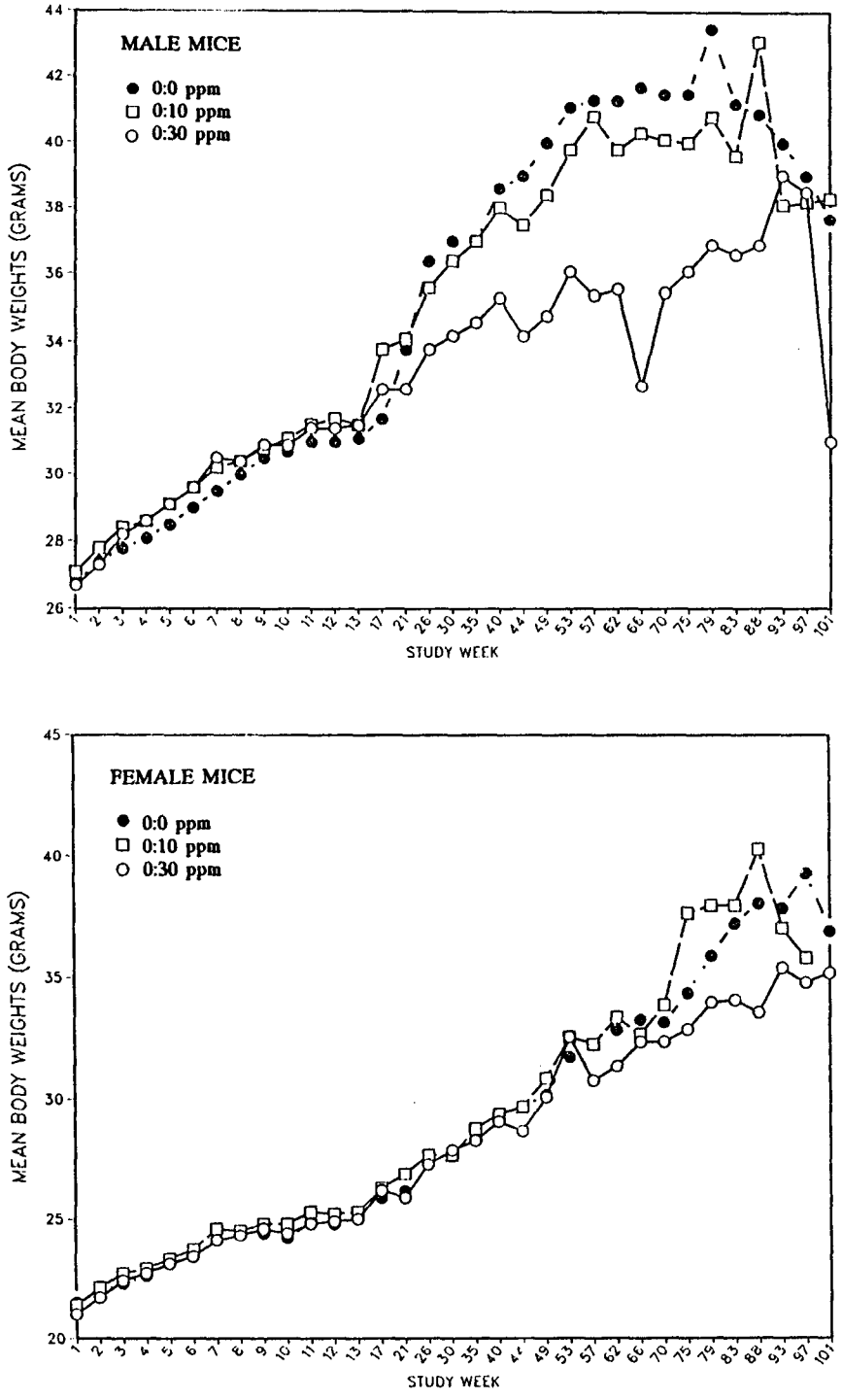

FIGURE 5a
Growth Curves for Mice Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 0:10, and 0:30 ppm Groups

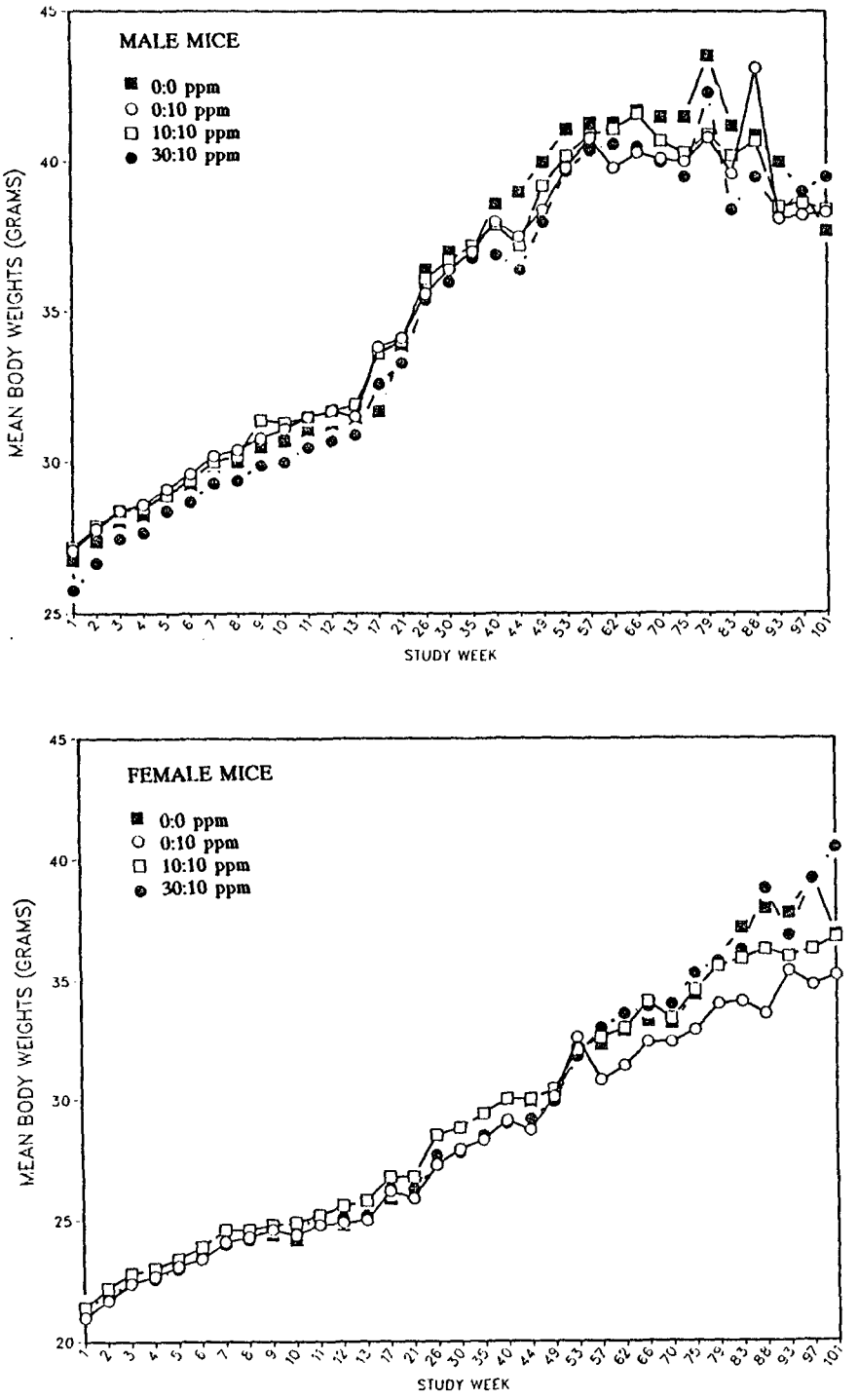

FIGURE 5b
Growth Curves for Mice Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 0:10, 10:10, and 30:10 ppm Groups

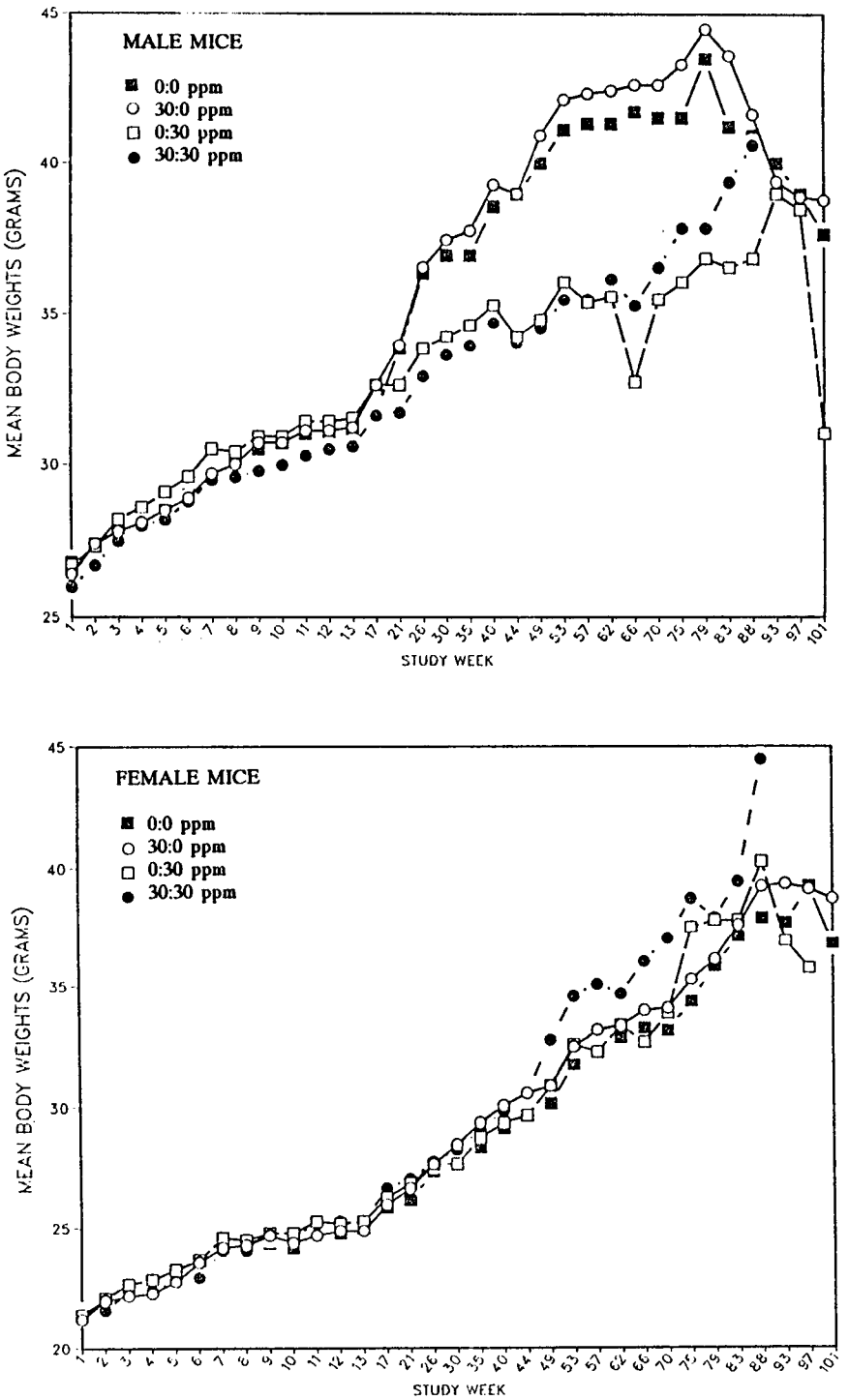

FIGURE 5c
Growth Curves for Mice Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 30:0, 0:30, and 30:30 ppm Groups

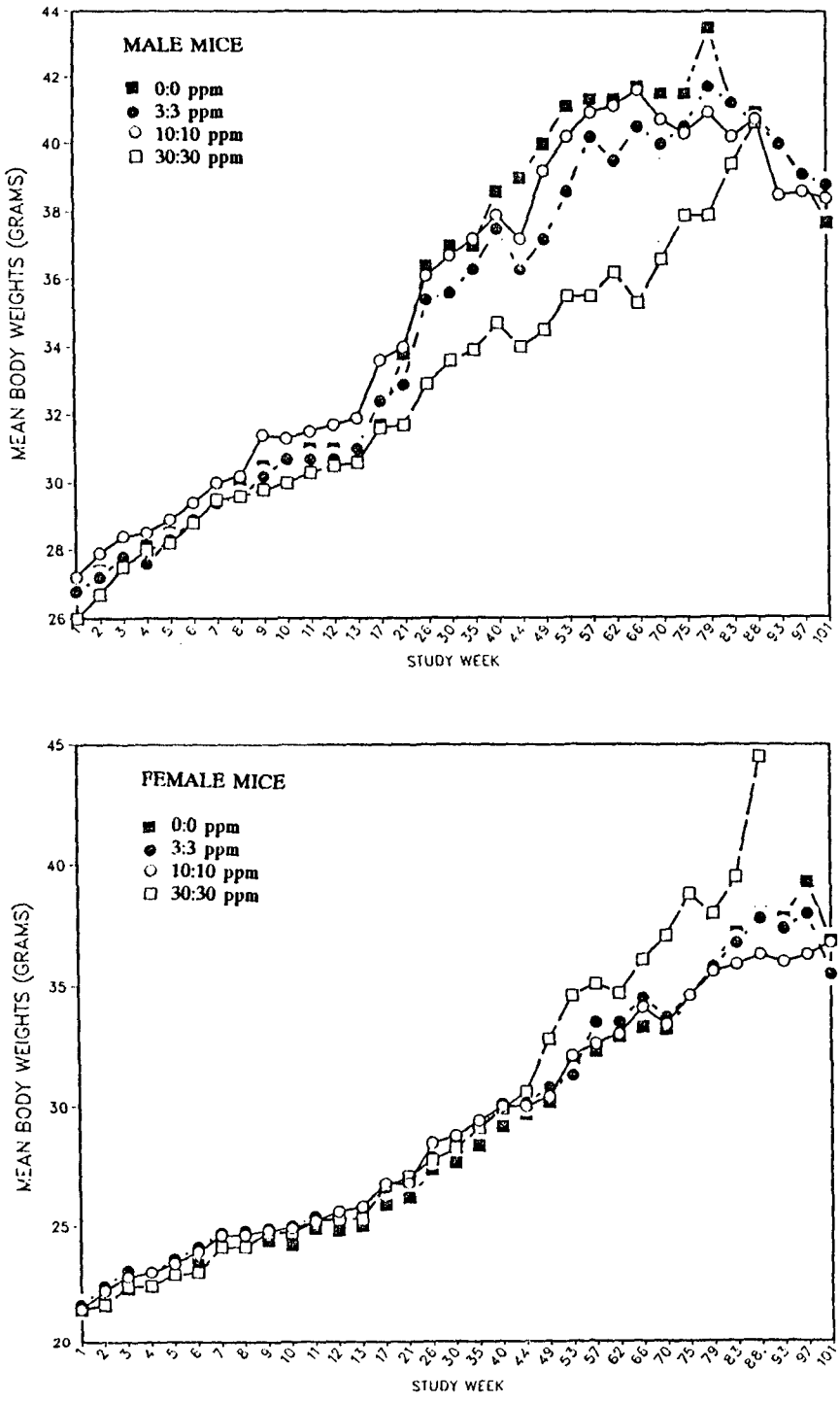

FIGURE 5d
Growth Curves for Mice Administered Polybrominated Biphenyls in Feed for 2 Years: 0:0, 3:3, 10:10, and 30:30 ppm Groups

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of lymphoma and neoplasms or nonneoplastic lesions of the liver, kidney, uterus, bone marrow, spleen, thyroid gland, and lung in mice.

Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendixes C for male mice and D for female mice. Historical incidences of neoplasms in control mice are given in Tables C3 for males and D3 for females.

Effects of Adult-Only Exposure of Mice to Polybrominated Biphenyls

The neoplastic and nonneoplastic effects of adult-only exposure were determined by comparison of the incidences of lesions in the 0:0, 0:10, and 0:30 ppm groups, which correspond to a standard carcinogenicity study.

Liver: The incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) were increased in 0:10 and 0:30 ppm mice (Table 25). While generally a single hepatocellular adenoma or carcinoma occurred in the liver of control mice, multiple adenomas, carcinomas, or both adenomas and carcinomas were often present in exposed mice. The incidences of various nonneoplastic lesions were also increased in exposed males and females. The increased incidences of some of these nonneoplastic lesions (coagulative necrosis and thrombosis) were associated with the increased incidences of liver neoplasms in exposed mice. Other lesions including cytomegaly (hypertrophy), fatty change (cytoplasmic vacuolization), eosinophilic and clear cell foci, necrosis of individual hepatocytes, and bile duct hyperplasia were related to polybrominated biphenyls treatment. In mice, bile duct hyperplasia consisted of both proliferation of oval cells similar to that described for rats and an increase in the number of small, well-formed bile ducts.

Kidney: The incidence of chronic nephropathy was increased in 0:30 ppm males (9/50, 10/50, 42/50) and females (0/50, 3/50, 16/46). No renal tubule cell neoplasms occurred in the kidney of any group of mice.

Other Lesions: The incidences of endometrial cystic hyperplasia of the uterus (46/49, 32/50, 2/48) and sternal myelofibrosis of the bone marrow (42/50, 11/50, 0/49) were decreased in female mice; increased hematopoiesis occurred in the spleen of 0:30 ppm males (10/50, 8/49, 41/49) and females (4/48, 5/50, 37/44). The incidence of thyroid follicular cell hyperplasia was increased in 0:30 ppm males (0/50, 0/49, 9/46). The incidences of lymphoma were decreased in 0:30 ppm male and female mice (males: 10/50, 4/50, 2/50; females: 6/50, 7/50, 0/49), and the incidence of alveolar/bronchiolar neoplasms was decreased in 0:30 ppm male mice (13/50, 10/50, 2/50).

TABLE 25
Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups

	0:0 ppm	0:10 ppm	0:30 ppm
Male			
Cytomegaly			
Overall rate[a]	0/50 (0%)	49/49 (100%)**	50/50 (100%)**
Fatty Change			
Overall rate	2/50 (4%)	45/49 (92%)**	50/50 (100%)**
Clear Cell Focus			
Overall rate	0/50 (0%)	8/49 (16%)**	1/50 (2%)
Eosinophilic Focus			
Overall rate	3/50 (6%)	16/49 (33%)**	6/50 (12%)
Hepatocyte Necrosis			
Overall rate	0/50 (0%)	4/49 (8%)	1/50 (2%)
Bile Duct Hyperplasia			
Overall rate	0/50 (0%)	0/49 (0%)	34/50 (68%)**
Hepatocellular Adenoma			
Overall rate	9/50 (18%)	48/49 (98%)	42/50 (84%)
Adjusted rate[b]	26.1%	100.0%	100.0%
Terminal rate[c]	7/31 (23%)	34/34 (100%)	0/0 (0%)
First incidence (days)	531	505	420
Logistic regression test[d]	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma, Multiple			
Overall rate	1/50 (2%)	48/49 (98%)**	37/50 (74%)**
Hepatocellular Carcinoma			
Overall rate	8/50 (16%)	30/49 (61%)	36/50 (72%)
Adjusted rate	21.3%	72.8%	100.0%
Terminal rate	4/31 (13%)	23/34 (68%)	0/0 (0%)
First incidence (days)	447	561	464
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple			
Overall rate	4/50 (8%)	13/49 (27%)*	22/50 (44%)**
Hepatocellular Adenoma or Carcinoma[e]			
Overall rate	16/50 (32%)	48/49 (98%)	48/50 (96%)
Adjusted rate	42.9%	100.0%	100.0%
Terminal rate	11/31 (35%)	34/34 (100%)	0/0 (0%)
First incidence (days)	447	505	420
Logistic regression test	P<0.001	P<0.001	P<0.001

(continued)

TABLE 25
Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

	0:0 ppm	0:10 ppm	0:30 ppm
Female			
Cytomegaly			
Overall rate	0/50 (0%)	49/50 (98%)**	48/48 (100%)**
Fatty Change			
Overall rate	0/50 (0%)	39/50 (78%)**	46/48 (96%)**
Clear Cell Focus			
Overall rate	2/50 (4%)	22/50 (44%)**	1/48 (2%)
Eosinophilic Focus			
Overall rate	1/50 (2%)	18/50 (36%)**	4/48 (8%)
Hepatocyte Necrosis			
Overall rate	0/50 (0%)	23/50 (46%)**	10/48 (21%)*
Bile Duct Hyperplasia			
Overall rate	0/50 (0%)	9/50 (18%)**	39/48 (81%)**
Hepatocellular Adenoma			
Overall rate	4/50 (8%)	39/50 (78%)	46/48 (96%)
Adjusted rate	10.3%	86.6%	100.0%
Terminal rate	4/39 (10%)	32/38 (84%)	0/0 (0%)
First incidence (days)	729 (T)	617	359
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma, Multiple			
Overall rate	1/50 (2%)	28/50 (56%)**	41/48 (85%)**
Hepatocellular Carcinoma			
Overall rate	1/50 (2%)	22/50 (44%)	35/48 (73%)
Adjusted rate	2.6%	52.2%	95.5%
Terminal rate	1/39 (3%)	18/38 (47%)	0/0 (0%)
First incidence (days)	729 (T)	629	484
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple			
Overall rate	0/50 (0%)	8/50 (16%)**	21/48 (44%)**
Hepatocellular Adenoma or Carcinoma[f]			
Overall rate	5/50 (10%)	42/50 (84%)	47/48 (98%)
Adjusted rate	12.8%	93.3%	100.0%
Terminal rate	5/39 (13%)	35/38 (92%)	0/0 (0%)
First incidence (days)	729 (T)	617	359
Logistic regression test	P<0.001	P<0.001	P<0.001

(continued)

TABLE 25
Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

* Significantly different (P≤0.05) from the 0:0 ppm group by the logistic regression test
** P≤0.01
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.
[e] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 363/1,114 (32.6% ± 13.6%), range 10%–68%
[f] Historical incidence: 153/1,113 (13.7% ± 8.6%), range 3%–34%

Effects of Perinatal-Only Exposure of Mice to Polybrominated Biphenyls

The neoplastic and nonneoplastic effects of perinatal-only exposure were determined by comparison of the incidences of lesions in the 0:0 and 30:0 ppm groups.

Liver: The incidences of hepatocellular adenomas, carcinomas, and adenomas or carcinomas (combined) were increased in mice receiving perinatal exposure to 30:0 ppm (Table 26). The incidences of a number of nonneoplastic liver lesions observed in the continuous exposure groups (cytomegaly, eosinophilic focus, and clear cell focus) were increased in male and female mice exposed only during the perinatal period to 30 ppm. Fatty change (cytoplasmic vacuolization), bile duct hyperplasia, and necrosis of individual hepatocytes, lesions seen in the chronic exposure groups, did not occur in females receiving only perinatal exposure; the incidences of these lesions were not increased in males. One lesion that was seen primarily in male mice and only in the mice from the 30:0 ppm perinatal exposure was cytologic alteration of hepatocytes. These hepatocytes were enlarged and contained a crystalline to irregular-shaped eosinophilic material within cytoplasmic vacuoles.

TABLE 26
Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0 and 30:0 ppm Groups

	0:0 ppm	30:0 ppm
Male		
Cytomegaly		
Overall rate[a]	0/50 (0%)	36/50 (72%)**
Fatty Change		
Overall rate	2/50 (4%)	2/50 (4%)
Clear Cell Focus		
Overall rate	0/50 (0%)	13/50 (26%)**
Eosinophilic Focus		
Overall rate	3/50 (6%)	20/50 (40%)**
Hepatocyte Necrosis		
Overall rate	0/50 (0%)	3/50 (6%)
Cytologic Alteration		
Overall rate	0/50 (0%)	21/50 (42%)**
Hepatocellular Adenoma		
Overall rate	9/50 (18%)	31/50 (62%)
Adjusted rate[b]	26.1%	77.1%
Terminal rate[c]	7/31 (23%)	23/32 (72%)
First incidence (days)	531	581
Logistic regression test[d]		P<0.001
Hepatocellular Adenoma, Multiple		
Overall rate	1/50 (2%)	22/50 (44%)**
Hepatocellular Carcinoma		
Overall rate	8/50 (16%)	17/50 (34%)
Adjusted rate	21.3%	42.8%
Terminal rate	4/31 (13%)	10/32 (31%)
First incidence (days)	447	516
Logistic regression test		P=0.033
Hepatocellular Carcinoma, Multiple		
Overall rate	4/50 (8%)	6/50 (12%)
Hepatocellular Adenoma or Carcinoma		
Overall rate	16/50 (32%)	40/50 (80%)
Adjusted rate	42.9%	88.8%
Terminal rate	11/31 (35%)	27/32 (84%)
First incidence (days)	447	516
Logistic regression test		P<0.001

(continued)

TABLE 26
Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0 and 30:0 ppm Groups (continued)

	0:0 ppm	30:0 ppm
Female		
Cytomegaly		
Overall rate	0/50 (0%)	6/50 (12%)**
Clear Cell Focus		
Overall rate	2/50 (4%)	5/50 (10%)
Eosinophilic Focus		
Overall rate	1/50 (2%)	3/50 (6%)
Cytologic Alteration		
Overall rate	0/50 (0%)	1/50 (2%)
Hepatocellular Adenoma		
Overall rate	4/50 (8%)	19/50 (38%)
Adjusted rate	10.3%	39.6%
Terminal rate	4/39 (10%)	16/45 (36%)
First incidence (days)	729 (T)	620
Logistic regression test		P<0.001
Hepatocellular Adenoma, Multiple		
Overall rate	1/50 (2%)	7/50 (14%)*
Hepatocellular Carcinoma		
Overall rate	1/50 (2%)	4/50 (8%)
Adjusted rate	2.6%	8.7%
Terminal rate	1/39 (3%)	3/45 (7%)
First incidence (days)	729 (T)	708
Logistic regression test		P=0.213
Hepatocellular Carcinoma, Multiple		
Overall rate	0/50 (0%)	0/50 (0%)
Hepatocellular Adenoma or Carcinoma		
Overall rate	5/50 (10%)	21/50 (42%)
Adjusted rate	12.8%	43.8%
Terminal rate	5/39 (13%)	18/45 (40%)
First incidence (days)	729 (T)	620
Logistic regression test		P<0.001

* Significantly different (P≤0.05) from the 0:0 ppm group by the logistic regression test
** (P≤0.01)
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.

Effects of Combined Perinatal and Adult Exposure of Mice to Polybrominated Biphenyls

The effects of combined perinatal and adult exposure were determined by comparison of the incidences of lesions in mice in the 0:10, 10:10, and 30:10 ppm exposure groups and in the 0:30 and 30:30 ppm exposure groups.

Liver: In groups receiving adult exposure to 10 ppm, the incidences of hepatocellular carcinoma were increased in males and females receiving perinatal exposure to 30 ppm (Table 27); the incidence of hepatocellular adenoma was increased in females receiving 30:10 ppm. There were increases in the number of 30:10 ppm females with multiple adenomas (females: 28/50, 31/50, 44/50) and the numbers of mice with multiple hepatocellular carcinomas (males: 13/49, 14/49, 21/50; females: 8/50, 13/50, 22/50). There were slight decreases in the incidences of clear cell and eosinophilic foci; there were no other clear effects of polybrominated biphenyls exposure on the incidences or severity of nonneoplastic lesions.

In mice receiving adult exposure to 30 ppm, the incidence of hepatocellular adenoma was significantly decreased in females receiving perinatal exposure to 30 ppm (Table 28). The incidence of hepatocellular adenoma was increased in males exposed to 30:30 ppm. The incidence of necrosis was slightly increased in males and the incidence of necrosis of individual hepatocytes was slightly decreased in females exposed to 30:30 ppm (Tables C4 and D4).

The combined incidences of hepatocellular adenomas and carcinomas for all exposure groups are shown in Table 29. A single logistic regression analysis applied to all eight experimental groups indicated that the incidences of hepatocellular neoplasms increased significantly ($P \leq 0.001$) with increasing F_0 and F_1 levels of polybrominated biphenyls (Piegorsch *et al.*, 1986). Because of the high liver neoplasm response in the adult-only and perinatal-only exposure groups, the enhancing effects of combined F_0 and F_1 exposure on hepatocellular neoplasms could not be assessed.

Kidney: The incidences of chronic nephropathy were increased in females and decreased in males exposed to 30:30 ppm (Tables C4 and D4).

Spleen: In animals receiving adult exposure to 10 ppm, the incidence of hematopoietic cell proliferation increased with the perinatal exposure level in females (5/50, 8/50, 23/50) and increased slightly in 30:10 ppm males (8/49, 8/50, 11/48). The incidences of hematopoietic cell proliferation were decreased in mice exposed to 30:30 ppm compared with those in the 0:30 ppm group (Tables C4 and D4), but the decreases were not statistically significant.

Thyroid Gland: The incidences of thyroid follicular cell hyperplasia were increased in males and females receiving adult exposure to 10 ppm; the incidence of follicular cell adenoma was increased in 30:10 ppm males (Table 30), and the incidence exceeds the historical control range of 0% to 4% in untreated males in NTP 2-year studies.

TABLE 27
Selected Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 10:10, and 30:10 ppm Groups

	0:10 ppm	10:10 ppm	30:10 ppm
Male			
Cytomegaly			
Overall rate[a]	49/49 (100%)	48/49 (98%)	48/50 (96%)
Fatty Change			
Overall rate	45/49 (92%)	43/49 (88%)	40/50 (80%)
Clear Cell Focus			
Overall rate	8/49 (16%)	8/49 (16%)	5/50 (10%)
Eosinophilic Focus			
Overall rate	16/49 (33%)	4/49 (8%)**	0/50 (0%)**
Hepatocyte Necrosis			
Overall rate	4/49 (8%)	1/49 (2%)	4/50 (8%)
Bile Duct Hyperplasia			
Overall rate	0/49 (0%)	0/49 (0%)	8/50 (16%)**
Hepatocellular Adenoma			
Overall rate	48/49 (98%)	46/49 (94%)	48/50 (96%)
Adjusted rate[b]	100%	97.9%	100%
Terminal rate[c]	34/34 (100%)	33/34 (97%)	29/29 (100%)
First incidence (days)	505	475	391
Logistic regression test[d]	P=0.705	P=0.464N	P=0.984N
Hepatocellular Adenoma, Multiple			
Overall rate	48/49 (98%)	41/49 (84%)	42/50 (84%)
Hepatocellular Carcinoma			
Overall rate	30/49 (61%)	31/49 (63%)	40/50 (80%)
Adjusted rate	72.8%	75.5%	92.9%
Terminal rate	23/34 (68%)	23/34 (71%)	26/29 (90%)
First incidence (days)	561	640	391
Logistic regression test	P=0.004	P=0.484	P=0.010
Hepatocellular Carcinoma, Multiple			
Overall rate	13/49 (27%)	14/49 (29%)	21/50 (42%)*
Hepatocellular Adenoma or Carcinoma			
Overall rate	48/49 (98%)	46/49 (94%)	48/50 (96%)
Adjusted rate	100.0%	97.9%	100.0%
Terminal rate	34/34 (100%)	33/34 (97%)	29/29 (100%)
First incidence (days)	505	475	391
Logistic regression test	P=0.705	P=0.464N	P=0.984N

(continued)

TABLE 27
Selected Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 10:10, and 30:10 ppm Groups (continued)

	0:10 ppm	10:10 ppm	30:10 ppm
Female			
Cytomegaly			
Overall rate	49/50 (98%)	49/50 (98%)	50/50 (100%)
Fatty Change			
Overall rate	39/50 (78%)	44/50 (88%)	43/50 (86%)
Clear Cell Focus			
Overall rate	22/50 (44%)	13/50 (26%)	4/50 (8%)**
Eosinophilic Focus			
Overall rate	18/50 (36%)	16/50 (32%)	4/50 (8%)**
Hepatocyte Necrosis			
Overall rate	23/50 (46%)	15/50 (30%)	4/50 (8%)**
Bile Duct Hyperplasia			
Overall rate	9/50 (18%)	9/50 (18%)	6/50 (12%)
Hepatocellular Adenoma			
Overall rate	39/50 (78%)	38/50 (76%)	47/50 (94%)
Adjusted rate	86.6%	94.9%	97.9%
Terminal rate	32/38 (84%)	31/33 (94%)	16/17 (94%)
First incidence (days)	617	563	435
Logistic regression test	P<0.001	P=0.546	P=0.005
Hepatocellular Adenoma, Multiple			
Overall rate	28/50 (56%)	31/50 (62%)	44/50 (88%)*
Hepatocellular Carcinoma			
Overall rate	22/50 (44%)	26/50 (52%)	44/50 (88%)
Adjusted rate	52.2%	63.1%	95.5%
Terminal rate	18/38 (47%)	18/33 (55%)	15/17 (88%)
First incidence (days)	629	563	435
Logistic regression test	P<0.001	P=0.215	P<0.001
Hepatocellular Carcinoma, Multiple			
Overall rate	8/50 (16%)	13/50 (26%)	22/50 (44%)*
Hepatocellular Adenoma or Carcinoma			
Overall rate	42/50 (84%)	44/50 (88%)	50/50 (100%)
Adjusted rate	93.3%	97.8%	100.0%
Terminal rate	35/38 (92%)	32/33 (97%)	17/17 (100%)
First incidence (days)	617	563	435
Logistic regression test	P<0.001	P=0.192	P<0.001

* Significantly different (P≤0.05) from the 0:10 ppm group by the logistic regression test
** P≤0.01
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 0:10 ppm group are the P values associated with the trend test. Beneath the 10:10 and 30:10 ppm groups are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by N.

TABLE 28
Selected Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 30:30 ppm Groups

	0:30	30:30
Male		
Hepatocellular Adenoma		
Overall rate[a]	42/50 (84%)	48/50 (96%)
Adjusted rate[b]	100.0%	100.0%
Terminal rate[c]	0/0 (0%)	0/0 (0%)
First incidence (days)	420	314
Logistic regression test[d]		P=0.007
Hepatocellular Adenoma, Multiple		
Overall rate	37/50 (74%)	43/50 (86%)*
Hepatocellular Carcinoma		
Overall rate	36/50 (72%)	35/50 (70%)
Adjusted rate	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)
First incidence (days)	464	314
Logistic regression test		P=0.223
Hepatocellular Carcinoma, Multiple		
Overall rate	22/50 (44%)	17/50 (34%)
Hepatocellular Adenoma or Carcinoma		
Overall rate	48/50 (96%)	50/50 (100%)
Adjusted rate	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)
First incidence (days)	420	314
Logistic regression test		P=0.053

(continued)

TABLE 28
Selected Liver Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 30:30 ppm Groups (continued)

	0:30	30:30
Female		
Hepatocellular Adenoma		
Overall rate	46/48 (96%)	41/47 (87%)
Adjusted rate	100.0%	94.9%
Terminal rate	0/0 (0%)	0/0 (0%)
First incidence (days)	359	291
Logistic regression test		P=0.022N
Hepatocellular Adenoma, Multiple		
Overall rate	41/48 (85%)	36/47 (77%)
Hepatocellular Carcinoma		
Overall rate	35/48 (73%)	29/47 (62%)
Adjusted rate	95.5%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)
First incidence (days)	484	291
Logistic regression test		P=0.484N
Hepatocellular Carcinoma, Multiple		
Overall rate	21/48 (44%)	9/47 (19%)**
Hepatocellular Adenoma or Carcinoma		
Overall rate	47/48 (98%)	47/47 (100%)
Adjusted rate	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)
First incidence (days)	359	291
Logistic regression test		P=0.529

* Significantly different (P≤0.05) from the 0:30 ppm group by the logistic regression test
** P≤0.01
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by N.

TABLE 29
Hepatocellular Adenomas and Carcinomas in Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration (ppm)	F_1 Concentration (ppm) 0	3	10	30
Male				
0	16/50	–[b]	48/49**	48/50**
3	–	31/48**	–	–
10	–	–	46/49**	–
30	40/50**	–	48/50**▲▲	50/50**▲▲
Female				
0	5/50	–	42/50**	47/48**
3	–	7/50	–	–
10	–	–	44/50**	–
30	21/50**	–	50/50**▲▲	47/47**▲▲

** Significantly different (P≤0.01) from the 0:0 ppm group by the logistic regression test
▲▲ Significantly different (P≤0.01) from the 30:0 ppm group by the logistic regression test
[a] Incidences are given as the number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Animals were not exposed at these concentrations.

TABLE 30
Selected Thyroid Gland Lesions in Mice in the 2-Year Feed Study of Polybrominated Biphenyls

	0:10 ppm	10:10 ppm	30:10 ppm
Male			
Follicular Cell Hyperplasia (Nodular)			
Overall rate[a]	0/49 (0%)	3/48 (6%)	16/48 (33%)**
Follicular Cell Adenoma			
Overall rate	0/49 (0%)	0/48 (0%)	5/48 (10%)
Adjusted rate[b]	0.0%	0.0%	16.1%
Terminal rate[c]	0/34 (0%)	0/34 (0%)	4/28 (14%)
First incidence (days)	–[e]	–	548
Logistic regression test[d]	P=0.003	–	P=0.029
Female			
Follicular Cell Hyperplasia (Nodular)			
Overall rate	6/50 (12%)	11/50 (22%)	15/50 (30%)**
Follicular Cell Adenoma			
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.6%	3.0%	11.8%
Terminal rate	1/38 (3%)	1/33 (3%)	2/17 (12%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Logistic regression test	P=0.156	P=0.730	P=0.233

** Significantly different (P≤0.01) from the 0:10 ppm group by the logistic regression test
(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.
[e] Not applicable; no neoplasms in animal group

DISCUSSION AND CONCLUSIONS

The present studies were designed to compare the carcinogenicity of polybrominated biphenyls given in a combined perinatal and adult exposure regimen with that of polybrominated biphenyls given in a conventional bioassay protocol. The combined perinatal and adult protocol included exposure of maternal animals prior to breeding, through gestation, lactation, and weaning, followed by continued dietary exposure of offspring beginning at 8 weeks of age for 2 years. The conventional protocol involved exposure to polybrominated biphenyls in the diet for 2 years beginning at 8 weeks of age.

GESTATIONAL STUDIES: PERINATAL TOXICITY

The selection of dietary levels of 10 and 30 ppm polybrominated biphenyls for the adult exposure portion of the studies was based on the findings of gavage studies with polybrominated biphenyls reported by Gupta *et al.* (1983). The gestational studies were undertaken to determine the maximum exposure levels that when given to the dams during the perinatal period would not have adverse effects on embryonic, fetal, or neonatal development. The exposure levels evaluated for this portion of the studies were 1 to 30 ppm in the diet. In both rats and mice, there were no chemical-related effects observed on implantation number, fertility, or litter-yield. There were also no chemical effects on preweaning or postweaning survival of the F_1 generation. However, in contrast to the results of the mouse study, the preweaning litter weights of rats in the 30 ppm groups were reduced to less than 80% of the respective control group means at days 0, 4, and 12. At weaning, the mean weight of litters in the 30 ppm group was 80% of the control group mean. The final body weights of male and female rats exposed to 30 ppm were also decreased more than 10% compared to those of the controls. Histopathologic changes in the liver, seen in rats exposed to 10 or 30 ppm, were limited to hepatocellular hypertrophy of insufficient severity to influence exposure selection. Therefore, because of the body weight decreases in rats in the 30 ppm groups, 10 ppm was selected as the highest exposure level for the perinatal portion of the study in rats; 30 ppm was selected as the highest perinatal exposure level for mice. These exposure levels were much lower than ones previously reported to cause embryonic toxicity or overt toxicity in offspring of female mice and rats exposed to polybrominated biphenyls in the diet at 100 and 200 ppm, respectively (Corbett *et al.*, 1975; Preache *et al.*, 1976; McCormack *et al.*, 1981).

The following combinations of perinatal and adult exposures were evaluated in these studies:

Rats (ppm)		Mice (ppm)	
F_0	F_1	F_0	F_1
0	0	0	0
0	10	0	10
0	30	0	30
1	3	3	3
3	10	10	10
10	0	30	0
10	10	30	10
10	30	30	30

CHRONIC TOXICITY

Polybrominated biphenyl toxicity has been reported in a number of short-term studies in laboratory animals exposed for up to six months. Toxicity findings included decreased body weights, survival, and feed consumption, hepatomegaly, histopathologic changes in the liver (e.g. hypertrophy, enlarged nuclei, and altered hepatic foci), induction of liver mixed-function oxidases, decreased thyroxine and triiodothyronine, increased serum cholesterol and gamma-glutamyl transpeptidase activity, and decreased hepatic vitamin A (Dent *et al.*, 1976; Sleight and Sanger, 1976; Kimbrough *et al.*, 1978; Gupta and Moore, 1979; McCormack *et al.*, 1981; Gupta *et al.*, 1983). Animals evaluated at 9 months during these NTP studies had decreased body weights, hepatomegaly, histopathologic changes in the liver (hypertrophy, vacuolization, and altered cellular foci), mild anemia, increases in serum cholesterol, and decreases in serum triglycerides, representing the expected pattern of chronic toxicity. After 9 months of exposure to the F_1 generation, there were no differences to the general features of polybrominated biphenyls toxicity due to perinatal exposure.

At the end of the 2-year study, mean body weights were decreased more than 10% compared to those of the controls for all groups of rats exposed to 10 or 30 ppm during the adult period except females receiving an F_0:F_1 exposures of 0:10 and 3:10 ppm. Survival rates were also decreased in male rats exposed to 3:10, 10:10, or 10:30 ppm polybrominated biphenyls. The increased incidences of mononuclear cell leukemia and liver neoplasms in exposed animals could have contributed to reduced survival in these groups. The 2-year survival of mice exposed to polybrominated biphenyls was most adversely affected, as all the mice in 10:30 and 0:30 ppm groups died before the end of the study. Again, the increased mortality was probably due to the high incidences of liver neoplasms in these animals. In general, there was not a marked difference in mean body weights or survival between groups of mice receiving a combination of perinatal and adult exposure and groups receiving adult exposure only.

Positive serologic reactions for a number of murine viruses were observed, particularly in mice (Table I1). Rao *et al.* (1989) have shown that viral infections in mice generally do not influence body weights, survival, or spontaneous liver neoplasm rates.

Negative trends in the incidences of myelofibrosis in the bone marrow and cystic endometrial hyperplasia in female mice may have been related to decreased survival, as these lesions are commonly seen in aged female mice. However, the incidences of both lesions have been exacerbated with administration of estrogens (McLachlan *et al.*, 1980; Sass and Montal, 1980). Because the incidence of these lesions was also reduced in the 0:10 ppm group, which did not have reduced survival compared to the controls, it is possible that polybrominated biphenyls may have had a direct effect on these organs or a secondary effect related to altered (enhanced) estrogen metabolism by the liver.

The slight increase in proliferative follicular cell lesions in the thyroid gland of mice may have been related to increased degradative metabolism of triiodothyronine and thyroxine by the liver. Increased thyroid gland weights and decreased serum triiodothyronine concentrations for rats in prechronic studies have been reported previously (Gupta and Moore, 1979; Akoso *et al.*, 1982; Gupta *et al.*, 1983), but no thyroid gland effects have been identified in chronic studies. The presence of this slight increase in the incidence of proliferative lesions in the thyroid gland of mice is in contrast to the results seen in rats, which had minimal hypertrophy and hyperplasia of the thyroid follicular gland cells at the 9-month interim evaluation but no evidence of an increase in thyroid gland proliferative lesions after 2 years. Increased splenic hematopoiesis occurred in the 0:30 ppm mice, which all died during the 2-year study.

There was no evidence of depletion of bone marrow and a compensatory response in the spleen. Similarly, there were no chronic, severe, inflammatory lesions or skin ulcerations to account for the increased splenic hematopoiesis. The significance of the unusual cytologic alteration in the liver of male mice and one female mouse given only perinatal exposure to 30:0 ppm is not known. Similar hepatocellular changes have been described in mice in the 2-year study of pentachloroanisole (NTP, 1993) with increased incidences of hepatocellular changes occurring more frequently in males than in females.

CARCINOGENICITY OF POLYBROMINATED BIPHENYLS

Adult-Only Exposure

The liver was the principal organ showing a carcinogenic response to polybrominated biphenyls in rats and mice. The administration of polybrominated biphenyls in the diet to adult rats and mice resulted in increased incidences of hepatocellular adenomas, carcinomas, or adenomas and carcinomas (combined). The combined incidence of liver neoplasms in male and female rats ranged from 24% at 0:10 ppm to 82% at 0:30 ppm. The historical rates for these neoplasms are 0.7% for male rats and 0.2% for female rats (Haseman *et al.*, 1985). Although the combined incidences of adenoma and carcinoma were similar for males and females, there were more carcinomas in males (19 carcinomas) than females (4 carcinomas) at the highest exposure level. Mice appeared more sensitive to carcinogenicity induced by polybrominated biphenyls than rats in that hepatocellular adenomas occurred after as few as 9 months of exposure, and the combined incidence of liver neoplasms ranged from 84% to 98% at the 0:10 and 0:30 ppm exposure levels at the end of the studies. At the 9-month interim evaluation, the polybrominated biphenyls residues in the livers of mice given the 0:30 ppm diet were about two times higher for males and three to four times higher for females than in the livers of rats given the same diet, suggesting a possible association between the concentration of polybrominated biphenyls in liver and the incidence of liver neoplasms. The incidences of liver neoplasms in mice at the end of the study suggest that polybrominated biphenyls concentrations lower than 0:10 ppm are required to establish a dose-response relationship for liver neoplasms.

The observations in the mouse study of centrilobular hepatocellular hypertrophy in the perinatal dose determination study, hepatocellular adenoma at the 9-month interim evaluation, and progression to a preponderance of hepatocellular carcinomas at 2 years is similar to the spectrum of hepatocellular proliferative lesions described by Maronpot *et al.* (1987) for a number of known hepatocarcinogens such as chlordane, di(2-ethylhexyl)phthalate, and hexachlorodibenzo-*p*-dioxin. The previous studies of polybrominated biphenyls carcinogenicity in rats and mice, which involved exposures of as long as 6 months followed by an observation period of up to 24 months, have adequately demonstrated that the liver is the major site of carcinogenicity induced by polybrominated biphenyls (Kimbrough *et al.*, 1978; Bernert *et al.*, 1983; Gupta *et al.*, 1983; NTP, 1983). However, the current studies have shown that the liver neoplasm incidences were much higher in the animals exposed for up to 2 years than in the studies with exposures of a shorter duration reported previously.

Decreased incidences of adrenal gland pheochromocytoma and of pituitary gland, mammary gland, and preputial gland neoplasms occurred in those groups of rats with significantly reduced body weight gains. Similar reduced incidences for these types of neoplasms have been reported in rats with reduced body weights (Rao *et al.*, 1987, 1990a).

In mice, negative trends for lymphoma and alveolar/bronchiolar neoplasms appeared to be treatment related. Decreased incidences of lymphoma and alveolar/bronchiolar neoplasms in 0:30 ppm mice may have been related to decreased survival. Significantly decreased body weights in the 0:30 ppm mice may also have contributed to the reduced incidences of these two relatively common, spontaneously occurring neoplasms (Rao *et al.*, 1987, 1990b).

Perinatal-Only Exposure

The groups of male and female mice that received only perinatal exposure at the maternal dietary exposure of 30:0 ppm had increased incidences of liver neoplasms compared to the control groups; a marginal increase in liver adenomas was also observed in male rats exposed to 10:0 ppm during the perinatal period. As with adult-only exposure, mice appeared more sensitive to the development of liver neoplasms than were rats exposed perinatally, although it is difficult to directly compare the species response because the mice received a higher exposure. In rats, only five exposed males had hepatocellular adenomas (one control male also had a hepatocellular adenoma), while in perinatally exposed mice, the combined incidences of neoplasms were 40 of 50 for males and 21 of 50 for females, with control incidences of 16 of 50 for males and 5 of 50 for females. The species differences in the incidences of liver neoplasms induced by polybrominated biphenyls agreed with the liver residue values of polybrominated biphenyls determined at the 9-month interim evaluations. The polybrominated biphenyls concentrations were about 20 times higher in the livers of mice than in the livers of rats (0.89 to 2.93 ppm in rats versus 22.33 to 24.57 ppm in mice; Appendix G).

Groce and Kimbrough (1984) previously reported a weak carcinogenic effect of Firemaster FF-1® (polybrominated biphenyls) in the offspring of Sherman rats exposed on days 7 and 14 of gestation to 200 mg/kg Firemaster FF-1® by gavage. At 2 years of age, 3 of 51 females and 4 of 41 males had liver neoplasms. There were no liver neoplasms in controls.

Combined Perinatal and Adult Exposure

Agents that are carcinogenic for animals during postnatal life and are capable of crossing placental barriers can be expected to act as transplacental carcinogens (Rice, 1981). This was clearly true for polybrominated biphenyls in that the combined perinatal and adult dietary exposure to polybrominated biphenyls also caused increased hepatocellular neoplasms in rats and mice. The perinatal and adult exposure protocol for detection of the carcinogenic potential of polybrominated biphenyls or other chemicals was based on the premise that combining these exposures would increase the sensitivity of the bioassay. This increased sensitivity was expected to be expressed in terms of a higher incidence of neoplasms, a reduction in the latency period, a different spectrum of neoplasms, or a combination of these effects. As in the adult exposure studies, the major site of polybrominated biphenyls carcinogenicity in the perinatal and adult exposure studies was the liver. In male rats, there were no enhancing effects of combined perinatal and adult exposure. However, perinatal exposure enhanced the susceptibility of female rats receiving 10 or 30 ppm polybrominated biphenyls as adults to the induction of liver neoplasms. This finding is in contrast with the findings that females receiving perinatal exposure only had no increased incidences of liver neoplasms, while males receiving perinatal exposure only had marginally increased incidences in hepatocellular adenomas. It is not known whether the enhancing effect of perinatal exposure on the liver neoplasm incidence in female rats was due to an increased sensitivity of the animals or due simply to the longer duration of exposure.

It was not possible to assess the possible enhancing effect of combined perinatal and adult exposure on hepatocellular neoplasms in male and female mice, because adult-only exposure to 10 or 30 ppm polybrominated biphenyls resulted in such high (84% to 98%) liver neoplasm rates. Thus, changes in the overall neoplasm incidences among these groups could not be demonstrated. There was some indication that liver neoplasms were occurring earlier in the 30:30 ppm groups as compared to the 0:30 ppm groups (Table 31). In addition, there is some evidence for perinatal enhancement of liver neoplasms in the 10 ppm groups (Table 27) where there were increases in multiple neoplasms, particularly in females receiving a high perinatal exposure level. Because of the high neoplasm rates, the statistical comparisons of survival among these groups (see Table 22 and Figure 3) are essentially equivalent to an evaluation of time to death with neoplasm. However, because cause of death determinations were not made and the actual times of neoplasm onset were unknown, it is unclear whether the earlier mortality in the higher exposure groups resulted from a shortened neoplasm latency or was merely reflecting an increased generalized toxicity, which would result in the liver neoplasms being observed, but not necessarily developing, earlier.

TABLE 31
Mean Time to Death with Liver Neoplasms in Mice Administered Polybrominated Biphenyls[a]

F_0 Concentration (ppm)	F_1 Concentration (ppm)	
	10	30
Male		
0	728	630
10	728	–[b]
30	727	512
Female		
0	731	575
10	730	–
30	700	501

[a] Mean times to death are given in days.
[b] Animals were not exposed at these concentrations.

The incidences of mononuclear cell leukemia were increased in male and female rats receiving chronic exposure to polybrominated biphenyls, and this increase was enhanced when perinatal exposure was combined with chronic exposure. In untreated control rats in NTP 2-year studies, the mean historical incidences of mononuclear cell leukemia in 25- to 26-month-old male and female F344/N rats are 48% and 27%, respectively. The mean historical incidence of mononuclear cell leukemia for studies performed at the study laboratory are similar: 51.5% for males and 28% for females (Tables A3b and B3b). In both male and female rats, all groups receiving chronic exposure to polybrominated biphenyls had a higher incidence of animals with mononuclear cell leukemia; groups with chronic exposure to 10 or 30 ppm polybrominated biphenyls with perinatal exposure to 10 ppm had significantly higher incidences of mononuclear cell leukemia (Table 15). The incidences in males were as high as 82% and exceeded the upper historical control range of 62%. In females, the incidences were as high as 54% and exceeded the overall upper historical control range of 52% and the laboratory upper historical control range of 28%. The term "mononuclear cell leukemia," as used in this report, refers to a neoplastic disease of F344/N rats that is characterized by infiltration of pleomorphic blastlike mononuclear cells in numerous organs. This type of leukemia appears to originate in the spleen but later infiltrates the liver, lung, bone marrow, lymph nodes, and other organs (Stefanski *et al.*, 1990). Mononuclear cell leukemia has also been described as large, granular, lymphocytic leukemia (Losco and Ward, 1984; Stromberg, 1985). None of the studies in the literature on polybrominated biphenyls carcinogenicity reported an association of leukemia with polybrominated biphenyls exposure. This could be due to the shorter durations of exposure to polybrominated biphenyls in those studies.

MECHANISM OF POLYBROMINATED BIPHENYLS TOXICITY AND CARCINOGENICITY

Polybrominated biphenyls are potent inducers of hepatic microsomal enzymes and produce a mixed type of induction; that is, they cause the formation of enzymes typical of those induced by phenobarbital and 3-methylcholanthrene (Dent *et al.*, 1976). In a study of the major components of a polybrominated biphenyls mixture, Aust *et al.* (1981) reported that congeners with no ortho bromines have a toxicity similar to that associated with TCDD (dioxin) and induce liver microsomal enzyme activity similar to that induced by 3-methylcholanthrene. The TCDD-like toxicity can be related to polybrominated biphenyls having a planar configuration, and a mechanism of toxicity of halogenated aryl hydrocarbons, including

polybrominated biphenyls, may be initial binding to a cytosolic receptor protein, followed by the toxic responses in target tissues (Zacharewski *et al.*, 1988).

Polybrominated biphenyl compounds have been assayed for mutagenic and clastogenic activity in a variety of bacterial and mammalian systems and were generally found to be nongenotoxic (Haworth *et al.*, 1983; Galloway *et al.*, 1987). It has been shown that polybrominated biphenyls are liver neoplasm promoters in a two-stage model of hepatocarcinogenesis (Jensen *et al.*, 1982, 1984; Sleight, 1985). Polybrominated biphenyls also inhibited intercellular communication in Chinese hamster V79 cells in culture and rat epithelial cells, a characteristic of neoplasm promoters (Tsushimoto *et al.*, 1982; Rezabek *et al.*, 1988). Polybrominated biphenyls did not induce DNA repair synthesis in rat, mouse, or hamster hepatocytes in primary cultures and did not cause mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in rat liver epithelial cells or in human fibroblasts, supporting further that polybrominated biphenyls possibly act as promoters (Williams *et al.*, 1984). Wasito and Sleight (1989) have shown that polybrominated biphenyls may also promote the development of neoplasms at nonhepatic sites. The number of tracheal papillomas in hamsters given *N*-nitrosodiethylamine (NDEA) as an initiator and polybrominated biphenyls as a promotor were significantly increased compared to the number of papillomas in hamsters who were given only NDEA.

Mirsalis and Steinmetz (1990) have reported that genotoxic hepatocarcinogens interact *in vivo* with DNA and increase S-phase synthesis in rodent hepatocytes. Furthermore, the nongenotoxic hepatocarcinogens also induce S-phase synthesis in the liver and the increases correlate with hepatocarcinogenic activity of the chemicals. Polybrominated biphenyls were studied by Mirsalis *et al.* (1989) for unscheduled DNA synthesis and S-phase synthesis *in vitro* in rodent hepatocytes, following *in vivo* treatment. As expected, the results with polybrominated biphenyls for unscheduled DNA synthesis were negative in both rats and mice. The results for S-phase synthesis were positive in mice; the induction of S-phase synthesis were three to six times higher in females than in males, depending on the dose levels of polybrominated biphenyls. The current mouse study correlates well with that observation, because the percentage of dosed animals with liver neoplasms was much higher in female mice than in male mice compared to the controls. However, the S-phase synthesis induction in rats reported by Mirsalis was equivocal; this does not correlate well with the results from the present rat study, where polybrominated biphenyls were clearly hepatocarcinogenic in rats. This may also disagree with the hypothesis that increased cell turnover is a requirement for nongenotoxic hepatocarcinogenesis.

CONCLUSIONS

Adult-Only Exposure

Under the conditions of these 2-year, adult-only, dietary exposure studies, there was *clear evidence of carcinogenic activity** for polybrominated biphenyls in male and female F344/N rats and male and female B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms.

Perinatal-Only Exposure

Perinatal exposure alone (through dietary administration of 10:0 ppm polybrominated biphenyls to the dams) had no effect on the incidences of neoplasms in female F344/N rats, but in male F344/N rats, perinatal exposure was associated with a marginally increased incidence of hepatocellular adenomas that may have been related to chemical administration. In male and female B6C3F$_1$ mice, perinatal exposure to 30:0 ppm polybrominated biphenyls resulted in significantly increased incidences of hepatocellular neoplasms. The incidences of a number of nonneoplastic lesions in the liver (cytomegaly, eosinophilic focus, and clear cell focus) were increased in male and female B6C3F$_1$ mice.

Combined Perinatal and Adult Exposure

Combined perinatal and adult dietary exposure to polybrominated biphenyls confirmed the findings of the adult-only exposures for the increased incidences of hepatocellular neoplasms in F344/N rats and B6C3F$_1$ mice. In male rats, there were no enhancing effects of combined perinatal and adult exposure. However, perinatal exposure enhanced the susceptibility of female F344/N rats receiving adult exposure of 10 or 30 ppm to the induction of liver neoplasms.

For male and female F344/N rats, a combined analysis of the incidences of leukemia in the adult-only, perinatal-only, and combined perinatal and adult exposure groups revealed an apparent association

between increasing incidences of mononuclear cell leukemia and exposure to polybrominated biphenyls.

In male and female B6C3F$_1$ mice, it was not possible to adequately assess the enhancing effects of combined perinatal and adult exposure on hepatocellular neoplasms, because adult-only exposure to 10 or 30 ppm polybrominated biphenyls resulted in high incidences (84% to 98%) of liver neoplasms. However, with increased perinatal exposure, there were increases in the numbers of B6C3F$_1$ mice with hepatocellular carcinomas and in the numbers of B6C3F$_1$ mice with multiple hepatocellular adenomas, which suggests an enhancement of polybrominated biphenyls-related hepatocellular carcinogenicity associated with perinatal exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 11.

REFERENCES

Adams, J., and Buelke-Sam, J. (1981). Behavioral assessment of the postnatal animal: Testing and methods development. In *Developmental Toxicology* (C.A. Kimmel and J. Buelke-Sam, Eds.), pp. 233-258. Raven Press, New York.

Akoso, B.T., Sleight, S.D., Nachreiner, R.F., and Aust, S.D. (1982). Effects of purified polybrominated biphenyl congeners on the thyroid and pituitary glands in rats. *J. Am. Coll. Toxicol.* **1**, 23-36.

Alexandrov, V.A. (1983). Role of the maternal organism in transplacental carcinogenesis. In *Modulators of Experimental Carcinogenesis* (V. Turosov and R. Montesano, Eds.). International Agency for Research on Cancer, Lyon, France.

Amin-Zaki, L., Elhassani, S., Majeed, M.A., Clarkson, T.W., Doherty, R.A., and Greenwood, M. (1974). Intra-uterine methylmercury poisoning in Iraq. *Pediatrics* **54**, 587-595.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Arundel, S.E., and Kinnier-Wilson, L.M. (1986). Parental occupations and cancer: A review of the literature. *J. Epidemiol. Community Health* **40**, 30-36.

Aust, S.D., Dannan, G.A., Sleight, S.D., Fraker, P.J., Ringer, R.K., and Polin, D. (1981). Toxicology of polybrominated biphenyls. *Toxicol. Halogenated Hydrocarbons: Health Ecol. Eff. (paper symposium)*, 73-96.

Bekesi, J.G., Anderson, H.A., Roboz, J.P., Roboz, J., Fischbein, A., Selikoff, I.J., and Holland, J.F. (1979). Immunologic dysfunction among PBB-exposed Michigan dairy farmers. *Ann. N.Y. Acad. Sci.* **320**, 717-728.

Bekesi, J.G., Roboz, J.P., Fischbein, A., and Mason, P. (1987). Immunotoxicology: Environmental contamination by polybrominated biphenyls and immune dysfunction among residents of the state of Michigan. *Cancer Detect. Prev. Suppl.* **1**, 29-37.

Bernert, J.T., Jr., Groce, D.F., and Kimbrough, R.D. (1983). Long-term effects of a single oral dose of polybrominated biphenyls on serum and liver lipids in rats. *Toxicol. Appl. Pharmacol.* **68**, 424-433.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Brilliant, L.B., Van Amburg, G., Isbister, J., Humphrey, H., Wilcox, K., Eyster, J., Bloomer, A.W., and Price, H. (1978). Breast-milk monitoring to measure Michigan's contamination with polybrominated biphenyls. *Lancet* (September 23), 643-646.

Brown, G.G., Preisman, R.C., Anderson, M.D., Nixon, R.K., Isbister, J.L., and Price, H.A. (1981). Memory performance of chemical workers exposed to polybrominated biphenyls. *Science* **212**, 1413-1415.

Chhabra, R.S., Huff, J.E., Schwetz, B.S., and Selkirk, J. (1990). An overview of prechronic and chronic toxicity/carcinogenicity experimental study designs and criteria used by the National Toxicology Program. *Environ. Health Perspect.* **86**, 313-321.

Cook, H., Helland, D.R., VanderWeele, B.H., and DeJong, R.J. (1978). Histotoxic effects of polybrominated biphenyls in Michigan dairy cattle. *Environ. Res.* **15**, 82-89.

Corbett, T.H., Beaudoin, A.R., Cornell, R.G., Anver, M.R., Schumacher, R., Endres, J., and Szwabowska, M. (1975). Toxicity of polybrominated biphenyls (Firemaster BP-6) in rodents. *Environ. Res.* **10**, 390-396.

Cox, D.R. (1972). Regression models and life tables. *J. R. Stat. Soc.* **B34**, 187-220.

Dent, J.G., Netter, K.J., and Gibson, J.E. (1976). Effects of chronic administration of polybrominated biphenyls on parameters associated with hepatic drug metabolism. *Res. Commun. Chem. Pathol. Pharmacol.* **13** (No. 1), 75-82.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

Druckery, H., Ivanokovic, S., and Preussmann, R. (1966). Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. *Nature* **210**, 1378-1379.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1095-1121.

Eyster, J.T., Humphrey, H.E.B., and Kimbrough, R.D. (1983). Partitioning of polybrominated biphenyls (PBBs) in serum, adipose tissue, breast milk, placenta, cord blood, biliary fluid, and feces. *Arch. Environ. Health* **38**, 47-53.

Fraker, P.J. (1980). The antibody-mediated and delayed type hypersensitivity response of mice exposed to polybrominated biphenyls. *Toxicol. Appl. Pharmacol.* **53**, 1-7.

Fries, G.F. (1985). The PBB episode in Michigan: An overall appraisal. *CRC Crit. Rev. Toxicol.* **16**, 105-156.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* **62**, 957-974.

Garthoff, L.H., Friedman, L., Farber, T.M., Locke, K.K., Sobotka, T.J., Green, S., Hurley, N.E., Peters, E.L., Story, G.E., Moreland, F.M., Graham, C.H., Keys, J.E., Taylor, M.J., Scalera, J.V., Rothlein, J.E., Marks, E.M., Cerra, F.E., Rodi, S.B., and Sporn, E.M. (1977). Biochemical and cytogenetic effects in rats caused by short-term ingestion of Aroclor 1254 or Firemaster BP6. *J. Toxicol. Environ. Health* **3**, 769-796.

Gause, E.M., Ross, D.H., Hamilton, M.G., Leal, B.Z., Seifter, J., and Geller, I. (1979). Correlation of systematic and biochemical effects of PBB with behavioral effects. *Neurobehav. Toxicol.* **1**, 269-274.

Groce, D.F., and Kimbrough, R.D. (1984). Stunted growth, increased mortality, and liver tumors in offspring of polybrominated biphenyl (PBB) dosed Sherman rats. *J. Toxicol. Environ. Health* **14**, 695-706.

Grufferman, S., Delzell, E.S., Maile, M.C., and Michalopoulos, G. (1983). Parents' cigarette smoking and childhood cancer. *Med. Hypotheses* **12**, 17-20.

Gupta, B.N., and Moore, J.A. (1979). Toxicologic assessments of a commercial polybrominated biphenyl mixture in the rat. *Am. J. Vet. Res.* **40**, 1458-1468.

Gupta, B.N., McConnell, E.E., Moore, J.A., and Haseman, J.K. (1983). Effects of a polybrominated biphenyl mixture in the rat and mouse. II. Lifetime study. *Toxicol. Appl. Pharmacol.* **68**, 19-35.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* **75**, 975-984.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.

Herbst, A.L., Ulfelder, H., and Poskanzer, D.C. (1971). Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N. Engl. J. Med.* **284**, 878-881.

Herbst, A.L., Poskanzer, D.C., Robboy, S.J., Friedlander, L., and Scully, R.E. (1975). Prenatal exposure to stilbestrol. A prospective comparison of exposed female offspring with unexposed controls. *N. Engl. J. Med.* **292**, 334-339.

International Agency for Research on Cancer (IARC) (1973). (L. Tomatis, U. Mohr, and W. Davis, Eds.). IARC Scientific Publications on Transplacental Carcinogenesis No. 4. IARC, World Health Organization, Lyon, France.

International Agency for Research on Cancer (IARC) (1986). Some halogenated hydrocarbons and pesticide exposures. *IARC Monogr. Evaluation Carcinog. Risks Hum.* **41**, 261-292.

International Agency for Research on Cancer (IARC) (1987). Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs* Vol. 1 to 42 (Suppl. 7), 321-322.

Jensen, R.K., Sleight, S.D., Goodman, J.I., Aust, S.D., and Trosko, J.E. (1982). Polybrominated biphenyls as promoters in experimental hepatocarcinogenesis in rats. *Carcinogenesis* **3**, 1183-1186.

Jensen, R.K., Sleight, S.D., and Aust, S.D. (1984). Effect of varying the length of exposure to polybrominated biphenyls on the development of gamma-glutamyl transpeptidase enzyme-altered foci. *Carcinogenesis* **5**, 63-66.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kimbrough, R.D., Burse, V.W., and Liddle, J.A. (1978). Persistent liver lesions in rats after a single oral dose of polybrominated biphenyls (FireMaster FF-1) and concomitant PBB tissue levels. *Environ. Health Perspect.* **23**, 265-273.

Kimbrough, R.D., Groce, D.F., Korver, M.P., and Burse, V.W. (1981). Induction of liver tumors in female Sherman strain rats by polybrominated biphenyls. *JNCI* **66**, 535-542.

Klein, M. (1952). The transplacental effect of urethan on lung tumorigenesis in mice. *J. Natl. Cancer Inst.* **12**, 1003-1010.

Lambrecht, L.K., Barsotti, D.A., and Allen, J.R. (1978). Responses of nonhuman primates to a polybrominated biphenyl mixture. *Environ. Health Perspect.* **23**, 139-145.

Larsen, C.D. (1947). Pulmonary-tumor induction by transplacental exposure to urethane. *J. Natl. Cancer Inst.* **8**, 63-70.

Lewerenz, H.J. (1982). Xenobiotics in the environment of the fetus and the food of the infant and consequences for later life. *Bibl. Nutr. Dieta* **31**, 83-94.

Losco, P.E., and Ward, J.M. (1984). The early stages of large granular lymphocyte leukemia in the F344 rat. *Vet. Pathol.* **21**, 286-291.

Lowengart, R.A., Peters, J.M., Cicioni, C., Buckley, J., Bernstein, L., Preston-Martin, S., and Rappaport, E. (1987). Childhood leukemia and parents' occupational and home exposures. *JNCI* **79**, 39-46.

Lu, L.-J.W., Disher, R.M., Reddy, M.V., and Randerath, K. (1986). ^{32}P-postlabeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl, and benzo(*a*)pyrene. *Cancer Res.* **46**, 3046-3054.

Luster, M.I., Faith, R.E., and Moore, J.A. (1978). Effects of polybrominated biphenyls (PBB) on immune response in rodents. *Environ. Health Perspect.* **23**, 227-232.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Maronpot, R.R., Haseman, J.K., Boorman, G.A., Eustis, S.E., Rao, G.N., and Huff, J.E. (1987). Liver lesions in B6C3F1 mice: The National Toxicology Program, experience and position. *Arch. Toxicol.* (Suppl. 10), 10-26.

Matthews, H.B., and Kato, S. (1979). The metabolism and disposition of halogenated aromatics. *Ann. N.Y. Acad. Sci.* **320**, 131-137.

Matthews, H.B., Kato, S., Morales, N.M., and Tuey, D.B. (1977). Distribution and excretion of 2,4,5,2′,4′,5′-hexabromobiphenyl, the major component of Firemaster BP-6. *J. Toxicol. Environ. Health* **3**, 599-605.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

McCormack, K.M., Lepper, L.F., Wilson, D.M., and Hook, J.B. (1981). Biochemical and physiological sequelae to perinatal exposure to polybrominated biphenyls: A multigeneration study in rats. *Toxicol. Appl. Pharmacol.* **59**, 300-313.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

McLachlan, J.A., Newbold, R.R., and Bullock, B.C. (1980). Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res.* **40**, 3988-3999.

McLachlan, J.A., Newbold, R.R., Korach, K.S., Lamb, J.C., IV, and Suzuki, Y. (1981). Transplacental toxicology: Prenatal factors influencing postnatal fertility. In *Developmental Toxicology* (C.A. Kimmel and J. Buelke-Sam, Eds.), pp. 213-232. Raven Press, New York.

Miller, R.K. (1983). Perinatal toxicology: Its recognition and fundamentals. *Am. J. Ind. Med.* **4**, 205-244.

Millis, C.D., Mills, R.A., Sleight, S.D., and Aust, S.D. (1985). Toxicity of 3,4,5,3′,4′,5′-hexabrominated biphenyl and 3,4,3′,4′-tetrabrominated biphenyl. *Toxicol. Appl. Pharmacol.* **78**, 88-95.

Millischer, R., Girault, F., Heywood, R., Clarke, G., Hossack, D., and Clair, M. (1979). Décabromobiphényle: Etude toxicologique. *Toxicol. Eur. Res.* **2**, 155-161.

Mills, R.A., Millis, C.D., Dannan, G.A., Guengerich, F.P., and Aust, S.D. (1985). Studies on the structure-activity relationships for the metabolism of polybrominated biphenyls by rat liver microsomes. *Toxicol. Appl. Pharmacol.* **78**, 96-104.

Mirsalis, J.C., and Steinmetz, K.L. (1990). The role of hyperplasia in liver carcinogenesis. *Prog. Clin. Biol. Res.* **331**, 149-161.

Mirsalis, J.C., Tyson, C.K., Loh, E.N., Steinmetz, K.L., Bakke, J.P., Hamilton, C.M., Spak, D.K., and Spalding, J.W. (1985). Induction of hepatic cell proliferation and unscheduled DNA synthesis in mouse hepatocytes following *in vivo* treatment. *Carcinogenesis* **6**, 1521-1524.

Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.N., Hamilton, C.M., Bakke, J.P., and Spalding, J.W. (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds. *Environ. Mol. Mutagen.* **14**, 155-164.

National Cancer Institute (NCI) (1979). Perinatal Carcinogenesis. NCI Monograph 51. DHEW Publication No. (NIH) 79-1633. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1983). Carcinogenesis Studies of Polybrominated Biphenyl Mixture (Firemaster FF-1) (CAS No. 67774-32-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 244. NIH Publication No. 83-1800. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1992). Perinatal Toxicology and Carcinogenesis Studies of Ethylene Thiourea (CAS No. 96-45-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 388. NIH Publication No. 92-2843. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1993). Toxicology and Carcinogenesis Studies of Pentachloroanisole (CAS No. 1825-21-4) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 414. NIH Publication No. 93-3145. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Needham, L.L., Hill, R.H., Jr., Orti, D.L., Patterson, D.G., Kimbrough, R.D., Groce, D.F., and Liddle, J.A. (1982). Investigation of hyperkeratotic activity of polybrominated biphenyls in Firemaster FF-1. *J. Toxicol. Environ. Health* **9**, 877-887.

Nomura, T. (1982). Parental exposure to X rays and chemicals induces heritable tumours and anomalies in mice. *Nature* **296**, 575-577.

Pershagen, G. (1989). Childhood cancer and malignancies other than lung cancer related to passive smoking. *Mutat. Res.* **222**, 129-135.

Peters, J.M., Preston-Martin, S., and Yu, M.C. (1981). Brain tumors in children and occupational exposure of parents. *Science* **213**, 235-237.

Piegorsch, W.W., Weinberg, C.R., and Haseman, J.K. (1986). Testing for simple independent action between two factors for dichotomous response data. *Biometrics* **42**, 413-419.

Pietra, G., Spencer, K., and Shubik, P. (1959). Response of newly born mice to a chemical carcinogen. *Nature* **183**, 1689.

Pietra, G., Rappaport, H., and Shubik, P. (1961). The effects of carcinogenic chemicals in newborn mice. *Cancer* **14**, 308-317.

Preache, M.M., Cagen, S.Z., and Gibson, J.E. (1976). Perinatal toxicity in mice following maternal dietary exposure to polybrominated biphenyls. *Toxicol. Appl. Pharmacol.* **37**, 171. (Abst.)

Rao, G.N., Piegorsch, W.W., and Haseman, J.K. (1987). Influence of body weight on the incidence of spontaneous tumors in rats and mice of long-term studies. *Am. J. Clin. Nutr.* **45**, 252-260.

Rao, G.N., Piegorsch, W.W., Crawford, D.D., Edmondson, J., and Haseman, J.K. (1989). Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F1 (C57BL/6N × C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

Rao, G.N., Haseman, J.K., Grumbein, S., Crawford, D.D., and Eustis, S.L. (1990a). Growth, body weight, survival, and tumor trends in F344/N rats during an eleven-year period. *Toxicol. Pathol.* **18**, 61-69.

Rao, G.N., Haseman, J.K., Grumbein, S., Crawford, D.D., and Eustis, S.L. (1990b). Growth, body weight, survival, and tumor trends in (C57BL/6 × C3H/HeN) F1 (B6C3F1) mice during a nine-year period. *Toxicol. Pathol.* **18**, 71-77.

Rezabek, M.S., Trosko, J.E., Jone, C., and Sleight, S.D. (1988). Effects of hepatic tumor promoters phenobarbital and polybrominated biphenyls on intercellular communication between rat liver epithelial cells. *In Vitro Toxicol.* **2**, 45-58.

Rice, J.M. (1981). Effects of prenatal exposure to chemical carcinogens and methods for their detection. In *Developmental Toxicology* (C.A. Kimmel and J. Buelke-Sam, Eds.), pp. 191-212. Raven Press, New York.

Rickert, D.E., Dent, J.G., Cagen, S.Z., McCormack, K.M., Melrose, P., and Gibson, J.E. (1978). Distribution of polybrominated biphenyls after dietary exposure in pregnant and lactating rats and their offspring. *Environ. Health Perspect.* **23**, 63-66.

Roberts, D.W., and Chapman, J.R. (1981). Concepts essential to the assessment of toxicity to the developing immune system. In *Developmental Toxicology* (C.A. Kimmel and J. Buelke-Sam, Eds.), pp. 167-185. Raven Press, New York.

Sass, B., and Montali, R.J. (1980). Spontaneous fibro-osseous lesions in aging female mice. *Lab. Anim. Sci.* **30**, 907-909.

Schardein, J.L., and Keller, K.A. (1989). Potential human developmental toxicants and the role of animal testing in their identification and characterization. *CRC Crit. Rev. Toxicol.* **19**, 251-339.

Sleight, S.D. (1979). Polybrominated biphenyls: A recent environmental pollutant. In *Animals as Monitors of Environmental Pollutants*, pp. 366-374. Natl. Acad. Sciences, ISBN 0-309-02871-x.

Sleight, S.D. (1985). Effects of PCBs and related compounds on hepatocarcinogenesis in rats and mice. *Environ. Health Perspect.* **60**, 35-39.

Sleight, S.D., and Sanger, V.L. (1976). Pathologic features of polybrominated biphenyl toxicosis in the rat and Guinea pig. *J. Am. Vet. Med. Assoc.* **169**, 1231-1235.

Stefanski, S.A., Elwell, M.R., and Stromberg, P.C. (1990). Spleen, lymph nodes, and thymus. In *Pathology of the Fischer Rat: Reference and Atlas* (G.A. Boorman, S.L. Eustis, M.R. Elwell, C.A. Montgomery, Jr., and W.F. MacKenzie, Eds.), pp. 369-393. Academic Press, San Diego, CA.

Stjernfeldt, M., Berglund, K., Lindsten, J., and Ludvigsson, J. (1986). Maternal smoking during pregnancy and risk of childhood cancer. *Lancet* **1**, 1350-1352.

Stromberg, P.C. (1985). Animal model of human disease: Large granular lymphocyte leukemia in F344 rats. Model for human Tγ lymphoma, malignant histiocytosis, and T-cell chronic lymphocytic leukemia. *Am. J. Pathol.* **119**, 517-519.

Stross, J.K., Nixon, R.K., and Anderson, M.D. (1979). Neuropsychiatric findings in patients exposed to polybrominated biphenyls. *Ann. N.Y. Acad. Sci.* **320**, 368-372.

Swenberg, J.A. (1979). Incorporation of transplacental exposure into routine carcinogenicity bioassays. *Natl. Cancer Inst. Monogr.* **51**, 265-268.

Swenberg, J.A., Koestner, A., Wechsler, W., and Denlinger, R.H. (1972). Quantitative aspects of transplacental tumor induction with ethylnitrosourea in rats. *Cancer Res.* **32**, 2656-2660.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Stasiewicz, S., and Spalding, J.W. (1986). Comparison of multiple parameters of rodent carcinogenicity and in vitro genetic toxicity. *Environ. Mutagen.* **8**, 205-227.

Tilson, H.A., and Cabe, P.A. (1979). IV. Neurologic and behavioral abnormalities. Studies on the neurobehavioral effects of polybrominated biphenyls in rats. *Ann. N.Y. Acad. Sci.* **320**, 325-336.

Tomatis, L. (1979). Prenatal exposure to chemical carcinogens and its effect on subsequent generations. *Natl. Cancer Inst. Monogr.* **51**, 159-184.

Tomatis, L. (1988). Prenatal carcinogenesis. *IARC Monogr.* **92**, 121-132.

Tsushimoto, G., Trosko, J.E., Chang, C., and Aust, S.D. (1982). Inhibition of metabolic cooperation in Chinese hamster V79 cells in culture by various polybrominated biphenyl (PBB) congeners. *Carcinogenesis (London)* **3**, 181-185.

Tuey, D.B., and Matthews, H.B. (1980). Distribution and excretion of 2,2′,4,4′,5,5′-hexabromobiphenyl in rats and man: Pharmacokinetic model predictions. *Toxicol. Appl. Pharmacol.* **53**, 420-431.

Valciukas, J.A., Lilis, R., Anderson, H.A., Wolff, M.S., and Petrocci, M. (1979). The neurotoxicity of polybrominated biphenyls: Results of a medical field survey. *Ann. N.Y. Acad. Sci.* **320**, 337-367.

Vesselinovitch, S.D., Rao, K.V.N., and Mihailovich, N. (1979). Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. *Natl. Cancer Inst. Monogr.* **51**, 239-249.

Wasito and Sleight, S.D. (1989). Promoting effect of polybrominated biphenyls on tracheal papillomas in Syrian golden hamsters. *J. Toxicol. Environ. Health* **27**, 173-187.

Wechsler, W., Rice, J.M., and Vesselinovitch, S.D. (1979). Transplacental and neonatal induction of neurogenic tumors in mice: Comparison with related species and with human pediatric neoplasms. *Natl. Cancer Inst. Monogr.* **51**, 219-226.

Wertz, G.F., and Ficsor, G. (1978). Cytogenetic and teratogenic test of polybrominated biphenyls in rodents. *Environ. Health Perspect.* **23**, 129-132.

Williams, G.M., Tong, C., and Telang, S. (1984). Polybrominated biphenyls are nongenotoxic and produce an epigenetic membrane effect in cultured liver cells. *Environ. Res.* **34**, 310-320.

Wolff, M.S., and Selikoff, I.J. (1979). Variation of polybrominated biphenyl homolog peaks in blood of rats following treatment with Firemaster FF-1. *Bull. Environ. Contam. Toxicol.* **21**, 771-774.

Wong, O., Brocker, W., Davis, H.V., and Nagle, G.S. (1984). Mortality of workers potentially exposed to organic and inorganic brominated chemicals, DBCP, TRIS, PBB, and DDT. *Br. J. Ind. Med.* **41**, 15-24.

Yamasaki, H., Hollstein, M., Martel, N., Cabral, J.R.P., Galendo, D., and Tomatis, L. (1987). Transplacental induction of a specific mutation in fetal Ha-*ras* and its critical role in post-natal carcinogenesis. *Int. J. Cancer* **40**, 818-822.

Zacharewski, T., Harris, M., Safe, S., Thoma, H., and Hutzinger, O. (1988). Applications of the *in vitro* aryl hydrocarbon hydroxylase induction assay for determining "2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents": Pyrolyzed brominated flame retardants. *Toxicology* **51**, 177-189.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987). *Salmonella* mutagenicity tests. III. Results from the testing of 255 chemicals. *Environ. Mutagen.* **9** (Suppl. 9), 1-110.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF POLYBROMINATED BIPHENYLS

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	16	16	15	21	25	26
Natural deaths	5	4	1	6	5	4
Survivors						
Died last week of study		1		1	1	1
Terminal sacrifice	29	29	34	22	19	19
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(48)	(20)	(16)	(26)	(30)	(29)
Intestine large, colon	(49)	(21)	(16)	(27)	(31)	(30)
Intestine large, rectum	(49)	(21)	(16)	(27)	(30)	(30)
Intestine small, duodenum	(48)	(21)	(16)	(27)	(31)	(30)
Intestine small, ileum	(49)	(19)	(16)	(28)	(29)	(29)
Adenocarcinoma	1 (2%)			1 (4%)		
Intestine small, jejunum	(49)	(20)	(16)	(27)	(30)	(30)
Adenocarcinoma				1 (4%)		
Liver	(50)	(50)	(50)	(49)	(50)	(50)
Adenocarcinoma, metastatic, prostate		1 (2%)				
Adenocarcinoma, metastatic, intestine small				1 (2%)		
Fibrosarcoma, metastatic, skin	1 (2%)					
Hepatocellular carcinoma			1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hepatocellular adenoma	1 (2%)	4 (8%)	3 (6%)	4 (8%)	6 (12%)	10 (20%)
Hepatocellular adenoma, multiple		1 (2%)	2 (4%)	6 (12%)	7 (14%)	6 (12%)
Mesentery		(6)		(3)	(1)	(2)
Adenocarcinoma, metastatic, prostate		1 (17%)				
Lipoma						1 (50%)
Pancreas	(49)	(21)	(16)	(27)	(32)	(31)
Acinus, adenoma	1 (2%)		1 (6%)	1 (4%)	3 (9%)	1 (3%)
Salivary glands	(50)	(21)	(16)	(27)	(30)	(30)
Schwannoma malignant	1 (2%)					
Stomach, forestomach	(50)	(49)	(48)	(49)	(50)	(50)
Squamous cell carcinoma				1 (2%)		
Squamous cell papilloma		1 (2%)		1 (2%)		
Stomach, glandular	(50)	(48)	(48)	(48)	(50)	(49)
Carcinoid tumor malignant		1 (2%)				
Tongue					(1)	
Squamous cell carcinoma					1 (100%)	
Tooth				(1)		
Gingiva, squamous cell carcinoma				1 (100%)		
Cardiovascular System						
Heart	(50)	(21)	(16)	(27)	(31)	(31)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	23	24
Natural deaths	3	7
Survivors		
Died last week of study	1	1
Terminal sacrifice	23	18
Animals examined microscopically	50	50
Alimentary System		
Intestine small, jejunum	(48)	(48)
Leiomyoma		2 (4%)
Leiomyosarcoma	1 (2%)	
Leiomyosarcoma, metastatic, mesentery	1 (2%)	
Liver	(50)	(50)
Hepatocellular carcinoma	12 (24%)	13 (26%)
Hepatocellular carcinoma, multiple	7 (14%)	10 (20%)
Hepatocellular adenoma	2 (4%)	3 (6%)
Hepatocellular adenoma, multiple	36 (72%)	35 (70%)
Squamous cell carcinoma, metastatic, stomach	1 (2%)	
Mesentery	(5)	(2)
Fibrosarcoma	1 (20%)	
Hemangiosarcoma		1 (50%)
Squamous cell carcinoma, metastatic, stomach	1 (20%)	
Pancreas	(49)	(50)
Squamous cell carcinoma, metastatic, stomach	1 (2%)	
Stomach, forestomach	(50)	(50)
Squamous cell carcinoma	1 (2%)	2 (4%)
Cardiovascular System		
Heart	(50)	(49)
Schwannoma malignant		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Endocrine System						
Adrenal gland, cortex	(50)	(21)	(15)	(28)	(31)	(30)
Adrenal gland, medulla	(49)	(22)	(16)	(29)	(32)	(30)
Pheochromocytoma malignant	1 (2%)			1 (3%)	1 (3%)	1 (3%)
Pheochromocytoma complex		1 (5%)	1 (6%)			
Pheochromocytoma benign	10 (20%)	4 (18%)	1 (6%)	1 (3%)	2 (6%)	3 (10%)
Bilateral, pheochromocytoma benign	1 (2%)					
Islets, pancreatic	(49)	(21)	(16)	(27)	(31)	(30)
Adenoma	4 (8%)	1 (5%)		1 (4%)	1 (3%)	
Carcinoma	3 (6%)					
Parathyroid gland	(43)	(21)	(16)	(27)	(31)	(29)
Adenoma				1 (4%)		2 (7%)
Pituitary gland	(50)	(27)	(22)	(32)	(30)	(34)
Pars distalis, adenoma	13 (26%)	7 (26%)	9 (41%)	11 (34%)	9 (30%)	11 (32%)
Pars distalis, adenoma, multiple				1 (3%)		
Pars nervosa, astrocytoma malignant, metastatic, brain		1 (4%)				
Thyroid gland	(49)	(23)	(17)	(28)	(31)	(30)
Bilateral, C-cell, adenoma	1 (2%)	1 (4%)		1 (4%)		
Bilateral, follicular cell, adenoma						1 (3%)
C-cell, adenoma	16 (33%)	3 (13%)	4 (24%)	8 (29%)	3 (10%)	8 (27%)
C-cell, carcinoma	1 (2%)	2 (9%)	1 (6%)		3 (10%)	
Follicular cell, adenoma	1 (2%)			1 (4%)	2 (6%)	
Follicular cell, carcinoma		2 (9%)		1 (4%)		
General Body System						
Tissue NOS		(1)				
Chordoma		1 (100%)				
Genital System						
Epididymis	(50)	(20)	(16)	(28)	(30)	(30)
Adenocarcinoma, metastatic, prostate		1 (5%)				
Preputial gland	(47)	(25)	(20)	(32)	(34)	(32)
Adenoma	4 (9%)	1 (4%)	1 (5%)		1 (3%)	
Carcinoma	3 (6%)	3 (12%)	3 (15%)	4 (13%)	4 (12%)	2 (6%)
Duct, squamous cell papilloma	1 (2%)					
Prostate	(50)	(21)	(16)	(28)	(30)	(31)
Adenocarcinoma		1 (5%)				
Testes	(50)	(49)	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	43 (86%)	41 (84%)	43 (86%)	45 (90%)	37 (74%)	42 (84%)
Interstitial cell, adenoma	6 (12%)	5 (10%)	4 (8%)	4 (8%)	9 (18%)	5 (10%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Endocrine System		
Adrenal gland, cortex	(50)	(50)
Adenoma		1 (2%)
Adrenal gland, medulla	(50)	(50)
Pheochromocytoma benign	2 (4%)	3 (6%)
Islets, pancreatic	(49)	(50)
Adenoma	2 (4%)	1 (2%)
Parathyroid gland	(49)	(47)
Adenoma		2 (4%)
Pituitary gland	(48)	(49)
Pars distalis, adenoma	4 (8%)	9 (18%)
Pars intermedia, adenoma		1 (2%)
Thyroid gland	(49)	(49)
C-cell, adenoma	8 (16%)	11 (22%)
C-cell, adenoma, multiple	1 (2%)	
C-cell, carcinoma	1 (2%)	1 (2%)
General Body System		
None		
Genital System		
Epididymis	(50)	(50)
Preputial gland	(44)	(49)
Adenoma	1 (2%)	1 (2%)
Carcinoma		5 (10%)
Testes	(50)	(50)
Bilateral, interstitial cell, adenoma	37 (74%)	36 (72%)
Interstitial cell, adenoma	9 (18%)	10 (20%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Hematopoietic System						
Bone marrow	(49)	(21)	(15)	(27)	(31)	(30)
Lymph node	(50)	(23)	(18)	(27)	(33)	(31)
Mediastinal, adenocarcinoma, metastatic, prostate		1 (4%)				
Mediastinal, fibrosarcoma, metastatic, skin					1 (3%)	
Lymph node, mesenteric	(2)	(3)	(2)	(9)	(8)	(4)
Adenocarcinoma, metastatic, prostate		1 (33%)				
Fibrosarcoma, metastatic, skin	1 (50%)					
Osteosarcoma, metastatic, uncertain primary site	1 (50%)					
Spleen	(50)	(50)	(49)	(49)	(50)	(50)
Fibrosarcoma			1 (2%)			
Osteosarcoma, metastatic, bone			1 (2%)			
Thymus	(47)	(17)	(15)	(22)	(24)	(27)
Integumentary System						
Mammary gland	(39)	(14)	(14)	(23)	(20)	(18)
Fibroadenoma	1 (3%)		2 (14%)	4 (17%)		
Skin	(50)	(26)	(22)	(31)	(32)	(32)
Basosquamous tumor benign						1 (3%)
Fibroma				1 (3%)		
Keratoacanthoma	2 (4%)		1 (5%)			
Squamous cell papilloma	1 (2%)				1 (3%)	
Trichoepithelioma				1 (3%)	1 (3%)	
Sebaceous gland, adenoma			1 (5%)			
Subcutaneous tissue, fibroma	5 (10%)	2 (8%)	6 (27%)	1 (3%)	4 (13%)	2 (6%)
Subcutaneous tissue, fibroma, multiple	1 (2%)					
Subcutaneous tissue, fibrosarcoma	2 (4%)	4 (15%)	3 (14%)	2 (6%)	1 (3%)	1 (3%)
Subcutaneous tissue, neurofibrosarcoma				1 (3%)		
Musculoskeletal System						
Bone	(50)	(21)	(16)	(28)	(31)	(31)
Femur, hemangiosarcoma						1 (3%)
Femur, osteosarcoma			1 (6%)			
Lumbar, osteosarcoma						1 (3%)
Rib, osteosarcoma		1 (5%)				
Nervous System						
Brain	(50)	(21)	(16)	(29)	(31)	(31)
Astrocytoma malignant		1 (5%)				
Glioma malignant					1 (3%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Hematopoietic System		
Bone marrow	(50)	(50)
Lymph node	(49)	(49)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (2%)	
Lymph node, mesenteric	(4)	(2)
Spleen	(50)	(50)
Osteosarcoma, metastatic, bone		1 (2%)
Thymus	(40)	(28)
Integumentary System		
Skin	(50)	(50)
Squamous cell carcinoma	1 (2%)	
Squamous cell papilloma	1 (2%)	2 (4%)
Subcutaneous tissue, fibroma	2 (4%)	3 (6%)
Subcutaneous tissue, fibroma, multiple	2 (4%)	
Subcutaneous tissue, fibrosarcoma	2 (4%)	
Subcutaneous tissue, hemangioma	1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)	
Subcutaneous tissue, neurofibroma	1 (2%)	
Musculoskeletal System		
Bone	(50)	(50)
Femur, osteosarcoma		1 (2%)
Skeletal muscle		(1)
Rhabdomyosarcoma		1 (100%)
Nervous System		
Brain	(50)	(50)
Oligodendroglioma malignant		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Respiratory System						
Lung	(50)	(21)	(17)	(28)	(32)	(31)
Adenocarcinoma, metastatic, prostate		1 (5%)				
Alveolar/bronchiolar adenoma	2 (4%)		2 (12%)	1 (4%)		1 (3%)
Alveolar/bronchiolar carcinoma			1 (6%)	1 (4%)	1 (3%)	
Carcinoma adenosquamous					1 (3%)	1 (3%)
Chordoma, metastatic, uncertain primary site			1 (6%)			
Fibrosarcoma, metastatic, skin			1 (6%)		1 (3%)	
Osteosarcoma, metastatic, bone			1 (6%)			
Osteosarcoma, metastatic, uncertain primary site	1 (2%)					
Pheochromocytoma complex, metastatic, adrenal gland			1 (6%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (6%)				
Nose	(50)	(21)	(16)	(27)	(31)	(30)
Submucosa, glands, adenocarcinoma		2 (10%)				
Trachea	(50)	(21)	(16)	(27)	(31)	(31)
Peritracheal tissue, fibrosarcoma, metastatic, skin					1 (3%)	
Special Senses System						
Zymbal's gland	(1)					
Carcinoma	1 (100%)					
Urinary System						
Kidney	(50)	(24)	(20)	(32)	(32)	(37)
Medulla, sarcoma	1 (2%)					
Renal tubule, adenoma		1 (4%)			2 (6%)	
Renal tubule, carcinoma	1 (2%)					
Urinary bladder	(49)	(20)	(17)	(27)	(31)	(30)
Adenocarcinoma, metastatic, prostate		1 (5%)				
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	25 (50%)	31 (62%)	32 (64%)	33 (66%)	41 (82%)	37 (74%)
Lymphoma malignant histiocytic		1 (2%)				
Lymphoma malignant lymphocytic		1 (2%)				1 (2%)
Mesothelioma benign	1 (2%)					1 (2%)
Mesothelioma malignant	1 (2%)	5 (10%)		2 (4%)	1 (2%)	3 (6%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Respiratory System		
Lung	(50)	(49)
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)
Hepatocellular carcinoma, metastatic, liver		3 (6%)
Squamous cell carcinoma, metastatic, stomach	1 (2%)	
Special Senses System		
None		
Urinary System		
Kidney	(50)	(50)
Renal tubule, adenoma		1 (2%)
Systemic Lesions		
Multiple organs	(50)	(50)
Leukemia mononuclear	31 (62%)	37 (74%)
Mesothelioma malignant	1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Neoplasm Summary						
Total animals with primary neoplasms[c]	50	50	50	50	50	50
Total primary neoplasms	156	129	124	145	144	144
Total animals with benign neoplasms	50	47	48	50	47	48
Total benign neoplasms	115	72	80	94	88	95
Total animals with malignant neoplasms	34	41	39	41	43	41
Total malignant neoplasms	41	57	44	51	56	49
Total animals with metastatic neoplasms	2	3	5	1	1	
Total metastatic neoplasms	4	9	6	1	3	
Total animals with malignant neoplasms of uncertain primary site	1		1			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Neoplasm Summary		
Total animals with primary neoplasms	50	50
Total primary neoplasms	171	196
Total animals with benign neoplasms	49	49
Total benign neoplasms	113	123
Total animals with malignant neoplasms	42	47
Total malignant neoplasms	58	73
Total animals with metastatic neoplasms	3	4
Total metastatic neoplasms	6	4

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

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	12/49 (24%)	1/29 (3%)[e]	2/50 (4%)
Adjusted rate[b]	33.3%		8.3%
Terminal rate[c]	6/28 (21%)		2/24 (8%)
First incidence (days)	651		727 (T)
Life table test[d]			P=0.014N
Logistic regression test[d]			P=0.006N
Fisher exact test[d]			P=0.003N
Adrenal Medulla: Pheochromocytoma (Benign, Complex, or Malignant)			
Overall rate	12/49 (24%)	2/29 (7%)[e]	2/50 (4%)
Adjusted rate	33.3%		8.3%
Terminal rate	6/28 (21%)		2/24 (8%)
First incidence (days)	651		727 (T)
Life table test			P=0.014N
Logistic regression test			P=0.006N
Fisher exact test			P=0.003N
Liver: Hepatocellular Adenoma			
Overall rate	1/50 (2%)	10/49 (20%)	38/50 (76%)
Adjusted rate	3.4%	38.3%	92.5%
Terminal rate	1/29 (3%)	7/23 (30%)	21/24 (88%)
First incidence (days)	727 (T)	716	588
Life table test	P<0.001	P=0.002	P<0.001
Logistic regression test	P<0.001	P=0.002	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test		P=0.003	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	0/50 (0%)	2/49 (4%)	19/50 (38%)
Adjusted rate	0.0%	5.3%	61.6%
Terminal rate	0/29 (0%)	0/23 (0%)	13/24 (54%)
First incidence (days)	-[f]	678	604
Life table test	P<0.001	P=0.228	P<0.001
Logistic regression test	P<0.001	P=0.237	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.242	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	1/50 (2%)	12/49 (24%)	41/50 (82%)
Adjusted rate	3.4%	41.6%	97.6%
Terminal rate	1/29 (3%)	7/23 (30%)	23/24 (96%)
First incidence (days)	727 (T)	678	588
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	2/50 (4%)	1/28 (4%)[e]	3/50 (6%)
Adjusted rate	6.9%		12.5%
Terminal rate	2/29 (7%)		3/24 (13%)
First incidence (days)	727 (T)		727 (T)
Life table test			P=0.413
Logistic regression test			P=0.413
Fisher exact test			P=0.500
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	2/50 (4%)	2/28 (7%)[e]	3/50 (6%)
Adjusted rate	6.9%		12.5%
Terminal rate	2/29 (7%)		3/24 (13%)
First incidence (days)	727 (T)		727 (T)
Life table test			P=0.413
Logistic regression test			P=0.413
Fisher exact test			P=0.500
Mammary Gland: Fibroadenoma			
Overall rate	1/50 (2%)	4/50 (8%)	0/50 (0%)
Adjusted rate	3.4%	15.0%	0.0%
Terminal rate	1/29 (3%)	2/23 (9%)	0/24 (0%)
First incidence (days)	727 (T)	693	–
Life table test	P=0.326N	P=0.129	P=0.538N
Logistic regression test	P=0.311N	P=0.150	P=0.538N
Cochran-Armitage test	P=0.272N		
Fisher exact test		P=0.181	P=0.500N
Pancreatic Islets: Adenoma			
Overall rate	4/49 (8%)	1/27 (4%)[e]	2/49 (4%)
Adjusted rate	12.2%		8.3%
Terminal rate	3/29 (10%)		2/24 (8%)
First incidence (days)	507		727 (T)
Life table test			P=0.411N
Logistic regression test			P=0.332N
Fisher exact test			P=0.339N
Pancreatic Islets: Carcinoma			
Overall rate	3/49 (6%)	0/27 (0%)[e]	0/49 (0%)
Adjusted rate	10.3%		0.0%
Terminal rate	3/29 (10%)		0/24 (0%)
First incidence (days)	727 (T)		–
Life table test			P=0.155N
Logistic regression test			P=0.155N
Fisher exact test			P=0.121N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Pancreatic Islets: Adenoma or Carcinoma			
Overall rate	7/49 (14%)	1/27 (4%)[e]	2/49 (4%)
Adjusted rate	22.3%		8.3%
Terminal rate	6/29 (21%)		2/24 (8%)
First incidence (days)	507		727 (T)
Life table test			P=0.124N
Logistic regression test			P=0.088N
Fisher exact test			P=0.080N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	13/50 (26%)	12/32 (38%)[e]	4/48 (8%)
Adjusted rate	35.4%		13.5%
Terminal rate	7/29 (24%)		2/24 (8%)
First incidence (days)	657		493
Life table test			P=0.047N
Logistic regression test			P=0.021N
Cochran-Armitage test			
Fisher exact test			P=0.019N
Preputial Gland: Adenoma			
Overall rate	4/47 (9%)	0/32 (0%)[e]	1/44 (2%)
Adjusted rate	13.5%		2.4%
Terminal rate	3/28 (11%)		0/23 (0%)
First incidence (days)	710		631
Life table test			P=0.238N
Logistic regression test			P=0.212N
Cochran-Armitage test			
Fisher exact test			P=0.202N
Preputial Gland: Carcinoma			
Overall rate	3/47 (6%)	4/32 (13%)[e]	0/44 (0%)
Adjusted rate	10.7%		0.0%
Terminal rate	3/28 (11%)		0/23 (0%)
First incidence (days)	727 (T)		–
Life table test			P=0.156N
Logistic regression test			P=0.156N
Cochran-Armitage test			
Fisher exact test			P=0.133N
Preputial Gland: Adenoma or Carcinoma			
Overall rate	7/47 (15%)	4/32 (13%)[e]	1/44 (2%)
Adjusted rate	23.9%		2.4%
Terminal rate	6/28 (21%)		0/23 (0%)
First incidence (days)	710		631
Life table test			P=0.057N
Logistic regression test			P=0.049N
Cochran-Armitage test			
Fisher exact test			P=0.036N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Squamous Cell Carcinoma			
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.3%	4.3%	8.3%
Terminal rate	3/29 (10%)	1/23 (4%)	2/24 (8%)
First incidence (days)	727 (T)	727 (T)	727 (T)
Life table test	P=0.567N	P=0.390N	P=0.587N
Logistic regression test	P=0.567N	P=0.390N	P=0.587N
Cochran-Armitage test	P=0.500N		
Fisher exact test		P=0.309N	P=0.500N
Skin (Subcutaneous Tissue): Fibroma			
Overall rate	6/50 (12%)	1/50 (2%)	4/50 (8%)
Adjusted rate	19.1%	4.3%	16.7%
Terminal rate	5/29 (17%)	1/23 (4%)	4/24 (17%)
First incidence (days)	651	727 (T)	727 (T)
Life table test	P=0.524N	P=0.097N	P=0.489N
Logistic regression test	P=0.510N	P=0.070N	P=0.459N
Cochran-Armitage test	P=0.434N		
Fisher exact test		P=0.056N	P=0.370N
Skin (Subcutaneous Tissue): Fibroma or Neurofibroma			
Overall rate	6/50 (12%)	1/50 (2%)	5/50 (10%)
Adjusted rate	19.1%	4.3%	20.0%
Terminal rate	5/29 (17%)	1/23 (4%)	4/24 (17%)
First incidence (days)	651	727 (T)	725
Life table test	P=0.503	P=0.097N	P=0.626N
Logistic regression test	P=0.515	P=0.070N	P=0.601N
Cochran-Armitage test	P=0.595		
Fisher exact test		P=0.056N	P=0.500N
Skin (Subcutaneous Tissue): Fibrosarcoma or Neurofibrosarcoma			
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.4%	8.0%	6.2%
Terminal rate	0/29 (0%)	0/23 (0%)	1/24 (4%)
First incidence (days)	571	568	588
Life table test	P=0.599N	P=0.484	P=0.668
Logistic regression test	P=0.533N	P=0.531	P=0.659N
Cochran-Armitage test	P=0.582N		
Fisher exact test		P=0.500	P=0.691N
Skin (Subcutaneous Tissue): Fibroma, Neurofibroma, Neurofibrosarcoma, or Fibrosarcoma			
Overall rate	8/50 (16%)	4/50 (8%)	7/50 (14%)
Adjusted rate	22.6%	12.0%	25.6%
Terminal rate	5/29 (17%)	1/23 (4%)	5/24 (21%)
First incidence (days)	571	568	588
Life table test	P=0.516	P=0.258N	P=0.595
Logistic regression test	P=0.546N	P=0.162N	P=0.520N
Cochran-Armitage test	P=0.552N		
Fisher exact test		P=0.178N	P=0.500N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Testes: Adenoma			
Overall rate	49/50 (98%)	49/50 (98%)	46/50 (92%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	29/29 (100%)	23/23 (100%)	24/24 (100%)
First incidence (days)	505	503	536
Life table test	P=0.342	P=0.165	P=0.293
Logistic regression test	P=0.105N	P=0.761	P=0.205N
Cochran-Armitage test	P=0.091N		
Fisher exact test		P=0.753N	P=0.181N
Thyroid Gland (C-cell): Adenoma			
Overall rate	17/49 (35%)	9/28 (32%)[e]	9/49 (18%)
Adjusted rate	45.4%		30.5%
Terminal rate	10/29 (34%)		5/24 (21%)
First incidence (days)	651		682
Life table test			P=0.155N
Logistic regression test			P=0.075N
Fisher exact test			P=0.054N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	17/49 (35%)	9/28 (32%)[e]	10/49 (20%)
Adjusted rate	45.4%		34.2%
Terminal rate	10/29 (34%)		6/24 (25%)
First incidence (days)	651		682
Life table test			P=0.220N
Logistic regression test			P=0.119N
Fisher exact test			P=0.087N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma			
Overall rate	1/49 (2%)	2/28 (7%)[e]	0/49 (0%)
Adjusted rate	3.1%		0.0%
Terminal rate	0/29 (0%)		0/24 (0%)
First incidence (days)	710		–
Life table test			P=0.541N
Logistic regression test			P=0.515N
Fisher exact test			P=0.500N
All Organs: Mononuclear Cell Leukemia			
Overall rate	25/50 (50%)	33/50 (66%)	31/50 (62%)
Adjusted rate	59.3%	79.3%	70.5%
Terminal rate	12/29 (41%)	15/23 (65%)	12/24 (50%)
First incidence (days)	505	503	517
Life table test	P=0.115	P=0.043	P=0.083
Logistic regression test	P=0.208	P=0.075	P=0.161
Cochran-Armitage test	P=0.199		
Fisher exact test		P=0.078	P=0.157

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
All Organs: Benign Neoplasms			
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	29/29 (100%)	23/23 (100%)	24/24 (100%)
First incidence (days)	505	503	493
Life table test	P=0.234	P=0.167	P=0.201
Logistic regression test	P=0.327N	–[g]	P=0.527N
Cochran-Armitage test	P=0.296N		
Fisher exact test		P=1.000N	P=0.500N
All Organs: Malignant Neoplasms			
Overall rate	35/50 (70%)	41/50 (82%)	42/50 (84%)
Adjusted rate	75.9%	86.8%	89.0%
Terminal rate	18/29 (62%)	17/23 (74%)	19/24 (79%)
First incidence (days)	505	503	517
Life table test	P=0.067	P=0.073	P=0.049
Logistic regression test	P=0.094	P=0.132	P=0.079
Cochran-Armitage test	P=0.083		
Fisher exact test		P=0.121	P=0.077
All Organs: Benign or Malignant Neoplasms			
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	29/29 (100%)	23/23 (100%)	24/24 (100%)
First incidence (days)	505	503	493
Life table test	P=0.190	P=0.167	P=0.165
Logistic regression test	–	–	–
Cochran-Armitage test	–		
Fisher exact test		P=1.000N	P=1.000N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not appropriate.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed

TABLE A2b
Statistical Analysis of Selected Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 1:3, 3:10, 10:10, and 10:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	1 ppm 3 ppm	3 ppm 10 ppm	10 ppm 10 ppm	10 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate[a]	1/50 (2%)	6/50 (12%)	14/50 (28%)	16/50 (32%)	41/50 (82%)
Adjusted rate[b]	3.4%	16.4%	55.9%	50.4%	100.0%
Terminal rate[c]	1/29 (3%)	4/34 (12%)	10/20 (50%)	7/20 (35%)	19/19 (100%)
First incidence (days)	727 (T)	702	484	531	515
Life table test[d]	P<0.001	P=0.092	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P=0.068	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001				
Fisher exact test[d]		P=0.056	P<0.001	P<0.001	P<0.001
All Organs: Mononuclear Cell Leukemia					
Overall rate	25/50 (50%)	32/50 (64%)	41/50 (82%)	37/50 (74%)	37/50 (74%)
Adjusted rate	59.3%	71.0%	93.0%	80.9%	84.7%
Terminal rate	12/29 (41%)	21/34 (62%)	17/20 (85%)	12/20 (60%)	13/19 (68%)
First incidence (days)	505	520	484	397	461
Life table test	P<0.001	P=0.344	P<0.001	P=0.002	P=0.001
Logistic regression test	P=0.020	P=0.112	P<0.001	P=0.018	P=0.012
Cochran-Armitage test	P=0.012				
Fisher exact test		P=0.113	P<0.001	P=0.011	P=0.011

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE A2c
Statistical Analysis of Selected Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 3:10, and 10:10 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	12/49 (24%)	14/50 (28%)	16/50 (32%)
Adjusted rate[b]	41.6%	55.9%	50.4%
Terminal rate[c]	7/23 (30%)	10/20 (50%)	7/20 (35%)
First incidence (days)	678	484	531
Life table test[d]	P=0.145	P=0.242	P=0.147
Logistic regression test[d]	P=0.184	P=0.263	P=0.200
Cochran-Armitage test[d]	P=0.253		
Fisher exact test[d]		P=0.433	P=0.272
All Organs: Mononuclear Cell Leukemia			
Overall rate	33/50 (66%)	41/50 (82%)	37/50 (74%)
Adjusted rate	79.3%	93.0%	80.9%
Terminal rate	15/23 (65%)	17/20 (85%)	12/20 (60%)
First incidence (days)	503	484	397
Life table test	P=0.196	P=0.036	P=0.134
Logistic regression test	P=0.363	P=0.038	P=0.336
Cochran-Armitage test	P=0.353		
Fisher exact test		P=0.055	P=0.257

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE A2d
Statistical Analysis of Selected Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 10:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate[a]	41/50 (82%)	41/50 (82%)
Adjusted rate[b]	97.6%	100.0%
Terminal rate[c]	23/24 (96%)	19/19 (100%)
First incidence (days)	588	515
Life table test[d]		P=0.129
Logistic regression test[d]		P=0.204
Fisher exact test[d]		P=0.602N
All Organs: Mononuclear Cell Leukemia		
Overall rate	31/50 (62%)	37/50 (74%)
Adjusted rate	70.5%	84.7%
Terminal rate	12/24 (50%)	13/19 (68%)
First incidence (days)	517	461
Life table test		P=0.064
Logistic regression test		P=0.144
Fisher exact test		P=0.142

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE A2e
Statistical Analysis of Selected Primary Neoplasms in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 10:0, 10:10, and 10:30 ppm Groups

F_0 Concentration F_1 Concentration	10 ppm 0 ppm	10 ppm 10 ppm	10 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	5/50 (10%)	16/50 (32%)	41/50 (82%)
Adjusted rate[b]	16.7%	50.4%	100.0%
Terminal rate[c]	5/30 (17%)	7/20 (35%)	19/19 (100%)
First incidence (days)	727 (T)	531	515
Life table test[d]	P<0.001	P=0.001	P<0.001
Logistic regression test[d]	P<0.001	P=0.005	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P=0.006	P<0.001
All Organs: Mononuclear Cell Leukemia			
Overall rate	32/50 (64%)	37/50 (74%)	37/50 (74%)
Adjusted rate	77.8%	80.9%	84.7%
Terminal rate	21/30 (70%)	12/20 (60%)	13/19 (68%)
First incidence (days)	580	397	461
Life table test	P=0.030	P=0.028	P=0.015
Logistic regression test	P=0.200	P=0.175	P=0.145
Cochran-Armitage test	P=0.208		
Fisher exact test		P=0.194	P=0.194

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE A3a
Historical Incidence of Hepatocellular Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	4/50	3/50	5/50
5,5′-Diphenylhydantoin (Phenytoin)	0/50	0/50	0/50
Ethylene thiourea	0/50	0/50	0/50
Polybrominated biphenyls	1/50	0/50	1/50
Manganese sulfate	0/52	0/52	0/52
Triamterene	0/50	0/50	0/50
Overall Historical Incidence			
Total	26/1,001 (2.6%)	9/1,001 (0.9%)	32/1,001 (3.2%)
Standard deviation	3.2%	1.7%	3.6%
Range	0%–10%	0%–6%	0%–10%

[a] Data as of 17 December 1991

TABLE A3b
Historical Incidence of Leukemia in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratories	
2,4-Dichlorophenol	31/50
5,5′-Diphenylhydantoin (Phenytoin)	25/50
Ethylene thiourea	22/50
Polybrominated biphenyls	25/50
Manganese sulfate	32/52
Triamterene	22/50
Overall Historical Incidence	
Total	483/1,002 (48.2%)
Standard deviation	8.7%
Range	32%-62%

[a] Data as of 17 December 1991 for lymphocytic, monocytic, mononuclear cell, or undifferentiated leukemias

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	16	16	15	21	25	26
Natural deaths	5	4	1	6	5	4
Survivors						
Died last week of study		1		1	1	1
Terminal sacrifice	29	29	34	22	19	19
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(48)	(20)	(16)	(26)	(30)	(29)
Dilatation		1 (5%)		1 (4%)		
Inflammation, chronic active					1 (3%)	
Ulcer					1 (3%)	
Intestine large, colon	(49)	(21)	(16)	(27)	(31)	(30)
Dilatation		1 (5%)				
Parasite metazoan	3 (6%)	1 (5%)			1 (3%)	
Intestine large, rectum	(49)	(21)	(16)	(27)	(30)	(30)
Parasite metazoan	3 (6%)		1 (6%)	1 (4%)	2 (7%)	
Intestine small, duodenum	(48)	(21)	(16)	(27)	(31)	(30)
Ulcer						1 (3%)
Intestine small, ileum	(49)	(19)	(16)	(28)	(29)	(29)
Parasite metazoan						1 (3%)
Liver	(50)	(50)	(50)	(49)	(50)	(50)
Angiectasis		4 (8%)	1 (2%)			
Basophilic focus	27 (54%)	19 (38%)	36 (72%)	28 (57%)	8 (16%)	15 (30%)
Clear cell focus	1 (2%)	1 (2%)	1 (2%)			1 (2%)
Congestion			1 (2%)			
Degeneration, cystic	18 (36%)	12 (24%)	12 (24%)	5 (10%)	3 (6%)	7 (14%)
Eosinophilic focus	18 (36%)	29 (58%)	40 (80%)	44 (90%)	42 (84%)	47 (94%)
Hepatodiaphragmatic nodule		4 (8%)	1 (2%)			
Hyperplasia, focal	2 (4%)	1 (2%)				
Hyperplasia, multifocal	2 (4%)					
Hypertrophy		3 (6%)	34 (68%)	27 (55%)	29 (58%)	27 (54%)
Infarct		1 (2%)				
Inflammation, chronic		4 (8%)				2 (4%)
Inflammation, chronic, multifocal		1 (2%)	13 (26%)	6 (12%)	4 (8%)	4 (8%)
Inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)	1 (2%)	
Necrosis		2 (4%)	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Vacuolization cytoplasmic	5 (10%)	18 (36%)	42 (84%)	28 (57%)	28 (56%)	32 (64%)
Bile duct, hyperplasia	48 (96%)	45 (90%)	49 (98%)	48 (98%)	47 (94%)	49 (98%)
Centrilobular, degeneration	1 (2%)					
Centrilobular, necrosis		1 (2%)				
Oval cell, hyperplasia		2 (4%)	4 (8%)	11 (22%)	13 (26%)	23 (46%)
Portal vein, thrombosis			1 (2%)			
Mesentery		(6)		(3)	(1)	(2)
Inflammation, chronic active		1 (17%)		1 (33%)		
Artery, inflammation, chronic active				2 (67%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	23	24
Natural deaths	3	7
Survivors		
Died last week of study	1	1
Terminal sacrifice	23	18
Animals examined microscopically	50	50
Alimentary System		
Intestine large, colon	(48)	(47)
Parasite metazoan	2 (4%)	
Intestine large, rectum	(48)	(46)
Parasite metazoan	1 (2%)	
Intestine small, duodenum	(48)	(48)
Ulcer		1 (2%)
Liver	(50)	(50)
Angiectasis	1 (2%)	
Basophilic focus	8 (16%)	5 (10%)
Clear cell focus	1 (2%)	1 (2%)
Congestion		1 (2%)
Degeneration, cystic	1 (2%)	3 (6%)
Eosinophilic focus	46 (92%)	46 (92%)
Fibrosis		2 (4%)
Hypertrophy	17 (34%)	15 (30%)
Infarct		2 (4%)
Inflammation, chronic, multifocal	4 (8%)	5 (10%)
Metaplasia	1 (2%)	1 (2%)
Necrosis	1 (2%)	4 (8%)
Vacuolization cytoplasmic	34 (68%)	37 (74%)
Bile duct, cyst		1 (2%)
Bile duct, hyperplasia	49 (98%)	49 (98%)
Oval cell, hyperplasia	37 (74%)	41 (82%)
Serosa, fibrosis		1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Alimentary System (continued)						
Pancreas	(49)	(21)	(16)	(27)	(32)	(31)
Fibrosis			1 (6%)			
Inflammation, chronic active			1 (6%)			
Necrosis						1 (3%)
Acinus, atrophy	23 (47%)	7 (33%)	8 (50%)	13 (48%)	12 (38%)	16 (52%)
Acinus, cytomegaly, focal				1 (4%)		
Acinus, hyperplasia	2 (4%)				1 (3%)	
Duct, hyperplasia				1 (4%)		
Stomach, forestomach	(50)	(49)	(48)	(49)	(50)	(50)
Acanthosis		4 (8%)	3 (6%)	7 (14%)	7 (14%)	8 (16%)
Cyst epithelial inclusion	1 (2%)			1 (2%)		
Erosion						1 (2%)
Foreign body	1 (2%)					
Inflammation, chronic					1 (2%)	1 (2%)
Inflammation, chronic active		1 (2%)	2 (4%)	6 (12%)	5 (10%)	5 (10%)
Mineralization					1 (2%)	
Ulcer		1 (2%)		5 (10%)	3 (6%)	4 (8%)
Stomach, glandular	(50)	(48)	(48)	(48)	(50)	(49)
Dysplasia					1 (2%)	
Hyperplasia, focal						1 (2%)
Inflammation, chronic active		1 (2%)			1 (2%)	
Mineralization					1 (2%)	1 (2%)
Ulcer		1 (2%)		1 (2%)		1 (2%)
Cardiovascular System						
Heart	(50)	(21)	(16)	(27)	(31)	(31)
Degeneration, chronic	50 (100%)	12 (57%)	9 (56%)	23 (85%)	22 (71%)	23 (74%)
Thrombosis		1 (5%)	1 (6%)			1 (3%)
Endocrine System						
Adrenal gland, cortex	(50)	(21)	(15)	(28)	(31)	(30)
Atrophy						1 (3%)
Degeneration, cystic					1 (3%)	1 (3%)
Hyperplasia	19 (38%)	4 (19%)	4 (27%)	9 (32%)	5 (16%)	4 (13%)
Hypertrophy	8 (16%)	1 (5%)		1 (4%)		2 (7%)
Necrosis			1 (7%)		1 (3%)	
Vacuolization cytoplasmic	11 (22%)	10 (48%)	7 (47%)	6 (21%)	15 (48%)	14 (47%)
Adrenal gland, medulla	(49)	(22)	(16)	(29)	(32)	(30)
Hyperplasia	13 (27%)	3 (14%)	1 (6%)	6 (21%)	1 (3%)	3 (10%)
Islets, pancreatic	(49)	(21)	(16)	(27)	(31)	(30)
Hyperplasia	1 (2%)					1 (3%)
Necrosis						1 (3%)
Parathyroid gland	(43)	(21)	(16)	(27)	(31)	(29)
Hyperplasia						1 (3%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Alimentary System (continued)		
Pancreas	(49)	(50)
Acinus, atrophy	35 (71%)	29 (58%)
Acinus, cytomegaly	1 (2%)	
Acinus, hyperplasia	2 (4%)	1 (2%)
Stomach, forestomach	(50)	(50)
Acanthosis	11 (22%)	6 (12%)
Acanthosis, focal	1 (2%)	
Cyst epithelial inclusion	1 (2%)	
Erosion		1 (2%)
Inflammation, chronic active	9 (18%)	3 (6%)
Ulcer	6 (12%)	2 (4%)
Stomach, glandular	(50)	(49)
Erosion		1 (2%)
Hyperplasia, focal	1 (2%)	
Mineralization	1 (2%)	
Ulcer		3 (6%)
Cardiovascular System		
Heart	(50)	(49)
Degeneration, chronic	40 (80%)	34 (69%)
Atrium, thrombosis		1 (2%)
Endocrine System		
Adrenal gland	(50)	(50)
Capsule, spindle cell, hyperplasia		1 (2%)
Adrenal gland, cortex	(50)	(50)
Hyperplasia	17 (34%)	13 (26%)
Hypertrophy	7 (14%)	2 (4%)
Necrosis	1 (2%)	
Vacuolization cytoplasmic	10 (20%)	15 (30%)
Capsule, ectopic tissue	1 (2%)	
Adrenal gland, medulla	(50)	(50)
Hyperplasia	4 (8%)	5 (10%)
Islets, pancreatic	(49)	(50)
Hyperplasia		4 (8%)
Parathyroid gland	(49)	(47)
Cyst	1 (2%)	
Hyperplasia	2 (4%)	
Hypertrophy	1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Endocrine System (continued)						
Pituitary gland	(50)	(27)	(22)	(32)	(30)	(34)
Pars distalis, cyst	3 (6%)	3 (11%)	1 (5%)		2 (7%)	1 (3%)
Pars distalis, hemorrhage						1 (3%)
Pars distalis, hyperplasia	9 (18%)	5 (19%)	1 (5%)	8 (25%)	6 (20%)	8 (24%)
Pars intermedia, hyperplasia, glandular		1 (4%)				
Thyroid gland	(49)	(23)	(17)	(28)	(31)	(30)
C-cell, hyperplasia	48 (98%)	20 (87%)	16 (94%)	28 (100%)	30 (97%)	29 (97%)
Follicle, cyst			1 (6%)			1 (3%)
Follicular cell, hyperplasia						1 (3%)
Follicular cell, hypertrophy	2 (4%)		1 (6%)			
General Body System						
None						
Genital System						
Epididymis	(50)	(20)	(16)	(28)	(30)	(30)
Inflammation, chronic active						2 (7%)
Preputial gland	(47)	(25)	(20)	(32)	(34)	(32)
Hyperplasia	3 (6%)	1 (4%)				
Inflammation, chronic active	37 (79%)	15 (60%)	14 (70%)	27 (84%)	23 (68%)	25 (78%)
Duct, dilatation	1 (2%)	3 (12%)		2 (6%)	1 (3%)	1 (3%)
Duct, hyperplasia, squamous					3 (9%)	
Prostate	(50)	(21)	(16)	(28)	(30)	(31)
Inflammation, acute					1 (3%)	
Inflammation, chronic active	11 (22%)	4 (19%)	2 (13%)	8 (29%)	5 (17%)	10 (32%)
Metaplasia, squamous	11 (22%)				1 (3%)	3 (10%)
Seminal vesicle		(2)	(3)		(1)	(1)
Dilatation		1 (50%)	2 (67%)		1 (100%)	
Epithelium, hyperplasia		1 (50%)				
Testes	(50)	(49)	(50)	(50)	(50)	(50)
Interstitial cell, hyperplasia	8 (16%)	3 (6%)	1 (2%)	3 (6%)	9 (18%)	4 (8%)
Seminiferous tubule, atrophy	3 (6%)	2 (4%)	5 (10%)		1 (2%)	3 (6%)
Seminiferous tubule, mineralization	1 (2%)		3 (6%)			2 (4%)
Hematopoietic System						
Bone marrow	(49)	(21)	(15)	(27)	(31)	(30)
Femoral, atrophy			1 (7%)			
Femoral, hyperplasia, reticulum cell	1 (2%)					
Femoral, myelofibrosis						1 (3%)
Lymph node	(50)	(23)	(18)	(27)	(33)	(31)
Mandibular, hemorrhage	1 (2%)					
Mandibular, hyperplasia, lymphoid						3 (10%)
Mediastinal, congestion						1 (3%)
Mediastinal, hemorrhage			1 (6%)			
Mediastinal, hyperplasia, macrophage		1 (4%)				
Lymph node, mesenteric	(2)	(3)	(2)	(9)	(8)	(4)
Angiectasis				1 (11%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Endocrine System (continued)		
Pituitary gland	(48)	(49)
Pars distalis, hyperplasia	10 (21%)	10 (20%)
Pars distalis, vacuolization cytoplasmic	1 (2%)	
Thyroid gland	(49)	(49)
Inflammation, chronic	1 (2%)	
C-cell, hyperplasia	47 (96%)	48 (98%)
Follicle, cyst		1 (2%)
Follicle, hyperplasia, cystic		1 (2%)
Follicular cell, cytoplasmic alteration	1 (2%)	
Follicular cell, hyperplasia		1 (2%)
Follicular cell, hypertrophy	1 (2%)	1 (2%)
General Body System		
None		
Genital System		
Epididymis	(50)	(50)
Inflammation, chronic active	3 (6%)	3 (6%)
Preputial gland	(44)	(49)
Inflammation, chronic active	34 (77%)	36 (73%)
Duct, dilatation	3 (7%)	6 (12%)
Duct, hyperplasia, squamous	2 (5%)	
Prostate	(49)	(50)
Dilatation	1 (2%)	
Inflammation, chronic active	7 (14%)	10 (20%)
Metaplasia, squamous	3 (6%)	8 (16%)
Testes	(50)	(50)
Interstitial cell, hyperplasia	7 (14%)	12 (24%)
Seminiferous tubule, atrophy	6 (12%)	11 (22%)
Seminiferous tubule, mineralization	1 (2%)	
Hematopoietic System		
Bone marrow	(50)	(50)
Femoral, hyperplasia, neutrophil		1 (2%)
Lymph node	(49)	(49)
Mandibular, hyperplasia, lymphoid	1 (2%)	
Mandibular, inflammation, chronic, multifocal	1 (2%)	
Sinus, mandibular, ectasia		1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Hematopoietic System (continued)						
Spleen	(50)	(50)	(49)	(49)	(50)	(50)
Depletion lymphoid			1 (2%)			
Fibrosis	1 (2%)	2 (4%)	6 (12%)	4 (8%)	2 (4%)	
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)	1 (2%)		
Inflammation, chronic active					1 (2%)	
Necrosis			1 (2%)	2 (4%)		
Thrombosis				1 (2%)		
Capsule, cyst						1 (2%)
Capsule, hemorrhage		1 (2%)				
Thymus	(47)	(17)	(15)	(22)	(24)	(27)
Depletion lymphoid						1 (4%)
Inflammation, chronic active			1 (7%)			
Necrosis			1 (7%)			1 (4%)
Integumentary System						
Mammary gland	(39)	(14)	(14)	(23)	(20)	(18)
Hyperplasia, cystic	38 (97%)	14 (100%)	12 (86%)	19 (83%)	20 (100%)	18 (100%)
Skin	(50)	(26)	(22)	(31)	(32)	(32)
Acanthosis				1 (3%)		
Necrosis	1 (2%)					
Face, cyst epithelial inclusion		1 (4%)				
Subcutaneous tissue, fibrosis	1 (2%)					
Musculoskeletal System						
None						
Nervous System						
Brain	(50)	(21)	(16)	(29)	(31)	(31)
Compression	3 (6%)		2 (13%)		1 (3%)	4 (13%)
Gliosis		1 (5%)				
Hemorrhage	1 (2%)	2 (10%)		4 (14%)	2 (6%)	1 (3%)
Hydrocephalus			2 (13%)		2 (6%)	4 (13%)
Spinal cord	(2)	(1)	(1)			
Hemorrhage		1 (100%)				
Nerve, demyelination	2 (100%)	1 (100%)				
Respiratory System						
Lung	(50)	(21)	(17)	(28)	(32)	(31)
Alveolar epithelium, hyperplasia	1 (2%)	1 (5%)		1 (4%)		1 (3%)
Interstitium, inflammation, chronic active	3 (6%)	2 (10%)			1 (3%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Hematopoietic System (continued)		
Spleen	(50)	(50)
Fibrosis	10 (20%)	6 (12%)
Hematopoietic cell proliferation		1 (2%)
Thrombosis, multiple	1 (2%)	
Integumentary System		
Mammary gland	(39)	(30)
Hyperplasia, cystic	37 (95%)	30 (100%)
Musculoskeletal System		
None		
Nervous System		
Brain	(50)	(50)
Compression		1 (2%)
Hydrocephalus	1 (2%)	
Respiratory System		
Lung	(50)	(49)
Alveolar epithelium, hyperplasia	4 (8%)	2 (4%)
Bronchiole, epithelium, hyperplasia		1 (2%)
Interstitium, inflammation, chronic active	2 (4%)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Respiratory System (continued)						
Nose	(50)	(21)	(16)	(27)	(31)	(30)
Foreign body					1 (3%)	
Mucosa, inflammation, acute					1 (3%)	
Mucosa, inflammation, chronic active	1 (2%)	1 (5%)	1 (6%)		2 (6%)	1 (3%)
Nasolacrimal duct, inflammation, chronic active	1 (2%)					
Special Senses System						
Eye		(1)	(4)	(7)	(4)	(3)
Cornea, inflammation, chronic active						1 (33%)
Lens, cataract		1 (100%)	2 (50%)	7 (100%)	4 (100%)	2 (67%)
Retina, atrophy		1 (100%)	3 (75%)	7 (100%)	4 (100%)	2 (67%)
Urinary System						
Kidney	(50)	(24)	(20)	(32)	(32)	(37)
Hydronephrosis	1 (2%)	2 (8%)				
Nephropathy, chronic	50 (100%)	23 (96%)	19 (95%)	32 (100%)	32 (100%)	36 (97%)
Cortex, cyst		1 (4%)	1 (5%)	2 (6%)	1 (3%)	1 (3%)
Urinary bladder	(49)	(20)	(17)	(27)	(31)	(30)
Calculus micro observation only			1 (6%)			
Hemorrhage	1 (2%)	1 (5%)				1 (3%)
Inflammation, acute					1 (3%)	
Mucosa, hyperplasia						1 (3%)
Mucosa, ulcer	1 (2%)					

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Respiratory System (continued)		
Nose	(49)	(50)
Hemorrhage, subacute		1 (2%)
Mucosa, inflammation, chronic active	3 (6%)	5 (10%)
Special Senses System		
Eye	(5)	(4)
Cornea, inflammation, chronic active	1 (20%)	
Lens, cataract	3 (60%)	1 (25%)
Retina, atrophy	3 (60%)	1 (25%)
Harderian gland		(1)
Acinus, atrophy		1 (100%)
Urinary System		
Kidney	(50)	(50)
Inflammation, chronic, multifocal	1 (2%)	
Nephropathy, chronic	50 (100%)	50 (100%)
Cortex, cyst	1 (2%)	1 (2%)
Urinary bladder	(48)	(50)
Mucosa, hyperplasia		1 (2%)

[a] Number of animals with any tissue examined microscopically

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF POLYBROMINATED BIPHENYLS

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	10	10	9	12	8	10
Natural deaths		1	1	2	3	2
Survivors						
Died last week of study			1			
Terminal sacrifice	40	39	39	36	39	38
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(50)	(10)	(11)	(13)	(10)	(12)
Leiomyosarcoma					1 (10%)	
Intestine small, duodenum	(50)	(12)	(11)	(13)	(10)	(12)
Schwannoma malignant		1 (8%)				
Serosa, adenocarcinoma, metastatic, uterus		1 (8%)				
Liver	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocellular carcinoma				2 (4%)	1 (2%)	7 (14%)
Hepatocellular carcinoma, multiple						1 (2%)
Hepatocellular adenoma			2 (4%)	6 (12%)	12 (24%)	15 (30%)
Hepatocellular adenoma, multiple			1 (2%)	4 (8%)	10 (20%)	20 (40%)
Hepatocholangiocarcinoma					1 (2%)	
Mesentery	(2)	(1)	(1)	(1)	(1)	(4)
Hepatocellular carcinoma, metastatic, liver						1 (25%)
Pancreas	(49)	(12)	(11)	(13)	(9)	(12)
Adenocarcinoma, metastatic, uterus		1 (8%)				
Stomach	(50)	(49)	(50)	(50)	(50)	(50)
Serosa, adenocarcinoma, metastatic, uterus		1 (2%)				
Stomach, forestomach	(50)	(49)	(50)	(50)	(50)	(49)
Squamous cell carcinoma				1 (2%)		
Stomach, glandular	(50)	(48)	(50)	(50)	(50)	(50)
Sarcoma				1 (2%)		
Tongue		(1)	(1)	(1)	(1)	(1)
Carcinoma, metastatic, Zymbal's gland						1 (100%)
Squamous cell carcinoma				1 (100%)	1 (100%)	
Squamous cell papilloma		1 (100%)	1 (100%)			
Cardiovascular System						
Heart	(50)	(11)	(12)	(14)	(11)	(12)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	10	15
Natural deaths	5	2
Survivors		
Died last week of study	1	1
Terminal sacrifice	34	32
Animals examined microscopically	50	50
Alimentary System		
Liver	(50)	(50)
Hepatocellular carcinoma	4 (8%)	16 (32%)
Hepatocellular carcinoma, multiple		6 (12%)
Hepatocellular adenoma	1 (2%)	1 (2%)
Hepatocellular adenoma, multiple	37 (74%)	44 (88%)
Hepatocholangiocarcinoma	1 (2%)	
Pancreas	(50)	(50)
Stomach, forestomach	(48)	(50)
Squamous cell papilloma	1 (2%)	
Cardiovascular System		
Heart	(49)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Endocrine System						
Adrenal gland	(50)	(11)	(12)	(14)	(9)	(12)
Capsule, adenocarcinoma, metastatic, uterus		1 (9%)				
Adrenal gland, cortex	(50)	(10)	(12)	(14)	(9)	(12)
Adenoma	1 (2%)	1 (10%)				
Adrenal gland, medulla	(48)	(10)	(12)	(13)	(9)	(12)
Pheochromocytoma malignant			1 (8%)			
Pheochromocytoma benign	2 (4%)		1 (8%)			
Bilateral, pheochromocytoma benign	1 (2%)					
Islets, pancreatic	(49)	(12)	(11)	(13)	(9)	(12)
Carcinoma		1 (8%)				
Parathyroid gland	(46)	(9)	(11)	(14)	(9)	(10)
Adenoma	2 (4%)					
Pituitary gland	(50)	(34)	(27)	(29)	(28)	(27)
Pars distalis, adenoma	21 (42%)	19 (56%)	19 (70%)	18 (62%)	12 (43%)	12 (44%)
Pars intermedia, adenoma				2 (7%)	1 (4%)	
Thyroid gland	(50)	(12)	(15)	(14)	(10)	(13)
Bilateral, C-cell, adenoma	1 (2%)					
C-cell, adenoma	10 (20%)	5 (42%)	4 (27%)	3 (21%)		2 (15%)
C-cell, carcinoma		1 (8%)	2 (13%)	1 (7%)		
General Body System						
Tissue NOS						(1)
Liposarcoma						1 (100%)
Genital System						
Clitoral gland	(50)	(15)	(12)	(16)	(10)	(13)
Adenoma	3 (6%)	1 (7%)		1 (6%)		1 (8%)
Bilateral, adenoma		1 (7%)				
Ovary	(50)	(23)	(19)	(22)	(14)	(20)
Granulosa-theca tumor malignant			1 (5%)			
Periovarian tissue, adenocarcinoma, metastatic, uterus		1 (4%)				
Periovarian tissue, leiomyoma				1 (5%)		
Uterus	(50)	(17)	(18)	(18)	(21)	(20)
Adenocarcinoma	2 (4%)	1 (6%)	2 (11%)			
Polyp stromal	7 (14%)	4 (24%)	2 (11%)	3 (17%)	6 (29%)	4 (20%)
Sarcoma stromal				1 (6%)		
Schwannoma malignant	1 (2%)					
Epithelium, adenoma	1 (2%)					

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Endocrine System		
Adrenal gland, cortex	(50)	(50)
Adenoma		1 (2%)
Bilateral, adenoma	1 (2%)	
Adrenal gland, medulla	(49)	(49)
Pituitary gland	(50)	(50)
Pars distalis, adenoma	2 (4%)	5 (10%)
Pars distalis, adenoma, mild	1 (2%)	
Pars distalis, adenoma, moderate	1 (2%)	
Thyroid gland	(48)	(48)
Bilateral, C-cell, adenoma		1 (2%)
C-cell, adenoma	2 (4%)	7 (15%)
C-cell, carcinoma	1 (2%)	
General Body System		
None		
Genital System		
Clitoral gland	(47)	(46)
Carcinoma	1 (2%)	1 (2%)
Ovary	(48)	(49)
Leiomyosarcoma	1 (2%)	
Uterus	(49)	(50)
Adenocarcinoma		1 (2%)
Leiomyosarcoma		1 (2%)
Polyp stromal	7 (14%)	4 (8%)
Sarcoma stromal		1 (2%)
Vagina		(2)
Leiomyosarcoma		1 (50%)
Polyp		1 (50%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Hematopoietic System						
Bone marrow	(50)	(11)	(11)	(13)	(9)	(11)
Lymph node	(50)	(11)	(11)	(14)	(12)	(12)
Mediastinal, adenocarcinoma, metastatic, uterus		1 (9%)				
Lymph node, mesenteric	(4)	(2)	(3)	(1)	(1)	(3)
Adenocarcinoma, metastatic, uterus		1 (50%)				
Spleen	(50)	(50)	(50)	(50)	(49)	(49)
Thymus	(44)	(9)	(11)	(11)	(9)	(10)
Integumentary System						
Mammary gland	(48)	(16)	(13)	(21)	(16)	(11)
Adenoma					1 (6%)	
Carcinoma		1 (6%)				
Fibroadenoma	4 (8%)	7 (44%)	2 (15%)	10 (48%)	7 (44%)	2 (18%)
Fibroadenoma, multiple			1 (8%)	1 (5%)		
Skin	(50)	(11)	(12)	(13)	(11)	(14)
Basal cell adenoma	1 (2%)					
Squamous cell papilloma	1 (2%)					
Trichoepithelioma	1 (2%)					
Subcutaneous tissue, fibroma		1 (9%)				
Subcutaneous tissue, fibrosarcoma			1 (8%)			3 (21%)
Subcutaneous tissue, sarcoma	1 (2%)					
Musculoskeletal System						
Skeletal muscle	(1)					
Nervous System						
Brain	(50)	(11)	(11)	(14)	(10)	(12)
Astrocytoma malignant	2 (4%)			1 (7%)		
Respiratory System						
Lung	(50)	(11)	(11)	(14)	(9)	(11)
Adenocarcinoma, metastatic, uterus		1 (9%)	1 (9%)			
Hepatocellular carcinoma, metastatic, liver						1 (9%)
Special Senses System						
Zymbal's gland				(1)		(1)
Carcinoma				1 (100%)		1 (100%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Hematopoietic System		
Bone marrow	(48)	(47)
Lymph node	(49)	(47)
Lymph node, mesenteric	(5)	
Spleen	(50)	(50)
Thymus	(41)	(42)
Integumentary System		
Mammary gland	(49)	(48)
Adenoma		1 (2%)
Fibroadenoma	1 (2%)	4 (8%)
Fibroadenoma, multiple	1 (2%)	
Skin	(49)	(50)
Squamous cell papilloma	1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)	1 (2%)
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(47)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)	
Squamous cell carcinoma, multiple, metastatic, uncertain primary site		1 (2%)
Squamous cell carcinoma, metastatic, clitoral gland	1 (2%)	
Special Senses System		
None		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Urinary System						
Kidney	(49)	(11)	(11)	(14)	(11)	(12)
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	14 (28%)	13 (26%)	20 (40%)	22 (44%)	17 (34%)	27 (54%)
Lymphoma malignant lymphocytic			1 (2%)			
Mesothelioma malignant	1 (2%)					
Neoplasm Summary						
Total animals with primary neoplasms[c]	40	37	35	42	41	49
Total primary neoplasms	77	58	61	80	70	96
Total animals with benign neoplasms	34	30	25	33	34	41
Total benign neoplasms	56	40	33	49	49	56
Total animals with malignant neoplasms	20	16	25	28	20	31
Total malignant neoplasms	21	18	28	31	21	40
Total animals with metastatic neoplasms		1	1			2
Total metastatic neoplasms		8	1			3

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Urinary System		
Kidney	(50)	(50)
Transitional epithelium, carcinoma		1 (2%)
Urinary bladder	(49)	(50)
Papilloma		1 (2%)
Systemic Lesions		
Multiple organs	(50)	(50)
Leukemia mononuclear	23 (46%)	25 (50%)
Lymphoma malignant histiocytic		1 (2%)
Neoplasm Summary		
Total animals with primary neoplasms	47	50
Total primary neoplasms	90	125
Total animals with benign neoplasms	41	46
Total benign neoplasms	59	71
Total animals with malignant neoplasms	27	36
Total malignant neoplasms	31	54
Total animals with metastatic neoplasms	2	1
Total metastatic neoplasms	2	1
Total animals with malignant neoplasms of uncertain primary site		1

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

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	3/48 (6%)	0/13 (0%)[e]	0/49 (0%)
Adjusted rate[b]	7.9%		0.0%
Terminal rate[c]	3/38 (8%)		0/34 (0%)
First incidence (days)	729 (T)		–[f]
Life table test[d]			P=0.141N
Logistic regression test[d]			P=0.141N
Fisher exact test[d]			P=0.117N
Clitoral Gland: Adenoma			
Overall rate	3/50 (6%)	1/16 (6%)[e]	0/47 (0%)
Adjusted rate	7.5%		0.0%
Terminal rate	3/40 (8%)		0/34 (0%)
First incidence (days)	729 (T)		–
Life table test			P=0.151N
Logistic regression test			P=0.151N
Fisher exact test			P=0.133N
Clitoral Gland: Adenoma or Carcinoma			
Overall rate	3/50 (6%)	1/16 (6%)[e]	1/47 (2%)
Adjusted rate	7.5%		2.9%
Terminal rate	3/40 (8%)		1/34 (3%)
First incidence (days)	729 (T)		729 (T)
Life table test			P=0.365N
Logistic regression test			P=0.365N
Fisher exact test			P=0.332N
Liver: Hepatocellular Adenoma			
Overall rate	0/50 (0%)	10/50 (20%)	38/50 (76%)
Adjusted rate	0.0%	26.6%	90.4%
Terminal rate	0/40 (0%)	9/36 (25%)	31/35 (89%)
First incidence (days)	–	565	593
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.001	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test		P<0.001	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	5.6%	10.6%
Terminal rate	0/40 (0%)	2/36 (6%)	3/35 (9%)
First incidence (days)	–	729 (T)	614
Life table test	P=0.038	P=0.215	P=0.052
Logistic regression test	P=0.045	P=0.215	P=0.063
Cochran-Armitage test	P=0.047		
Fisher exact test		P=0.247	P=0.059

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	0/50 (0%)	12/50 (24%)	39/50 (78%)
Adjusted rate	0.0%	32.0%	90.6%
Terminal rate	0/40 (0%)	11/36 (31%)	31/35 (89%)
First incidence (days)	–	565	593
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Mammary Gland: Fibroadenoma			
Overall rate	4/50 (8%)	11/50 (22%)	2/50 (4%)
Adjusted rate	10.0%	27.7%	5.7%
Terminal rate	4/40 (10%)	8/36 (22%)	2/35 (6%)
First incidence (days)	729 (T)	661	729 (T)
Life table test	P=0.228N	P=0.034	P=0.400N
Logistic regression test	P=0.193N	P=0.043	P=0.400N
Cochran-Armitage test	P=0.168N		
Fisher exact test		P=0.045	P=0.339N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	21/50 (42%)	18/29 (62%)	4/50 (8%)
Adjusted rate	46.3%	82.8%	11.4%
Terminal rate	16/40 (40%)	12/15 (80%)	4/35 (11%)
First incidence (days)	523	576	729 (T)
Life table test	P<0.001N	P=0.030	P<0.001N
Logistic regression test	P<0.001N	P=0.046	P<0.001N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.068	P<0.001N
Skin: Squamous Cell Papilloma, Trichoepithelioma, or Basal Cell Adenoma			
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	7.1%	0.0%	2.9%
Terminal rate	2/40 (5%)	0/36 (0%)	1/35 (3%)
First incidence (days)	656	–	729 (T)
Life table test	P=0.328N	P=0.139N	P=0.351N
Logistic regression test	P=0.299N	P=0.121N	P=0.311N
Cochran-Armitage test	P=0.294N		
Fisher exact test		P=0.121N	P=0.309N
Thyroid Gland (C-cell): Adenoma			
Overall rate	11/50 (22%)	3/14 (21%)	2/48 (4%)
Adjusted rate	27.5%	6.5%	5.0%
Terminal rate	11/40 (28%)	0/1 (0%)	1/35 (3%)
First incidence (days)	729 (T)	588	614
Life table test	P=0.013N	P=0.224	P=0.016N
Logistic regression test	P=0.008N	P=0.531	P=0.013N
Cochran-Armitage test	P=0.008N		
Fisher exact test		P=0.638N	P=0.009N

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	11/50 (22%)	4/14 (29%)	3/48 (6%)
Adjusted rate	27.5%	100.0%	7.8%
Terminal rate	11/40 (28%)	1/1 (100%)	2/35 (6%)
First incidence (days)	729 (T)	588	614
Life table test	P=0.028N	P=0.040	P=0.038N
Logistic regression test	P=0.020N	P=0.262	P=0.032N
Cochran-Armitage test	P=0.019N		
Fisher exact test		P=0.424	P=0.025N
Uterus: Stromal Polyp			
Overall rate	7/50 (14%)	3/50 (6%)	7/50 (14%)
Adjusted rate	17.5%	8.3%	20.0%
Terminal rate	7/40 (18%)	3/36 (8%)	7/35 (20%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table test	P=0.383	P=0.202N	P=0.508
Logistic regression test	P=0.383	P=0.202N	P=0.508
Cochran-Armitage test	P=0.473		
Fisher exact test		P=0.159N	P=0.613N
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rate	7/50 (14%)	4/50 (8%)	7/50 (14%)
Adjusted rate	17.5%	11.1%	20.0%
Terminal rate	7/40 (18%)	4/36 (11%)	7/35 (20%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table test	P=0.408	P=0.322N	P=0.508
Logistic regression test	P=0.408	P=0.322N	P=0.508
Cochran-Armitage test	P=0.500		
Fisher exact test		P=0.262N	P=0.613N
All Organs: Mononuclear Cell Leukemia			
Overall rate	14/50 (28%)	22/50 (44%)	23/50 (46%)
Adjusted rate	33.3%	48.0%	49.2%
Terminal rate	12/40 (30%)	13/36 (36%)	12/35 (34%)
First incidence (days)	678	485	491
Life table test	P=0.048	P=0.059	P=0.035
Logistic regression test	P=0.071	P=0.072	P=0.052
Cochran-Armitage test	P=0.064		
Fisher exact test		P=0.072	P=0.048
All Organs: Benign Neoplasms			
Overall rate	34/50 (68%)	33/50 (66%)	41/50 (82%)
Adjusted rate	75.4%	71.5%	95.3%
Terminal rate	29/40 (73%)	23/36 (64%)	33/35 (94%)
First incidence (days)	523	565	593
Life table test	P=0.018	P=0.439	P=0.016
Logistic regression test	P=0.037	P=0.500N	P=0.045
Cochran-Armitage test	P=0.054		
Fisher exact test		P=0.500N	P=0.083

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
All Organs: Malignant Neoplasms			
Overall rate	20/50 (40%)	28/50 (56%)	27/50 (54%)
Adjusted rate	44.2%	59.0%	58.1%
Terminal rate	15/40 (38%)	17/36 (47%)	16/35 (46%)
First incidence (days)	334	485	491
Life table test	P=0.101	P=0.070	P=0.075
Logistic regression test	P=0.176	P=0.080	P=0.129
Cochran-Armitage test	P=0.147		
Fisher exact test		P=0.080	P=0.115
All Organs: Benign and Malignant Neoplasms			
Overall rate	40/50 (80%)	42/50 (84%)	47/50 (94%)
Adjusted rate	83.3%	84.0%	97.9%
Terminal rate	32/40 (80%)	28/36 (78%)	34/35 (97%)
First incidence (days)	334	485	491
Life table test	P=0.027	P=0.235	P=0.022
Logistic regression test	P=0.032	P=0.402	P=0.038
Cochran-Armitage test	P=0.030		
Fisher exact test		P=0.398	P=0.036

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not appropriate.
[f] Not applicable; no neoplasms in animal group

TABLE B2b
Statistical Analysis of Selected Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 1:3, 3:10, 10:10, and 10:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	1 ppm 3 ppm	3 ppm 10 ppm	10 ppm 10 ppm	10 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate[a]	0/50 (0%)	3/50 (6%)	22/50 (44%)	39/50 (78%)	47/50 (94%)
Adjusted rate[b]	0.0%	7.2%	54.8%	88.5%	100.0%
Terminal rate[c]	0/40 (0%)	2/40 (5%)	21/39 (54%)	33/38 (87%)	33/33 (100%)
First incidence (days)	–[e]	719	625	529	312
Life table test[d]	P<0.001	P=0.127	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P=0.127	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001				
Fisher exact test[d]		P=0.121	P<0.001	P<0.001	P<0.001
All Organs: Mononuclear Cell Leukemia					
Overall rate	14/50 (28%)	20/50 (40%)	17/50 (34%)	27/50 (54%)	25/50 (50%)
Adjusted rate	33.3%	43.4%	38.9%	58.5%	53.6%
Terminal rate	12/40 (30%)	14/40 (35%)	13/39 (33%)	19/38 (50%)	12/33 (36%)
First incidence (days)	678	592	574	511	543
Life table test	P=0.002	P=0.172	P=0.317	P=0.008	P=0.013
Logistic regression test	P=0.013	P=0.154	P=0.336	P=0.007	P=0.020
Cochran-Armitage test	P=0.006				
Fisher exact test		P=0.146	P=0.333	P=0.007	P=0.020

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[e] Not applicable; no neoplasms in animal group

TABLE B2c
Statistical Analysis of Selected Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 3:10, and 10:10 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	12/50 (24%)	22/50 (44%)	39/50 (78%)
Adjusted rate[b]	32.0%	54.8%	88.5%
Terminal rate[c]	11/36 (31%)	21/39 (54%)	33/38 (87%)
First incidence (days)	565	625	529
Life table test[d]	P<0.001	P=0.045	P<0.001
Logistic regression test[d]	P<0.001	P=0.030	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P=0.028	P<0.001
All Organs: Mononuclear Cell Leukemia			
Overall rate	22/50 (44%)	17/50 (34%)	27/50 (54%)
Adjusted rate	48.0%	38.9%	58.5%
Terminal rate	13/36 (36%)	13/39 (33%)	19/38 (50%)
First incidence (days)	485	574	511
Life table test	P=0.158	P=0.175N	P=0.293
Logistic regression test	P=0.104	P=0.249N	P=0.220
Cochran-Armitage test	P=0.108		
Fisher exact test		P=0.206N	P=0.212

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE B2d
Statistical Analysis of Selected Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 10:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate[a]	39/50 (78%)	47/50 (94%)
Adjusted rate[b]	90.6%	100.0%
Terminal rate[c]	31/35 (89%)	33/33 (100%)
First incidence (days)	593	312
Life table test[d]		P=0.045
Logistic regression test[d]		P=0.016
Fisher exact test[d]		P=0.020
All Organs: Mononuclear Cell Leukemia		
Overall rate	23/50 (46%)	25/50 (50%)
Adjusted rate	49.2%	53.6%
Terminal rate	12/35 (34%)	12/33 (36%)
First incidence (days)	491	543
Life table test		P=0.411
Logistic regression test		P=0.300
Fisher exact test		P=0.421

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates.

TABLE B2e
Statistical Analysis of Selected Primary Neoplasms in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 10:0, 10:10, and 10:30 ppm Groups

F_0 Concentration F_1 Concentration	10 ppm 0 ppm	10 ppm 10 ppm	10 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	0/50 (0%)	39/50 (78%)	47/50 (94%)
Adjusted rate[b]	0.0%	88.5%	100.0%
Terminal rate[c]	0/39 (0%)	33/38 (87%)	33/33 (100%)
First incidence (days)	–[e]	529	312
Life table test[d]	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P<0.001	P<0.001
All Organs: Mononuclear Cell Leukemia			
Overall rate	13/50 (26%)	27/50 (54%)	25/50 (50%)
Adjusted rate	29.5%	58.5%	53.6%
Terminal rate	9/39 (23%)	19/38 (50%)	12/33 (36%)
First incidence (days)	515	511	543
Life table test	P=0.018	P=0.007	P=0.010
Logistic regression test	P=0.032	P=0.005	P=0.015
Cochran-Armitage test	P=0.029		
Fisher exact test		P=0.004	P=0.011

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver. For other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[e] Not applicable; no neoplasms in animal group

TABLE B3a
Historical Incidence of Hepatocellular Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	0/50	0/50	0/50
5,5′-Diphenylhydantoin (Phenytoin)	0/50	0/50	0/50
Ethylene thiourea	0/50	0/50	0/50
Polybrominated biphenyls	0/50	0/50	0/50
Manganese sulfate	0/50	0/50	0/50
Triamterene	0/50	0/50	0/50
Overall Historical Incidence			
Total	5/1,000 (0.5%)	1/1,000 (0.1%)	6/1,000 (0.6%)
Standard deviation	1.4%	0.5%	1.5%
Range	0%–6%	0%–2%	0%–6%

[a] Data as of 17 December 1991

TABLE B3b
Historical Incidence of Leukemia in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratories	
2,4-Dichlorophenol	11/50
5,5′-Diphenylhydantoin (Phenytoin)	13/50
Ethylene thiourea	18/50
Polybrominated biphenyls	14/50
Maganese sulfate	19/50
Triamterene	8/50
Overall Historical Incidence	
Total	263/1,000 (26.3%)
Standard deviation	8.8%
Range	14%-52%

[a] Data as of 17 December 1991 for lymphocytic, monocytic, mononuclear cell, or undifferentiated leukemias

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	10	10	9	12	8	10
Natural deaths		1	1	2	3	2
Survivors						
Died last week of study			1			
Terminal sacrifice	40	39	39	36	39	38
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Intestine large, cecum	(50)	(10)	(11)	(13)	(10)	(12)
Inflammation, chronic active	1 (2%)					
Necrosis						1 (8%)
Intestine large, colon	(50)	(11)	(12)	(13)	(11)	(12)
Diverticulum			1 (8%)			
Parasite metazoan	2 (4%)				1 (9%)	
Intestine large, rectum	(50)	(11)	(11)	(13)	(9)	(12)
Parasite metazoan	1 (2%)			1 (8%)		
Liver	(50)	(50)	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		4 (8%)	1 (2%)	
Basophilic focus	47 (94%)	44 (88%)	40 (80%)	37 (74%)	36 (72%)	23 (46%)
Clear cell focus	3 (6%)					
Cytomegaly			1 (2%)			
Degeneration, cystic		2 (4%)			2 (4%)	3 (6%)
Eosinophilic focus	3 (6%)	19 (38%)	19 (38%)	47 (94%)	45 (90%)	45 (90%)
Eosinophilic focus, multiple					1 (2%)	
Hematopoietic cell proliferation	1 (2%)					1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	3 (6%)	2 (4%)			1 (2%)
Hyperplasia, focal			1 (2%)			
Hypertrophy			9 (18%)	32 (64%)	39 (78%)	40 (80%)
Inflammation, chronic, multifocal				21 (42%)	8 (16%)	7 (14%)
Inflammation, chronic active	34 (68%)	12 (24%)	19 (38%)	13 (26%)	12 (24%)	8 (16%)
Necrosis	3 (6%)		1 (2%)	5 (10%)	5 (10%)	5 (10%)
Vacuolization cytoplasmic	3 (6%)	6 (12%)	18 (36%)	47 (94%)	43 (86%)	43 (86%)
Bile duct, cyst					1 (2%)	1 (2%)
Bile duct, hyperplasia	14 (28%)	18 (36%)	34 (68%)	39 (78%)	38 (76%)	44 (88%)
Oval cell, hyperplasia				12 (24%)	19 (38%)	34 (68%)
Periportal, infiltration cellular, lymphocyte					2 (4%)	
Subserosa, congestion, multifocal				1 (2%)		
Mesentery	(2)	(1)	(1)	(1)	(1)	(4)
Inflammation, chronic active	1 (50%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	3 (75%)
Pancreas	(49)	(12)	(11)	(13)	(9)	(12)
Ectopic liver				1 (8%)		
Acinus, atrophy	15 (31%)	2 (17%)	3 (27%)	4 (31%)	4 (44%)	3 (25%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	10	15
Natural deaths	5	2
Survivors		
Died last week of study	1	1
Terminal sacrifice	34	32
Animals examined microscopically	50	50
Alimentary System		
Intestine large, colon	(48)	(50)
Parasite metazoan		1 (2%)
Intestine large, rectum	(47)	(49)
Parasite metazoan	6 (13%)	1 (2%)
Liver	(50)	(50)
Basophilic focus	11 (22%)	13 (26%)
Cytomegaly		1 (2%)
Eosinophilic focus	48 (96%)	47 (94%)
Hypertrophy	17 (34%)	6 (12%)
Inflammation, chronic, multifocal	31 (62%)	22 (44%)
Inflammation, chronic active	3 (6%)	2 (4%)
Inflammation, multifocal		1 (2%)
Necrosis	1 (2%)	3 (6%)
Thrombosis		1 (2%)
Vacuolization cytoplasmic	50 (100%)	40 (80%)
Bile duct, cyst	2 (4%)	5 (10%)
Bile duct, fibrosis	3 (6%)	19 (38%)
Bile duct, hyperplasia	50 (100%)	15 (30%)
Bile duct, metaplasia	1 (2%)	
Oval cell, hyperplasia	42 (84%)	44 (88%)
Pancreas	(50)	(50)
Focal cellular change, multiple		1 (2%)
Acinus, atrophy	29 (58%)	25 (50%)
Perivascular, inflammation, chronic active	1 (2%)	
Salivary glands	(49)	(49)
Cytomegaly, focal	1 (2%)	
Acinus, atrophy	1 (2%)	2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Alimentary System (continued)						
Stomach, forestomach	(50)	(49)	(50)	(50)	(50)	(49)
Acanthosis	1 (2%)	2 (4%)		3 (6%)	2 (4%)	1 (2%)
Diverticulum			1 (2%)			
Inflammation, chronic active		1 (2%)		1 (2%)		
Ulcer				1 (2%)		1 (2%)
Submucosa, foreign body				1 (2%)		
Stomach, glandular	(50)	(48)	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)			1 (2%)		
Inflammation, chronic active			1 (2%)			
Mineralization			5 (10%)	3 (6%)	6 (12%)	10 (20%)
Ulcer			1 (2%)	1 (2%)		3 (6%)
Cardiovascular System						
Heart	(50)	(11)	(12)	(14)	(11)	(12)
Degeneration, chronic	42 (84%)	3 (27%)	4 (33%)	3 (21%)	1 (9%)	1 (8%)
Endocrine System						
Adrenal gland, cortex	(50)	(10)	(12)	(14)	(9)	(12)
Cyst					1 (11%)	
Hyperplasia	39 (78%)	6 (60%)	2 (17%)	3 (21%)	1 (11%)	2 (17%)
Hypertrophy	15 (30%)	1 (10%)	3 (25%)	1 (7%)		1 (8%)
Necrosis			2 (17%)	1 (7%)	1 (11%)	1 (8%)
Vacuolization cytoplasmic	17 (34%)	4 (40%)	3 (25%)	6 (43%)	1 (11%)	6 (50%)
Adrenal gland, medulla	(48)	(10)	(12)	(13)	(9)	(12)
Hyperplasia	6 (13%)		1 (8%)			
Pituitary gland	(50)	(34)	(27)	(29)	(28)	(27)
Hyperplasia				1 (3%)		
Pars distalis, cyst	20 (40%)	9 (26%)	1 (4%)	5 (17%)	8 (29%)	3 (11%)
Pars distalis, hyperplasia	13 (26%)	7 (21%)	6 (22%)	3 (10%)	6 (21%)	8 (30%)
Thyroid gland	(50)	(12)	(15)	(14)	(10)	(13)
Atrophy					1 (10%)	
Infiltration cellular, lymphocyte					1 (10%)	
C-cell, hyperplasia	50 (100%)	11 (92%)	12 (80%)	12 (86%)	10 (100%)	12 (92%)
General Body System						
None						
Genital System						
Clitoral gland	(50)	(15)	(12)	(16)	(10)	(13)
Hyperplasia		1 (7%)	1 (8%)	1 (6%)		1 (8%)
Inflammation, chronic active	24 (48%)	13 (87%)	10 (83%)	6 (38%)	3 (30%)	5 (38%)
Duct, dilatation	19 (38%)		3 (25%)	8 (50%)	3 (30%)	4 (31%)
Ovary	(50)	(23)	(19)	(22)	(14)	(20)
Infiltration cellular, lymphocyte	3 (6%)					
Follicle, cyst	3 (6%)	1 (4%)				1 (5%)
Periovarian tissue, cyst	9 (18%)	11 (48%)	8 (42%)	9 (41%)	3 (21%)	8 (40%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Alimentary System (continued)		
Stomach, forestomach	(48)	(50)
Acanthosis		5 (10%)
Inflammation, chronic active	1 (2%)	3 (6%)
Ulcer	1 (2%)	1 (2%)
Stomach, glandular	(49)	(50)
Dysplasia, focal		1 (2%)
Cardiovascular System		
Heart	(49)	(50)
Degeneration, chronic	21 (43%)	11 (22%)
Myocardium, inflammation, chronic active	1 (2%)	
Endocrine System		
Adrenal gland, cortex	(50)	(50)
Cyst		2 (4%)
Hyperplasia	19 (38%)	21 (42%)
Hypertrophy	9 (18%)	9 (18%)
Vacuolization cytoplasmic	18 (36%)	25 (50%)
Adrenal gland, medulla	(49)	(49)
Hyperplasia	1 (2%)	1 (2%)
Parathyroid gland	(46)	(49)
Hyperplasia	1 (2%)	
Pituitary gland	(50)	(50)
Pars distalis, cyst	1 (2%)	1 (2%)
Pars distalis, hyperplasia	13 (26%)	13 (26%)
Thyroid gland	(48)	(48)
C-cell, hyperplasia	43 (90%)	47 (98%)
Follicle, cyst	1 (2%)	
General Body System		
None		
Genital System		
Clitoral gland	(47)	(46)
Inflammation, chronic active	22 (47%)	13 (28%)
Duct, dilatation	26 (55%)	23 (50%)
Ovary	(48)	(49)
Follicle, cyst	1 (2%)	
Periovarian tissue, cyst	11 (23%)	9 (18%)
Rete ovarii, hyperplasia	1 (2%)	
Oviduct		(1)
Dilatation		1 (100%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Genital System (continued)						
Uterus	(50)	(17)	(18)	(18)	(21)	(20)
Dilatation	7 (14%)		1 (6%)	1 (6%)		
Fibrosis			1 (6%)			
Hemorrhage					2 (10%)	
Inflammation, acute					1 (5%)	
Necrosis					1 (5%)	
Cervix, cyst	1 (2%)		2 (11%)			3 (15%)
Endometrium, hyperplasia, cystic, glandular		4 (24%)	4 (22%)	1 (6%)	7 (33%)	6 (30%)
Hematopoietic System						
Lymph node	(50)	(11)	(11)	(14)	(12)	(12)
Mandibular, hyperplasia	1 (2%)					
Mandibular, necrosis						1 (8%)
Mediastinal, infiltration cellular, histiocyte	1 (2%)					
Lymph node, mesenteric	(4)	(2)	(3)	(1)	(1)	(3)
Angiectasis					1 (100%)	
Cyst		1 (50%)				
Hyperplasia, lymphoid	1 (25%)					
Infiltration cellular, histiocyte	1 (25%)					
Sinus, ectasia	2 (50%)					
Spleen	(50)	(50)	(50)	(50)	(49)	(49)
Depletion lymphoid			1 (2%)		1 (2%)	
Fibrosis				1 (2%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation			1 (2%)	1 (2%)	1 (2%)	
Necrosis						1 (2%)
Capsule, hemorrhage					1 (2%)	
Integumentary System						
Mammary gland	(48)	(16)	(13)	(21)	(16)	(11)
Hyperplasia, cystic	46 (96%)	10 (63%)	10 (77%)	13 (62%)	10 (63%)	9 (82%)
Skin	(50)	(11)	(12)	(13)	(11)	(14)
Subcutaneous tissue, lip, foreign body			1 (8%)			
Subcutaneous tissue, lip, inflammation, chronic active			1 (8%)			
Musculoskeletal System						
Bone	(50)	(11)	(11)	(14)	(11)	(12)
Cranium, fracture			1 (9%)			
Femur, hyperostosis			1 (9%)		1 (9%)	2 (17%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm		10 ppm 30 ppm	
Genital System (continued)				
Uterus	(49)		(50)	
Dilatation			1 (2%)	
Cervix, cyst			2 (4%)	
Cervix, mucosa, hyperplasia, squamous			1 (2%)	
Endometrium, hyperplasia, cystic, glandular	12 (24%)		11 (22%)	
Hematopoietic System				
Lymph node	(49)		(47)	
Mandibular, angiectasis	1 (2%)			
Mandibular, infiltration cellular, plasma cell			1 (2%)	
Mediastinal, angiectasis	1 (2%)			
Lymph node, mesenteric	(5)			
Angiectasis	1 (20%)			
Spleen	(50)		(50)	
Hematopoietic cell proliferation	1 (2%)			
Integumentary System				
Mammary gland	(49)		(48)	
Hyperplasia, cystic	47 (96%)		43 (90%)	
Skin	(49)		(50)	
Acanthosis	1 (2%)			
Subcutaneous tissue, inflammation, chronic active		1 (2%)		
Musculoskeletal System				
Bone	(49)		(50)	
Femur, hyperostosis	1 (2%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	10 ppm 0 ppm	1 ppm 3 ppm	0 ppm 10 ppm	3 ppm 10 ppm	10 ppm 10 ppm
Nervous System						
Brain	(50)	(11)	(11)	(14)	(10)	(12)
Compression	7 (14%)	3 (27%)	3 (27%)	3 (21%)	3 (30%)	1 (8%)
Hemorrhage			1 (9%)			
Hydrocephalus		3 (27%)	3 (27%)	3 (21%)	3 (30%)	1 (8%)
Necrosis	1 (2%)					
Spinal cord	(2)	(2)		(1)	(1)	(1)
Nerve, demyelination	2 (100%)	2 (100%)		1 (100%)	1 (100%)	1 (100%)
Respiratory System						
Lung	(50)	(11)	(11)	(14)	(9)	(11)
Interstitium, inflammation, chronic active				1 (7%)		1 (9%)
Nose	(50)	(11)	(11)	(13)	(10)	(12)
Mucosa, inflammation, chronic active				1 (8%)		
Special Senses System						
Eye	(4)	(3)	(2)	(4)	(3)	(5)
Choroid, hemorrhage		1 (33%)				
Lens, cataract	4 (100%)	2 (67%)	1 (50%)	4 (100%)	3 (100%)	5 (100%)
Retina, atrophy	4 (100%)	2 (67%)	1 (50%)	4 (100%)	3 (100%)	5 (100%)
Urinary System						
Kidney	(49)	(11)	(11)	(14)	(11)	(12)
Hydronephrosis		1 (9%)				
Nephropathy, chronic	25 (51%)	7 (64%)	8 (73%)	11 (79%)	8 (73%)	8 (67%)
Cortex, infarct				1 (7%)		
Renal tubule, mineralization	3 (6%)			2 (14%)		
Urinary bladder	(50)	(11)	(11)	(14)	(10)	(12)
Calculus micro observation only						1 (8%)
Mucosa, hyperplasia, papillary		1 (9%)				1 (8%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	10 ppm 30 ppm
Nervous System		
Brain	(49)	(50)
Compression		1 (2%)
Respiratory System		
Lung	(47)	(50)
Inflammation, focal, granulomatous	1 (2%)	
Metaplasia, focal, osseous		1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)	
Interstitium, inflammation, chronic active	1 (2%)	
Nose	(48)	(50)
Mucosa, inflammation, chronic active	4 (8%)	2 (4%)
Special Senses System		
Eye	(2)	(5)
Lens, cataract	1 (50%)	4 (80%)
Retina, atrophy	1 (50%)	4 (80%)
Urinary System		
Kidney	(50)	(50)
Nephropathy, chronic	37 (74%)	42 (84%)
Renal tubule, inflammation, acute, multifocal		1 (2%)
Renal tubule, mineralization	2 (4%)	
Urinary bladder	(49)	(50)
Hemorrhage		1 (2%)

[a] Number of animals with any tissue examined microscopically

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF POLYBROMINATED BIPHENYLS

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	59	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	9	5	2	5	2	3
Natural deaths	10	13	5	11	14	18
Survivors						
Died last week of study			1		1	2
Terminal sacrifice	31	32	41	34	33	27
Animals examined microscopically	50	50	49	50	50	50
Alimentary System						
Esophagus	(50)	(50)	(49)	(50)	(50)	(49)
Gallbladder	(38)	(43)	(45)	(43)	(39)	(36)
Intestine large, cecum	(47)	(49)	(44)	(49)	(49)	(48)
Intestine large, rectum	(50)	(49)	(44)	(48)	(48)	(47)
Intestine small, duodenum	(49)	(49)	(45)	(49)	(49)	(47)
Adenoma			1 (2%)			
Intestine small, ileum	(47)	(47)	(45)	(46)	(49)	(43)
Intestine small, jejunum	(48)	(45)	(47)	(46)	(48)	(47)
Adenocarcinoma	1 (2%)					
Liver	(50)	(50)	(48)	(49)	(49)	(50)
Fibrosarcoma, metastatic, uncertain primary site		1 (2%)				
Hemangiosarcoma	3 (6%)	5 (10%)	3 (6%)	5 (10%)	3 (6%)	2 (4%)
Hepatocellular carcinoma				1 (2%)		1 (2%)
Hepatocellular carcinoma, multiple	4 (8%)	6 (12%)	2 (4%)	13 (27%)	14 (29%)	21 (42%)
Hepatocellular carcinoma, single	4 (8%)	11 (22%)	6 (13%)	16 (33%)	17 (35%)	18 (36%)
Hepatocellular adenoma, multiple	1 (2%)	22 (44%)	12 (25%)	48 (98%)	41 (84%)	42 (84%)
Hepatocellular adenoma, single	8 (16%)	9 (18%)	17 (35%)		5 (10%)	6 (12%)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)			
Ito cell tumor malignant, multiple		1 (2%)				
Sarcoma		2 (4%)		1 (2%)		1 (2%)
Mesentery	(5)	(3)				(2)
Pancreas	(50)	(50)	(47)	(49)	(49)	(48)
Salivary glands	(50)	(49)	(49)	(49)	(50)	(49)
Stomach, forestomach	(48)	(49)	(47)	(50)	(49)	(47)
Squamous cell papilloma	1 (2%)					
Stomach, glandular	(48)	(48)	(47)	(50)	(49)	(47)
Adenoma	1 (2%)					
Tongue		(1)				
Squamous cell carcinoma		1 (100%)				
Tooth		(4)	(4)	(3)	(6)	(1)
Cardiovascular System						
Heart	(50)	(49)	(49)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		1 (2%)		
Histiocytic sarcoma		1 (2%)				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	16	21
Natural deaths	34	29
Animals examined microscopically	50	50
Alimentary System		
Liver	(50)	(50)
Hepatocellular carcinoma, multiple	22 (44%)	17 (34%)
Hepatocellular carcinoma, single	14 (28%)	18 (36%)
Hepatocellular adenoma, multiple	37 (74%)	43 (86%)
Hepatocellular adenoma, single	5 (10%)	5 (10%)
Stomach, forestomach	(50)	(50)
Squamous cell papilloma	1 (2%)	
Cardiovascular System		
None		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Endocrine System						
Adrenal gland	(39)	(50)	(48)	(49)	(50)	(50)
Adrenal gland, cortex	(39)	(50)	(47)	(49)	(50)	(50)
Adrenal gland, medulla	(38)	(50)	(47)	(49)	(49)	(50)
Pheochromocytoma benign	2 (5%)	2 (4%)	2 (4%)	1 (2%)		1 (2%)
Bilateral, pheochromocytoma benign				1 (2%)	1 (2%)	
Parathyroid gland	(45)	(48)	(46)	(48)	(47)	(45)
Pituitary gland	(44)	(44)	(45)	(46)	(44)	(41)
Pars distalis, adenoma						1 (2%)
Thyroid gland	(50)	(49)	(47)	(49)	(48)	(48)
Follicular cell, adenoma	1 (2%)					5 (10%)
General Body System						
None						
Genital System						
Epididymis	(50)	(50)	(48)	(50)	(49)	(50)
Prostate	(50)	(50)	(48)	(49)	(49)	(49)
Seminal vesicle	(1)		(1)	(2)		
Testes	(50)	(50)	(49)	(50)	(49)	(50)
Hematopoietic System						
Bone marrow	(50)	(50)	(49)	(49)	(50)	(49)
Femoral, hemangiosarcoma	2 (4%)			2 (4%)		
Sternal, hemangiosarcoma	1 (2%)			1 (2%)	1 (2%)	
Lymph node	(48)	(50)	(48)	(48)	(48)	(46)
Lymph node, mesenteric	(32)	(34)	(34)	(37)	(35)	(31)
Histiocytic sarcoma		1 (3%)		1 (3%)	1 (3%)	
Spleen	(50)	(50)	(47)	(49)	(50)	(48)
Hemangiosarcoma	3 (6%)	2 (4%)	5 (11%)	3 (6%)	1 (2%)	1 (2%)
Histiocytic sarcoma				1 (2%)		
Thymus	(29)	(40)	(40)	(37)	(29)	(25)
Thymoma benign			1 (3%)			
Integumentary System						
Skin	(50)	(50)	(49)	(49)	(50)	(49)
Subcutaneous tissue, fibrosarcoma		1 (2%)		1 (2%)	2 (4%)	
Subcutaneous tissue, hemangioma					1 (2%)	
Subcutaneous tissue, hemangiosarcoma					2 (4%)	
Subcutaneous tissue, sarcoma				2 (4%)		
Musculoskeletal System						
Skeletal muscle	(1)	(2)		(1)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Endocrine System		
Adrenal gland, cortex	(48)	(46)
Thyroid gland	(46)	(45)
Follicular cell, adenoma	1 (2%)	2 (4%)
General Body System		
None		
Genital System		
None		
Hematopoietic System		
Lymph node, mesenteric	(10)	(10)
Spleen	(49)	(48)
Hemangiosarcoma	1 (2%)	1 (2%)
Integumentary System		
None		
Musculoskeletal System		
None		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls *(continued)*

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Nervous System						
Brain	(50)	(50)	(49)	(50)	(50)	(49)
Respiratory System						
Lung	(50)	(50)	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	3 (6%)	3 (6%)	3 (6%)	2 (4%)	
Alveolar/bronchiolar adenoma, single	9 (18%)	8 (16%)	8 (16%)	6 (12%)	11 (22%)	10 (20%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	2 (4%)		1 (2%)		
Alveolar/bronchiolar carcinoma, single	2 (4%)	2 (4%)	1 (2%)			
Hepatoblastoma, metastatic, uncertain primary site		1 (2%)				
Hepatocellular carcinoma, metastatic, liver	2 (4%)	4 (8%)	1 (2%)	3 (6%)	4 (8%)	9 (18%)
Histiocytic sarcoma		1 (2%)				
Nose	(50)	(50)	(49)	(50)	(50)	(50)
Special Senses System						
Harderian gland	(3)	(4)	(5)	(1)	(1)	(3)
Adenoma	2 (67%)	3 (75%)	5 (100%)		1 (100%)	3 (100%)
Urinary System						
Kidney	(50)	(50)	(49)	(50)	(49)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)					
Hepatoblastoma, metastatic, uncertain primary site		1 (2%)				
Histiocytic sarcoma			1 (2%)			
Urinary bladder	(49)	(50)	(48)	(48)	(48)	(50)
Hemangiosarcoma			1 (2%)			
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(49)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Lymphoma malignant histiocytic			1 (2%)			
Lymphoma malignant lymphocytic	3 (6%)	3 (6%)			1 (2%)	1 (2%)
Lymphoma malignant mixed	1 (2%)	1 (2%)				1 (2%)
Lymphoma malignant undifferentiated cell	6 (12%)	5 (10%)	6 (12%)	4 (8%)	8 (16%)	1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Nervous System		
None		
Respiratory System		
Lung	(50)	(46)
Alveolar/bronchiolar adenoma, single	1 (2%)	3 (7%)
Alveolar/bronchiolar carcinoma, single	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	6 (12%)	6 (13%)
Special Senses System		
Harderian gland		(1)
Adenoma		1 (100%)
Urinary System		
None		
Systemic Lesions		
Multiple organs	(50)	(50)
Lymphoma malignant lymphocytic	2 (4%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Neoplasm Summary						
Total animals with primary neoplasms[c]	37	47	42	48	47	49
Total primary neoplasms	59	91	75	111	112	115
Total animals with benign neoplasms	21	37	37	48	47	48
Total benign neoplasms	26	47	49	59	62	68
Total animals with malignant neoplasms	23	29	20	34	37	44
Total malignant neoplasms	33	44	26	52	50	47
Total animals with metastatic neoplasms	3	5	1	3	4	9
Total metastatic neoplasms	3	7	1	3	4	9
Total animals with malignant neoplasms of uncertain primary site		2				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Neoplasm Summary		
Total animals with primary neoplasms	48	50
Total primary neoplasms	85	91
Total animals with benign neoplasms	42	48
Total benign neoplasms	45	54
Total animals with malignant neoplasms	37	35
Total malignant neoplasms	40	37
Total animals with metastatic neoplasms	6	6
Total metastatic neoplasms	6	6

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

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	2/38 (5%)	2/49 (4%)	0/49 (0%)
Adjusted rate[b]	7.7%	5.9%	0.0%
Terminal rate[c]	1/21 (5%)	2/34 (6%)	0/0 (0%)
First incidence (days)	714	727 (T)	–[e]
Life table test[d]	P=0.734N	P=0.542N	–
Logistic regression test[d]	P=0.520N	P=0.511N	P=0.797N
Cochran-Armitage test[d]	P=0.128N		
Fisher exact test[d]		P=0.590N	P=0.188N
Liver: Hepatocellular Adenoma			
Overall rate	9/50 (18%)	48/49 (98%)	42/50 (84%)
Adjusted rate	26.1%	100.0%	100.0%
Terminal rate	7/31 (23%)	34/34 (100%)	0/0 (0%)
First incidence (days)	531	505	420
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	8/50 (16%)	30/49 (61%)	36/50 (72%)
Adjusted rate	21.3%	72.8%	100.0%
Terminal rate	4/31 (13%)	23/34 (68%)	0/0 (0%)
First incidence (days)	447	561	464
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	16/50 (32%)	48/49 (98%)	48/50 (96%)
Adjusted rate	42.9%	100.0%	100.0%
Terminal rate	11/31 (35%)	34/34 (100%)	0/0 (0%)
First incidence (days)	447	505	420
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Liver: Hemangiosarcoma			
Overall rate	3/50 (6%)	5/49 (10%)	0/50 (0%)
Adjusted rate	8.9%	12.2%	0.0%
Terminal rate	2/31 (6%)	2/34 (6%)	0/0 (0%)
First incidence (days)	658	561	–
Life table test	P=0.505N	P=0.415	P=0.704N
Logistic regression test	P=0.111N	P=0.339	P=0.318N
Cochran-Armitage test	P=0.087N		
Fisher exact test		P=0.346	P=0.121N

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	10/50 (20%)	9/50 (18%)	1/50 (2%)
Adjusted rate	29.0%	26.5%	2.6%
Terminal rate	8/31 (26%)	9/34 (26%)	0/0 (0%)
First incidence (days)	531	727 (T)	531
Life table test	P=0.566N	P=0.416N	P=0.574N
Logistic regression test	P=0.025N	P=0.449N	P=0.013N
Cochran-Armitage test	P=0.004N		
Fisher exact test		P=0.500N	P=0.004N
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	9.2%	2.8%	33.3%
Terminal rate	2/31 (6%)	0/34 (0%)	0/0 (0%)
First incidence (days)	705	723	703
Life table test	P=0.428	P=0.267N	P=0.108
Logistic regression test	P=0.720	P=0.268N	P=0.622
Cochran-Armitage test	P=0.272N		
Fisher exact test		P=0.309N	P=0.309N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	13/50 (26%)	10/50 (20%)	2/50 (4%)
Adjusted rate	37.1%	28.5%	35.0%
Terminal rate	10/31 (32%)	9/34 (26%)	0/0 (0%)
First incidence (days)	531	723	531
Life table test	P=0.543	P=0.234N	P=0.478
Logistic regression test	P=0.027N	P=0.258N	P=0.020N
Cochran-Armitage test	P=0.002N		
Fisher exact test		P=0.318N	P=0.002N
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma			
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	7.6%	0.0%
Terminal rate	0/31 (0%)	1/34 (3%)	0/0 (0%)
First incidence (days)	–	607	–
Life table test	P=0.579	P=0.141	–
Logistic regression test	P=0.587N	P=0.119	–
Cochran-Armitage test	P=0.500N		
Fisher exact test		P=0.121	–
Spleen: Hemangiosarcoma			
Overall rate	3/50 (6%)	3/49 (6%)	1/49 (2%)
Adjusted rate	8.5%	7.7%	3.2%
Terminal rate	1/31 (3%)	1/34 (3%)	0/0 (0%)
First incidence (days)	658	604	587
Life table test	P=0.564	P=0.608N	P=0.628
Logistic regression test	P=0.288N	P=0.660	P=0.421N
Cochran-Armitage test	P=0.241N		
Fisher exact test		P=0.651	P=0.316N

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
All Organs: Hemangiosarcoma			
Overall rate	5/50 (10%)	7/50 (14%)	1/50 (2%)
Adjusted rate	14.6%	17.2%	3.2%
Terminal rate	3/31 (10%)	3/34 (9%)	0/0 (0%)
First incidence (days)	658	561	587
Life table test	P=0.545	P=0.459	P=0.628
Logistic regression test	P=0.130N	P=0.390	P=0.274N
Cochran-Armitage test	P=0.070N		
Fisher exact test		P=0.380	P=0.102N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rate	10/50 (20%)	4/50 (8%)	2/50 (4%)
Adjusted rate	27.3%	9.7%	13.0%
Terminal rate	6/31 (19%)	1/34 (3%)	0/0 (0%)
First incidence (days)	554	604	461
Life table test	P=0.379N	P=0.060N	P=0.616
Logistic regression test	P=0.017N	P=0.072N	P=0.040N
Cochran-Armitage test	P=0.014N		
Fisher exact test		P=0.074N	P=0.014N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma			
Overall rate	11/50 (22%)	5/50 (10%)	2/50 (4%)
Adjusted rate	29.3%	12.0%	13.0%
Terminal rate	6/31 (19%)	1/34 (3%)	0/0 (0%)
First incidence (days)	554	604	461
Life table test	P=0.338N	P=0.069N	P=0.661N
Logistic regression test	P=0.010N	P=0.084N	P=0.023N
Cochran-Armitage test	P=0.008N		
Fisher exact test		P=0.086N	P=0.007N
All Organs: Benign Neoplasms			
Overall rate	21/50 (42%)	48/50 (96%)	42/50 (84%)
Adjusted rate	55.7%	100.0%	100.0%
Terminal rate	15/31 (48%)	34/34 (100%)	0/0 (0%)
First incidence (days)	531	505	420
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
All Organs: Malignant Neoplasms			
Overall rate	23/50 (46%)	34/50 (68%)	37/50 (74%)
Adjusted rate	55.4%	77.0%	100.0%
Terminal rate	13/31 (42%)	24/34 (71%)	0/0 (0%)
First incidence (days)	447	561	461
Life table test	P<0.001	P=0.114	P<0.001
Logistic regression test	P<0.001	P=0.029	P=0.001
Cochran-Armitage test	P=0.006		
Fisher exact test		P=0.021	P=0.004

TABLE C2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
All Organs: Benign or Malignant Neoplasms			
Overall rate	37/50 (74%)	48/50 (96%)	48/50 (96%)
Adjusted rate	82.0%	100.0%	100.0%
Terminal rate	23/31 (74%)	34/34 (100%)	0/0 (0%)
First incidence (days)	447	505	420
Life table test	P<0.001	P=0.157	P<0.001
Logistic regression test	P<0.001	P=0.003	P<0.001
Cochran-Armitage test	P=0.002		
Fisher exact test		P=0.002	P=0.002

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE C2b
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 3:3, 10:10, 30:10, and 30:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	3 ppm 3 ppm	10 ppm 10 ppm	30 ppm 10 ppm	30 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate[a]	16/50 (32%)	31/48 (65%)	46/49 (94%)	48/50 (96%)	50/50 (100%)
Adjusted rate[b]	42.9%	68.8%	97.9%	100.0%	100.0%
Terminal rate[c]	11/31 (35%)	28/42 (67%)	33/34 (97%)	29/29 (100%)	0/0
First incidence (days)	447	307	475	391	314
Life table test[d]	P<0.001	P=0.065	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P=0.002	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001				
Fisher exact test[d]		P=0.001	P<0.001	P<0.001	P<0.001

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE C2c
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 10:10, and 30:10 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	48/49 (98%)	46/49 (94%)	48/50 (96%)
Adjusted rate[b]	100.0%	97.9%	100.0%
Terminal rate[c]	34/34 (100%)	33/34 (97%)	29/29 (100%)
First incidence (days)	505	475	391
Life table test[d]	P=0.090	P=0.438N	P=0.145
Logistic regression test[d]	P=0.705	P=0.464N	P=0.984N
Cochran-Armitage test[d]	P=0.529N		
Fisher exact test[d]		P=0.309N	P=0.508N

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

TABLE C2d
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 30:30 ppm Groups

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate[a]	48/50 (96%)	50/50 (100%)
Adjusted rate[b]	100.0%	100.0%
Terminal rate[c]	0/0	0/0
First incidence (days)	420	314
Life table test[d]		P<0.001
Logistic regression test[d]		P=0.053
Fisher exact test[d]		P=0.247

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates.

TABLE C2e
Statistical Analysis of Liver Neoplasms in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 30:0, 30:10, and 30:30 ppm Groups

F_0 Concentration	30 ppm	30 ppm	30 ppm
F_1 Concentration	0 ppm	10 ppm	30 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	40/50 (80%)	48/50 (96%)	50/50 (100%)
Adjusted rate[b]	88.8%	100.0%	100.0%
Terminal rate[c]	27/32 (84%)	29/29 (100%)	0/0
First incidence (days)	516	391	314
Life table test[d]	P<0.001	P=0.035	P<0.001
Logistic regression test[d]	P<0.001	P=0.003	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P=0.014	P<0.001

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposuregroup incidence are the P values corresponding to pairwise comparisons between the controls and that exposuregroup. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE C3
Historical Incidence of Hepatocellular Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	4/50	7/50	10/50
5,5'-Diphenylhydantoin (Phenytoin)	19/50	13/50	29/50
Ethylene thiourea	11/49	13/49	20/49
Polybrominated biphenyls	9/50	8/50	16/50
Manganese sulfate	30/50	9/50	34/50
Pentachlorophenol (Dowicide EC-7)	5/35	1/35	6/35
Pentachlorophenol (technical grade)	5/32	2/32	7/32
Triamterene	17/50	5/50	20/50
Triamterene	21/50	9/50	25/50
Overall Historical Incidence			
Total	226/1,114 (20.3%)	169/1,114 (15.2%)	363/1,114 (32.6%)
Standard deviation	13.2%	7.1%	13.6%
Range	4%–60%	3%–27%	10%–68%

[a] Data as of 17 December 1991

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	59	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	9	5	2	5	2	3
Natural deaths	10	13	5	11	14	18
Survivors						
Died last week of study			1		1	2
Terminal sacrifice	31	32	41	34	33	27
Animals examined microscopically	50	50	49	50	50	50
Alimentary System						
Gallbladder	(38)	(43)	(45)	(43)	(39)	(36)
Epithelium, hyperplasia	1 (3%)	1 (2%)				
Epithelium, inclusion body intracytoplasmic		2 (5%)	1 (2%)			
Lumen, crystals		1 (2%)				
Intestine large, cecum	(47)	(49)	(44)	(49)	(49)	(48)
Parasite metazoan		1 (2%)	1 (2%)	1 (2%)		1 (2%)
Ulcer	1 (2%)			2 (4%)		
Intestine large, colon	(47)	(49)	(46)	(49)	(50)	(49)
Parasite metazoan	1 (2%)	2 (4%)	2 (4%)	4 (8%)	9 (18%)	14 (29%)
Intestine large, rectum	(50)	(49)	(44)	(48)	(48)	(47)
Parasite metazoan	3 (6%)	4 (8%)	1 (2%)	4 (8%)	5 (10%)	2 (4%)
Intestine small, duodenum	(49)	(49)	(45)	(49)	(49)	(47)
Inflammation, chronic active					1 (2%)	
Intestine small, ileum	(47)	(47)	(45)	(46)	(49)	(43)
Parasite metazoan		2 (4%)		2 (4%)		2 (5%)
Ulcer	1 (2%)			2 (4%)		
Intestine small, jejunum	(48)	(45)	(47)	(46)	(48)	(47)
Hyperplasia, lymphoid				1 (2%)		
Inflammation, chronic active	2 (4%)					
Parasite metazoan					1 (2%)	
Ulcer		1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Liver	(50)	(50)	(48)	(49)	(49)	(50)
Abscess			1 (2%)			
Amyloid deposition		1 (2%)				
Bacterium			1 (2%)			
Basophilic focus	2 (4%)	6 (12%)	1 (2%)	3 (6%)	7 (14%)	8 (16%)
Clear cell focus		13 (26%)	15 (31%)	8 (16%)	8 (16%)	5 (10%)
Cyst	1 (2%)	1 (2%)		1 (2%)		
Cytologic alterations		21 (42%)				
Cytomegaly		36 (72%)	44 (92%)	49 (100%)	48 (98%)	48 (96%)
Eosinophilic focus	3 (6%)	20 (40%)	8 (17%)	16 (33%)	4 (8%)	
Fatty change	2 (4%)	2 (4%)	4 (8%)	45 (92%)	43 (88%)	40 (80%)
Granuloma	1 (2%)					1 (2%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)				
Necrosis, coagulative	1 (2%)	9 (18%)	5 (10%)	17 (35%)	16 (33%)	23 (46%)
Parasite metazoan						1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	16	21
Natural deaths	34	29
Animals examined microscopically	50	50
Alimentary System		
Gallbladder	(41)	(32)
Hemorrhage	1 (2%)	
Inflammation, chronic active	1 (2%)	
Intestine large, cecum	(43)	(49)
Cyst	1 (2%)	
Parasite metazoan	2 (5%)	1 (2%)
Intestine large, colon	(50)	(49)
Parasite metazoan	23 (46%)	20 (41%)
Ulcer		1 (2%)
Intestine large, rectum	(47)	(49)
Parasite metazoan	3 (6%)	3 (6%)
Intestine small, duodenum	(50)	(47)
Ulcer	1 (2%)	
Epithelium, metaplasia, squamous		1 (2%)
Intestine small, ileum	(39)	(40)
Parasite metazoan	3 (8%)	1 (3%)
Intestine small, jejunum	(43)	(47)
Parasite metazoan	1 (2%)	
Liver	(50)	(50)
Basophilic focus	1 (2%)	7 (14%)
Clear cell focus	1 (2%)	2 (4%)
Cytomegaly	50 (100%)	50 (100%)
Eosinophilic focus	6 (12%)	4 (8%)
Fatty change	50 (100%)	48 (96%)
Hematopoietic cell proliferation	2 (4%)	3 (6%)
Infiltration cellular, mononuclear cell		1 (2%)
Infiltration cellular, mixed cell		1 (2%)
Inflammation, acute		1 (2%)
Necrosis, coagulative	24 (48%)	29 (58%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Alimentary System (continued)						
Liver (continued)	(50)	(50)	(48)	(49)	(49)	(50)
Thrombosis		6 (12%)	1 (2%)	3 (6%)	1 (2%)	6 (12%)
Bile duct, dilatation			1 (2%)			
Bile duct, hyperplasia						8 (16%)
Hepatocyte, necrosis		3 (6%)	3 (6%)	4 (8%)	1 (2%)	4 (8%)
Oval cell, hyperplasia					2 (4%)	
Oval cell, proliferation						1 (2%)
Mesentery	(5)	(3)				(2)
Inflammation, acute	1 (20%)					
Inflammation, chronic	1 (20%)	1 (33%)				2 (100%)
Inflammation, necrotizing	2 (40%)					
Pancreas	(50)	(50)	(47)	(49)	(49)	(48)
Necrosis	1 (2%)					
Acinus, atrophy					1 (2%)	2 (4%)
Artery, inflammation, chronic	1 (2%)			2 (4%)		2 (4%)
Duct, ectasia					1 (2%)	1 (2%)
Interlobular, inflammation, chronic	1 (2%)	1 (2%)				
Salivary glands	(50)	(49)	(49)	(49)	(50)	(49)
Abscess					1 (2%)	
Bacterium					1 (2%)	
Duct, fibrosis			1 (2%)			
Stomach, forestomach	(48)	(49)	(47)	(50)	(49)	(47)
Acanthosis	2 (4%)	1 (2%)				
Cyst epithelial inclusion						1 (2%)
Hyperkeratosis	2 (4%)	1 (2%)				
Inflammation, chronic						1 (2%)
Inflammation, necrotizing	1 (2%)					
Ulcer	1 (2%)					
Stomach, glandular	(48)	(48)	(47)	(50)	(49)	(47)
Ectopic tissue	1 (2%)					
Inflammation, acute		1 (2%)				
Inflammation, chronic	1 (2%)				1 (2%)	
Ulcer					1 (2%)	
Mucosa, hyperplasia			1 (2%)			
Mucosa, mineralization						1 (2%)
Tooth		(4)	(4)	(3)	(6)	(1)
Gingiva, inflammation, chronic active		2 (50%)	2 (50%)		1 (17%)	
Pulp, inflammation, chronic active		2 (50%)	2 (50%)	2 (67%)	4 (67%)	1 (100%)
Pulp, inflammation, suppurative				1 (33%)		
Cardiovascular System						
Heart	(50)	(49)	(49)	(50)	(50)	(50)
Ectopic tissue						1 (2%)
Coronary artery, inflammation, chronic	2 (4%)	1 (2%)		1 (2%)	1 (2%)	
Epicardium, inflammation, chronic	2 (4%)					
Myocardium, inflammation, chronic, multifocal	1 (2%)	2 (4%)	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Myocardium, mineralization, multifocal				1 (2%)		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Alimentary System (continued)		
Liver (continued)	(50)	(50)
Thrombosis	8 (16%)	7 (14%)
Bile duct, hyperplasia	34 (68%)	26 (52%)
Hepatocyte, necrosis	1 (2%)	3 (6%)
Vein, inflammation, subacute	1 (2%)	
Pancreas	(49)	(49)
Hemorrhage	1 (2%)	
Infarct	3 (6%)	1 (2%)
Acinus, atrophy		1 (2%)
Artery, inflammation, chronic	8 (16%)	1 (2%)
Duct, ectasia	1 (2%)	
Salivary glands	(46)	(47)
Abscess	1 (2%)	
Bacterium	1 (2%)	
Stomach, forestomach	(50)	(50)
Acanthosis		2 (4%)
Hemorrhage	1 (2%)	1 (2%)
Hyperkeratosis		2 (4%)
Stomach, glandular	(50)	(50)
Hemorrhage	1 (2%)	
Mucosa, hyperplasia	2 (4%)	1 (2%)
Cardiovascular System		
Blood vessel	(3)	(2)
Aorta, inflammation, chronic active	2 (67%)	2 (100%)
Aorta, thrombosis	1 (33%)	
Heart	(50)	(46)
Necrosis	1 (2%)	
Coronary artery, inflammation, chronic	1 (2%)	
Myocardium, inflammation, chronic, multifocal	3 (6%)	
Valve, inflammation, chronic active	1 (2%)	2 (4%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Endocrine System						
Adrenal gland	(39)	(50)	(48)	(49)	(50)	(50)
Subcapsular, hyperplasia	4 (10%)	3 (6%)		1 (2%)	1 (2%)	
Adrenal gland, cortex	(39)	(50)	(47)	(49)	(50)	(50)
Amyloid deposition		1 (2%)				
Hyperplasia	1 (3%)	3 (6%)			1 (2%)	2 (4%)
Hypertrophy		1 (2%)				
Infarct	1 (3%)					
Zona glomerulosa, cytoplasmic alteration	1 (3%)					
Adrenal gland, medulla	(38)	(50)	(47)	(49)	(49)	(50)
Hyperplasia	2 (5%)	2 (4%)	2 (4%)			1 (2%)
Islets, pancreatic	(48)	(49)	(46)	(48)	(48)	(45)
Hyperplasia		1 (2%)	1 (2%)		1 (2%)	
Pituitary gland	(44)	(44)	(45)	(46)	(44)	(41)
Pars distalis, hyperplasia, nodular					2 (5%)	
Thyroid gland	(50)	(49)	(47)	(49)	(48)	(48)
Inflammation, chronic	1 (2%)					
Follicle, hyperplasia, nodular					3 (6%)	16 (33%)
General Body System						
None						
Genital System						
Epididymis	(50)	(50)	(48)	(50)	(49)	(50)
Granuloma sperm		1 (2%)			1 (2%)	
Inflammation, chronic						2 (4%)
Inflammation, chronic active					1 (2%)	
Preputial gland	(3)	(1)		(1)	(2)	
Abscess	2 (67%)			1 (100%)	2 (100%)	
Duct, dilatation	1 (33%)	1 (100%)				
Prostate	(50)	(50)	(48)	(49)	(49)	(49)
Inflammation, chronic active		1 (2%)			1 (2%)	1 (2%)
Inflammation, suppurative				1 (2%)	3 (6%)	
Serosa, inflammation, necrotizing	1 (2%)					
Testes	(50)	(50)	(49)	(50)	(49)	(50)
Seminiferous tubule, atrophy		1 (2%)	1 (2%)			1 (2%)
Seminiferous tubule, hypospermia						1 (2%)
Tunic, inflammation, necrotizing	1 (2%)					
Hematopoietic System						
Bone marrow	(50)	(50)	(49)	(49)	(50)	(49)
Myeloid cell, hyperplasia		1 (2%)				

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Endocrine System		
Adrenal gland	(49)	(46)
Subcapsular, hyperplasia	1 (2%)	
Adrenal gland, cortex	(48)	(46)
Hyperplasia		1 (2%)
Infarct	1 (2%)	1 (2%)
Adrenal gland, medulla	(49)	(46)
Hyperplasia	3 (6%)	
Infarct		1 (2%)
Thyroid gland	(46)	(45)
Follicle, hyperplasia	1 (2%)	
Follicle, hyperplasia, nodular	8 (17%)	9 (20%)
General Body System		
None		
Genital System		
Epididymis	(49)	(50)
Granuloma sperm	1 (2%)	
Preputial gland		(1)
Duct, dilatation		1 (100%)
Prostate	(50)	(47)
Inflammation, suppurative	2 (4%)	
Hematopoietic System		
Bone marrow	(50)	(50)
Femoral, myelofibrosis		2 (4%)
Myeloid cell, hyperplasia	3 (6%)	1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Hematopoietic System (continued)						
Lymph node	(48)	(50)	(48)	(48)	(48)	(46)
Deep cervical, hyperplasia, plasma cell		1 (2%)				
Deep cervical, sinus, ectasia	1 (2%)					
Lumbar, hyperplasia, plasma cell					1 (2%)	
Mandibular, abscess	1 (2%)	1 (2%)		1 (2%)		
Mandibular, bacterium	1 (2%)			1 (2%)		
Mandibular, hyperplasia, lymphoid		2 (4%)	1 (2%)	1 (2%)		
Mandibular, hyperplasia, plasma cell				1 (2%)	1 (2%)	
Mandibular, infiltration cellular, histiocyte		1 (2%)		1 (2%)		
Mediastinal, hematopoietic cell proliferation	1 (2%)					
Mediastinal, hyperplasia, plasma cell	1 (2%)					
Mediastinal, sinus, ectasia						1 (2%)
Pancreatic, hematopoietic cell proliferation					1 (2%)	
Pancreatic, hyperplasia, lymphoid				1 (2%)		
Renal, hyperplasia, lymphoid				1 (2%)		
Sinus, mandibular, ectasia	1 (2%)					1 (2%)
Lymph node, mesenteric	(32)	(34)	(34)	(37)	(35)	(31)
Angiectasis		1 (3%)				
Hematopoietic cell proliferation	13 (41%)	12 (35%)	14 (41%)	20 (54%)	13 (37%)	14 (45%)
Hyperplasia, lymphoid	1 (3%)		8 (24%)	8 (22%)	5 (14%)	2 (6%)
Hyperplasia, plasma cell	1 (3%)					1 (3%)
Infiltration cellular, histiocyte		4 (12%)	6 (18%)	8 (22%)	9 (26%)	2 (6%)
Inflammation, granulomatous	1 (3%)	1 (3%)				
Artery, thrombosis			1 (3%)			
Sinus, ectasia	9 (28%)	18 (53%)	16 (47%)	14 (38%)	19 (54%)	24 (77%)
Spleen	(50)	(50)	(47)	(49)	(50)	(48)
Amyloid deposition		1 (2%)		1 (2%)		
Depletion lymphoid	1 (2%)			2 (4%)		
Fibrosis						1 (2%)
Hematopoietic cell proliferation	10 (20%)	9 (18%)	1 (2%)	8 (16%)	8 (16%)	11 (23%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	7 (15%)	5 (10%)	2 (4%)	
Infiltration cellular, plasma cell	1 (2%)	1 (2%)				
Integumentary System						
Mammary gland			(2)			(1)
Duct, ectasia			1 (50%)			
Skin	(50)	(50)	(49)	(49)	(50)	(49)
Abscess		1 (2%)				
Acanthosis	29 (58%)	35 (70%)	32 (65%)	38 (78%)	38 (76%)	31 (63%)
Cyst epithelial inclusion				1 (2%)		
Hyperkeratosis	24 (48%)	35 (70%)	29 (59%)	37 (76%)	38 (76%)	33 (67%)
Inflammation, chronic		1 (2%)				1 (2%)
Inflammation, necrotizing		2 (4%)				
Parasite metazoan	23 (46%)	35 (70%)	29 (59%)	36 (73%)	36 (72%)	29 (59%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Hematopoietic System (continued)		
Lymph node	(44)	(42)
Hyperplasia, lymphoid		1 (2%)
Mandibular, abscess	1 (2%)	
Mandibular, fibrosis	1 (2%)	
Mandibular, hyperplasia, lymphoid	1 (2%)	
Mandibular, infiltration cellular, histiocyte	1 (2%)	
Pancreatic, hematopoietic cell proliferation		1 (2%)
Sinus, mandibular, ectasia		1 (2%)
Lymph node, mesenteric	(10)	(10)
Hematopoietic cell proliferation	2 (20%)	4 (40%)
Sinus, ectasia	7 (70%)	6 (60%)
Spleen	(49)	(48)
Hematopoietic cell proliferation	41 (84%)	32 (67%)
Hyperplasia, lymphoid		1 (2%)
Integumentary System		
Skin	(50)	(49)
Acanthosis	26 (52%)	31 (63%)
Erosion		1 (2%)
Hyperkeratosis	29 (58%)	34 (69%)
Parasite metazoan	18 (36%)	21 (43%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Integumentary System (continued)						
Skin (continued)	(50)	(50)	(49)	(49)	(50)	(49)
Ulcer		1 (2%)				
Dermis, metaplasia, osseous		1 (2%)				
Sebaceous gland, ectasia				1 (2%)		
Subcutaneous tissue, fat, necrosis	2 (4%)					
Musculoskeletal System						
Bone	(49)	(50)	(49)	(50)	(50)	(50)
Coccygeal, fracture healed			1 (2%)			
Femur, osteopetrosis			1 (2%)			
Tarsal, hyperostosis	5 (10%)	5 (10%)	10 (20%)		1 (2%)	2 (4%)
Nervous System						
Brain	(50)	(50)	(49)	(50)	(50)	(49)
Bacterium						1 (2%)
Inflammation, acute			1 (2%)			
Hypothalamus, compression						1 (2%)
Respiratory System						
Lung	(50)	(50)	(49)	(50)	(50)	(50)
Abscess		1 (2%)				
Bacterium		1 (2%)				
Alveolar epithelium, hyperplasia	3 (6%)		1 (2%)	1 (2%)	2 (4%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	2 (4%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Alveolus, inflammation, acute						2 (4%)
Artery, thrombosis	1 (2%)			1 (2%)	1 (2%)	1 (2%)
Bronchus, foreign body	1 (2%)					
Interstitium, inflammation, chronic	2 (4%)	1 (2%)		2 (4%)	1 (2%)	
Mediastinum, inflammation, chronic active	1 (2%)					
Special Senses System						
Eye		(1)	(1)			
Synechia			1 (100%)			
Anterior chamber, inflammation, suppurative		1 (100%)				
Harderian gland	(3)	(4)	(5)	(1)	(1)	(3)
Hyperplasia, nodular				1 (100%)		
Inflammation, necrotizing	1 (33%)					
Duct, dilatation				1 (100%)		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration	**0 ppm**	**30 ppm**
F_1 Concentration	**30 ppm**	**30 ppm**
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(50)	(46)
Alveolus, infiltration cellular, histiocyte		1 (2%)
Alveolus, inflammation, acute	1 (2%)	
Bronchus, metaplasia, squamous	1 (2%)	
Mediastinum, inflammation, chronic active	1 (2%)	
Special Senses System		
None		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Urinary System						
Kidney	(50)	(50)	(49)	(50)	(49)	(48)
Abscess		1 (2%)				
Bacterium				1 (2%)		
Cyst		6 (12%)	3 (6%)	1 (2%)	3 (6%)	
Hydronephrosis		1 (2%)				
Infarct	1 (2%)	1 (2%)			1 (2%)	1 (2%)
Infiltration cellular, lymphocyte			1 (2%)			
Inflammation, acute				1 (2%)		
Metaplasia, osseous					2 (4%)	3 (6%)
Nephropathy, chronic	9 (18%)	8 (16%)	8 (16%)	10 (20%)	11 (22%)	5 (10%)
Glomerulus, amyloid deposition		1 (2%)				
Glomerulus, inflammation, membranoproliferative					1 (2%)	2 (4%)
Glomerulus, nephropathy, chronic					1 (2%)	
Urinary bladder	(49)	(50)	(48)	(48)	(48)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)			1 (2%)
Parasite metazoan	1 (2%)					

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration	**0 ppm**		**30 ppm**	
F_1 Concentration	**30 ppm**		**30 ppm**	
Urinary System				
Kidney	(50)		(49)	
Inflammation, acute	1 (2%)			
Nephropathy, chronic	42 (84%)		29 (59%)	
Artery, inflammation, subacute			2 (4%)	
Fat, necrosis	1 (2%)			
Glomerulus, infiltration cellular, mononuclear cell			1 (2%)	
Glomerulus, inflammation, membranoproliferative	1 (2%)		1 (2%)	
Urinary bladder	(47)		(47)	
Inflammation, chronic active	1 (2%)			
Mucosa, hyperplasia	1 (2%)			

[a] Number of animals with any tissue examined microscopically

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF POLYBROMINATED BIPHENYLS

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	7	3	5	2	4	8
Natural deaths	4	2	13	10	13	25
Survivors						
Terminal sacrifice	39	45	32	38	33	17
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Gallbladder	(45)	(50)	(45)	(46)	(38)	(45)
Intestine large, cecum	(47)	(50)	(49)	(50)	(46)	(50)
Intestine large, rectum	(49)	(50)	(48)	(50)	(46)	(50)
Intestine small, duodenum	(48)	(50)	(49)	(50)	(50)	(47)
Adenoma					1 (2%)	
Intestine small, ileum	(46)	(50)	(46)	(50)	(47)	(45)
Intestine small, jejunum	(47)	(50)	(47)	(49)	(47)	(44)
Adenocarcinoma	1 (2%)			1 (2%)		
Histiocytic sarcoma						1 (2%)
Liver	(50)	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	1 (2%)	4 (8%)	
Hepatocellular carcinoma, multiple				8 (16%)	13 (26%)	22 (44%)
Hepatocellular carcinoma, single	1 (2%)	4 (8%)	2 (4%)	14 (28%)	13 (26%)	22 (44%)
Hepatocellular adenoma, multiple	1 (2%)	7 (14%)		28 (56%)	31 (62%)	44 (88%)
Hepatocellular adenoma, single	3 (6%)	12 (24%)	5 (10%)	11 (22%)	7 (14%)	3 (6%)
Histiocytic sarcoma	1 (2%)	1 (2%)	3 (6%)	1 (2%)		1 (2%)
Mesentery	(2)	(1)	(4)	(3)	(2)	(1)
Hemangioma			1 (25%)			
Pancreas	(48)	(50)	(49)	(50)	(50)	(48)
Stomach, forestomach	(49)	(50)	(47)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	2 (4%)	2 (4%)			2 (4%)
Stomach, glandular	(47)	(49)	(48)	(50)	(49)	(50)
Cardiovascular System						
Heart	(50)	(50)	(50)	(50)	(50)	(50)
Endocrine System						
Adrenal gland	(50)	(49)	(50)	(50)	(50)	(50)
Capsule, histiocytic sarcoma			1 (2%)			
Adrenal gland, cortex	(50)	(49)	(50)	(49)	(50)	(50)
Adrenal gland, medulla	(50)	(49)	(50)	(49)	(50)	(49)
Pheochromocytoma benign	1 (2%)					
Islets, pancreatic	(48)	(49)	(47)	(50)	(47)	(43)
Adenoma						1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	16	15
Natural deaths	34	35
Survivors		
Animals examined microscopically	49	49
Alimentary System		
Liver	(48)	(47)
Hepatocellular carcinoma, multiple	21 (44%)	9 (19%)
Hepatocellular carcinoma, single	14 (29%)	20 (43%)
Hepatocellular adenoma, multiple	41 (85%)	36 (77%)
Hepatocellular adenoma, single	5 (10%)	5 (11%)
Stomach, forestomach	(45)	(46)
Squamous cell papilloma	1 (2%)	
Cardiovascular System		
None		
Endocrine System		
Adrenal gland, medulla	(43)	(47)
Pheochromocytoma benign		1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Endocrine System (continued)						
Pituitary gland	(48)	(47)	(49)	(48)	(47)	(44)
Pars distalis, adenoma	5 (10%)	6 (13%)	10 (20%)	10 (21%)	11 (23%)	3 (7%)
Pars intermedia, adenoma				1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)	(50)	(50)
Bilateral, follicular cell, adenoma		1 (2%)				1 (2%)
Follicular cell, adenoma		2 (4%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
General Body System						
None						
Genital System						
Clitoral gland			(2)			(1)
Carcinoma			1 (50%)			1 (100%)
Ovary	(47)	(49)	(48)	(48)	(49)	(49)
Cystadenoma	1 (2%)					
Granulosa cell tumor benign		2 (4%)			1 (2%)	
Granulosa-theca tumor benign				1 (2%)		
Luteoma		1 (2%)	1 (2%)		2 (4%)	
Mixed tumor benign		1 (2%)			1 (2%)	
Osteosarcoma, metastatic			1 (2%)			
Thecoma benign	1 (2%)					
Oviduct		(1)	(2)			
Uterus	(49)	(50)	(49)	(50)	(49)	(49)
Adenocarcinoma				1 (2%)		
Hamartoma				1 (2%)		
Hemangioma					1 (2%)	
Histiocytic sarcoma	1 (2%)					1 (2%)
Leiomyosarcoma				1 (2%)		
Polyp stromal	1 (2%)		1 (2%)	1 (2%)		
Sarcoma stromal	1 (2%)		2 (4%)			
Hematopoietic System						
Bone marrow	(50)	(50)	(50)	(50)	(50)	(50)
Femoral, hemangiosarcoma			3 (6%)	1 (2%)	2 (4%)	
Femoral, histiocytic sarcoma			1 (2%)			
Sternal, hemangiosarcoma				2 (4%)		
Sternal, histiocytic sarcoma			1 (2%)			
Lymph node	(48)	(49)	(48)	(50)	(49)	(47)
Mandibular, histiocytic sarcoma			1 (2%)			
Mediastinal, carcinoma, metastatic	1 (2%)					
Mediastinal, histiocytic sarcoma		1 (2%)				1 (2%)
Pancreatic, histiocytic sarcoma			1 (2%)			
Renal, histiocytic sarcoma			1 (2%)	1 (2%)		
Lymph node, mesenteric	(8)	(21)	(15)	(21)	(23)	(20)
Histiocytic sarcoma		1 (5%)	1 (7%)			1 (5%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration	**0 ppm**	**30 ppm**
F_1 Concentration	**30 ppm**	**30 ppm**
General Body System		
None		
Genital System		
Ovary	(45)	(46)
Teratoma	1 (2%)	
Hematopoietic System		
None		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Hematopoietic System (continued)						
Spleen	(48)	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Histiocytic sarcoma			1 (2%)	1 (2%)		
Thymus	(43)	(43)	(31)	(38)	(29)	(27)
Histiocytic sarcoma			1 (3%)			
Integumentary System						
Mammary gland	(41)	(44)	(34)	(32)	(37)	(26)
Adenocarcinoma			2 (6%)		1 (3%)	
Skin	(49)	(50)	(49)	(49)	(50)	(50)
Subcutaneous tissue, fibrosarcoma	1 (2%)		2 (4%)			
Subcutaneous tissue, hemangioma	1 (2%)					1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	2 (4%)	1 (2%)	
Musculoskeletal System						
Bone	(50)	(50)	(49)	(50)	(50)	(50)
Osteoma				1 (2%)		
Osteosarcoma	1 (2%)		1 (2%)			
Skeletal muscle	(1)	(1)	(1)			(1)
Nervous System						
Brain	(50)	(50)	(50)	(50)	(50)	(50)
Peripheral nerve	(1)					
Respiratory System						
Lung	(50)	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma, single		2 (4%)	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Alveolar/bronchiolar carcinoma, single	1 (2%)	1 (2%)	3 (6%)		1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver				2 (4%)	2 (4%)	3 (6%)
Histiocytic sarcoma						1 (2%)
Osteosarcoma, metastatic, bone			1 (2%)			
Nose	(50)	(50)	(50)	(50)	(49)	(50)
Special Senses System						
Eye	(1)	(1)				
Harderian gland	(6)	(1)	(6)	(2)	(2)	(1)
Adenoma	3 (50%)	1 (100%)	4 (67%)	2 (100%)	2 (100%)	
Zymbal's gland	(1)					
Carcinoma	1 (100%)					

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Integumentary System		
Skin	(49)	(47)
Subcutaneous tissue, hemangioma		1 (2%)
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(46)	(44)
Alveolar/bronchiolar adenoma, single	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	4 (9%)
Special Senses System		
None		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Urinary System						
Kidney	(50)	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)			1 (2%)
Urinary bladder	(48)	(50)	(49)	(48)	(49)	(49)
Systemic Lesions						
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	3 (6%)	2 (4%)		2 (4%)
Lymphoma malignant lymphocytic	2 (4%)	2 (4%)	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Lymphoma malignant mixed	3 (6%)	2 (4%)	3 (6%)	3 (6%)	2 (4%)	
Lymphoma malignant undifferentiated cell	1 (2%)	3 (6%)	6 (12%)	2 (4%)		1 (2%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	25	35	36	46	49	50
Total primary neoplasms	33	51	63	102	101	111
Total animals with benign neoplasms	16	28	22	39	42	47
Total benign neoplasms	18	37	27	59	59	59
Total animals with malignant neoplasms	13	13	26	31	32	45
Total malignant neoplasms	15	14	36	43	42	52
Total animals with metastatic neoplasms	1		1	2	2	4
Total metastatic neoplasms	1		2	2	2	4

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls
(continued)

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Urinary System		
None		
Systemic Lesions		
None		
Neoplasm Summary		
Total animals with primary neoplasms	47	47
Total primary neoplasms	84	73
Total animals with benign neoplasms	46	41
Total benign neoplasms	49	44
Total animals with malignant neoplasms	35	29
Total malignant neoplasms	35	29
Total animals with metastatic neoplasms	1	4
Total metastatic neoplasms	1	4

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

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Harderian Gland: Adenoma			
Overall rate[a]	3/50 (6%)	2/50 (4%)	0/49 (0%)
Adjusted rate[b]	7.5%	5.3%	0.0%
Terminal rate[c]	2/39 (5%)	2/38 (5%)	0/0 (0%)
First incidence (days)	723	729 (T)	–[e]
Life table test[d]	P=0.688N	P=0.514N	–
Logistic regression test[d]	P=0.647N	P=0.505N	P=0.999N
Cochran-Armitage test[d]	P=0.093N		
Fisher exact test[d]		P=0.500N	P=0.125N
Liver: Hepatocellular Adenoma			
Overall rate	4/50 (8%)	39/50 (78%)	46/48 (96%)
Adjusted rate	10.3%	86.6%	100.0%
Terminal rate	4/39 (10%)	32/38 (84%)	0/0 (0%)
First incidence (days)	729 (T)	617	359
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	1/50 (2%)	22/50 (44%)	35/48 (73%)
Adjusted rate	2.6%	52.2%	95.5%
Terminal rate	1/39 (3%)	18/38 (47%)	0/0 (0%)
First incidence (days)	729 (T)	629	484
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	5/50 (10%)	42/50 (84%)	47/48 (98%)
Adjusted rate	12.8%	93.3%	100.0%
Terminal rate	5/39 (13%)	35/38 (92%)	0/0 (0%)
First incidence (days)	729 (T)	617	359
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	5/48 (10%)	10/48 (21%)	0/36 (0%)
Adjusted rate	13.2%	25.2%	0.0%
Terminal rate	5/38 (13%)	7/36 (19%)	0/0 (0%)
First incidence (days)	729 (T)	662	–
Life table test	P=0.207	P=0.118	–
Logistic regression test	P=0.562	P=0.138	–
Cochran-Armitage test	P=0.061N		
Fisher exact test		P=0.130	P=0.055N

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
Spleen: Hemangiosarcoma			
Overall rate	1/48 (2%)	3/50 (6%)	0/44 (0%)
Adjusted rate	2.6%	6.9%	0.0%
Terminal rate	1/39 (3%)	1/38 (3%)	0/0 (0%)
First incidence (days)	729 (T)	640	–
Life table test	P=0.599	P=0.318	–
Logistic regression test	P=0.480N	P=0.295	–
Cochran-Armitage test	P=0.320N		
Fisher exact test		P=0.324	P=0.522N
All Organs: Hemangiosarcoma			
Overall rate	1/50 (2%)	4/50 (8%)	0/49 (0%)
Adjusted rate	2.6%	9.4%	0.0%
Terminal rate	1/39 (3%)	2/38 (5%)	0/0 (0%)
First incidence (days)	729 (T)	640	–
Life table test	P=0.446	P=0.189	–
Logistic regression test	P=0.589N	P=0.171	–
Cochran-Armitage test	P=0.278N		
Fisher exact test		P=0.181	P=0.505N
All Organs: Hemangioma or Hemangiosarcoma			
Overall rate	2/50 (4%)	4/50 (8%)	0/49 (0%)
Adjusted rate	5.1%	9.4%	0.0%
Terminal rate	2/39 (5%)	2/38 (5%)	0/0 (0%)
First incidence (days)	729 (T)	640	–
Life table test	P=0.584	P=0.344	–
Logistic regression test	P=0.458N	P=0.337	–
Cochran-Armitage test	P=0.163N		
Fisher exact test		P=0.339	P=0.253N
All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rate	6/50 (12%)	6/50 (12%)	0/49 (0%)
Adjusted rate	14.6%	14.4%	0.0%
Terminal rate	4/39 (10%)	3/38 (8%)	0/0 (0%)
First incidence (days)	647	662	–
Life table test	P=0.629N	P=0.611	P=0.872N
Logistic regression test	P=0.138N	P=0.604N	P=0.313N
Cochran-Armitage test	P=0.016N		
Fisher exact test		P=0.620N	P=0.014N
All Organs: Malignant Lymphoma and Histiocytic Sarcoma			
Overall rate	7/50 (14%)	8/50 (16%)	0/49 (0%)
Adjusted rate	16.5%	18.6%	0.0%
Terminal rate	4/39 (10%)	4/38 (11%)	0/0 (0%)
First incidence (days)	575	630	–
Life table test	P=0.550N	P=0.505	P=0.515N
Logistic regression test	P=0.038N	P=0.477	P=0.053N
Cochran-Armitage test	P=0.009N		
Fisher exact test		P=0.500	P=0.007N

TABLE D2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 0:10, and 0:30 ppm Groups (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm
All Organs: Benign Neoplasms			
Overall rate	16/50 (32%)	39/50 (78%)	46/49 (94%)
Adjusted rate	38.9%	86.6%	100.0%
Terminal rate	14/39 (36%)	32/38 (84%)	0/0 (0%)
First incidence (days)	548	617	359
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
All Organs: Malignant Neoplasms			
Overall rate	13/50 (26%)	31/50 (62%)	35/49 (71%)
Adjusted rate	29.3%	65.9%	95.5%
Terminal rate	8/39 (21%)	22/38 (58%)	0/0 (0%)
First incidence (days)	451	629	484
Life table test	P<0.001	P=0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
All Organs: Benign or Malignant Neoplasms			
Overall rate	25/50 (50%)	46/50 (92%)	47/49 (96%)
Adjusted rate	55.4%	95.8%	100.0%
Terminal rate	19/39 (49%)	36/38 (95%)	0/0 (0%)
First incidence (days)	451	617	359
Life table test	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE D2b
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:0, 3:3, 10:10, 30:10, and 30:30 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	3 ppm 3 ppm	10 ppm 10 ppm	30 ppm 10 ppm	30 ppm 30 ppm
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate[a]	5/50 (10%)	7/50 (16%)	44/50 (88%)	50/50 (100%)	47/47 (100%)
Adjusted rate[b]	12.8%	22.4%	97.8%	100.0%	100.0%
Terminal rate[c]	5/39 (13%)	6/32 (19%)	32/33 (97%)	17/17 (100%)	0/0
First incidence (days)	729 (T)	621	563	435	291
Life table test[d]	P<0.001	P=0.183	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P=0.249	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001				
Fisher exact test[d]		P=0.277	P<0.001	P<0.001	P<0.001

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE D2c
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:10, 10:10, and 30:10 ppm Groups

F_0 Concentration F_1 Concentration	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	42/50 (84%)	44/50 (88%)	50/50 (100%)
Adjusted rate[b]	93.3%	97.8%	100.0%
Terminal rate[c]	35/38 (92%)	32/33 (97%)	17/17 (100%)
First incidence (days)	617	563	435
Life table test[d]	P<0.001	P=0.094	P<0.001
Logistic regression test[d]	P<0.001	P=0.192	P<0.001
Cochran-Armitage test[d]	P=0.004		
Fisher exact test[d]		P=0.387	P=0.003

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE D2d
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 0:30 and 30:30 ppm Groups

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate[a]	47/48 (98%)	47/47 (100%)
Adjusted rate[b]	100.0%	100.0%
Terminal rate[c]	0/0	0/0
First incidence (days)	359	291
Life table test[d]		P<0.001
Logistic regression test[d]		P=0.529
Fisher exact test[d]		P=0.505

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates.

TABLE D2e
Statistical Analysis of Liver Neoplasms in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls: Comparison of the 30:0, 30:10, and 30:30 ppm Groups

F_0 Concentration	30 ppm	30 ppm	30 ppm
F_1 Concentration	0 ppm	10 ppm	30 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate[a]	21/50 (42%)	50/50 (100%)	47/47 (100%)
Adjusted rate[b]	43.8%	100.0%	100.0%
Terminal rate[c]	18/45 (40%)	17/17 (100%)	0/0
First incidence (days)	620	435	291
Life table test[d]	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001		
Fisher exact test[d]		P<0.001	P<0.001

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposure group incidence are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE D3
Historical Incidence of Hepatocellular Neoplasms in Untreated Female $B6C3F_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
2,4-Dichlorophenol	0/50	2/50	2/50
5,5′-Diphenylhydantoin (Phenytoin)	5/48	0/48	5/48
Ethylene thiourea	2/50	2/50	4/50
Polybrominated biphenyls	4/50	1/50	5/50
Manganese sulfate	12/51	3/51	13/51
Pentachlorophenol (Dowicide EC-7)	1/34	0/34	1/34
Pentachlorophenol (technical grade)	3/33	0/33	3/33
Triamterene	10/50	4/50	13/50
Triamterene	7/50	5/50	10/50
Overall Historical Incidence			
Total	110/1,113 (9.9%)	54/1,113 (4.9%)	153/1,113 (13.7%)
Standard deviation	7.2%	4.7%	8.6%
Range	0%–28%	0%–20%	3%–34%

[a] Data as of 17 December 1991

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Disposition Summary						
Animals initially in study	60	60	60	60	60	60
9-Month interim evaluation	10	10	10	10	10	10
Early deaths						
Moribund	7	3	5	2	4	8
Natural deaths	4	2	13	10	13	25
Survivors						
Terminal sacrifice	39	45	32	38	33	17
Animals examined microscopically	50	50	50	50	50	50
Alimentary System						
Gallbladder	(45)	(50)	(45)	(46)	(38)	(45)
Hemorrhage						1 (2%)
Inflammation, chronic active	1 (2%)					
Epithelium, inclusion body intracytoplasmic			1 (2%)		1 (3%)	
Intestine large, cecum	(47)	(50)	(49)	(50)	(46)	(50)
Parasite metazoan		1 (2%)		2 (4%)	2 (4%)	5 (10%)
Mucosa, hyperplasia					1 (2%)	
Intestine large, colon	(48)	(50)	(49)	(49)	(47)	(50)
Parasite metazoan	2 (4%)	2 (4%)	1 (2%)	2 (4%)	5 (11%)	7 (14%)
Intestine large, rectum	(49)	(50)	(48)	(50)	(46)	(50)
Parasite metazoan	1 (2%)	1 (2%)		3 (6%)	2 (4%)	4 (8%)
Ulcer			1 (2%)			
Intestine small, duodenum	(48)	(50)	(49)	(50)	(50)	(47)
Hemorrhage				1 (2%)		
Hyperplasia, lymphoid	1 (2%)					
Inflammation, chronic active			1 (2%)	1 (2%)	1 (2%)	
Ulcer			2 (4%)		1 (2%)	
Peyer's patch, infiltration cellular, histiocyte						1 (2%)
Intestine small, ileum	(46)	(50)	(46)	(50)	(47)	(45)
Hyperplasia	1 (2%)					
Parasite metazoan						1 (2%)
Intestine small, jejunum	(47)	(50)	(47)	(49)	(47)	(44)
Hyperplasia	1 (2%)					
Inflammation, chronic active					1 (2%)	
Parasite metazoan				1 (2%)		
Ulcer	1 (2%)		1 (2%)		1 (2%)	
Liver	(50)	(50)	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)				1 (2%)	1 (2%)
Basophilic focus	1 (2%)			2 (4%)	8 (16%)	3 (6%)
Clear cell focus	2 (4%)	5 (10%)		22 (44%)	13 (26%)	4 (8%)
Cytomegaly		6 (12%)	14 (28%)	49 (98%)	49 (98%)	50 (100%)
Cytoplasmic alteration		1 (2%)				
Eosinophilic focus	1 (2%)	3 (6%)	2 (4%)	18 (36%)	16 (32%)	4 (8%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Disposition Summary		
Animals initially in study	60	60
9-Month interim evaluation	10	10
Early deaths		
Moribund	16	15
Natural deaths	34	35
Animals examined microscopically	49	49
Alimentary System		
Intestine large, cecum	(45)	(45)
Parasite metazoan	2 (4%)	5 (11%)
Intestine large, colon	(44)	(45)
Parasite metazoan	22 (50%)	17 (38%)
Intestine large, rectum	(45)	(46)
Parasite metazoan	2 (4%)	2 (4%)
Intestine small, ileum	(39)	(41)
Parasite metazoan	2 (5%)	2 (5%)
Liver	(48)	(47)
Clear cell focus	1 (2%)	3 (6%)
Cyst	2 (4%)	
Cytomegaly	48 (100%)	47 (100%)
Eosinophilic focus	4 (8%)	8 (17%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Alimentary System (continued)						
Liver (continued)	(50)	(50)	(50)	(50)	(50)	(50)
Fatty change			2 (4%)	39 (78%)	44 (88%)	43 (86%)
Hematopoietic cell proliferation	1 (2%)		1 (2%)	3 (6%)	1 (2%)	1 (2%)
Infiltration cellular, mixed cell	1 (2%)					
Necrosis, coagulative	1 (2%)	2 (4%)	5 (10%)	18 (36%)	9 (18%)	31 (62%)
Thrombosis		1 (2%)		1 (2%)	1 (2%)	7 (14%)
Bile duct, cyst					1 (2%)	
Bile duct, hyperplasia				9 (18%)	9 (18%)	6 (12%)
Hepatocyte, necrosis			2 (4%)	23 (46%)	15 (30%)	4 (8%)
Sinusoid, congestion				1 (2%)		
Mesentery	(2)	(1)	(4)	(3)	(2)	(1)
Hyperplasia, lymphoid	1 (50%)					
Inflammation, chronic				2 (67%)	1 (50%)	
Inflammation, necrotizing	1 (50%)		2 (50%)	1 (33%)		
Pancreas	(48)	(50)	(49)	(50)	(50)	(48)
Hemorrhage						1 (2%)
Hypoplasia	1 (2%)			1 (2%)		
Necrosis				2 (4%)		
Acinus, atrophy	1 (2%)	3 (6%)	2 (4%)	1 (2%)	2 (4%)	
Artery, inflammation, chronic				1 (2%)	2 (4%)	6 (13%)
Duct, ectasia		3 (6%)	1 (2%)	4 (8%)		2 (4%)
Interlobular, inflammation, chronic			1 (2%)	1 (2%)	2 (4%)	1 (2%)
Salivary glands	(50)	(49)	(49)	(49)	(48)	(47)
Abscess	2 (4%)		1 (2%)	1 (2%)		
Bacterium	2 (4%)		1 (2%)			
Mucocele				1 (2%)		
Stomach, forestomach	(49)	(50)	(47)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)			1 (2%)	1 (2%)
Hyperplasia					1 (2%)	
Inflammation, chronic				1 (2%)	1 (2%)	
Ulcer					2 (4%)	
Stomach, glandular	(47)	(49)	(48)	(50)	(49)	(50)
Hemorrhage				1 (2%)		
Inflammation, chronic					1 (2%)	
Ulcer				1 (2%)		
Epithelium, metaplasia, squamous					1 (2%)	
Cardiovascular System						
Blood vessel			(1)	(1)	(2)	
Aorta, inflammation, chronic active				1 (100%)	2 (100%)	
Pulmonary artery, inflammation, chronic			1 (100%)			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Alimentary System (continued)		
Liver (continued)	(48)	(47)
Fatty change	46 (96%)	47 (100%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)
Necrosis, coagulative	34 (71%)	20 (43%)
Thrombosis	6 (13%)	6 (13%)
Bile duct, cyst	1 (2%)	
Bile duct, hyperplasia	39 (81%)	37 (79%)
Hepatocyte, necrosis	10 (21%)	3 (6%)
Mesentery	(1)	
Inflammation, chronic	1 (100%)	
Pancreas	(41)	(47)
Hemorrhage		1 (2%)
Infarct	1 (2%)	
Artery, inflammation, chronic	8 (20%)	4 (9%)
Interlobular, inflammation, chronic		1 (2%)
Stomach, forestomach	(45)	(46)
Acanthosis		1 (2%)
Hyperkeratosis	1 (2%)	1 (2%)
Stomach, glandular	(44)	(45)
Ulcer		1 (2%)
Cardiovascular System		
Blood vessel	(1)	
Aorta, inflammation, chronic active	1 (100%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Cardiovascular System (continued)						
Heart	(50)	(50)	(50)	(50)	(50)	(50)
Coronary artery, inflammation, chronic	1 (2%)		1 (2%)		2 (4%)	
Myocardium, inflammation, acute, multifocal				2 (4%)		
Myocardium, inflammation, chronic, multifocal	1 (2%)		1 (2%)			1 (2%)
Myocardium, mineralization, multifocal				1 (2%)		
Valve, bacterium	1 (2%)					
Valve, inflammation, chronic active			1 (2%)			
Endocrine System						
Adrenal gland	(50)	(49)	(50)	(50)	(50)	(50)
Subcapsular, hyperplasia	4 (8%)	5 (10%)		1 (2%)		
Adrenal gland, cortex	(50)	(49)	(50)	(49)	(50)	(50)
Cyst	1 (2%)					
Hematopoietic cell proliferation			3 (6%)	3 (6%)		
Hyperplasia	1 (2%)	2 (4%)	3 (6%)		1 (2%)	
Zona reticularis, vacuolization cytoplasmic	1 (2%)	2 (4%)	4 (8%)	1 (2%)	3 (6%)	
Adrenal gland, medulla	(50)	(49)	(50)	(49)	(50)	(49)
Hyperplasia	2 (4%)		2 (4%)		2 (4%)	1 (2%)
Pituitary gland	(48)	(47)	(49)	(48)	(47)	(44)
Angiectasis	1 (2%)					
Pars distalis, angiectasis			1 (2%)			
Pars distalis, cyst		1 (2%)				
Pars distalis, hyperplasia, nodular	12 (25%)	16 (34%)	9 (18%)	7 (15%)	8 (17%)	4 (9%)
Thyroid gland	(49)	(50)	(50)	(50)	(50)	(50)
Inflammation, chronic		2 (4%)	1 (2%)		1 (2%)	
Inflammation, subacute		1 (2%)				
Follicle, hyperplasia, nodular	2 (4%)	5 (10%)	3 (6%)	6 (12%)	11 (22%)	15 (30%)
General Body System						
None						
Genital System						
Clitoral gland			(2)			(1)
Abscess			1 (50%)			
Ovary	(47)	(49)	(48)	(48)	(49)	(49)
Angiectasis			1 (2%)			
Cyst	1 (2%)					
Inflammation, chronic active			3 (6%)	1 (2%)		
Mineralization		1 (2%)		1 (2%)		1 (2%)
Thrombosis			1 (2%)		1 (2%)	
Bilateral, periovarian tissue, cyst		2 (4%)				
Follicle, cyst	3 (6%)	3 (6%)	2 (4%)	6 (13%)	4 (8%)	2 (4%)
Periovarian tissue, cyst	11 (23%)	12 (24%)	8 (17%)	4 (8%)	9 (18%)	4 (8%)
Rete ovarii, cyst	1 (2%)	1 (2%)	3 (6%)		2 (4%)	2 (4%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Cardiovascular System (continued)		
Heart	(45)	(45)
Atrium, dilatation	1 (2%)	
Coronary artery, inflammation, chronic	1 (2%)	
Myocardium, inflammation, chronic, multifocal	2 (4%)	
Endocrine System		
Adrenal gland	(44)	(47)
Subcapsular, hyperplasia	3 (7%)	4 (9%)
Adrenal gland, cortex	(43)	(47)
Hematopoietic cell proliferation	1 (2%)	2 (4%)
Infarct	1 (2%)	
Thyroid gland	(43)	(44)
Follicle, hyperplasia, nodular	4 (9%)	9 (20%)
General Body System		
None		
Genital System		
Ovary	(45)	(46)
Mineralization	1 (2%)	
Follicle, cyst	1 (2%)	
Periovarian tissue, cyst	1 (2%)	1 (2%)
Rete ovarii, cyst	2 (4%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Genital System (continued)						
Uterus	(49)	(50)	(49)	(50)	(49)	(49)
Angiectasis			1 (2%)	1 (2%)		
Dilatation		6 (12%)				
Inflammation, acute			2 (4%)			
Inflammation, chronic					1 (2%)	
Inflammation, granulomatous			1 (2%)			
Inflammation, suppurative				1 (2%)		
Thrombosis					1 (2%)	
Endometrium, hyperplasia, cystic	46 (94%)	42 (84%)	38 (78%)	32 (64%)	24 (49%)	11 (22%)
Endometrium, endothelium, metaplasia, squamous				1 (2%)		
Hematopoietic System						
Bone marrow	(50)	(50)	(50)	(50)	(50)	(50)
Calvarium, myelofibrosis	6 (12%)	21 (42%)	13 (26%)	5 (10%)		
Femoral, myelofibrosis	13 (26%)	21 (42%)	13 (26%)	9 (18%)	5 (10%)	1 (2%)
Myeloid cell, hyperplasia		1 (2%)				
Sternal, myelofibrosis	42 (84%)	1 (2%)	1 (2%)	11 (22%)	11 (22%)	2 (4%)
Lymph node	(48)	(49)	(48)	(50)	(49)	(47)
Lumbar, hyperplasia, plasma cell			1 (2%)			
Lumbar, infiltration cellular, histiocyte	1 (2%)		1 (2%)			
Mandibular, abscess				1 (2%)		
Mandibular, bacterium				1 (2%)		
Mandibular, hyperplasia, lymphoid		1 (2%)		1 (2%)		
Mandibular, infiltration cellular, histiocyte	1 (2%)					
Mediastinal, hematopoietic cell proliferation		1 (2%)				
Mediastinal, hyperplasia, lymphoid	1 (2%)					
Mediastinal, hyperplasia, plasma cell	1 (2%)					
Mediastinal, infiltration cellular, histiocyte		1 (2%)				
Renal, infiltration cellular, histiocyte				1 (2%)		
Sinus, mandibular, ectasia		2 (4%)	3 (6%)		1 (2%)	
Lymph node, mesenteric	(8)	(21)	(15)	(21)	(23)	(20)
Hematopoietic cell proliferation		2 (10%)	4 (27%)	5 (24%)	8 (35%)	7 (35%)
Hyperplasia, lymphoid	2 (25%)	3 (14%)		5 (24%)	3 (13%)	3 (15%)
Hyperplasia, plasma cell			1 (7%)	2 (10%)		1 (5%)
Infiltration cellular, histiocyte	1 (13%)		1 (7%)	1 (5%)	4 (17%)	
Inflammation, granulomatous					1 (4%)	
Sinus, ectasia	4 (50%)	10 (48%)	7 (47%)	6 (29%)	15 (65%)	13 (65%)
Spleen	(48)	(50)	(50)	(50)	(50)	(50)
Depletion lymphoid					1 (2%)	
Granuloma					1 (2%)	
Hematopoietic cell proliferation	4 (8%)	2 (4%)	9 (18%)	5 (10%)	8 (16%)	23 (46%)
Hyperplasia, lymphoid	14 (29%)	9 (18%)	2 (4%)	3 (6%)	2 (4%)	
Necrosis, acute			1 (2%)			
Pigmentation		2 (4%)				

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Genital System (continued)		
Uterus	(48)	(48)
Pigmentation	1 (2%)	
Endometrium, hyperplasia, cystic	2 (4%)	2 (4%)
Hematopoietic System		
Bone marrow	(49)	(49)
Myeloid cell, hyperplasia		1 (2%)
Lymph node	(42)	(41)
Mandibular, hematopoietic cell proliferation		1 (2%)
Mandibular, hyperplasia, lymphoid	1 (2%)	1 (2%)
Pancreatic, sinus, ectasia	1 (2%)	
Sinus, mandibular, ectasia	1 (2%)	1 (2%)
Lymph node, mesenteric	(7)	(10)
Hematopoietic cell proliferation	2 (29%)	8 (80%)
Infiltration cellular, histiocyte	1 (14%)	
Sinus, ectasia	5 (71%)	2 (20%)
Spleen	(44)	(47)
Hematopoietic cell proliferation	37 (84%)	29 (62%)
Hyperplasia, lymphoid	1 (2%)	
Pigmentation	1 (2%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Hematopoietic System (continued)						
Thymus	(43)	(43)	(31)	(38)	(29)	(27)
Amyloid deposition			1 (3%)			
Atrophy		1 (2%)				
Hyperplasia, lymphoid	1 (2%)					
Integumentary System						
Skin	(49)	(50)	(49)	(49)	(50)	(50)
Acanthosis	31 (63%)	37 (74%)	28 (57%)	44 (90%)	44 (88%)	43 (86%)
Hyperkeratosis	23 (47%)	29 (58%)	28 (57%)	46 (94%)	42 (84%)	46 (92%)
Infiltration cellular, mast cell						1 (2%)
Inflammation, necrotizing		2 (4%)	1 (2%)	1 (2%)	1 (2%)	
Parasite metazoan	24 (49%)	28 (56%)	26 (53%)	43 (88%)	38 (76%)	41 (82%)
Subcutaneous tissue, granuloma	1 (2%)					
Musculoskeletal System						
Bone	(50)	(50)	(49)	(50)	(50)	(50)
Tarsal, hyperostosis		1 (2%)				
Nervous System						
Brain	(50)	(50)	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)				
Infarct	1 (2%)					
Artery, inflammation, chronic			1 (2%)			
Artery, necrosis, fibrinoid			1 (2%)			
Cerebrum, compression				2 (4%)		
Hypothalamus, compression	1 (2%)	1 (2%)	1 (2%)	2 (4%)	3 (6%)	
Peripheral nerve	(1)					
Sciatic, degeneration	1 (100%)					
Respiratory System						
Lung	(50)	(50)	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)		
Alveolar epithelium, hyperplasia						1 (2%)
Alveolus, infiltration cellular, histiocyte					1 (2%)	1 (2%)
Alveolus, inflammation, acute				1 (2%)		
Artery, thrombosis					1 (2%)	2 (4%)
Bronchus, granuloma			1 (2%)			
Interstitium, inflammation, chronic	1 (2%)			1 (2%)	1 (2%)	
Lymphatic, infiltration cellular, polymorphonuclear						1 (2%)
Vein, thrombosis						1 (2%)
Nose	(50)	(50)	(50)	(50)	(49)	(50)
Nerve, inflammation, chronic active				1 (2%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 30 ppm	30 ppm 30 ppm
Hematopoietic System (continued)		
Thymus	(20)	(32)
Ectopic parathyroid gland	1 (5%)	
Integumentary System		
Skin	(49)	(47)
Acanthosis	38 (78%)	30 (64%)
Hyperkeratosis	39 (80%)	33 (70%)
Parasite metazoan	33 (67%)	31 (66%)
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(46)	(44)
Inflammation, chronic		1 (2%)
Alveolus, infiltration cellular, histiocyte	1 (2%)	
Interstitium, inflammation, chronic	1 (2%)	1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	30 ppm 0 ppm	3 ppm 3 ppm	0 ppm 10 ppm	10 ppm 10 ppm	30 ppm 10 ppm
Special Senses System						
Ear					(1)	
Middle ear, abscess					1 (100%)	
Middle ear, bacterium					1 (100%)	
Harderian gland	(6)	(1)	(6)	(2)	(2)	(1)
Atrophy			1 (17%)			
Hyperplasia, nodular	2 (33%)		1 (17%)			1 (100%)
Urinary System						
Kidney	(50)	(50)	(50)	(50)	(50)	(50)
Cyst			1 (2%)			
Infarct		1 (2%)	1 (2%)			
Infiltration cellular, lymphocyte	2 (4%)					
Infiltration cellular, mixed cell	1 (2%)					
Inflammation, chronic					1 (2%)	
Metaplasia, osseous			2 (4%)	1 (2%)		1 (2%)
Mineralization				2 (4%)		
Nephropathy, chronic			3 (6%)	3 (6%)	6 (12%)	5 (10%)
Glomerulus, inflammation, membranoproliferative						2 (4%)
Urinary bladder	(48)	(50)	(49)	(48)	(49)	(49)
Inflammation, acute					1 (2%)	
Inflammation, chronic active				1 (2%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration	0 ppm	30 ppm
F_1 Concentration	30 ppm	30 ppm
Special Senses System		
Harderian gland	(1)	
Hyperplasia, nodular	1 (100%)	
Urinary System		
Kidney	(46)	(49)
Metaplasia, osseous	1 (2%)	
Nephropathy, chronic	16 (35%)	29 (59%)
Glomerulus, inflammation, membranoproliferative	1 (2%)	
Glomerulus, nephropathy, chronic	2 (4%)	
Urinary bladder	(45)	(47)
Parasite metazoan	1 (2%)	

[a] Number of animals with any tissue examined microscopically

APPENDIX E
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE E1
Hematology and Clinical Chemistry Data for Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study of Polybrominated Biphenyls[a]

F_0 Concentration F_1 Concentration	0 ppm 0 ppm	0 ppm 10 ppm	0 ppm 30 ppm	10 ppm 0 ppm	10 ppm 10 ppm	10 ppm 30 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	48.4 ± 0.5	45.7 ± 0.5*	44.1 ± 0.6**	48.1 ± 0.3	46.9 ± 0.7	44.1 ± 0.6**
Hemoglobin (g/dL)	15.4 ± 0.1	14.2 ± 0.1**	13.9 ± 0.1**	15.1 ± 0.1	14.5 ± 0.1*	13.9 ± 0.1**
Erythrocytes ($10^6/\mu L$)	9.39 ± 0.07	9.24 ± 0.10	8.91 ± 0.13*	9.40 ± 0.07	9.26 ± 0.07	9.00 ± 0.09*
Mean cell volume (fL)	51.0 ± 0.3	49.7 ± 0.4	49.6 ± 0.4	51.0 ± 0.3	50.8 ± 0.6	49.3 ± 0.4
Platelets ($10^3/\mu L$)	511.2 ± 21.9	511.4 ± 31.7	487.0 ± 28.7	475.0 ± 12.0	523.4 ± 16.7	497.2 ± 31.5
Reticulocytes ($10^6/\mu L$)	1.23 ± 0.16	1.41 ± 0.16	1.50 ± 0.16	1.34 ± 0.22	1.54 ± 0.24	1.88 ± 0.22
Leukocytes ($10^3/\mu L$)	6.04 ± 0.35	5.78 ± 0.23	5.04 ± 0.26	5.06 ± 0.24	5.58 ± 0.34	4.67 ± 0.21*
Clinical Chemistry						
Cholesterol (mg/dL)	67 ± 2	86 ± 4*	105 ± 3**	69 ± 2	74 ± 3	95 ± 5**
Triglycerides (mg/dL)	124 ± 9	67 ± 4*	57 ± 5**	145 ± 9	50 ± 4**	59 ± 3**
Female						
n	10	10	9	10	10	10
Hematology						
Hematocrit (%)	49.4 ± 0.5	49.4 ± 0.4	47.4 ± 0.9	48.6 ± 0.8	47.7 ± 0.7	44.1 ± 0.9**
Hemoglobin (g/dL)	15.5 ± 0.1	15.3 ± 0.1	14.4 ± 0.3*	15.4 ± 0.2	14.8 ± 0.2	13.7 ± 0.3**
Erythrocytes ($10^6/\mu L$)	8.61 ± 0.10	8.82 ± 0.07	8.38 ± 0.14	8.47 ± 0.14	8.50 ± 0.10	7.88 ± 0.17**
Mean cell volume (fL)	57.5 ± 0.3	56.0 ± 0.4	56.4 ± 0.6	57.4 ± 0.4	55.9 ± 0.4	56.1 ± 0.4
Platelets ($10^3/\mu L$)	458.6 ± 14.9	391.1 ± 31.0	302.7 ± 12.0**	446.3 ± 21.3	383.8 ± 18.9	309.5 ± 20.4**
Reticulocytes ($10^6/\mu L$)	1.48 ± 0.15	1.87 ± 0.26	2.08 ± 0.26	1.40 ± 0.16	1.66 ± 0.17	2.27 ± 0.25
Leukocytes ($10^3/\mu L$)	3.77 ± 0.17	3.78 ± 0.26	3.93 ± 0.31	3.71 ± 0.22	3.72 ± 0.24	4.16 ± 0.65
Clinical Chemistry						
Cholesterol (mg/dL)	92 ± 2	108 ± 2*	118 ± 4**	94 ± 2	106 ± 3	127 ± 4**
Triglycerides (mg/dL)	76 ± 7	72 ± 5	55 ± 5	68 ± 6	57 ± 9	51 ± 5

TABLE E1
Hematology and Clinical Chemistry Data for Rats at the 9-Month Interim Evaluations in the 2-Year Feed Study of Polybrominated Biphenyls (continued)

F_0 Concentration F_1 Concentration	3 ppm 10 ppm	1 ppm 3 ppm
Male		
n	10	10
Hematology		
Hematocrit	45.5 ± 0.4*	47.7 ± 0.3
Hemoglobin (g/dL)	14.3 ± 0.1**	15.1 ± 0.1
Erythrocytes (10^6/μL)	9.14 ± 0.10	9.40 ± 0.09
Mean cell volume (fL)	49.9 ± 0.2	50.6 ± 0.5
Platelets (10^3/μL)	485.8 ± 22.5	509.2 ± 10.5
Reticulocytes (10^6/μL)	2.00 ± 0.23	1.55 ± 0.21
Leukocytes (10^3/μL)	5.48 ± 0.30	5.27 ± 0.15
Clinical Chemistry		
Cholesterol (mg/dL)	72 ± 3	64 ± 2
Triglycerides (mg/dL)	53 ± 4**	92 ± 7
Female		
n	10	10
Hematology		
Hematocrit (%)	48.3 ± 0.6	51.0 ± 0.5
Hemoglobin (g/dL)	15.0 ± 0.2	15.9 ± 0.1
Erythrocytes (10^6/μL)	8.63 ± 0.13	8.87 ± 0.06
Mean cell volume (fL)	56.1 ± 0.5	57.4 ± 0.3
Platelets (10^3/μL)	405.8 ± 22.0	400.0 ± 19.4
Reticulocytes (10^6/μL)	1.59 ± 0.23	1.24 ± 0.21
Leukocytes (10^3/μL)	4.40 ± 0.40	4.11 ± 0.23
Clinical Chemistry		
Cholesterol (mg/dL)	108 ± 4	92 ± 4
Triglycerides (mg/dL)	62 ± 6	81 ± 8

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
** $P \leq 0.01$
[a] Mean ± standard error

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF POLYBROMINATED BIPHENYLS

Polybrominated biphenyls were obtained from Monsanto Corporation (St. Louis, MO) in one lot (FF1312-FT), which was used throughout the studies. Purity and identity analyses were conducted at the study laboratory, Battelle Columbus Laboratories (Columbus, OH). The reports on analyses performed in support of the polybrominated biphenyls studies are on file at the National Institute of Environmental Health Sciences.

The chemical was identified as polybrominated biphenyls by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with those expected for the structure of polybrominated biphenyls (Figures F1 and F2). The infrared spectrum was characteristic of an aromatic compound containing an isolated hydrogen on the ring, and was typical of a highly halogenated substance. The NMR spectrum was characteristic of either two compounds, each containing a single proton, present in nearly equal amounts, or a single compound containing two isolated hydrogens.

The purity of polybrominated biphenyls was determined by elemental analyses, Karl Fischer water analysis (performed at Galbraith Laboratories, Knoxville, TN), ashing to determine inorganic content, gas chromatography, high-performance liquid chromatography (HPLC), and gas chromatography/mass spectroscopy. Gas chromatography was performed with a 3% OV-17 on 100/120 mesh Gas Chrom Q column with two systems:

1) a flame ionization detector (FID), oven temperature of 280° C, with a helium carrier gas at 30 mL/minute with chloroform as a solvent, and
2) an electron capture detector, oven temperature 270° C, with a nitrogen carrier gas at 30 mL/minute with hexane as a solvent.

HPLC was performed with a Lichrosorb RP-18 column and a mobile phase of 90% acetonitrile in water (v/v) at a flow rate of 1 mL/minute. Ultraviolet detection was at 254 nm. Gas chromatography/mass spectroscopy was performed on a 30 m SE-52 glass capillary column with an oven temperature program of 70° to 320° C, at 5° C/minute, with a hydrogen carrier gas at a flow rate of 2 mL/minute.

Elemental analyses for carbon and hydrogen were slightly higher and analysis for bromine was slightly lower than the theoretical values for hexabromobiphenyl. Ashing indicated 1.4% inorganic material. Karl Fischer analysis indicated 0.15% water. Gas chromatographic analysis indicated 9 components with an electron capture detector and 10 components with an FID. HPLC indicated 8 components in the polybrominated biphenyl sample. Gas chromatography/mass spectroscopy indicated 9 components, with the major component identified as a hexa isomer, and detected the presence of biphenyl (Figure F3 and Table F1).

Stability studies performed using gas chromatography with the first system described for the purity analyses indicated that polybrominated biphenyls were stable for 2 weeks at temperatures up to 60° C. Periodic reanalysis of polybrominated biphenyls by spectroscopy and gas chromatography with system 1, but with nitrogen as the carrier gas and hexane as the solvent, indicated no significant deterioration during the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared weekly by mixing the appropriate quantities of polybrominated biphenyls with feed in a Patterson-Kelley twin-shell blender (Table F2). The formulations were stored in plastic-lined tin cans for no longer than 1 week.

Stability and homogeneity studies were conducted by the study laboratory. Feed samples were extracted with hexane and evaporated to dryness, then dissolved in 0.5 mL petroleum ether. The samples were then pushed through a Florisil Sep-Pak column with activated sodium sulfate, and petroleum ether was added. The eluate was evaporated to dryness and the residue was diluted with hexane and analyzed by gas chromatography with system 2 described for the purity analyses. The results of these studies indicated that the formulations were stable for at least 7 days and that they should be stored at or below room temperature. Due to the low concentrations of polybrominated biphenyls in the feed formulations, achieving homogeneous feed blends was difficult. Only after the premix procedure was changed to include the use of a feed flour were homogeneous blends achieved.

Dose formulations of polybrominated biphenyls were analyzed by the study laboratory approximately every 2 months using the system described for the stability studies; 43 of the 56 dose formulations were within 10% of the target concentrations. Results of the dose formulation analyses for the 2-year studies are presented in Table F3.

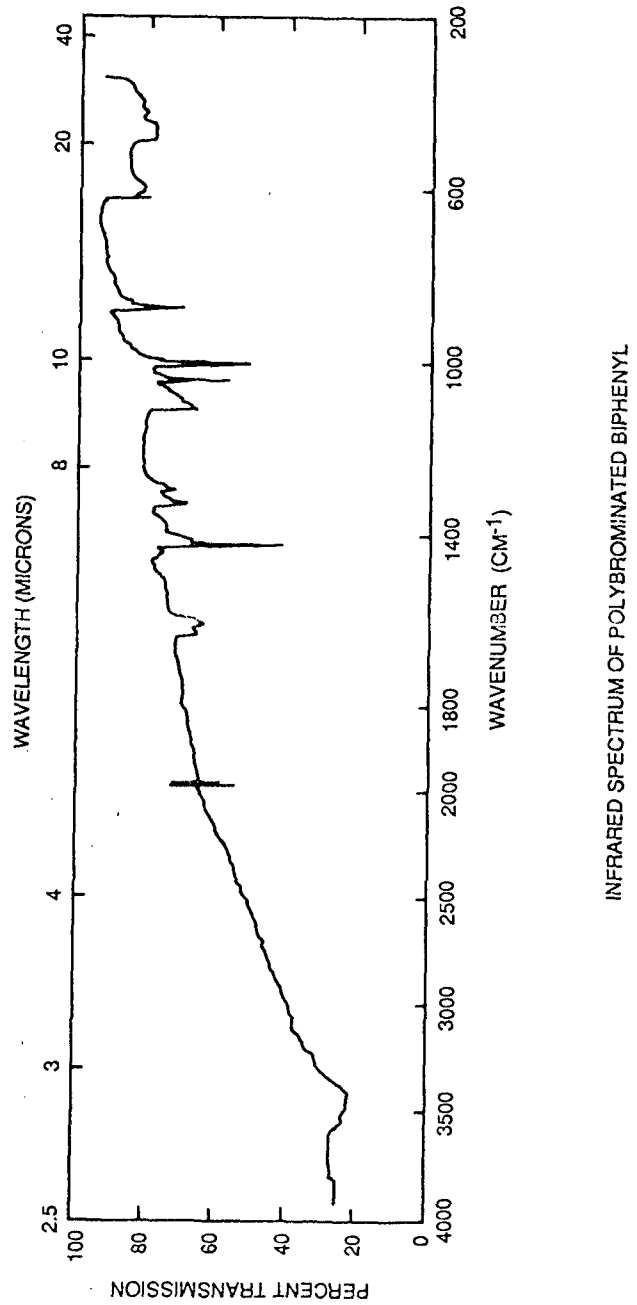


FIGURE F1
Infrared Absorption Spectrum of Polybrominated Biphenyls

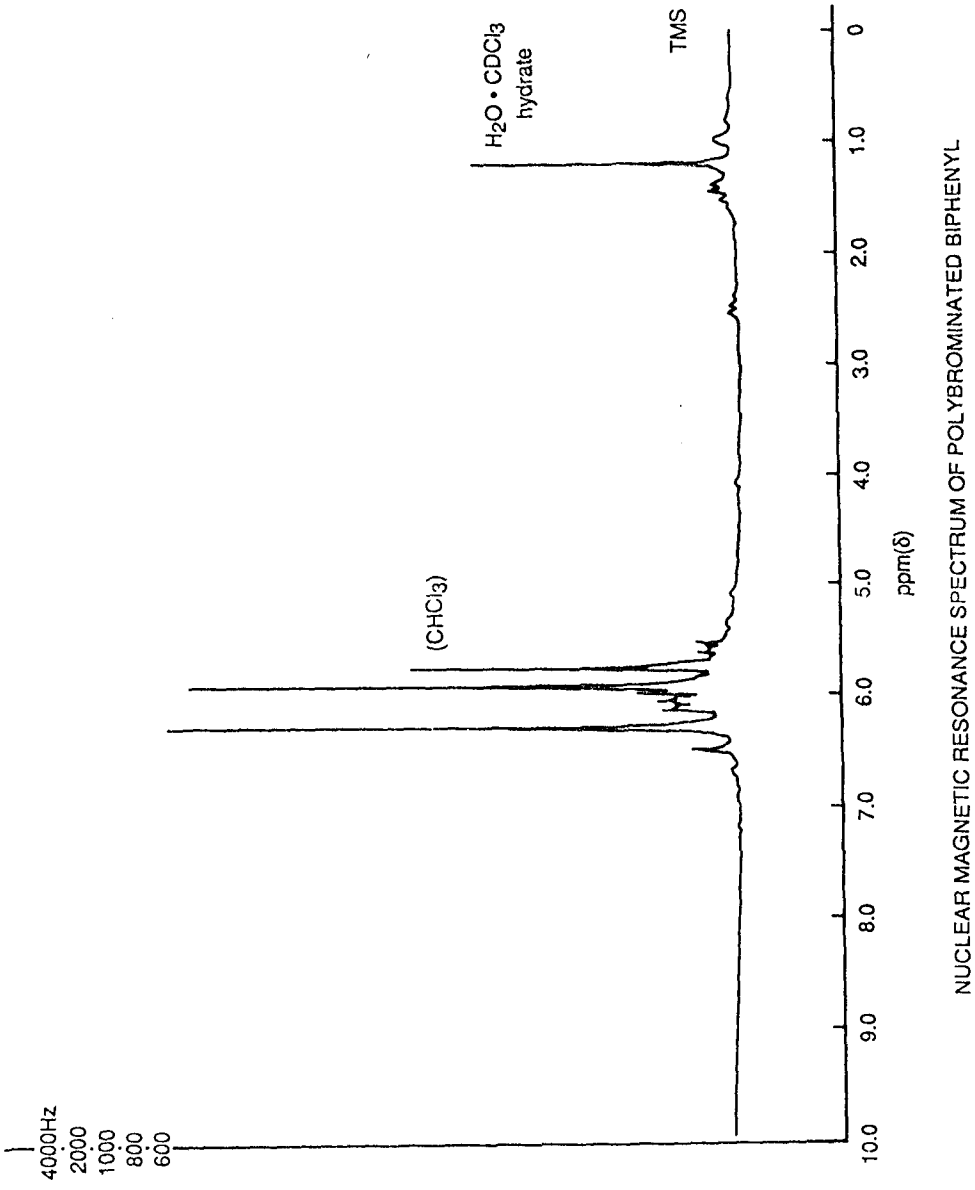
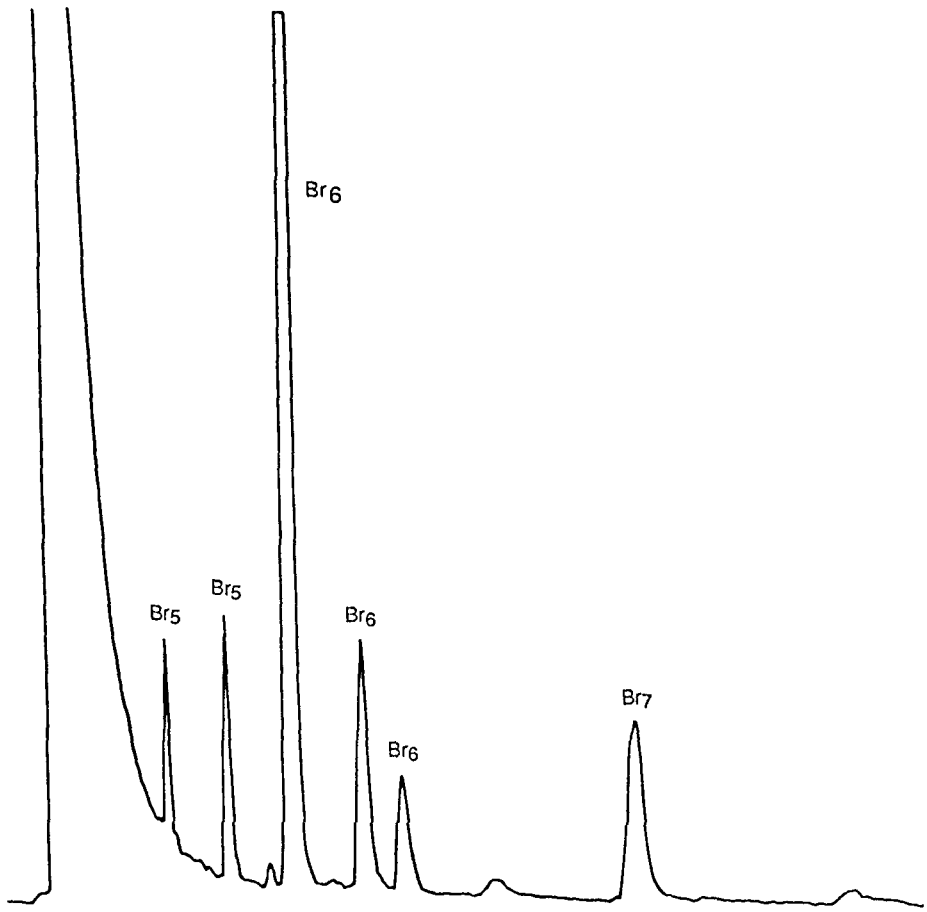


FIGURE F2
Nuclear Magnetic Resonance Spectrum of Polybrominated Biphenyls



CHROMATOGRAM OF POLYBROMINATED BIPHENYL (Br_i i designates number of Br on the biphenyl ring)

FIGURE F3
Gas Chromatogram of Polybrominated Biphenyls

TABLE F1
Gas Chromatography/Mass Spectroscopy Analysis of Polybrominated Biphenyls

Isomer	Order of Chromatographic Elution	Percentage
Pentabromo[a]	1	2–3
Pentabromo	2	4–6
Hexabromo	3	75–80
Hexabromo	4	5–7
Hexabromo	5	2–3
Heptabromo	6	7–9

[a] Substitution pattern could not be determined.

TABLE F2
Preparation and Storage of Dose Formulations in the Feed Studies of Polybrominated Biphenyls

Preparation
A premix with polybrominated biphenyls and feed was prepared by blending with a spatula; the premix and remainder of feed were layered into a Patterson-Kelley twin-shell blender and mixed. The use of feed flour in the premix began on 24 July 1984. Dose formulations were prepared weekly.

Chemical Lot Number
FF1312-FT

Maximum Storage Time
14 days from date of preparation

Storage Conditions
In plastic-lined tin cans, at or below room temperature

Study Laboratory
Battelle Columbus Laboratories, Columbus, OH

TABLE F3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Polybrominated Biphenyls[a]

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[b] (ppm)	% Difference from Target
F_0 Female Mice				
13 December 1982	17 December 1982	3	1.9	–36
		10	5.7	–43
		30	29.2	–3
6 January 1983	13 January 1983	3[c]	1.9	–36
		3[d]	4.3	+42
		3[e]	2.8	–6
		10[c]	12.8	+28
		10[d]	7.1	–29
		10[e]	10.7	+7
		30[c]	25.7	–14
		30[d]	26.2	–13
		30[e]	45.3	+51
3 March 1983	8 March 1983	3	3.6	+20[f]
		10[c]	8.3	–17[f]
		10[d]	9.7	–3[f]
		10[e]	8.9	–11[f]
		30	30.6	+2
	29 March 1983[g]	3	4.2	+38
		10[c]	8.5	–15
		10[d]	10.2	+2
		10[e]	9.8	–2
F_0 Female Rats and Mice				
5 May 1983	11 May 1983	1	0.9	–8[f]
		3	5.9	+98[f]
	19 May 1983[g]	1	1.1	+7
		3	3.0	–1
6 May 1983	11 May 1983	10	9.8	–2[f]
		30	28.8	–4[f]
	19 May 1983[g]	10	8.8	–12
		30	27.6	–8
F_0 Female Rats and F_1 Mice				
24 June 1983	28 June 1983	1	1.1	+7
		3	2.2	–26
		10	9.9	–1
		30	29.0	–3

TABLE F3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Polybrominated Biphenyls (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
F_1 Rats and Mice				
22 August 1983	26 August 1983	10	9.8	–2
	26, 29, 31 August 1983	3[c]	2.9	–3
		3[d]	3.1	+2
		3[e]	3.6	+20
	26, 31 August 1983	30[c]	30.2	+1
		30[d]	28.6	–5
		30[e]	28.7	–4
14 October 1983	21 October 1983	3	2.9	–4
		10	10.5	+5
		30	28.8	–4
16 December 1983	21 December 1983	3	2.8	–6
		10	9.3	–7
		30	29.7	–1
9 February 1984	20 February 1984	3[e]	3.0	–1
		30	27.6	–8
	20, 22 February 1984	3[h]	2.9	–5
		3[i]	4.2	+39
		10	10.8	+8
6 April 1984	12 April 1984	3	3.9	+30
		10	10.5	+5
		30	30.3	+1
	18 April 1984[g]	3	4.1	+35
		10	9.5	–4
1 June 1984	7 June 1984	3	3.0	+1
		10	10.8	+8
		30	30.1	0
20 July 1984	25 July 1984	10	9.3	–7
		30	29.8	–1
24 July 1984	25 July 1984	3[c]	2.8	–7
		3[d]	2.8	–7
		3[e]	2.8	–7
21-25 September 1984	26 September 1984	3	2.8	–5
		10	10.5	+5
		30	32.1	+7
27 November 1984	27 November 1984	3	3.3	+9
		10	9.6	–4
		30	29.6	–1

TABLE F3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Polybrominated Biphenyls (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
14 January 1985	15 January 1985	3	3.3	+10
		10	10.5	+5
		30	31.7	+6
13 May 1985	23-24 May 1985	3	2.5	-17
		10	11.7	+17
		30	30.9	+3
17 June 1985	21 June 1985	10	9.2	-8
	21, 25 June 1985	3	2.6	-15
		30	26.9	-10
6 August 1985	11 August 1985	3	3.0	0
		10	9.8	-2
		30	29.7	-1

[a] Doses for F_0 rats: 0, 1, 3, and 10 ppm. Doses for F_0 mice and F_1 rats and mice: 0, 3, 10, and 30 ppm.
[b] Results of duplicate analyses
[c] Sample taken from upper left section of twin-shell blender
[d] Sample taken from upper right section of twin-shell blender
[e] Sample taken from bottom of twin-shell blender
[f] Reanalyzed due to inhomogeneity of samples or poor linearity of standards
[g] Results of reanalysis
[h] Sample taken from middle of twin-shell blender
[i] Sample taken from top of twin-shell blender

APPENDIX G
ANALYSIS OF TISSUE CONCENTRATIONS OF POLYBROMINATED BIPHENYLS

ANALYSIS OF TISSUE CONCENTRATIONS OF POLYBROMINATED BIPHENYLS

PREPARATION AND ANALYSIS OF TISSUE CONCENTRATIONS OF POLYBROMINATED BIPHENYLS

At the 9-month interim evaluation, the livers of three rats of each sex from the 0:0, 0:30, 10:0, and 10:30 ppm groups and three mice of each sex from the 0:0, 0:30, 30:0, and 30:30 ppm groups were excised, weighed, and frozen. The tissues were stored at −15° to −20° C until analysis for polybrominated biphenyl concentrations.

At the time of analysis, the livers from the three animals from each group were combined, passed through a hand tissue press, and homogenized with four parts by weight 0.9% sodium chloride solution. Aliquots of the pooled liver homogenates were extracted with hexane and separated by centrifuging, then the organic layer was evaporated to approximately 2 mL. The extraction and separation was repeated twice, then the hexane phases were combined, diluted with hexane, and passed through a Florisil Sep-Pak (Waters Associates, ME). Samples were then evaporated to a volume of 1 mL. Analysis of polybrominated biphenyl concentrations was conducted using gas chromatography with a 3% OV-17 on 100/120 mesh Chromosorb W-HP with electron capture detection, with a nitrogen carrier gas at 30 mL/minute. The oven temperature was 280° C. Results are given in Tables G1 and G2.

TABLE G1
Tissue Concentrations of Polybrominated Biphenyls in Rats at the 9-Month Interim Evaluation in the 2-Year Feed Study

F_0:F_1 Dose Concentration	Male Rats		Female Rats	
	Pooled Weights[a]	Tissue Concentration[b]	Pooled Weights	Tissue Concentration
0:0	23.96	1.23	11.72	1.24
0:30	32.99	111.65	15.69	78.58
10:0	31.69	0.89	10.72	2.93
10:30	36.12	138.39	12.58	79.03

[a] Pooled weight of three livers
[b] Average of duplicate analyses

TABLE G2
Tissue Concentrations of Polybrominated Biphenyls in Mice at the 9-Month Interim Evaluation in the 2-Year Feed Study

F_0:F_1 Dose Concentration	Male Mice		Female Mice	
	Pooled Weights[a]	Tissue Concentration[b]	Pooled Weights	Tissue Concentration
0:0	1.64	0	1.45	0
0:30	4.34	248.27	4.55	290.66
30:0	2.15	24.57	1.68	22.33
30:30	4.48	246.89	7.91	246.47

[a] Pooled weight of three livers
[b] Average of duplicate analyses

APPENDIX H
FEED CONSUMPTION

TABLE H1
Feed Consumption by Male Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

Month	0:0 ppm	10:0 ppm	1:3 ppm	0:10 ppm	3:10 ppm	10:10 ppm	0:30 ppm	10:30 ppm
1	15.4	14.8	15.4	15.3	16.0	15.7	15.5	15.9
2	14.2	14.5	15.2	16.3	16.8	17.2	16.8	15.8
3	16.7	17.8	14.8	15.4	15.8	15.0	14.7	14.7
4	16.6	16.3	16.6	15.4	16.5	17.1	15.3	17.1
5	15.7	16.3	14.1	15.9	16.3	16.3	14.0	16.1
6	17.9	17.2	16.5	17.4	18.9	17.3	16.3	15.8
7	17.3	18.2	20.7	18.1	17.9	17.7	17.8	15.9
8	16.4	16.7	15.3	16.3	16.5	16.6	16.0	16.3
9	17.3	18.0	17.2	18.2	17.8	17.7	17.1	17.1
10	17.1	18.3	16.4	16.9	16.3	17.4	16.1	16.5
11	17.0	16.8	16.8	17.2	17.3	17.0	16.5	15.9
12	17.7	19.0	17.0	17.2	17.3	17.2	16.9	16.5
13	16.2	16.7	16.9	16.4	16.8	16.5	16.4	16.0
14	15.4	17.4	17.5	17.6	16.8	17.2	16.4	16.4
15	16.8	17.3	17.0	17.5	18.6	17.6	16.6	16.3
16	15.6	15.8	16.0	15.8	16.1	16.3	15.4	14.9
17	16.0	17.3	15.5	15.1	16.1	15.0	14.8	15.3
18	15.7	16.0	15.9	15.7	16.8	16.1	15.6	15.7
19	16.3	16.2	16.1	16.5	17.1	16.4	16.0	14.7
20	16.2	17.4	15.4	15.4	16.5	16.3	15.9	15.4
21	15.9	16.5	15.8	16.8	17.0	16.3	15.6	14.5
22	16.0	17.8	15.3	15.8	15.8	16.3	16.2	15.9
23	15.2	16.5	15.4	15.6	17.3	17.9	17.1	14.5
24	15.7	16.7	16.8	16.7	16.6	16.5	16.6	17.4

[a] Feed consumption is given as grams of feed consumed per animal per day.

TABLE H2
Feed Consumption by Female Rats in the 2-Year Feed Study of Polybrominated Biphenyls[a]

Month	0:0 ppm	10:0 ppm	1:3 ppm	0:10 ppm	3:10 ppm	10:10 ppm	0:30 ppm	10:30 ppm
1	8.7	9.7	9.9	10.3	10.6	10.0	9.3	10.0
2	9.4	8.8	9.4	9.2	9.1	8.9	9.1	9.2
3	8.9	9.0	8.7	8.5	8.3	8.6	8.3	8.4
4	9.3	9.2	8.6	8.7	9.1	8.9	8.9	8.7
5	8.8	8.7	7.8	7.2	7.5	6.9	7.0	7.1
6	9.0	9.1	8.5	7.7	7.3	8.6	8.0	8.5
7	8.2	8.5	8.3	8.0	8.8	8.8	8.2	8.4
8	9.6	9.8	8.6	10.0	10.3	10.1	9.0	9.1
9	9.4	10.0	10.0	10.0	9.9	10.2	9.2	9.4
10	9.2	8.6	10.1	8.8	9.2	8.8	8.3	8.6
11	10.0	10.7	10.0	10.7	11.0	10.9	9.9	9.7
12	12.6	13.2	12.3	11.3	11.6	10.8	10.7	10.4
13	9.6	9.9	9.6	10.1	10.7	11.3	10.2	10.0
14	13.5	13.4	11.9	12.5	13.1	12.2	9.5	9.6
15	11.1	11.9	10.4	9.6	10.4	9.4	10.0	9.9
16	10.2	10.3	10.5	10.6	10.7	10.8	9.3	9.5
17	11.3	11.8	11.4	11.3	11.3	11.5	10.2	10.3
18	11.5	11.7	11.6	11.6	12.0	11.9	12.4	12.0
19	11.6	11.3	11.5	11.6	12.0	11.8	10.4	10.8
20	11.7	11.1	11.5	12.3	12.0	11.9	12.1	11.5
21	11.7	12.6	11.9	12.0	12.2	11.3	10.3	11.1
22	11.3	11.6	11.3	11.2	11.6	12.1	10.2	10.5
23	12.3	12.3	11.9	11.9	11.9	11.5	11.1	10.8
24	12.9	12.2	11.9	13.2	13.0	12.3	11.8	11.7

[a] Feed consumption is given as grams of feed consumed per animal per day.

TABLE H3
Feed Consumption by Male Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

Month	0:0 ppm	30:0 ppm	3:3 ppm	0:10 ppm	10:10 ppm	30:10 ppm	0:30 ppm	30:30 ppm
1	5.3	5.5	5.6	5.2	5.6	5.5	5.4	5.9
2	5.1	4.8	5.2	5.3	5.2	5.3	5.2	5.5
3	5.6	5.5	6.0	5.4	5.5	5.5	5.8	6.1
4	5.9	5.9	6.9	6.3	6.4	6.3	6.6	6.9
5	4.2	5.1	5.6	5.1	5.1	5.2	6.2	5.7
6	5.0	4.8	5.3	4.6	4.8	5.0	5.1	6.0
7	5.3	5.2	5.8	5.4	5.4	5.4	5.4	6.2
8	5.3	5.4	6.0	5.9	5.7	5.6	5.5	6.5
9	5.0	4.9	5.0	5.3	4.7	5.0	4.8	5.5
10	5.8	5.5	5.9	5.6	5.7	5.6	5.6	6.2
11	4.4	4.2	4.7	4.2	4.5	4.5	4.3	4.6
12	4.9	4.8	4.9	4.9	4.6	4.5	4.7	5.5
13	4.5	4.5	4.5	4.3	4.4	4.3	4.4	5.0
14	4.5	4.6	4.8	4.3	4.4	4.5	4.6	5.5
15	4.5	4.5	4.6	4.4	4.4	4.5	4.7	5.5
16	4.3	4.6	5.0	4.5	4.5	4.7	5.1	5.5
17	4.4	4.6	5.2	4.6	5.1	4.6	5.1	6.2
18	4.5	4.4	4.6	4.4	4.5	4.7	5.9	6.6
19	5.0	4.8	4.9	5.0	5.0	5.0	7.5	7.6
20	4.6	4.9	4.7	5.4	4.8	4.8	6.2	5.7
21	4.6	4.8	4.7	5.0	4.9	5.3	6.1	–[b]
22	4.8	4.8	4.8	5.0	5.2	5.5	6.3	–
23	4.7	4.8	4.7	5.2	5.2	5.5	7.4	–
24	4.9	5.1	4.8	5.2	5.6	5.5	–	–

[a] Feed consumption is given as grams of feed consumed per animal per day.
[b] No data due to 100% mortality in this group.

TABLE H4
Feed Consumption by Female Mice in the 2-Year Feed Study of Polybrominated Biphenyls[a]

Month	0:0 ppm	30:0 ppm	3:3 ppm	0:10 ppm	10:10 ppm	30:10 ppm	0:30 ppm	30:30 ppm
1	5.8	5.6	6.1	6.1	6.3	6.3	6.1	6.2
2	4.2	4.0	4.6	4.9	4.7	4.2	4.8	4.8
3	5.3	4.5	5.4	5.4	5.1	5.1	5.3	5.0
4	7.0	7.2	7.4	7.5	7.5	7.1	7.6	7.3
5	5.1	5.0	5.6	4.9	5.5	5.4	5.5	4.9
6	5.3	4.4	4.6	5.9	6.5	5.8	6.6	7.0
7	5.9	4.8	5.5	6.2	6.2	6.2	7.0	6.8
8	6.7	5.8	6.0	6.3	6.6	6.2	6.6	6.6
9	5.2	4.6	5.1	5.6	5.2	4.8	5.6	5.7
10	6.2	5.6	6.4	6.5	5.8	5.1	6.8	7.0
11	3.9	3.9	4.0	4.2	4.4	4.1	4.6	4.6
12	4.8	4.4	4.2	4.3	4.8	4.6	4.8	5.4
13	4.3	4.1	4.2	4.1	4.5	4.5	4.8	4.9
14	4.5	4.0	4.3	3.9	4.2	4.2	5.2	5.0
15	4.6	4.3	4.4	4.0	4.2	4.5	4.3	5.1
16	4.2	4.0	4.1	4.0	4.1	4.5	5.5	5.2
17	4.4	4.3	4.4	4.8	5.0	5.2	6.7	6.7
18	4.5	4.3	4.3	4.6	5.0	5.1	6.9	7.3
19	4.7	4.6	5.0	5.0	5.5	5.7	7.5	7.0
20	4.3	4.1	4.5	4.5	4.6	5.4	5.9	6.6
21	4.9	4.4	5.0	4.9	5.0	5.7	6.9	–[b]
22	4.7	4.7	5.3	5.0	5.5	5.9	6.4	–
23	4.7	4.7	5.0	4.9	5.6	6.0	–	–
24	5.1	5.0	5.4	5.2	5.8	6.2	–	–

[a] Feed consumption is given as grams of feed consumed per animal per day.
[b] No data due to 100% mortality in this group.

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These 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 chemical compounds.

Rats

During the 2-year studies, 30 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Ten animals of each designated sentinel group were killed at 6, 12, and 18 months into the study. Samples for viral screening at 24 months were collected from 10 diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Incorporated (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
ELISA	
Mycoplasma arthritidis	18 and 24 months
Mycoplasma pulmonis	18 and 24 months
PVM (pneumonia virus of mice)	18 and 24 months
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	6, 12, 18 and 24 months
Sendai	18 and 24 months
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	6, 12, 18, and 24 months
KRV (Kilham rat virus)	6, 12, 18, and 24 months
PVM	6 and 12 months
Sendai	6 and 12 months

Mice

During the 2-year studies, 30 B6C3F$_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Ten animals of each designated sentinel group were killed at 6, 12, and 18 months into the study. Samples for viral screening at 24 months were collected from 10 diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Incorporated for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
Mouse adenoma virus (mouse pox)	6 and 12 months
LCM (lymphocytic choriomeningitis virus)	6, 12, 18, and 24 months
ELISA	
Ectromelia virus	18 and 24 months
GDVII (mouse encephalomyelitis virus)	12, 18 and 24 months
MHV (mouse hepatitis virus)	6, 12, 18, and 24 months
Mouse adenoma virus	18 and 24 months
M. arthritidis	18 and 24 months
M. pulmonis	18 and 24 months
PVM	18 and 24 months
Reovirus 3	18 and 24 months
Sendai	18 and 24 months
Hemagglutination Inhibition	
Ectromelia virus	6 and 12 months
GDVII	6 months
K (papovavirus)	24 months
MVM (minute virus of mice)	6, 12, 18, and 24 months
Polyoma virus	6, 12, 18, and 24 months
PVM	6 and 12 months
Reovirus 3	6 and 12 months
Sendai	6 and 12 months
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	18 and 24 months

Test results are presented in Table I1.

TABLE I1
Murine Virus Antibody Determinations for Rats and Mice in the 2-Year Feed Studies of Polybrominated Biphenyls

	Interval (months)	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Rats			
	6	0/20	None positive
	12	0/20	None positive
	18	6/20	*M. arthritidis*
	24	1/20	*M. arthritidis*
Mice			
	6	20/20	MHV
	12	20/20	GDVII
		11/20	MHV
	18	14/19	EDIM
		18/19	GDVII
		19/19	MHV
	24	11/16	EDIM
		15/16	GDVII
		16/16	MHV

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF AUGUST 1993

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-Ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-Methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4′-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosponium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Pariffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PRINTED AS OF AUGUST 1993 (CONT.)

TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene

TR No.	CHEMICAL
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3′-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4-Diamino-2,2-Stilbenedisulfonic Acid
413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
416	*o*-Nitroanisole
417	*p*-Nitrophenol
418	*p*-Nitroaniline
419	HC Hellow 4
434	1,3-Butadiene

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NIH Publication No. 93-2853
August 1993