NTP TECHNICAL REPORT

ON THE

TOXICOLOGY STUDIES OF A PENTABROMODIPHENYL ETHER MIXTURE [DE-71 (TECHNICAL GRADE)]

(CAS NO. 32534-81-9)

IN F344/N RATS AND B6C3F1/N MICE

AND

TOXICOLOGY AND CARCINOGENESIS STUDIES OF A PENTABROMODIPHENYL ETHER MIXTURE [DE-71 (TECHNICAL GRADE)] IN WISTAR HAN [Crl:WI(Han)] RATS AND B6C3F1/N MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

February 2016

NTP TR 589

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

FOREWORD

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

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

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

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY STUDIES OF A PENTABROMODIPHENYL ETHER MIXTURE [DE-71 (TECHNICAL GRADE)]

(CAS NO. 32534-81-9)

IN F344/N RATS AND B6C3F1/N MICE AND TOXICOLOGY AND CARCINOGENESIS STUDIES OF A PENTABROMODIPHENYL ETHER MIXTURE [DE-71 (TECHNICAL GRADE)] IN WISTAR HAN [Crl:WI(Han)] RATS AND B6C3F1/N MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

February 2016

NTP TR 589

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

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

J.K. Dunnick, Ph.D., Study Scientist
A.E. Brix, D.V.M., Ph.D., Study Pathologist
Experimental Pathology Laboratories, Inc.
C.R. Blystone, Ph.D.
H.C. Cunny, Ph.D.
M.J. Devito, Ph.D.
P.M. Foster, Ph.D.
D.R. Germolec, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
M.J. Hooth, Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
B.S. McIntyre, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
B.A. Merrick, Ph.D.
A.R. Pandiri, B.V.Sc. & A.H., Ph.D.
J.M. Sanders, Ph.D.
S.L. Smith-Roe, Ph.D.
M.D. Stout, Ph.D.
G.S. Travlos, D.V.M.
M.K. Vallant, B.S., M.T.
S. Waidyanatha, Ph.D.
N.J. Walker, Ph.D.
K.L. Witt, M.S.

Southern Research Institute

Conducted studies and evaluated pathology findings

C.D. Hébert, Ph.D., Principal Investigator
R. Fulton, D.V.M., Ph.D.
S.D. Grimes, D.V.M., Ph.D.
G. Lathrop, Jr., D.V.M., M.S.
J.F. Mann, D.V.M.
J. Toomey, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
A.E. Brix, D.V.M., Ph.D.
M.M. Gruebbel, D.V.M., Ph.D.
R.R. Moore, D.V.M.

Gene Logic Laboratories, Inc.

Provided SCVCE analysis

B.J.T. Muir, Ph.D., Principal Investigator
B. Atkinson, M.Sc.

Dynamac Corporation

Prepared quality assessment audits

S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

NTP Pathology Working Group

Evaluated slides and contributed to pathology report on 2-year rats (November 19 & 21, 2013)

C.L. Johnson, D.V.M., Coordinator
Pathology Associates International, A Charles River Company
A.E. Brix, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.F. Cesta, D.V.M., Ph.D.
National Toxicology Program
S.A. Chandra, D.V.M., Ph.D.
GlaxoSmithKline
J.M. Cullen, V.M.D., Ph.D.
North Carolina State University
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
M.J. Hoenerhoff, D.V.M., Ph.D.
National Toxicology Program
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
R.R. Moore, D.V.M.
Experimental Pathology Laboratories, Inc.

NTP Pathology Working Group

Evaluated slides and contributed to pathology report on 2-year mice (December 17 & 19, 2013)

S.A. Hayes, D.V.M., Ph.D., Co-Coordinator
Pathology Associates International, A Charles River Company
J.P. Morrison, D.V.M., Co-Coordinator
Pathology Associates International, A Charles River Company
A.E. Brix, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.F. Cesta, D.V.M., Ph.D.
National Toxicology Program
J.M. Cullen, V.M.D.
North Carolina State University.
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
M.M. Gruebbel, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.J. Hoenerhoff, D.V.M., Ph.D.
National Toxicology Program
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
R.R. Moore, D.V.M.
Experimental Pathology Laboratories, Inc.
T. Osborne, D.V.M., Ph.D.
National Toxicology Program
E.M. Quist, D.V.M., M.S.
National Toxicology Program

Social & Scientific Systems, Inc.

Provided statistical analyses

M.V. Smith, Ph.D., Principal Investigator
L.J. Betz, M.S.
S.F. Harris, B.S.

Biotechnical Services, Inc.

Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
B.F. Hall, M.S.
L.M. Harper, B.S.
P.C. Nader, B.S.E.
E.S. Rathman, M.S.
D.C. Serbus, Ph.D.

CONTENTS

SUMMARY

Background

DE-71 (technical grade), a pentabromodiphenyl ether mixture, was used in the past as an additive flame retardant, often in furniture. We studied the effects of DE-71 on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We deposited solutions containing DE-71 in corn oil through a tube directly into the stomach to groups of 50 male and 50 female rats and mice 5 days per week for 2 years. Exposed rats received either 3, 15, or 50 milligrams (mg) of DE-71 per kilogram (kg) of body weight as *in utero*, postnatal, and adult exposure. Exposed mice received either 3, 30, or 100 mg/kg of body weight. Control animals received corn oil with no chemical added by the same method. At the end of the study, tissues from more than 40 sites were examined for every animal.

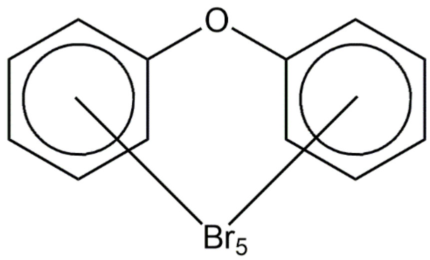
Results

Treated male and female rats and mice had liver cancer at the two highest dose levels. In male rats, there were also significant increases in thyroid gland tumors and pituitary gland tumors. In female rats, there were also a few benign uterine tumors that might have been related to treatment.

Conclusions

We conclude that DE-71 caused liver cancers in male and female rats and mice. Occurrences of thyroid gland and pituitary gland tumors in male rats were also considered to be related to treatment. Occurrences of uterine tumors in female rats may also have been related to exposure to DE-71.

ABSTRACT



Pentabromodiphenyl Ether Mixture

DE-71 (Technical Grade)

CAS No. 32534-81-9

Chemical Formula: $C_{12}H_5Br_5O$ Molecular Weight: 564.7

DE-71, a pentabromodiphenyl ether mixture, was used in the past as an additive flame retardant, often in furniture materials. Additive flame retardants are mixed into products, but they are not covalently bound to the polymers in the commercial products, and thus can leach out into the environment. Though use and sale of polybrominated diphenyl ethers (PBDEs) was banned in the European Union and production was voluntarily phased out in the United States around 2004, they remain in the environment as products produced before use was discontinued or as discarded products. PBDEs can be found in water, wildlife, and in humans, as well as in various food products including meat, poultry, and fish. The California Office of Environmental Health Hazard Assessment nominated individual PBDE congeners for study because they were considered a health risk and have been found in human and animal tissue in the United States. Because of limited availability of the individual PBDE congeners, DE-71, the flame retardant used in furniture, was evaluated in rats and mice to characterize the toxic and carcinogenic potential of PBDEs. Male and female F344/N rats and B6C3F1/N mice were administered DE-71 in corn oil by gavage for 3 months. Wistar Han [Crl:WI(Han)] dams (referred to as Wistar Han rats) were administered DE-71 in corn oil by gavage from gestational day (GD) 6 through postnatal day (PND) 20. Their pups were administered the same doses in corn oil by gavage from PND 12 through 2 years. Male and female B6C3F1/N mice were administered DE-71 in corn oil by gavage for 2 years. Genetic toxicology studies of DE-71 as well as three individual PBDEs were conducted in *Salmonella typhimurium* and *Escherichia coli*, mouse bone marrow cells, and mouse peripheral blood erythrocytes.

3-MONTH STUDY IN F344/N RATS

Groups of 10 male and 10 female rats were administered 0, 0.01, 5, 50, 100, or 500 mg DE-71/kg body weight in corn oil by gavage 5 days per week for 14 weeks. Groups of 10 male and 10 female special study rats were administered the same doses for 25 days. All rats survived to the end of the study. Mean body weights of 500 mg/kg males and females and 100 mg/kg females were significantly less than those of the vehicle controls.

Dose-related decreases in serum thyroxine (T_4) concentration occurred on days 4, 25, and 93 in males and females administered 5 mg/kg or greater. The decreases in T_4 were accompanied by increases in serum thyroid stimulating hormone concentrations, which occurred most consistently in the 100 and 500 mg/kg groups at 14 weeks. Serum cholesterol concentrations demonstrated dose-related increases at all time points in males and females administered 50 mg/kg or greater; the 0.01 and 5 mg/kg groups demonstrated an increase in cholesterol concentration at one or more time points.

At week 14, a small decrease in the circulating red cell mass, evidenced by decreases in hematocrit values and hemoglobin concentrations, occurred in 100 and 500 mg/kg males and females.

Absolute and relative liver weights of males and females administered 5 mg/kg or greater were significantly increased. Absolute and relative kidney weights were significantly greater than those of the vehicle controls in the 50, 100, and 500 mg/kg male groups. In females, absolute kidney weights were significantly increased in the groups administered 5 mg/kg or greater. Relative kidney weights were significantly greater than those of the vehicle control in all dosed groups of females. The absolute thymus weight in 500 mg/kg males and absolute and relative thymus weights in females administered 50 mg/kg or greater were significantly decreased.

In the liver, uridine diphosphate glucuronosyl transferase (UDPGT) activities were significantly increased in male rats administered 0.01 mg/kg on day 25 and in male and female rats administered 5 mg/kg or greater on day 25 and at week 14. 7-Ethoxyresorufin-*O*-deethylase (EROD) activities on day 25 displayed generally dose-related increases and significant increases were observed in males and females administered 5 mg/kg or greater. By week 14, EROD activity in 500 mg/kg males was induced approximately 105-fold, while in 500 mg/kg females, it was induced approximately 209-fold. Significant but smaller increases were observed in 50 and 100 mg/kg males and females administered 5 mg/kg or greater. On day 25, acetanilide-4-hydroxylase (A4H) activities were significantly increased in male rats administered 50 mg/kg or greater and in female rats administered 5 mg/kg or greater. At week 14, significant dose-related increases were observed in both male and female rats administered 5 mg/kg or greater. 7-Pentoxyresorufin-*O*-dealkylase (PROD) activities were increased in male and female rats administered 5 mg/kg or greater on day 25 and at week 14.

In the liver, there were significantly increased incidences of hepatocyte hypertrophy in males and females administered 5 mg/kg or greater. The incidences of cytoplasmic vacuolization of the hepatocytes were significantly increased in 50 mg/kg males and 100 and 500 mg/kg males and females. There were significantly increased incidences of thyroid gland follicle hypertrophy in females administered 50 mg/kg or greater and in 500 mg/kg males. In the 500 mg/kg groups, there were significantly increased incidences of epididymis hypospermia and glandular stomach erosion in males and thymus atrophy in females.

Epididymis and cauda epididymis weights were significantly decreased in 500 mg/kg males. The 500 mg/kg group also exhibited significantly decreased sperm per cauda and sperm per gram of cauda. In general, dosed males exhibited fewer total spermatids per testis and sperm per gram of testis were significantly decreased in the 100 and 500 mg/kg groups. Sperm motility was significantly decreased in the 500 mg/kg group. All 500 mg/kg females failed to cycle and remained in persistent diestrus throughout the examination period. Based on these findings, DE-71 exhibits the potential to be a reproductive toxicant in both male and female rats.

In males and females administered 5 mg/kg or greater, the concentrations of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153) in adipose and liver increased with increasing dose on day 25 and at week 14. The concentrations in adipose were higher than in liver suggesting preferential accumulation in the adipose. BDE-47 and BDE-99 concentrations in adipose were similar and were higher than the BDE-153 concentrations in both sexes; however, BDE-47, BDE-99, and BDE-153 concentrations were similar in the liver. Although there were no differences in BDE-153 concentrations on day 25 or at week 14 in the liver, BDE-47 and BDE-99 concentrations at week 14 were lower than on day 25, suggesting that BDE-47 and BDE-99 induce their own metabolism.

3-Month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 0.01, 5, 50, 100, or 500 mg DE-71/kg body weight in corn oil by gavage 5 days per week for 14 weeks. Survival of the 500 mg/kg groups was decreased. Mean body weights were significantly decreased in 100 and 500 mg/kg males and 500 mg/kg females.

For the surviving 500 mg/kg male and female mice, a small decrease in the circulating red cell mass, evidenced by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts, was observed.

The absolute and relative liver weights of 50 mg/kg males and 100 and 500 mg/kg males and females were significantly greater than those of the vehicle controls. The absolute kidney weight of 500 mg/kg males was significantly less (26%) than that of the vehicle controls. The absolute heart weights of 500 mg/kg males and females were significantly less (15% and 17%, respectively) than those of the vehicle controls. The absolute testis weight of 500 mg/kg males was significantly less than that of the vehicle controls. Males administered 100 mg/kg displayed significantly decreased left cauda epididymis weight and sperm motility, indicating that DE-71 exhibits the potential to be a reproductive toxicant in male mice.

UDPGT activities in the liver were significantly increased in all dosed groups of females. EROD activities were significantly increased in females administered 5 mg/kg or greater. A4H activities were significantly increased in males administered 50 mg/kg or greater, and in females administered 5 mg/kg or greater. PROD activities were significantly increased in male and female mice administered 5 mg/kg or greater.

In the liver, there were significantly increased incidences of hepatocyte hypertrophy in males administered 50 mg/kg or greater and in 100 and 500 mg/kg females. There were also significantly increased incidences of hepatocyte necrosis in 500 mg/kg males and females and hepatocyte cytoplasmic vacuolization in 500 mg/kg males. In the adrenal cortex, there were significantly increased incidences of fatty degeneration and hypertrophy of the zona fasciculata in males administered 500 mg/kg. There was a significantly increased incidence of atrophy of the thymus in 500 mg/kg males. In the testis, the incidence of abnormal residual bodies was significantly increased in males administered 500 mg/kg.

In male mice, concentrations of BDE-47, BDE-99, and BDE-153 in adipose increased linearly with dose up to 100 mg/kg, above which the increase in concentrations was more than proportional to the dose, indicating saturation of metabolism at or above 500 mg/kg. In females, the concentrations of all congeners increased proportionally with the dose. In general, the concentrations of BDE-99 were higher than those of the other two congeners; the concentrations of BDE-47 and BDE-153 were similar (except in 500 mg/kg males) suggesting a higher rate of accumulation of BDE-153 regardless of the lower percentage of BDE-153 in DE-71.

2-Year Study in Wistar Han Rats

Groups of 62 time-mated F_0 female rats were administered 0 or 50 mg DE-71/kg body weight in corn oil by gavage and groups of 52 time-mated F_0 female rats were administered 3 or 15 mg/kg daily from GD 6 until PND 20. F_1 offspring were administered the same doses as their dams by gavage starting on PND 12 until 105 weeks after weaning. Weaning occurred on the day the last litter reached PND 21. At weaning, litters were randomly standardized to two male and two female offspring, and groups of 60 males and 60 females (0 and 50 mg/kg) or 50 males and 50 females (3 and 15 mg/kg) were assigned to the 2-year study and dosed 5 days per week for the remainder of the study. Ten vehicle control and 10 50 mg/kg rats of each sex were evaluated at 3 months to allow comparison to 3-month endpoints in F344/N rats.

Administration of DE-71 had no biologically relevant effect on survival or body weights of pups or dams and no effects on the percentage of mated females producing pups, litter size, pup sex distribution, or weights of dams or male or female pups.

In the 2-year study, survival of 50 mg/kg males was significantly less than that of the vehicle controls. Mean body weights of dosed males were similar to those of the vehicle controls throughout the study. In 50 mg/kg females, there were increased incidences of thinness and the mean body weights were at least 10% less than those of the vehicle controls after week 37.

At the 3-month interim evaluation, organ weights were measured in vehicle control and 50 mg/kg rats. The absolute and relative liver weights of 50 mg/kg males and females were significantly greater than those of the vehicle controls. The absolute and relative kidney and absolute testis weights of 50 mg/kg males were significantly increased. The absolute thymus weight of 50 mg/kg females was significantly decreased.

In the liver at the 3-month interim evaluation, the incidences of hepatocyte hypertrophy were significantly increased in 50 mg/kg males and females. The incidence of fatty change was significantly increased in 50 mg/kg males. In the 2-year study, the incidences of liver neoplasms occurred with positive trends in males and females. The incidences of hepatocellular adenoma or carcinoma (combined) and hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (combined) were significantly increased in males and females administered 50 mg/kg. The incidences of hepatocholangioma, hepatocellular adenoma, and hepatocellular carcinoma were significantly increased in 50 mg/kg females. Cholangiocarcinoma occurred in two 50 mg/kg females. There was a significantly increased incidence of nodular hyperplasia in 50 mg/kg females. There were significantly increased incidences of eosinophilic focus and fatty change in 15 and 50 mg/kg male and female rats. There were significantly increased incidences of hepatocyte hypertrophy in all dosed groups of male and female rats. In 50 mg/kg females, there was a significantly increased incidence of oval cell hyperplasia.

In the thyroid gland at the 3-month interim evaluation, there were significantly increased incidences of follicle hypertrophy in 50 mg/kg males and females. At 2 years, there were increased incidences of follicular cell adenoma in 50 mg/kg males. Follicular cell carcinoma occurred in two 3 mg/kg males and one 15 mg/kg male. The incidence of follicular cell hyperplasia was significantly increased in 50 mg/kg females. There were significantly increased incidences of follicle hypertrophy in all dosed groups of males and in 15 and 50 mg/kg females.

At 2 years, there was a significantly increased incidence of adenoma in the pars distalis of the pituitary gland in 50 mg/kg males.

Uteri from the 2-year groups were examined both in an original cross sectional evaluation and in an additional residual longitudinal section evaluation. There were significantly increased incidences of stromal polyp or stromal sarcoma combined in 3 and 15 mg/kg females when both evaluations were combined. The occurrence of two polyps (multiple) in the vagina of 50 mg/kg females supported the findings for the uterus. There were also significantly increased incidences of squamous metaplasia of the uterus in the 15 and 50 mg/kg groups and of squamous hyperplasia of the cervix in the 50 mg/kg group when both evaluations were combined.

In the kidney, there were significantly increased incidences of hydronephrosis in 15 mg/kg males and 50 mg/kg males and females at 2 years. In the 2-year study, there were significantly increased incidences of atrophy and cytoplasmic vacuolization of the parotid salivary gland in 50 mg/kg male rats. In the 2-year study, there were significantly increased incidences of chronic active inflammation of the prostate gland in the 15 and 50 mg/kg males and ectasia of the preputial gland duct in 50 mg/kg males. In the 2-year study, there were significantly increased incidences of thymic atrophy and epithelial hyperplasia of the forestomach in 50 mg/kg males and adrenal cortex focal hyperplasia in 50 mg/kg females.

In adipose, liver, and plasma, at the end of the study, the concentrations of BDE-47, BDE-99 and BDE-153 increased with increasing dose and were higher than the corresponding vehicle control values. The concentrations were lowest in plasma and highest in adipose. In a given matrix, the concentrations of BDE-47, BDE-99, and BDE-153 were similar, suggesting a higher rate of accumulation of BDE-153 regardless of the lower percent of BDE-153 in DE-71.

2-Year Study in Mice

Groups of 50 male and 50 female mice were administered 0, 3, 30, or 100 mg DE-71/kg body weight in corn oil by gavage, 5 days per week for up to 105 weeks. Survival of 100 mg/kg males and females was significantly less than that of the vehicle controls, leading to these groups being removed from the study at 18 months. Mean body weights of 100 mg/kg males and females were at least 10% less than those of the vehicle control groups after weeks 17 and 21, respectively. The mean body weights of 30 mg/kg males were at least 10% less than those of the vehicle controls after week 87. Clinical findings included increased occurrences of distended abdomen, which correlated with liver neoplasms.

The incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were significantly increased in 30 and 100 mg/kg males and females (except carcinoma in 30 mg/kg females). There were also significantly increased incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in 30 and 100 mg/kg males. The incidence of hepatocellular adenoma was significantly increased in 3 mg/kg males, and the incidences of hepatoblastoma were significantly increased in 30 and 100 mg/kg males.

There were significantly increased incidences of centrilobular hepatocyte hypertrophy in all dosed groups of male and female mice. There were significantly increased incidences of eosinophilic focus in 30 and 100 mg/kg female mice. In 30 mg/kg males, there was a significantly increased incidence of clear cell focus. There were significantly increased incidences of fatty change in 30 and 100 mg/kg females. There were significantly increased incidences of focal necrosis in 30 mg/kg males and Kupffer cell pigmentation in all dosed groups of males and females.

There were significantly increased incidences of follicle hypertrophy of the thyroid gland in all dosed groups of male mice and in 30 and 100 mg/kg female mice. In the forestomach, there were significantly increased incidences of epithelial hyperplasia in 30 and 100 mg/kg males and in 100 mg/kg females and inflammation in 30 and 100 mg/kg males. In 100 mg/kg males and females, there were significantly increased incidences of diffuse hypertrophy of the adrenal cortex. The incidence of germinal epithelium atrophy was significantly increased in the testes of 100 mg/kg males.

Concentrations of BDE-47, BDE-99, and BDE-153 were determined in the adipose and liver of male and female mice at the end of the 2-year study, except for 30 mg/kg males. In both males and females, the tissue concentrations of all three congeners in adipose and liver increased with increasing dose and were higher in adipose than in liver, suggesting preferential accumulation in adipose. Regardless of the lower percentage of BDE-153 in DE-71 compared to the other two congeners, concentrations of BDE-153 were relatively higher in both adipose and liver, suggesting a higher rate of accumulation of BDE-153.

GENETIC TOXICOLOGY

DE-71 was tested for mutagenic activity in bacteria in three independent studies in three laboratories using a *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537, and *E. coli* strain WP2 *uvr*A/pKM101 with and without rat or hamster liver metabolic activation enzymes (S9), and no evidence of mutagenicity was observed in any of the tests.

Three related test BDE-47, BDE-99, and BDE-153 were tested for mutagenic activity in *S. typhimurium* strains TA98, TA100, and TA102 with and without rat liver S9 mix, and no evidence of mutagenicity was observed with any of the three test articles in any of the tests that were conducted.

In vivo, no increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood samples from male or female B6C3F1/N mice following administration of DE-71 for 3 months by corn oil gavage. In addition, no increases in micronucleated immature or mature erythrocytes were seen in peripheral blood samples from male B6C3F1/N mice administered DE-71 by gavage once daily for 3 days and evaluated using flow cytometric methods. In these same mice, bone marrow smears were also scored for frequency of micronucleated polychromatic erythrocytes, and no increases were observed. In none of the micronucleus tests were significant alterations in the percentage of immature erythrocytes (polychromatic erythrocytes or reticulocytes) seen over the dose range tested, suggesting no chemical-associated toxicity to the bone marrow.

CONCLUSIONS

Under the conditions of these 2-year oral gavage studies, there was *clear evidence of carcinogenic activity** of DE-71 in male Wistar Han rats based on increased incidences of hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (combined). Increased incidences of thyroid gland follicular cell adenoma and increased incidences of pituitary gland (pars distalis) adenoma were also considered to be related to exposure. There was *clear evidence of carcinogenic activity* of DE-71 in female Wistar Han rats based on increased incidences of hepatocholangioma, hepatocellular adenoma, and hepatocellular carcinoma. The occurrence of cholangiocarcinoma of the liver was also considered related to treatment. The incidences of stromal polyp or stromal sarcoma (combined) of the uterus may have been related to treatment. There was clear *evidence of carcinogenic activity* of DE-71 in male B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma. There was *clear evidence of carcinogenic activity* of DE-71 in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma and hepatocellular carcinoma.

Administration of DE-71 resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, kidney, parotid salivary gland, prostate gland, preputial gland, thymus, and forestomach of male rats; liver, thyroid gland, uterus, cervix, kidney, and adrenal cortex of female rats; liver, thyroid gland, forestomach, adrenal cortex, and testes of male mice; and liver, thyroid gland, forestomach, and adrenal cortex of female mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Peer Review Panel comments and the public discussion on this Technical Report appears on page 16.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Gavage and Perinatal and Postnatal Gavage Studies of DE-71

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in corn oil by gavage	0, 3, 15, or 50 mg/kg	0, 3, 15, or 50 mg/kg	0, 3, 30, or 100 mg/kg	0, 3, 30, or 100 mg/kg
Survival rates	36/50, 35/50, 38/50, 25/50	37/50, 39/50, 33/50, 28/50	29/50, 33/50, 31/50, 0/50	33/50, 35/50, 37/50, 0/50
Body weights	Dosed groups similar to the vehicle control group	50 mg/kg group at least 10% less than the vehicle control group after week 37	30 and 100 mg/kg groups at least 10% less than the vehicle control group after weeks 87 and 17, respectively	100 mg/kg group at least 10% less than the vehicle control group after week 21
Nonneoplastic effects	Liver: eosinophilic focus (3/49, 3/50, 12/50, 15/50); hepatocyte, hypertrophy (1/49, 44/50, 50/50, 50/50); fatty change (32/49, 37/50, 48/50, 48/50) Thyroid gland: follicle, hypertrophy (1/45, 26/45, 34/48, 23/46) Kidney: hydronephrosis (1/49, 5/46, 8/50, 10/50) Parotid salivary gland: atrophy (2/46, 2/48, 4/50, 13/50); cytoplasmic vacuolization (4/46, 4/48, 7/50, 17/50) Prostate gland: inflammation, chronic active (17/49, 20/50, 28/50, 27/50) Preputial gland: duct, ectasia (2/49, 2/49, 5/50, 15/50) Thymus: atrophy (14/45, 11/49, 15/49, 26/50) Forestomach: epithelium hyperplasia (8/49, 6/50, 5/50, 17/50)	Liver: hyperplasia, nodular (0/50, 0/49, 2/50, 7/47); eosinophilic focus (5/50, 7/49, 21/50, 31/47); hepatocyte, hypertrophy (0/50, 48/49, 49/50, 45/47); fatty change (15/50, 12/49, 28/50, 39/47); oval cell, hyperplasia (1/50, 3/49, 3/50, 10/47) Thyroid gland: follicle, hypertrophy (8/45, 17/49, 22/47, 35/42); follicular cell hyperplasia (1/45, 5/49, 4/47, 6/42) Uterus: squamous metaplasia (original and residual evaluations, combined – 0/50, 2/50, 5/50, 6/49) Cervix: squamous hyperplasia (original and residual evaluations, combined – 2/50, 3/50, 4/50, 8/49) Kidney: hydronephrosis (1/50, 1/50, 1/49, 6/47) Adrenal cortex: focal hyperplasia (8/50, 6/49, 12/50, 19/46)	Liver: centrilobular, hepatocyte, hypertrophy (0/50, 28/50, 46/50, 48/50); clear cell focus (10/50, 13/50, 20/50, 7/50); necrosis, focal (2/50, 2/50, 16/50, 2/50); Kupffer cell, pigmentation (5/50, 15/50, 33/50, 25/50) Thyroid gland: follicle, hypertrophy (25/50, 35/49, 41/50, 45/49) Forestomach: epithelium, hyperplasia (26/50, 19/50, 40/50, 29/50); inflammation (18/50, 18/50, 34/50, 19/50) Adrenal cortex: hypertrophy, diffuse (1/50, 0/50, 3/49, 20/48) Testes: germinal epithelium, atrophy (11/50, 8/50, 20/50, 13/49)	Liver: centrilobular, hepatocyte, hypertrophy (0/50, 7/49, 45/50, 47/49); eosinophilic focus (3/50, 2/49, 16/50, 15/49); fatty change (18/50, 18/49, 39/50, 20/49); Kupffer cell, pigmentation (3/50, 10/49, 24/50, 27/49) Thyroid gland: follicle, hypertrophy (24/50, 31/49, 37/48, 42/47) Forestomach: epithelium, hyperplasia (9/50, 5/50, 6/50, 16/49) Adrenal cortex: hypertrophy, diffuse (0/50, 0/50, 4/49, 8/47)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Gavage and Perinatal and Postnatal Gavage Studies of DE-71

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Neoplastic effects	Liver: hepatocellular adenoma or carcinoma (3/49, 2/50, 4/50, 9/50); hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (3/49, 2/50, 4/50, 11/50) Thyroid gland: follicular cell adenoma (1/45, 3/45, 2/48, 6/46) Pituitary gland (pars distalis): adenoma (19/49, 12/49, 22/50, 35/50)	Liver: cholangiocarcinoma (0/50, 0/49, 0/50, 2/47); hepatocholangioma (0/50, 0/49, 0/50, 8/47); hepatocellular adenoma (3/50, 2/49, 8/50, 16/47); hepatocellular carcinoma (0/50, 0/49, 1/50, 6/47); hepatocellular adenoma or carcinoma (3/50, 2/49, 8/50, 17/47); hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (3/50, 2/49, 8/50, 21/47)	Liver: hepatocellular adenoma (23/50, 35/50, 49/50, 40/50); hepatocellular carcinoma (18/50, 15/50, 30/50, 45/50); hepatocellular adenoma or carcinoma (31/50, 40/50, 49/50, 47/50); hepatoblastoma (1/50, 1/50, 16/50, 5/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (31/50, 40/50, 49/50, 47/50)	Liver: hepatocellular adenoma (5/50, 7/49, 32/50, 46/49); hepatocellular carcinoma (4/50, 2/49, 6/50, 27/49); hepatocellular adenoma or carcinoma (8/50, 8/49, 33/50, 47/49)
Equivocal findings		Uterus: stromal polyp or stromal sarcoma (original and residual evaluations, combined – 4/50, 12/50, 12/50, 9/49)		
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

Genetic toxicology

Bacterial gene mutations:	
DE-71:	Negative in *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 and *E. coli* with or without S9
BDE-47:	Negative in *S. typhimurium* strains TA98, TA100, and TA102
BDE-99:	Negative in *S. typhimurium* strains TA98, TA100, and TA102
BDE-153:	Negative in *S. typhimurium* strains TA98, TA100, and TA102
Micronucleated erythrocytes	
Mouse 3-month study peripheral blood *in vivo*:	Negative in males and females
Mouse 3-day study peripheral blood and bone marrow *in vivo*:	Negative in males

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on DE-71 on June 25, 2015, 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 the NTP studies:

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

Kenneth M. Portier, Ph.D., Chairperson
Statistics and Evaluation Center
American Cancer Society
Atlanta, GA

Russell C. Cattley, V.M.D., Ph.D.
Department of Pathobiology
College of Veterinary Medicine
Auburn University
Auburn, AL

Michael W. Conner, D.V.M.
Theravance, Inc.
San Francisco, CA

Willem D. Faber, Ph.D.
Willem Faber Toxicology Consulting, LLC
Victor, NY

Susan P. Felter, Ph.D.
Procter & Gamble
West Chester, OH

Gabriele Ludewig, Ph.D.
Department of Occupational and Environmental Health
College of Public Health
University of Iowa
Iowa City, IA

Donald G. Stump, Ph.D.
WIL Research
Ashland, OH

SUMMARY OF PEER REVIEW PANEL COMMENTS

On June 25, 2015, the draft Technical Report on the toxicology and carcinogenesis studies of DE-71 received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of DE-71 (technical grade), a mixture of pentabromodiphenyl ethers used as a flame retardant, by describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *clear evidence of carcinogenic activity* in male and female Wistar Han rats and *clear evidence of carcinogenic activity* in male and female B6C3F1/N mice.

Dr. Felter noted that the dose-selection range-finding study was conducted in the F344/N rat, while the 2-year bioassay was done in the Wistar Han. She asked what kind of experience the NTP has had in terms of being able to predict responses between the strains. Dr. Dunnick noted that the same switch had been done in two other NTP studies, including a study of tetrabromobisphenol A. Other scientists, such as those from the Environmental Protection Agency, have also been working with pentabromodiphenyl ethers in different strains of rats. Other studies have consistently found liver and thyroid gland toxicity across the different strains and species. Dr. N.J. Walker, NIEHS, said that during the transition in rat strains, the decision was made not to go back and redo many studies. He also noted that there are considerable data in the literature to support the dose selection for the study.

Dr. Felter asked whether the maximum tolerated dose (MTD) was exceeded in the male rats based on survival data. Dr. Dunnick said that the MTD was not exceeded because the decrease in survival in male rats at the high dose is due to the development of pituitary gland adenomas. Dr. Felter asked why the pituitary gland tumors were used as evidence that the MTD was not exceeded. She indicated that because they are benign tumors they are allowed no weight in assessing the conclusion of clear evidence of cancer. Dr. Dunnick said that the pituitary gland adenomas were considered "some evidence" for a carcinogenic effect. The mid-dose findings in the uterus are characterized as "may have been related to exposure" because these are primarily benign tumors and are not significantly different from controls at the high dose by pairwise comparison. Dr. Ludewig asked whether the conclusion would be different if the high dose results were removed in the analysis and only the data from the lower doses were used. Dr. G.E. Kissling, NIEHS statistician, replied that such an analysis would probably result in a marginally statistically significant increase.

Dr. Cattley, the first reviewer, asked for clarification of which specific endpoints were considered to reflect "minimal liver toxicity" for the lowest dose levels with respect to the various changes in organ weight, enzyme induction, histologic lesions, or clinical pathology findings. He asked for the diagnostic criteria used with the hepatocholangiomas and how they were distinguished from a "hepatocellular adenoma with some dilated, nonneoplastic bile ducts." He asked about distinguishing cholangiocarcinoma from cholangiofibrosis and suggested that if there is a published criterion for that differentiation, it should be included and cited. For the description of the mechanism of action, Dr. Cattley suggested adding a table listing evidence for the activation of different nuclear receptors by components of DE-71.

Regarding the conclusion that cholangiocarcinoma of the liver in female rats is related to exposure, Dr. Cattley noted the lack of historical controls. While he agreed with the overall conclusion that there is "clear evidence of carcinogenic activity" in the female rat, he asked whether the evidence concerning cholangiocarcinoma should be considered "equivocal" rather than "some evidence." In male rats, for the conclusion that increased incidences of thyroid gland follicular cell adenoma or carcinoma are related to exposure, Dr. Cattley noted there were no carcinomas in the high dose group. There is a limited number of historical controls for gavage studies in this strain, and he suggested limiting the conclusion to adenomas alone.

Dr. Conner, the second reviewer, agreed with Dr. Cattley's suggestion to reword the conclusion to reflect increased incidences of thyroid gland follicular cell adenoma alone. He noted that the report should state explicitly the significance level, which appears to be 0.05. For the conclusion of a carcinogenic effect based on hepatic tumors in male rats, he asked if the conclusion was referring to the combined incidences of "hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma."

Regarding dose selection for the 2-year study, Dr. Dunnick said that the lower doses were chosen to give a broader range of doses.

Study pathologist Dr. A.E. Brix, EPL, Inc., responded to Dr. Cattley's comments about the hepatocholangiomas. She said that hepatocholangiomas are thought to arise from cells that can differentiate into both hepatocytes and biliary cells. In this study, they were distinguished from hepatocellular adenomas with dilated, nonneoplastic bile ducts by the increased number of bile ducts within hepatocholangiomas. Hepatocellular adenomas typically lack bile ducts. In addition, the epithelium of the biliary component of the hepatocholangiomas was cuboidal, in contrast to the typically flattened epithelium found in biliary cysts. Regarding the cholangiocarcinomas, she said that distinguishing between cholangiofibrosis and cholangiocarcinoma is difficult and is primarily based on the extent of liver invasion. Dr. Brix noted that after extensive discussion within the NTP Pathology Working Group, some of the lesions were determined to be cholangiocarcinomas.

Regarding the thyroid gland, Dr. Brix said that progression from follicular cell adenomas to carcinomas, as with many endocrine proliferative lesions, is commonly seen in laboratory rodents. Thus, it made sense when interpreting the results from this study to consider those lesions together, even if it does not change the statistical conclusions.

Regarding Dr. Cattley's comment about the mechanism of action, Dr. Dunnick said that it was not possible to determine which component of the mixture might contribute to a particular effect. Many of the components have not been tested alone because it is difficult to acquire sufficient amounts of purified agents.

Regarding Dr. Conner's question about P values, Dr. Kissling said that statistical results are just one piece of evidence the team examines when interpreting results. Although P values are calculated, there is not a strict decision to accept or reject hypotheses, and P values are considered in the wider context of the biological issues. Dr. Dunnick added that cholangiocarcinomas were not seen in any of the previous studies using the Wistar Han rat. Dr. Walker noted that cholangiocarcinomas were quite rare in this study and were considered related to treatment, leading to the conclusion of "some evidence of carcinogenic activity."

Dr. Conner asked whether the hepatocholangiomas alone were being considered as evidence of carcinogenicity. Dr. Dunnick said that the conclusion is based on the combined occurrence of "hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma." He suggested including the term "combined" early in the report to clarify that point.

Dr. Cattley asked whether the conclusion regarding the liver cholangiocarcinomas should be "some evidence," as written, or "equivocal." Dr. D.E. Malarkey, NIEHS, noted that in the report, those tumors are "also considered related," which would mean the category of some evidence. Dr. Cattley drew a distinction between "may have been related" and "considered related." Dr. Malarkey said that the tumor has not been seen previously in NTP studies in thousands of animals in multiple strains, which adds to the conclusion of "some evidence." Dr. Cattley asked if there were any data on this strain and the incidence of the lesion. Dr. Malarkey said there are six studies, and the lesion is not seen in related controls. Dr. Cattley noted that two of those were gavage studies, and observed that "equivocal" does not mean not related. Dr. Walker confirmed that the cholangiocarcinomas are not combined with the hepatocellular tumors, which is why they stand alone in the conclusions. They were also considered related to treatment, leading to the "some evidence" conclusion.

Dr. Felter, the next reviewer, noted that the text indicated a positive trend for cholangiocarcinomas in the female rats, but this tumor was not listed in Table B2. She asked about dosing in the rats and whether the MTD was exceeded. She noted that many of the effects are only seen at the highest dose. She suggested that additional information be added to the section on survival.

Dr. Ludewig, the next reviewer, asked why the NTP-2000 diet was used for all studies and NIH-07 was used during pregnancy. She noted there can be strong effects based on diet. With respect to blood cells, she noted that there are decreases in reticulocytes and the ratio of polychromatic erythrocytes to micronucleated normochromatic erythrocytes; lower leukocytes, lymphocytes, neutrophils, and eosinophils; and more lymphomas. The report states that there is no bone marrow toxicity or hemotoxicity. She asked for further discussion in the report.

Dr. Ludewig also noted the increase in liver lipids in the F_1 rats, and thought it should be added to the Results. She did not find the citation for the studies of polybrominated diphenyl ether congeners that is mentioned. She also suggested adding several citations regarding AhR activation and inhibition, impurities in DE-71 as AhR agonists, and β-catenin in tumors.

Dr. Dunnick said that the cholangiocarcinoma would be added to table B2 as recommended by Dr. Felter. Regarding Dr. Ludewig's question about diet, she explained that the NIH-07 diet is used during lactation and development to provide adequate nutrition during those phases of the study, and the NIH-2000 diet is used as the maintenance diet in adult animals because reduced proteins levels reduce incidences of chronic nephropathy. Regarding blood cells, she said there was a downward trend in the ratio of polychromatic erythrocytes to micronucleated normochromatic erythrocytes; however, none of the dose groups differed significantly from the control group. The conclusion is that the small alteration is not biologically significant. Decreases in leukocyte and lymphocyte levels are considered to be stress-related.

Dr. Dunnick briefly explained the approach for measuring polybrominated diphenyl ether levels in tissues, noting that polybrominated diphenyl ether congeners are used as standards. She noted more information would be added regarding the standards and their purity. She thanked Dr. Ludewig for the suggested references.

Dr. Faber, the next reviewer, asked for greater detail regarding the necropsy schedule and schedule for obtaining the blood samples for thyroid hormone and thyroid stimulating hormone (TSH) levels, as those values can change depending on time of day. He pointed out the use of the term "perinatal" period for when the F_0 dams were in quarantine and suggested that "preimplantation" period was intended. He asked for clarification on descriptions of dose administration, which should have been continuous, rather than on a 5-day-per-week schedule reported. He said the report should state clearly that the body weight values were collected daily on the dams during gestation and lactation and should specify what body weight values were used to determine dosing volume. The same should be done for the pups.

Dr. Stump, the final reviewer, asked for an explanation in the Methods section for why dosing began in the pups on postnatal day 12; this design is different than what the NTP has used in the past. He noted the pregnancy rate seemed quite low in the study. The pregnancy rate looked to be about 85%, and he expected the rate to be at least 90%.

Dr. Dunnick said that blood was collected and the necropsies were conducted over approximately a 2-hour period in the morning from 8 to 10 a.m. The animals were necropsied by a randomized schedule across doses. Dams were quarantined throughout the perinatal period, which was upon arrival up to the end of lactation. The dams were dosed and weighed daily, and the weight from the previous day was used to calculate the dose on the following day. Regarding reproductive endpoints, she said that the NTP specifications were followed for sperm analysis and noted the small decrease in sperm motility was treatment related. She added that the Wistar Han rat strain did not have as high a pregnancy rate as the Sprague Dawley rat, which was one of the reasons for switching to the Sprague Dawley. She explained that dosing began at postnatal day 12 because that is when the pups begin to eat. NTP tried to make the exposures consistent with their natural physiology and behavioral patterns.

Dr. Portier requested a motion and second on the draft conclusions to initiate the panel discussion. Dr. Portier asked that one species and one sex at a time be considered. He asked for a motion to accept the conclusions on the male Wistar Han rats. Dr. Conner moved to accept the conclusions as written. Dr. Felter seconded the motion. Dr. Portier opened the panel discussion on those conclusions.

Dr. Cattley recommended deleting the words "or carcinoma" (regarding thyroid gland tumors) under conclusions for the male Wistar Han rat. Dr. Conner agreed. Dr. Walker said that the carcinomas were included because it was a plausible mechanism due to decreases in thyroxine concentrations, increases in TSH levels, and occurrences of thyroid gland nonneoplastic lesions. Including them fit in with the whole mechanism of thyroid gland carcinogenesis, with the carcinomas in the lower dose group almost certainly related to treatment.

Dr. Cattley said that dropping the "or carcinoma" phrase would not take away from the mechanism described by Dr. Walker. Dr. Conner said that the data associated with the carcinomas do not seem to indicate a treatment relationship. Dr. Walker noted that no follicular cell carcinomas were seen in the 295 historical controls assessed across all routes of exposure. The combination of mechanism and the historical control data led to the conclusion as written.

Dr. Felter asked whether the evidence for carcinoma is strong enough that the conclusion would be the same without reference to the adenomas. If not, she agreed that it should be deleted from the conclusion. Dr. C.R. Blystone, NIEHS, said that the carcinomas alone would not rise to an evidence category. Dr. Brix pointed out that there is decreased survival in the high dose.

Dr. Malarkey pointed out that a progression from adenoma to carcinoma is often seen. Also, a substantial percentage of animals had hypertrophy, so the thyroid gland follicular cell is a target. Dr. Stump suggested including an explanation that a potential reason the carcinomas were not seen in the high dose group is due to reduced survival, which would make a stronger case for including the carcinoma in the conclusion statement.

Dr. Ludewig said that the fact that the carcinoma is an extremely rare cancer in the control animals should be weighted heavily. Thus, there is evidence that the occurrence in the study animals was related to exposure. Different activation and antagonistic effects with respect to receptor action could be a mechanism to explain why no carcinomas were seen in the high doses. She would keep the conclusion as written.

Dr. Conner said that, in the absence of a dose response, it is less plausible that the carcinoma is a treatment-related effect. As a nongenotoxic mechanism, a dose-related increase in tumors would be expected.

There was further discussion on the meaning of "or" in the phrase "or carcinoma" and if adding "(combined)" at the end of the sentence would be better. The language for explaining the levels of evidence categories was also discussed.

Dr. Portier called for a vote on the original motion, which was to agree with the conclusions as written for male Wistar Han rats. There was one vote in favor of the motion and five votes against. Rather than ask for explanations of the no votes, Dr. Portier elected to ask for an alternative motion.

Dr. Conner moved to accept the conclusions with "or carcinoma" being struck. Dr. Cattley seconded the motion. Dr. Malarkey asked whether the conclusion regarding carcinoma should be changed from "some evidence" to "equivocal evidence" after being removed from the conclusion. Dr. Conner supported that change.

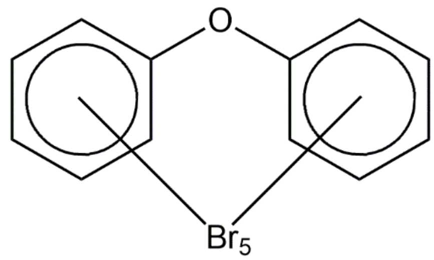
Dr. Portier called for a vote on the motion. The panel voted four in favor and two against the motion. Drs. Ludewig and Stump voted against the motion. Both explained that they preferred to keep the "adenomas or carcinomas" statement. Dr. Portier called for a motion on the female Wistar Han rat conclusions. Dr. Conner moved to accept the conclusions as written. Dr. Ludewig seconded the motion. The panel voted five in favor and one against the motion. Dr. Cattley explained he voted no because the evidence regarding cholangiocarcinoma fits an equivocal call.

Dr. Portier called for a motion on the male mice conclusion. Dr. Stump moved to accept the conclusions as written. Dr. Cattley seconded the motion. The panel voted unanimously to accept the motion.

Dr. Portier called for a motion on the female mouse conclusion. Dr. Faber moved to accept the draft language as written. Dr. Conner seconded the motion. Dr. Felter noted that the use of "and" versus "or" in the language of the conclusions should be clearer. The panel voted unanimously to accept the motion.

Dr. Portier asked the panel members if they had any final comments. Dr. Conner noted that the NTP Technical Reports are used widely and in recent years they have become more comprehensive.

INTRODUCTION



Pentabromodiphenyl Ether Mixture

DE-71 (Technical Grade)

CAS No. 32534-81-9

Chemical Formula: $C_{12}H_5Br_5O$ Molecular Weight: 564.7

CHEMICAL AND PHYSICAL PROPERTIES

Polybrominated diphenyl ether mixtures (PBDEs), which are flame retardant mixtures, have a common structure of a brominated diphenyl ether molecule with one to ten bromine atoms attached, and there are up to 209 possible congeners of PBDEs (ATSDR, 2004). This report focuses on DE-71, a technical grade pentabromodiphenyl ether mixture containing lower molecular weight PBDEs [e.g., 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153)]. Other PBDE formulations such as the octaBDE formulation (heptaBDE and octaBDE congeners, with secondary contributions by hexaBDE and nonaBDE congeners), and the decaBDE formulations [2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209), and small amounts of 2,2′,3,3′,4,4′,5,5′,6-nonabromodiphenyl ether (BDE-206), 2,2′,3,3′,4,4′,5,6,6′-nonabromodiphenyl ether (BDE-207), and 2,2′,3,3′,4,5,5′,6,6′-nonabromodiphenyl ether (BDE-208)] (USEPA, 2010a) are not discussed in this report. DE-71, a viscous sticky brown liquid, is dominated (by weight) by penta congeners with secondary contributions by tetra and hexa congeners (USEPA, 2010a).

PBDEs are lipophilic chemicals that bioaccumulate in the environment (ATSDR, 2004; USEPA, 2008a,b,c). The DE-71 flame retardant mixture used in the studies presented in this Technical Report had approximately 42% BDE-99, 36% BDE-47, 10% 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), 4% 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154), and 3% BDE-153 (Appendix J). Chemical and physical properties of these congeners are listed in Table 1 and structures are presented in Figure 1.

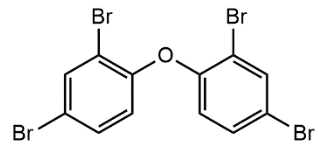
PRODUCTION, USE, AND HUMAN EXPOSURE

Lower molecular weight PBDEs (primarily pentaBDEs) were marketed as mixtures under several different trade names (e.g., DE-71, Bromkal 70-5, Tardex 50) (EFSA, 2011). PBDEs were used as additive flame retardants often in furniture materials (ATSDR, 2004). Additive flame retardants are mixed into products, but they are not covalently bound to the polymers in the commercial products, and thus, can leach out into the environment (Wu *et al.*, 2011).

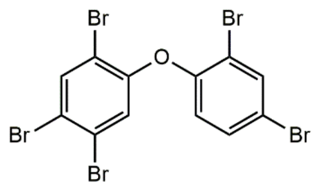
TABLE 1
Chemical and Physical Properties of Selected Polybrominated Diphenyl Ether Congeners Composing DE-71

	BDE-47[a]	**BDE-99**[b]	**BDE-100**[c]
Synonyms	Benzene,1,l′-oxybis [2,4-dibromo-]; 2,2′,4,4′-tetrabromodiphenyl ether	Benzene, l,2,4-tribromo-5-(2,4- dibromophenoxy)-; 2,2′,4,4′,5-pentabromodiphenyl ether	2,2′,4,4′,6-pentabromodiphenyl ether; 1,3,5-tribromo-2-(2,4-dibronphenoxy)-benzene
CAS No.	5436-43-1	60348-60-9	189084-64-8
Chemical formula	$C_{12}H_6Br_4O$	$C_{12}H_5Br_5O$	$C_{12}H_5Br_5O$
Molecular weight	485.8	564.7	564.7
Vapor pressure (Pa) at 25º C	2.5×10^{-4}	5×10^{-5}	2.86×10^{-5}
Melting point (ºC)	79-82	93	102 or 110
Solubility in water (µg/L)	11	2.4	40
Henry's law constant (Pa m^3 mol^{-1}) at 25º C	0.85	0.60	0.069 or 0.384
Log octanol/water partition coefficient (K_{OW}) at 25º C	6.81	6.5-8.4	6.86
Log octanol/air partition coefficient (K_{OA}) at 25º C	10.5	11.3	11.13
	BDE-153[d]	**BDE-154**[c]	
Synonyms	Benzene, 1,l′-oxybis-2,4,5-tribromo-; 2,2′,4,4′,5,5′-hexabromodiphenyl ether	2,2′,4,4′,5,6′-hexabromodiphenyl ether; 1,2,3-tribromo-2-(2,4,5-tribromophenoxy)-benzene	
CAS No.	68631-49-2	207122-15-4	
Chemical formula	$C_{12}H_4Br_6O$	$C_{12}H_4Br_6O$	
Molecular weight	643.6	643.6	
Vapor pressure (Pa) at 25º C	5.8×10^{-6}	3.80×10^{-6}	
Melting point (ºC)	183	131-132.5	
Solubility in water (µg/L)	0.9	8.70×10^{-4}	
Henry's law constant (Pa m^3 mol^{-1}) at 25º C	0.26	0.24	
Log octanol/water partition coefficient (K_{OW}) at 25º C	7.90	7.39	
Log octanol/air partition coefficient (K_{OA}) at 25º C	11.9	11.92	

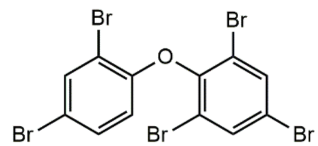
[a] USEPA, 2008a [b] USEPA, 2008b [c] Mackay *et al.*, 2006 [d] USEPA, 2008c



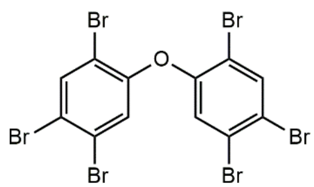
BDE-47



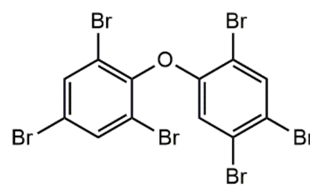
BDE-99



BDE-100



BDE-153



BDE-154

FIGURE 1
Chemical Structures of Selected Polybrominated Diphenyl Ethers in DE-71

The lower molecular weight PBDE congeners contained in DE-71 (e.g., BDE-47, BDE-99, and BDE-153) were used as flame retardants in polyester foams and may leach from the foams when they are deposited at waste dumps (Hale *et al.*, 2001, 2002, 2003). Microorganisms may dehalogenate higher molecular weight PBDEs to lower molecular weight PBDEs (van Pée and Unversucht, 2003; Lee and He, 2010; Lee *et al.*, 2011). Photolytic debromination may also occur (Söderström *et al.*, 2004; Rodenburg *et al.*, 2014).

PBDEs are present in water, wildlife (e.g., fish, seals, and birds) and in humans (Hale *et al.*, 2001, 2002, 2003; Chen and Hale, 2010), and in various food products including meat, poultry, and fish (Huwe and West, 2011). Total PBDE levels in fish caught in the United States can range up to 1,250 ng/g wet weight (Stahl *et al.*, 2013).

PBDEs are found in everyday products including butter wrappers (Schecter *et al.*, 2011) and plastic toys (Chen *et al.*, 2009). The most prevalent PBDEs found in household dust are BDE-47, BDE-99, and BDE-153 (Frederiksen *et al.*, 2010a). Uptake of PBDEs by plants growing near electronic waste sites has been reported (Huang *et al.*, 2011). Incineration of material containing PBDEs may result in the formation of brominated dioxins and furans and contribute to ambient air exposure to the PBDEs (Wyrzykowska-Ceradini *et al.*, 2011).

In a limited survey, PBDEs were found in 39% of couches purchased prior to 2005, but in only 2% of couches purchased after 2005 (Stapleton *et al.*, 2012). Other studies also present evidence that levels of PBDEs in the environment may be decreasing (Law *et al.*, 2014).

The potential for PBDE exposure, especially among the young, is a concern because of the widespread occurrence of these chemicals in the environment and in human tissues (Frederiksen *et al.*, 2010b; USEPA, 2010a). Exposure to the fetus and infant may occur from mother's milk, and children may be exposed to PBDEs adsorbed in house dust (Schecter *et al.*, 2006, 2010a; Frederiksen *et al.*, 2010a; Harrad *et al.*, 2010; Johnson *et al.*, 2010). Stapleton *et al.* (2014) found that flame retardants are often found on children's hands, and hand to mouth behavior in children can be an important route of flame retardant exposure. BDE-47 is usually the most prevalent PBDE congener found in human tissue (Petreas *et al.*, 2003; Sjödin *et al.*, 2004; Johnson-Restrepo *et al.*, 2005; Schecter *et al.*, 2010b,c). The higher blood PBDE concentrations in children (up to two to fivefold higher than that of their parents exposed to the same indoor concentrations) is thought to be due to higher rates of dust ingestion (Fischer *et al.*, 2006), higher PBDE dietary intake due to higher food intake per kilogram of body weight, and high levels of PBDEs in human milk (Schecter *et al.*, 2006).

The adult intake of total PBDEs (including higher molecular weight PBDEs such as BDE-209) in the United States is estimated to be 7.1 ng/kg body weight per day, resulting in a body burden of 31 ng/g lipid (USEPA, 2010a). Food is estimated to account for 10% of the total PBDE exposure in adults, and the remaining 90% of exposure is from household dust. Children's PBDE intakes are estimated to be 47.2 ng/kg per day for ages 1 to 5, 13.0 ng/kg per day for ages 6 to 11, and 8.3 ng/kg per day for ages 12 to 19. Infant PBDE daily intakes were estimated to be up to 141 ng/kg per day in part due to ingestion of PBDEs from mother's milk.

Levels of PBDEs in human serum/urine (including BDE-47, BDE-99, and BDE-153) are currently being followed as part of the National Health and Nutrition Examination Survey (NHANES) (Woodruff *et al.*, 2011). Concentrations of PBDEs in sera collected in 2006 were particularly high in certain occupational groups such as carpet installers (e.g., BDE-47 100 ng/g lipid in sera) and foam recyclers (e.g., 78 ng/g lipid in sera) (Stapleton *et al.*, 2008). PBDE levels were also found to be high in gymnasts (sera collected in 2012) (Carignan *et al.*, 2013). Daily intake of total PBDEs near electronic waste sites may be higher than in the general population. In a recent study in Asia, the total daily intake of PBDEs near electronic waste sites was 1,671 ng/day for adults (approximately 24 ng/kg body weight for a 70 kg man) as well as a daily intake of up to 24 ng/day of BDE-47 in a toddler (Jiang *et al.*, 2014). Another study estimated that the total daily PBDE intake near electronic waste sites was 130 ng/kg body weight in adults, and 614 ng/kg body weight in children (Labunska *et al.*, 2014). Total daily adult median intakes of DE-71 constituents, BDE-47, BDE-99, and BDE-153 were estimated at 14.3, 6.2, and 12.5 ng/kg body weight per day, respectively, but could range up to 73 to 84 ng/kg body weight per day. Total daily intakes of BDE-47, BDE-99, and BDE-153 for children were 53, 25, and 51 ng/kg body weight per day, respectively (ranging up to 263, 164, and 291 ng/kg per day, respectively) (Labunska *et al.*, 2014).

An analysis of NHANES data compared PBDE concentrations in pooled sera collected from 2005 to 2006 and 2007 to 2008 versus PBDE levels in individual sera collected from 2003 to 2004 to determine if concentrations have changed over time; even though PBDEs started to be phased out in 2004, no reduction in PBDE sera concentrations were detected by 2008 (Sjödin *et al.*, 2014). The mean serum concentrations (ng/g lipid) for BDE-47, BDE-85, BDE-99, BDE-100, and BDE-153 in the 2007 to 2008 NHANES data for people 12 to 19 years of age were 35.9 ± 8.0, 0.8 ± 0.2, 8.5 ± 2.5, 6.7 ± 1.6, and 12.0 ± 3.3, respectively. The mean serum concentrations (ng/g lipid) for BDE-47, BDE-85, BDE-99, BDE-100, and BDE-153 in people greater than 60 years of age were 39.9 ± 10.6, 0.9 ± 0.2, 8.7 ± 2.7, 8.1 ± 2.3, and 13.4 ± 3.3, respectively (Sjödin *et al.*, 2014).

Regulatory Status

The European Union banned the marketing and use of pentaBDE in 2003 (EPCEU, 2003). The United States manufacturers of pentaBDEs voluntarily phased out their production in 2004, and various individual states have developed regulations banning the sale of products containing pentaBDE flame retardants (USEPA, 2010a).

Based on evidence of long-range atmospheric transport, environmental persistence, and bioaccumulation in various species including humans, PBDE congeners were added to the United Nations Economic Commission for Europe lists of persistent organic pollutants protocol (UNEP, 2009a). The Stockholm Convention has initiated a global effort with more than 172 countries to manage the use and disposal of material containing persistent organic chemicals including the PBDEs (UNEP, 2008, 2009b).

The Agency for Toxic Substances and Disease Registry (ATSDR, 2004) has established minimal risk levels (MRLs) for pentaPBDEs. A MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. The pentaBDE acute (1 to 14 days) oral MRL is 0.03 mg/kg body weight per day based on endocrine disruption activity. The pentaBDE oral intermediate (14 to 364 days)

MRL is 0.007 mg/kg based on liver toxicity in a 90-day oral exposure study in rats. In 2014, ATSDR began a rereview of the pentaBDE MRLs.

A reference dose (RfD) is the United States Environmental Protection Agency's (USEPA) maximum acceptable oral dose of a toxic substance based upon critical toxic effects. RfDs for BDE-47, BDE-99, and BDE-153 are based on altered locomotor activity habituation in mice at 4 months of age following an acute oral dose of the PBDE congener on postnatal day 10 (USEPA, 2008a,b,c). The RfD for BDE-47 of 1.17×10^{-4} mg/kg per day (0.1 µg/kg per day) is based upon a point of departure of 0.35 mg/kg for neurotoxicity in mice (Eriksson *et al.*, 2001; USEPA, 2008a). The RfD for BDE-99 of 0.1 µg/kg per day was derived from a benchmark dose of 0.29 mg/kg per day, based on the effects of BDE-99 on spontaneous motor behavior in mice (Viberg *et al.*, 2004a,b; USEPA, 2008b). The RfD for BDE-153 of 1.5×10^{-4} mg/kg day (0.2 µg/kg per day) was based on a no-observed-adverse-effect-level of 0.45 mg/kg for neurotoxicity in mice (Viberg *et al.*, 2003; USEPA, 2008c).

The USEPA (*Fed. Regist.*, 2012) proposed to designate the processing of six PBDEs (tetraBDE, pentaBDE, hexaBDE, heptaBDE, octaBDE, and nonaBDE), or any combination of these chemical substances resulting from a chemical reaction, as a significant new use; designating manufacturing, importing, and processing of a seventh PBDE, decaBDE for any use that is not ongoing after December 31, 2013. Beginning January 2014, the state of California no longer required flame retardants to be incorporated into most furniture, or baby and infant products (CDCA, 2013).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Absorption, distribution, metabolism, and excretion studies have usually been done on individual PBDE congeners, not on the DE-71 mixture. The disposition and metabolism of several congeners in DE-71 has been reported in rodents (Örn and Klasson-Wehler, 1998; Hakk *et al.*, 2002, 2006, 2009; Staskal *et al.*, 2005, 2006a,b,c; Chen *et al.*, 2006; Darnerud and Risberg, 2006; Sanders *et al.*, 2006a,b). BDE-47, BDE-99, and BDE-153 are the most well studied of the congeners with some information also available for BDE-100 and BDE-154. However, very little information is available for other PBDE congeners in DE-71, which include two triBDEs (BDE-17 and BDE-28), a tetraBDE (BDE-66), a pentaBDE (BDE-85), a hexaBDE (BDE-138), and a heptaBDE (BDE-183), together contributing less than 4% of the total peak area. The disposition and metabolism data from these studies are in general agreement and some key studies are highlighted below.

Staskel *et al.* (2005) reported the effect of BDE-47 exposure in female C57BL/6 mice. Following a single oral dose (between 0.1 and 100 mg/kg) or an intratracheal dose (1 mg/kg), over 80% of the [^{14}C]BDE-47 dose was absorbed whereas approximately 62% of the dose was absorbed following dermal application (1 mg/kg). Radioactivity was distributed to tissues with the proportion of dose reaching the tissues dependent on the lipid content; the highest dose was observed in adipose (8% to 14%), with liver, skin, and muscle containing up to 3%. Repeated exposure to 1 mg/kg [^{14}C]BDE-47 for 10 days resulted in a higher concentration remaining in adipose tissue, suggesting its potential for bioaccumulation (Staskal *et al.*, 2006b). Following a single oral dose, the radioactivity was rapidly excreted in urine and feces totaling 65% to 81% of the administered dose (Staskal *et al.*, 2005). The urinary excretion was dose-dependent with 40%, 38%, 33%, and 14% of 0.1, 1.0, 10, and 100 mg/kg excreted at the end of 5 days, with parent BDE-47 detected as the major peak in urine. BDE-47 was eliminated in female mice in a biphasic pattern with an estimated whole body terminal half-life of 23 days and estimated terminal half-lives for tissues of 6 to13 days (Staskal *et al.*, 2005).

Disposition of multiple PBDE congeners was compared following a single 1 mg/kg intravenous dose of [^{14}C]-labeled BDE-47 (2.1 µmol/kg), BDE-99 (1.9 µmol/kg), BDE-100 (µmol/kg), or BDE-153 (1.8 µmol/kg) in female C57BL/6 mice (Staskal *et al.*, 2006c). Following administration, congeners were distributed with similar patterns to lipophilic tissues. The percent radioactivity in tissues examined 5 days following the dose administration were 26%, 39%, 55%, and 75% for BDE-47, BDE-99, BDE-100, and BDE-153, respectively, and were inversely related to excretion rates, demonstrating that the bromine substitution in these congeners played a role in disposition.

In a study where the disposition of BDE-47 was investigated in different stages of development in mice following gavage administration, the authors demonstrated that the pattern of disposition was similar, however, the concentration of BDE-47 was higher in pups compared to adults, suggesting the reduced capacity in pups to metabolize and excrete PBDEs (Staskal *et al.*, 2006a).

In a series of comparative studies, [^{14}C]-labeled doses (0.1 to 1,000 µmol/kg; range of 0.05 to 640 mg/kg) of BDE-47, BDE-99, and BDE-153 were rapidly, but not completely, absorbed in male and female F344 rats and B6C3F1 mice following gavage administration (Chen *et al.*, 2006; Sanders *et al.*, 2006a,b). Of the three congeners, BDE-153 was the least absorbed (70% of the total dose in rats and mice) and BDE-99 was absorbed to the greatest extent (85% of the total dose in rats and mice). Similar to studies by Staskal *et al.* (2005, 2006a,b,c) in mice, the radioactivity was distributed to all assayed tissues with the adipose tissue being the major depot for all three congeners; up to 40% of the total dose of BDE-47 was observed in female rats 24 hours after administration. Distribution of radioactivity to adipose and other tissues was dose proportional up to the highest doses administered for BDE-153 (100 µmol/kg) and BDE-47 and BDE-99 (1,000 µmol/kg). Most of the radioactivity in tissues consisted of parent material and was persistent over time, resulting in long elimination half-lives. These half-lives, likely representing elimination from lipid, are higher with increasing number of bromines (BDE-153>BDE-99>BDE-47) and generally followed log K_{OW} measurements (Braekevelt *et al.*, 2003; USEPA, 2008a). Similar to earlier reported studies (Staskal *et al.*, 2006b), the PBDEs accumulated in all assayed tissues following repeated dosing (Chen *et al.*, 2006; Sanders *et al.*, 2006a,b). The most prominent accumulation was observed in adipose, adrenal gland, skin, and thyroid gland following repeated doses of 0.1 and/or 1 µmol/kg BDE-47, BDE-99, or BDE-153 (Sanders *et al.*, 2006b). BDE-47 accumulated in the adipose tissue to the greatest extent when administered to male rats in an equimolar mixture (1 µmol/kg each) of the three congeners (Sanders *et al.*, 2006b). Relative accumulation in the liver was greatest for BDE-153. Congener-specific differences in initial concentrations of radioactivity in tissues in the rat correlated primarily to differences in the extent of metabolism prior to deposition in lipid (Chen *et al.*, 2006; Sanders *et al.*, 2006a,b). Similar to observations by previous investigators, the major tissue depot for PBDE congeners in mice was adipose tissue; doses were persistent and accumulating in tissues.

Some species differences in the excretion of PBDE congeners have been reported in rodents. The most striking difference was in the amounts of radioactivity excreted in the urine of PBDE-treated animals (Örn and Klasson-Wehler, 1998; Chen *et al.*, 2006; Sanders *et al.*, 2006a,b). Following gavage administration of 30 µmol/kg of [^{14}C]BDE-47 in Sprague Dawley rats, up to 0.5% of the dose was excreted in urine, whereas C57B1 mice excreted up to 20% of the administered dose in urine (Örn and Klasson-Wehler, 1998). Similarly, approximately 2% of a dose of 1 µmol/kg of BDE-47 or BDE-99 was excreted in the urine of rats within 24 hours after gavage administration, almost all as metabolites (Chen *et al.*, 2006; Sanders *et al.*, 2006a). In contrast, up to 40% of a BDE-47 dose or 10% of a BDE-99 dose (both 1 µmol/kg) was excreted unchanged in urine of mice. The difference was attributable to the affinity of the two congeners for a mouse-specific protein identified as the m-MUP-1 isoform (Staskal *et al.*, 2006c; Emond *et al.*, 2013). Consequently, the internal dose of BDE-47, and to a lesser extent BDE-99, was lower in mice than in rats receiving a comparable dose of either congener (Chen *et al.*, 2006; Sanders *et al.*, 2006a). BDE-153 had little apparent affinity for the carrier protein, as demonstrated by only up to 1% of the congener being excreted in urine of mice within 24 hours of dosing (Sanders *et al.*, 2006b).

The metabolism of the PBDE congeners in rodents appears to be similar (Örn and Klasson-Wehler, 1998; Chen *et al.*, 2006; Sanders *et al.*, 2006a,b; Qiu *et al.*, 2007). Following a single gavage dose, BDE-99 was metabolized to a greater extent than BDE-47 whereas BDE-153 was poorly metabolized (Chen *et al.*, 2006; Sanders *et al.*, 2006b). Repeated dosing resulted in increased metabolism of BDE-47 and BDE-99 but had little effect on BDE-153 metabolism. BDE-47 and BDE-99 appear to induce their own metabolism via increased expression of CYPs, and it is probable that concurrent exposure to BDE-153 contributes to this induction (Sanders *et al.*, 2005, 2006b). BDE-47- and BDE-99-derived metabolites isolated from bile consisted mostly of hydroxylated and conjugated species arising through formation of arene oxides, with a loss of bromine in some cases (Figure 2). Other metabolism studies of BDE-47 and BDE-99 in rats showed similar results (Örn and Klasson-Wehler, 1998; Hakk *et al.*, 2002; Malmberg *et al.*, 2005; Marsh *et al.*, 2006; Qiu *et al.*, 2007). A minor amount of BDE-47- and BDE-99-derived radioactivity was eliminated in urine of rats as catechols and conjugated tribromophenols, both arising from the cleavage of the ether linkage (Chen *et al.*, 2006; Sanders *et al.*, 2006a; Qiu *et al.*, 2007). Sufficient information on the metabolites of BDE-153 is not available in the literature.

Some information on the disposition and metabolism of other PBDEs found in DE-71, such as BDE-100 and BDE-154, following gavage administration is available in the literature. The pentaBDE congener, BDE-100, was readily absorbed following oral administration of [^{14}C]-labeled doses of 4.1 mg/kg to male Sprague Dawley rats (Hakk *et al.*, 2006). As with the congeners described above, the radiolabel was deposited into lipid and was persistent in tissues. Metabolites in bile and/or feces were identified as isomers of mono or dihydroxy tetraBDEs. Conjugated metabolites were suspected but were not confirmed. A second hexaBDE, BDE-154, in similar abundance to that of BDE-153 in DE-71, was rapidly absorbed and distributed to tissues following

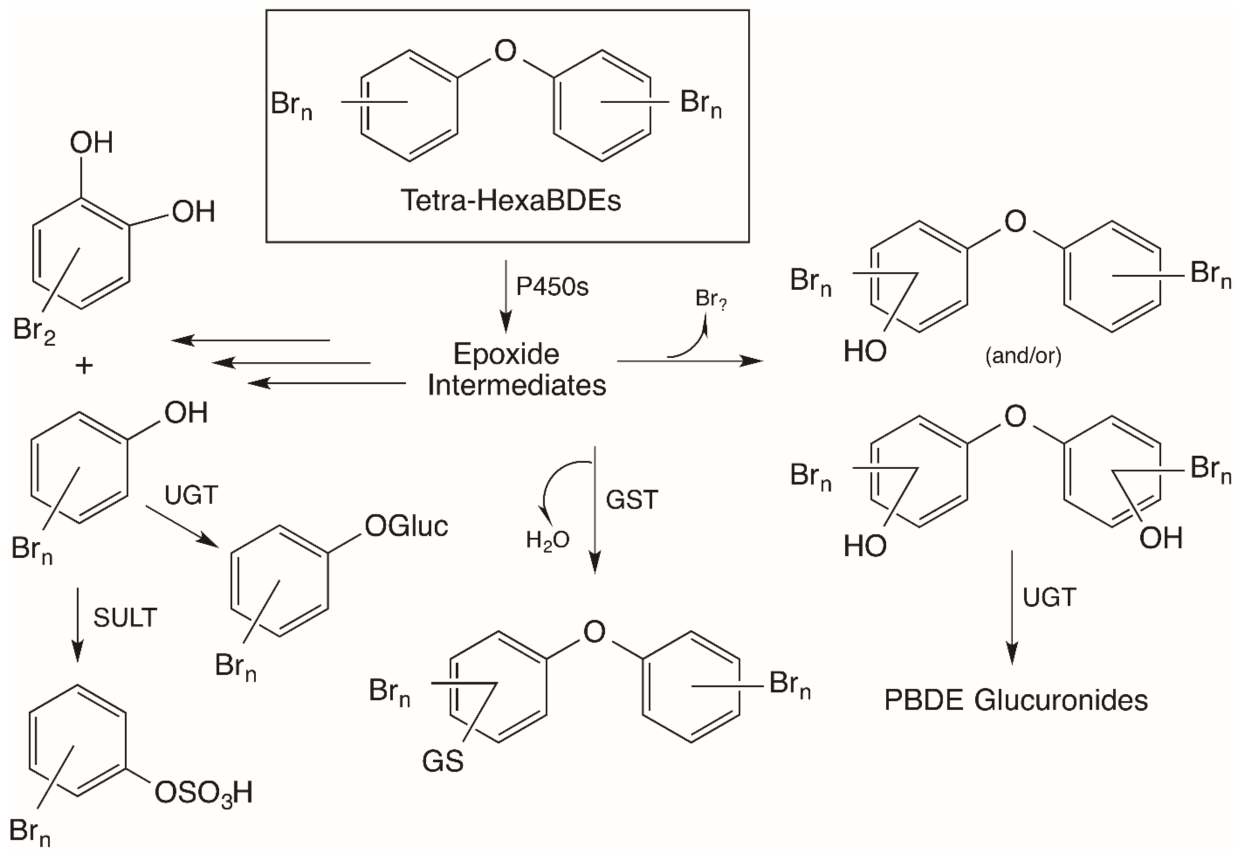


FIGURE 2
Possible Pathways of Metabolism of Tetra-hexabromo Diphenyl Ethers in Rodents
(adapted from Sanders *et al.*, 2006a,b; Chen *et al.*, 2006; Staskal *et al.*, 2006b; Hakk *et al.*, 2006, 2009).
GST = glutathione transferases, P450s = cytochromes P450, SULT = sulfotransferases,
UGT = UDP-glucuronosyltransferases, n=2 or 3

gavage administration of 1.9 mg/kg to male Sprague Dawley rats (Hakk *et al.*, 2009). BDE-154 was less bioaccumulative than BDE-153, and as with BDE-47 versus BDE-99, this observation appears to correlate to differences in the extent of metabolism. The dose was primarily excreted in feces, but in contrast to BDE-153, a large portion of the excreted radiolabel was in the form of metabolites, including multiple isomers of mono and dihydroxylated tetra, penta, and hexaBDEs.

Although pathways of metabolism, including hydroxylation, debromination, cleavage of the ether linkage, and conjugation (Figure 2), may be shared among these congeners, the number and substitution pattern of bromines on each phenyl ring influences the extent of metabolism and disposition and the potential for enzyme induction of the individual congeners. Several studies have shown that PBDEs have the potential to induce cytochrome P450s (CYPs) (Chen *et al.*, 2001, Zhou *et al.*, 2001; Sanders *et al.*, 2005, 2006b; Chen *et al.*, 2006). Studies conducted in male F344 rats by Sanders *et al.* (2005) showed that DE-71 and its individual congeners, BDE-47, BDE-99, and BDE-153 upregulated expression of CYP2B and CYP3B in a phenobarbital-like manner. The accompanying disposition and metabolism studies for BDE-47 and BDE-99 indicated that expression of the associated proteins increased, resulting in auto induction of metabolism (the congeners are inducers as well as substrates for the enzymes) (Chen *et al.*, 2006; Sanders *et al.*, 2006a). In contrast, BDE-153 upregulated the genes to the greatest extent and with similar potency as PCB-153, but appeared to be a poor substrate for the CYPs (Sanders *et al.*, 2006b).

Humans

Humans absorb specific PBDE congeners of DE-71 mixtures from the environment as evidenced by concentrations detected in tissues and fluids of populations living in highly populated as well as remote areas (Dallaire *et al.*, 2009; Eskenazi *et al.*, 2011; Hurley *et al.*, 2011; Park *et al.*, 2011). As with rodents, the major depot of lower molecular weight PBDEs is in lipid, particularly in adipose tissue (Johnson-Restrepo *et al.*, 2005). These PDBEs are persistent in humans with congener-specific half-lives ranging from months to years (Geyer *et al.*, 2004, Frederiksen *et al.*, 2010b; USEPA, 2010a). The PBDE congeners most often found in breast adipose tissue in California women include BDE-47, BDE-99, BDE-153, and BDE-154, and the mean levels of these congeners in adipose tissue were reported as 86, 35, 20, and 3 ng/g lipid, respectively (Petreas *et al.*, 2011). PBDE constituents of DE-71 were also found in maternal and cord blood (Guvenius *et al.*, 2003; Mazdai *et al.*, 2003; USEPA, 2010b); median cord blood concentrations of BDE-47, BDE-99, and BDE-100 were reported to be 11.2, 3.2, and 1.4 ng/g lipid, respectively (Herbstman *et al.*, 2010).

PBDE congeners may be hydroxylated when incubated with human microsomes or hepatocytes (Lupton *et al.*, 2009; Stapleton *et al.*, 2009; Erratico *et al.*, 2012, 2013; Feo *et al.*, 2013). This activity appears to be mediated primarily by CYP2B6 (Erratico *et al.*, 2012; Feo *et al.*, 2013). Further, hydroxylated PBDEs attributable to exposure to tetra-hexaBDEs have been detected in human serum including that obtained from maternal and cord blood in pregnant women (Athanasiadou *et al.*, 2008; Qiu *et al.*, 2009). Concentrations of hydroxylated BDE-47 and BDE-99 were higher in cord blood than in maternal blood in work conducted by Chen *et al.* (2013). BDE-47 and BDE-99, but not BDE-153, were metabolized to hydroxylated species by human microsomes (Lupton *et al.*, 2009); a dihydroxylated metabolite and 2,4-dibromobenzene were detected after incubation with BDE-47. BDE-99 exposure yielded a dihydroxylated metabolite, 2,4,5-tribromophenol, and 1,3-dibromobenzene. The presence of tribromophenol and the dibromobenzenes in these studies indicated that, as in rodents, cleavage of the ether linkage of tetra and pentaBDEs is possible in humans. Additional hydroxylated metabolites for BDE-47 and BDE-99 have been described in human microsome and human hepatocyte studies (Stapleton *et al.*, 2009; Erratico *et al.* 2012, 2013; Feo *et al.*, 2013). Comparative work conducted by Qiu *et al.* (2007, 2009) indicated differences in the profile of hydroxylated PBDE metabolites in humans and mice. For instance, following subcutaneous injection or oral administration of DE-71, the most abundant hydroxylated PBDE detected in plasma of mice was 4-OH-2,2′,3,4′-tetraBDE (indicating a bromine shift on BDE-47). In pregnant women, the hydroxlyated PBDEs found at the greatest concentration in blood (maternal and cord) were 5-OH BDE-47 and 5-OH BDE-99 with similar abundance. These two metabolites were not detected in DE-71-treated mice.

TOXICITY

Experimental Animals

Thyroid and Liver Toxicity

Hydroxy-BDE-47 interfered with thyroxine (T_4) for binding to the plasma transport protein transthyretin (TTR) (IC_{50}, 0.18 μM) (Hamers *et al.*, 2006). TTR binding activity was seen with BDE-47 ($IC_{50} > 25$ μM) but not with BDE-99 (Hamers *et al.*, 2006). Other studies show that decreased circulating concentrations of T_4 may be related to increased glucuronidation of T_4 after PBDE exposure (Richardson *et al.*, 2008). Hydroxylated PBDEs inhibited deiodinase from converting T_4 to triiodothyronine (T_3) (Butt *et al.*, 2011). Hydroxylated PBDEs (e.g., hydroxyBDE-47) were more effective estrogen receptor agonists than the parent PBDE (e.g., BDE-47), but still had many-fold lower activity levels than estradiol (Meerts *et al.*, 2001). Meerts *et al.* (2000) compared the interaction of 17 PBDE congeners with T_4 binding to TTR in an *in vitro* competitive binding assay, using human TTR and ^{125}I-T_4 as the displacement radioligand. Incubation of PBDEs with phenobarbital treated liver microsomes (mostly P450 2B enriched) in the presence of NADPH resulted in the formation of PBDE metabolites for use in the assay. PBDEs were able to compete with T_4-TTR binding only after metabolic conversion by rat liver microsomes to hydroxylated PBDEs. The TTR binding activity of BDE-47 was greater than that of BDE-99 (Meerts *et al.*, 2000).

Hamers *et al.* (2006) also measured interaction of 19 PBDEs and other flame retardants with the AhR, androgen receptor (AR), progesterone receptor (PR), and estrogen receptor (ER), and if these substances inhibited estradiol sulfation by sulfotransferase. No AhR, AR, or PR agonist activity was noted for most of the chemicals tested (including BDE-47, BDE-99, and BDE-153) compared to the positive controls (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, flutamide, or RU-486, respectively). Antagonist activity for BDEs (e.g., BDE-47) was found for the AhR ($IC_{50} = 2.7$ μM), AR ($IC_{50} = 1$ μM), and PR ($IC_{50} > 15$ μM) assays.

DE-71

DE-71 and its components (e.g., BDE-47) cause a number of toxic effects in rodents including alteration of thyroid homeostasis and liver toxicity (ATSDR, 2004; USEPA, 2008a,b,c).

In a 14-day study, when male Charles River CD rats were administered 0, 50, 500, or 5,000 mg/kg DE-71 by oral gavage, decreased survival was observed in the 5,000 mg/kg group (WHO, 1994). This 14-day study was followed by a 28-day study in which male and female Charles River CD rats were exposed to DE-71 in the diet (0, 100, or 1,000 mg/kg) (WHO, 1994). There were no treatment-related effects on survival or clinical signs. Liver weights were increased in 100 mg/kg females and 1,000 mg/kg males and females. Lesions included liver hypertrophy and thyroid gland hyperplasia in 100 and 1,000 mg/kg animals. In a 90-day DE-71 study in male and female CD Sprague Dawley rats administered 0, 2, 10, or 100 mg/kg by oral gavage, there were no treatment-related effects on survival or clinical signs; however, serum T_4 concentrations were decreased, relative liver weights increased, and hepatocytomegaly and thyroid gland hyperplasia occurred in the 10 and 100 mg/kg groups (WHO, 1994).

When DE-71 was administered at doses of 0, 1, 10, or 30 mg/kg by oral gavage to Long-Evans rat dams from gestational day (GD) 6 to postnatal day (PND) 21 there were no reported clinical signs and no effects on dam weight, litter size, sex ratio, or offspring viability or growth; pups did not receive direct dosing (Zhou *et al.*, 2002). There were decreases in serum T_4 concentrations in the 30 mg/kg dams on GD 20 (48% decrease), in fetuses on GD 20 (at least 15% decrease), and in pups on PND 4 and PND 14 (50% and 64% maximal decreases in the 10 and 30 mg/kg groups). T_4 rebounded by PND 36. No effect on serum T_4 concentrations occurred at 1 mg/kg. There were no changes in serum T_3 concentrations in dams. In 10 and 30 mg/kg pups on PNDs 4 and 14, ethoxyresorufin-*O*-deethylase (EROD; a marker of CYP1A1 activity) activity was increased up to 95-fold, pentoxyresorufin-*O*-dealkylase (PROD; a marker of CYP2B activity) activity was increased up to 26-fold, and uridine diphosphate glucuronosyl transferase (UDPGT) activity was increased up to 4.7-fold using T_4 as the substrate for glucuronidation activities in hepatic microsomes. EROD and PROD activities were increased in 10 and 30 mg/kg dams and UDPGT activity was increased in the 30 mg/kg dams on PND 22. When 28-day-old female Long-Evans rats were administered DE-71 by oral gavage for 4 days (0.1 to 300 mg/kg) serum T_4 was decreased a maximum of 80% (Zhou *et al.*, 2001). EROD and PROD liver enzyme concentration induction levels were increased up to 10- to 20-fold and up to 30- to 40-fold, respectively, in animals administered 10 mg/kg or greater. UDPGT activity was also increased. When male F344 rats were administered DE-71 (1.5, 15, or 150 mg/kg) orally on three consecutive days, liver CYP1A1, CYP2B, and CYP3A activities were increased in the 15 and 150 mg/kg groups (Sanders *et al.*, 2005). These three DE-71 rat studies (Zhou *et al.*, 2001, 2002; Sanders *et al.*, 2005) did not include a pathology evaluation for target organ lesions.

When pregnant Long-Evans rats were administered DE-71 from GD 6 to PND 21 (0, 1.7, 10.2, or 30.6 mg/kg) serum T_4 concentrations were decreased in the pups on PNDs 4 and 21 (Szabo *et al.*, 2009). Liver mRNA for CYP1A1, CYP2B1, and CYP2B2, and EROD, PROD, and UDPGT activities were increased in the pups on PNDs 4 and 21. Hepatic efflux transporters Mdr1 (multidrug resistance), Mrp2 (multidrug resistance–associated protein), and Mrp3 and influx transporter Oatp1a4 mRNA expression increased in the pups on PNDs 4 and 21. All responses were reversed by PND 60.

BDE-47

After a 4-day exposure of C57BL/6 mice to BDE-47 (3, 10, or 100 mg/kg), serum T_4 was decreased by 43% in 100 mg/kg mice, relative to controls, and liver (PROD) CYP2B concentrations, relative to controls, increased by 120%, 180%, and 480% in the 3, 10 and 100 mg/kg groups, respectively (Richardson *et al.*, 2008). Serum T_4 was decreased in C57BL/6N mice after BDE-47 exposure (18 mg/kg for 14 days) (Hallgren *et al.*, 2001). Developmental exposure to low doses of BDE-47 resulted in changes in thyroid gland histology and morphology in rats (Talsness *et al.*, 2008).

The BDE-47 upregulation of liver CYP2B (10 and 100 mg/kg) and CYP1A1 (100 mg/kg) after a 3-day exposure is thought to involve activation of both the constitutive activated/androstane receptor (CAR) (mouse and human) and pregnane X receptor (PXR) (human) (Sueyoshi *et al.*, 2014). This is based on the finding that BDE-47 increases CYP2B mRNA expression in wild mice but not in CAR knockout mice. In contrast, knocking out PXR in mice did not affect CYP2B mRNA expression related to BDE-47 exposure. However, in human primary hepatocytes, both CAR and PXR were involved in the PBDE effects on CYP2B concentrations (Sueyoshi *et al.*, 2014). The authors of this work concluded that BDE-47 works primarily through the CAR receptor in mice, and through both the CAR and PXR receptors in humans.

The PBDEs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, or BDE-183) did not induce EROD (a marker of CYP1A1 activity) in rodent hepatoma cell lines transfected with AhR (Peters *et al.*, 2006a). PBDEs

(BDE-47,-BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, or BDE-77) did not induce CYP1A1 in primary hepatocytes of the cynomolgus monkey (using EROD as a marker for CY1A) (Peters *et al.*, 2006b).

BDE-99

Liver CYP concentrations (1A1, 1A2, 2B1, and 2A2) were increased in Sprague Dawley rat pups on GD 20 when the dams were exposed to BDE-99 on GDs 6 to 19 (0, 0.5, 1, or 2 mg/kg) (Blanco *et al.*, 2012). When dams were administered BDE-99 from GD 6 to PND 21 and their pups evaluated for spatial learning task in a water maze, the pups of exposed dams showed a delay in this learning task. Serum T_3 was decreased by 14% and T_4 was decreased by 25% in 2 mg/kg pups on PND 21 (Blanco *et al.*, 2013). There was a decrease in genes in the AKT pathway in the liver of pups treated with BDE-99, suggesting that this PBDE induces changes in the metabolism of the pups (Blanco *et al.*, 2014).

Immunotoxicity

PBDEs are reported to be immunotoxicants in rodents. When female C57Bl/6 mice were given a single oral dose of DE-71 (0.8 to 500 mg/kg) or a 14-day DE-71 exposure (250 to 1,000 mg/kg), there was a depression in an anti-sheep red blood cell response in the plaque forming cell response assay in the 1,000 mg/kg group in the 14-day exposure (Fowles *et al.*, 1994). There was also a treatment-related decrease in the thymus weight. There was no effect on natural killer cell activity in YAC-1 target cells.

When DE-71 was administered orally to B6C3F1 mice (0.5 to 100 mg/kg), natural killer cell activity was decreased at 100 mg/kg at the end of the treatment period. There were some decreases in splenic CD4+CD8+ cells (Fair *et al.*, 2012).

Recently the NTP investigated the relative potency of a number of brominated dioxins and furans that are components of DE-71, with regard to their ability to suppress the humoral immune response (Frawley *et al*., 2014). To assess the relative potencies of polybrominated dibenzo-*p*-dioxins/dibenzofurans, female B6C3F1/N mice received a single oral exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,7,8-tetrabromodibenzofuran (TBDF), 1,2,3,7,8-pentabromodibenzofuran (1PeBDF), or 2,3,4,7,8-pentabromodibenzofuran (4PeBDF). Inhibition of the IgM antibody forming cell response was measured 4 days following immunization with sheep red blood cells. The reference compound for these studies, TCDD, induced a significant reduction in the number of antigen specific antibody forming cells at doses of 0.1 µg/kg or greater. Exposure to the three dibenzofurans resulted in a reduction in the antibody response against sheep red blood cells, although to a lesser degree than TCDD. TBDF and 4PeBDF suppressed the humoral immune response at doses of 3 µg/kg or greater. 1PeBDF was less potent and suppressed the total number of antibody forming cells per spleen at doses of 30 µg/kg or greater. Taken together, these studies suggest that DE-71 and its components have potent and persistent effects on the humoral immune system. Other immune cells and processes may also be targeted to a lesser degree.

Neurotoxicity

DE-71

When 4-month-old C57BL/6 mice were administered 30 mg/kg DE-71 orally by gavage for 30 days, there was deposition of PBDE congeners (including BDE-47, BDE-99, and BDE-153) in the brains and reductions in striatal dopamine and dopamine handling, as well as reductions in the striatal dopamine transporter and VMAT2, and a significant locomotor deficit (Bradner *et al.*, 2013).

BDE-47

When Sprague Dawley rats received a single oral dose (1, 5, or 10 mg/kg) of BDE-47 on PND 10, behavioral deficits in the Morris water maze were noted in all dosed groups (He *et al.*, 2009). At 10 mg/kg, ultrastructural changes at 2 months of age were observed in the CA1 hippocampal neurons by electron microscopy. The endoplasmic reticulum and mitochondria appeared swollen and/or degranulated; and neurons had puffed periplast, dissolved cell organelles, and vacuolized mitochondria. At 2 months of age, mRNA levels for caspase 3 and caspase 12 were elevated in the hippocampus in 5 and 10 mg/kg males and females. At 10 mg/kg, mRNA levels for cytochrome C were elevated in males and mRNA levels for DAPK were decreased in females and elevated in males.

Evidence for decreased organ-to-body-weight ratios for thyroid gland and uterus and decreased T_4 levels were seen in Sprague Dawley rats after a single oral dose (5 or 10 mg/kg) of BDE 47 on PND 10 (He *et al.*, 2011). PBDE exposure to rats has also been shown to disrupt estrogen-regulating genes (Ceccatelli *et al.*, 2006).

Neurotoxic effects were seen in 10-day-old male NMRI mice administered a single oral dose of BDE-47 (0.7 or 10.5 mg/kg) (Eriksson *et al.*, 2001). At 2 and 4 months of age, the habituation pattern for locomotor activity in a novel environment was delayed in mice administered 10.5 mg/kg. At 5 months of age, no learning deficit was observed in the Morris water maze.

Neurotoxic effects were seen in male C57BL/6 mice administered a single 6.8 mg/kg oral dose of BDE-47 on PND 10 (Dingemans *et al.*, 2007). Hippocampal slices were prepared from the mice on PND 17 and field potentials in the CA1 hippocampal region demonstrated a deficit in long-term potentiation with BDE-47 exposure indicative of postsynaptic effects. Protein levels for the NMDA receptor subunit NR2B, AMPA receptor subunit GluR1, and phospho-alpha CaMKII were decreased in postsynaptic densities.

Male C57BL/6 mice administered a single oral dose of BDE-47 on PND 10 (1, 10, or 30 mg/kg) showed no changes in motor activity at 2 months of age; but at 4 months of age, the overall activity level was elevated in all dosed groups (Gee and Moser, 2008).

PBDE neurotoxic effects occurring early may worsen as the animal ages, inducing persistent neurotoxic effects that are manifested later in life (Dingemans *et al.*, 2011).

BDE-99

Kuriyama *et al.* (2005) reported that when a single dose of BDE-99 (60 or 300 mg/kg) was given to pregnant Wistar rats on GD 6, increases in locomotor activity over a 24-hour time period on PND 34 for 300 mg/kg pups and on PND 71 for 60 and 300 mg/kg pups were observed. No changes were observed in developmental landmarks. Male offspring showed no impairment of sexual behavior. Serum T_4 levels were decreased on PND 22 (Kuriyama *et al.*, 2007).

When Sprague Dawley rats received oral gavage doses of BDE-99 (0 or 2 mg/kg) from GD 6 to PND 21, maturation of negative geotaxis (position orientation) was delayed, and latency on the Morris water maze was longer in the dosed group (Cheng *et al.*, 2009). On PND 37, activities of superoxide dismutase and glutathione peroxidase were decreased in the hippocampus with no change observed in the cerebellum or cortex. Electron spin resonance spectra of spin adducts were increased in the hippocampus but not in the cerebellum or the cortex. Tissue levels of BDE-99 were similar across the three brain regions.

When male and female C57BL/6 mice were administered BDE-99 by oral gavage (0.4, 0.8, 4, 8, or 16 mg/kg) on PND 10, there were lower locomotor activity levels and a deficit in habituation at 4 to 16 mg/kg at 2 and 4 months of age (Viberg *et al.*, 2004a). By 8 months of age, a lower activity level in the initial session was also observed at 0.8 mg/kg. A similar pattern was observed in female offspring. Further work by Viberg *et al.* (2004b, 2005) showed decreases in nicotinic and muscarinic receptor binding in the hippocampus of adult mice exposed to BDE-99 (8 to 16 mg/kg) on PND 10.

When 10-day old male NMRI mice were given one oral dose of BDE-99 (0.8 or 12.0 mg/kg), locomotor activity level was initially lower and a deficit in habituation was observed in the 12 mg/kg dose group at 2 months of age (Eriksson *et al.*, 2001). At 4 months of age, effects were seen in both dose groups. In the 12 mg/kg group, acquisition on the Morris water maze was not altered, but deficits were noted in performance when the mouse was required to learn on a new platform location.

When pregnant CD-1 mice received oral gavage doses of BDE-99 (0.6, 6, or 30 mg/kg) from GD 6 to PND 21 there were 15% to 20% decreases in litter size in the 6 and 30 mg/kg groups (Branchi *et al.*, 2002). No effects were observed on body weight, neurodevelopmental indices, ultrasonic vocalizations on PNDs 4, 8, or 12, or homing on PND 11. On PND 34, open-field activity was increased. On PND 60, habituation was diminished in the 0.6 and 6 mg/kg groups (Branchi *et al.*, 2004, 2005).

BDE-99 caused adult neurotoxic effects when administered during the neonatal period coinciding with the brain growth spurt, affecting brain proteins involved with growth, differentiation, and synaptogenesis in the cortex and hippocampus (CAMKII, GAP-43, synaptophysin and tar) (Viberg and Eriksson, 2011). A review of *in vitro* test results shows that hydoxylated PBDEs can affect voltage-gated Ca^{2+} channels and, thus, have the potential to alter calcium homeostasis and induce changes in neurotransmitter release (Westerink, 2014).

BDE-153

When NMRI mice were administered BDE-153 orally (0, 0.45, 0.9, or 9 mg/kg) on PND 10 there was a disruption in spontaneous behavior indicated by decreased habituation and impaired learning and memory capabilities when tested in the Morris water maze (lowest-observed-effect-level of 0.9 mg/kg) (Viberg *et al.*, 2003). A reduced density of nicotinic receptors in the hippocampus was observed at 6 months as well as decreases in locomotor activity habituation at 2, 4, and 6 months in the 9 mg/kg group. The Morris water maze test indicated learning but delayed performance in the 0.9 and 9.0 mg/kg groups.

Humans

Human exposures to low molecular weight PBDEs have been associated with alterations in thyroid gland homeostasis with varying results among the studies reported. Different BDE congeners may have been measured and different segments of the population may have been studied in the studies described below. Increased ΣPBDE serum levels (the sum of 10 low molecular weight PBDEs measured) were associated with decreased thyroid stimulating hormone (TSH) levels (but no correlation was found for T_4 levels) in pregnant mothers (Chevrier *et al.*, 2010). In another study, BDE-153 was associated with decreased cord blood T_4 levels (Herbstman *et al.*, 2008). In contrast, in another study there were positive associations between increased PBDE serum levels (27 PBDE congeners measured) and increased serum T_4 levels in pregnant women (Stapleton *et al.*, 2011). The occurrence of total PBDEs, BDE-47, and hydroxylated PBDEs has been associated with increased levels of TSH in women living in California (Zota *et al.*, 2011).

In adult males, there is a reported association between increased T_4 levels and increased BDE serum levels (BDE-47, BDE-99, BDE-100, and BDE-153) (Turyk *et al.*, 2008). Elevated serum levels of TSH were seen in workers exposed to PBDEs (total serum BDEs in control 158/ng/g lipid vs 382 ng/g lipid in the "exposed" group; data not presented as individual BDE congener data) at electronic waste sites in China (Yuan *et al.*, 2008).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

DE-71 and congeners contained in DE-71 (e.g., BDE-47 and BDE-99) have been shown to cause reproductive and developmental toxicity (Lasky *et al.*, 2002; Darras, 2008; EFSA, 2011). PBDEs may also cause reproductive toxicity in wildlife including fish, birds, and marine mammals (WHO, 2012).

DE-71

In a DE-71 oral gavage study (0, 3, 30, or 60 mg/kg) in adult Wistar rats, males were exposed from PNDs 23 to 53 and females from PNDs 22 to 41 (Stoker *et al.*, 2004). Liver to body weight ratios (at 30 and 60 mg/kg) were increased but no changes were seen in necropsy body weights. T_4 levels were decreased by approximately 70% in females exposed to 30 and 60 mg/kg after the 20 day exposure. T_4 levels were decreased approximately 85% and liver enzyme concentrations were increased in males exposed to 30 or 60 mg/kg for 31 days. Vaginal opening was delayed in rats exposed to 60 mg/kg (32.4 ± 4.2 days in vehicle controls versus 34.2 ± 7.3 days). Preputial separation was delayed in males by 1.7 and 2.1 days in the 30 and 60 mg/kg groups, respectively. Ventral prostate gland and seminal vesicle weights were significantly decreased in males at 60 mg/kg. Ovaries, uteri, testes, and epididymides were examined for treatment-related lesions after hematoxylin and eosin staining and none were found. However, there were treatment-related lesions in the thyroid gland consisting of decreased colloid area and increased follicular cell heights (indicative of the hypothyroid state) in 60 mg/kg females exposed for 20 days and 60 mg/kg males exposed for 31 days.

Stoker *et al.* (2005) examined competitive binding of DE-71, BDE-47, BDE-99, and BDE-100 with R1881 (also known as methyltrienolone, a synthetic androgen) for the androgen receptor in a rat ventral prostate cytosolic extract. DE-71 and BDE-100 both inhibited androgen receptor binding, with IC_{50}s of approximately 5 µM. In addition, DE-71, BDE-100, and BDE-47 inhibited dihydrotestosterone-induced transcriptional activation.

When Long-Evans rat dams were administered DE-71 by gavage (0, 1.7, 10.2, or 30.6 mg/kg) from GD 6 to weaning (except for day of birth) (Kodavanti *et al.*, 2010), there was a 5.5% (not statistically significant) decrease in anogenital distance on PND 7 in male pups from the 30.6 mg/kg group. Other findings in this dose group included an increase in the age of preputial separation attainment, and a 20% decrease in mean testosterone concentration on PND 60. In female pups, there was a reduction in mammary gland development on PND 21 in the 10.2 and 30.6 mg/kg groups.

Decreased epididymis, seminal vesicle, and prostate gland weights, as well as sperm head deformities and increased CYP17 levels were noted in male Wistar rats (7 weeks of age at start of dosing) exposed to DE-71 (0.27, 0.82, 2.47, 7.4, 22.2, 66.7, or 200 mg/kg) for 28 days; the bench mark dose for many of these effects was calculated to be 10 to 50 mg/kg (van der Ven *et al.*, 2008).

When Sprague Dawley rats were given a mixture of DE-71 and hexabromocylododecane (15:1, PBDE:HBCD) in the feed (estimated to deliver 0, 0.6, 20, or 60 mg/kg, assuming feed consumption of 80 g/kg body weight per day) 2 weeks prior to mating through GD 20, there was no effect on maternal health, litter size, or fetal viability, but the proportion of litters with fetuses that had anomalies increased (including soft tissue syndactyly and decreased ossification of the sixth sternebra) at all dose levels (Berger *et al.*, 2014). The lowest dose in this study was estimated to deliver the amount of flame retardant a child would ingest, 100 mg/day.

BDE-47

In female offspring of Wistar rat dams administered one dose of BDE-47 (140 or 700 µg/kg) by oral gavage on GD 6 and evaluated on PND 38, there were decreases in ovarian follicle numbers and serum estradiol concentration in the 700 µg/kg group (Talsness *et al.*, 2008). There was no change in ovarian aromatase activity. On PND 100, degeneration of thyroid gland follicular epithelium was noted. No evidence of altered reproductive performance or teratology findings was seen when F_1 females were mated to untreated males.

When pregnant Wistar rats were given an intravenous injection of BDE-47 (0.002 or 0.2 mg/kg) on GD 15 and every fifth day until PND 20 (6 total injections), there were no effects on litter size, developmental landmarks, vaginal opening, testis descent or preputial separation (Suvorov *et al.*, 2009a,b). Locomotor activity was increased in all dosed groups on PND 20, but no effect was seen on motor coordination (Suvorov *et al.*, 2009b). The effects were observed in both male and female offspring. Total serum T_4 levels were decreased on PND 27 in all dosed groups (Suvorov *et al.*, 2009b). Serum insulin-like growth factor 1 (IGF-1) levels were elevated on PND 27 in male offspring, but not in female offspring (Suvorov *et al.*, 2009b). After a bolus dose of glucose on PND 40 or PND 75, there were no BDE-47 related alterations in blood glucose levels (Suvorov *et al.*, 2009b).

PBDE flame retardants have been reported to be ER agonists, but only at concentrations six magnitudes greater than that of the positive control, estradiol [e.g., BDE-47 (EC_{50}=12 µM)] (Hamers *et al.*, 2006). 6-Hydroxy-BDE-47 and BDE-47 inhibited sulfotransferase. A study using assays similar to those used by Hamers *et al*. (2006) showed that BDE-47 had agonist activity in the ER assay, but only at concentrations many-fold higher than that of estradiol (Suzuki *et al.*, 2013).

BDE-99

When Wistar rat dams were administered a single dose of BDE-99 (60 or 300 µg/kg in peanut oil) on GD 6, alterations in degenerative changes in mitochondria were noted in the ovaries of female offspring (Talsness *et al.*, 2005). Mating of the F_1 females with untreated males resulted in an increased resorption rate in the dosed groups compared to controls. The same treatment protocol in male offspring showed reduced sperm and spermatid counts in the treated groups (Kuriyama *et al.*, 2005).

In another study, Long-Evans rat dams were exposed to BDE-99 by subcutaneous injection (1 or 10 mg/kg) from GD 10 through 18 (Ceccatelli *et al.*, 2006). There were no effects on reproductive endpoints. At 120 days of age, uterine mRNA levels were extracted from female offspring, and estrogen target genes were determined by real-time polymerase chain reaction. Progesterone receptor transcript was down-regulated at both dose levels, and ERα, ERβ, and IGF-1 were upregulated at the lower dose.

When Long-Evans rat dams were exposed by subcutaneous injection to BDE-99 (1 or 10 mg/kg) from GD 10 through 18, there were decreases in the circulating sex steroids 17β-estradiol and testosterone in male offspring at weaning and in adulthood, reduction of anogenital distance, and feminization of sexually dimorphic behavior (Lilienthal *et al.*, 2006). Puberty onset was delayed at the higher dose in female offspring, and a slight acceleration in puberty onset was detected in low-dose males. The number of primordial/primary ovarian follicles was reduced in females at the lower dose, whereas the decline of secondary follicles was more pronounced at the higher dose.

Studies in the literature suggest that PBDEs weakly bind to the estrogen receptor. Other studies show that PBDE estrogenic activity may be due in part to the ability to interact with sulfotransferases, resulting in prolongation of estrogen in the circulation by inhibiting its conjugation and excretion (USEPA, 2008c; Gosavi *et al.*, 2013).

Humans

The PBDE reproductive and developmental studies in humans reported in the literature were conducted on diverse study populations, and often did not provide enough data to quantify PBDE exposure or identify a no-effect level. Studies were often based on limited numbers of subjects.

PBDEs can pass through the placenta and may be found in umbilical cord plasma (Frederiksen *et al.*, 2009, 2010a,b). BDE-28, BDE-47, and BDE-99 have been found in umbilical cord blood samples and BDE-47 was the most abundant congener (56 ng/g lipid) (Foster *et al.*, 2011). PBDE exposure to the infant may also occur from mother's milk (Schecter *et al.*, 2006, 2010a), and children may continue to be exposed to PBDEs from ingestion of house dust (Lorber, 2008; Frederiksen *et al.*, 2010a; Harrad *et al.*, 2010).

Several studies suggest that *in utero* exposure to low molecular weight PBDEs may cause reproductive toxicity, alteration in hormone levels, or adverse effects on learning. Because of the structural similarity of PBDEs to the thyroid hormones, PBDEs (hydroxyl-PBDEs) bind to thyroid receptors α1 and β and may thus inhibit the release of TSH by the pituitary gland (Marsh *et al.*, 1998). This is of concern because maternal thyroid hormones play an essential role in fetal brain development (Haddow *et al.*, 1999; Ausó *et al.*, 2004). Hyperthyroidism during pregnancy has been linked to increased risk of miscarriage, premature birth, and intrauterine growth retardation (Lazarus, 2005a,b).

Total PBDE exposure and BDE-99 exposure have been associated with lower birth weight (Foster *et al.*, 2011; Lignell *et al.*, 2013). A positive association between the amount of 12 congeners of PBDE in milk and lower birth weight, length, and head and chest circumference in newborns was reported (after adjusting for maternal age, prepregnant body mass index, and parity) (Chao *et al.*, 2007). PBDE exposure (BDE-47, BDE-99, BDE-100, and BDE-153) was associated with delayed time to pregnancy in a group of women enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study (Harley *et al.*, 2010).

Children exposed prenatally to PBDEs (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154) may have altered learning parameters when these endpoints are measured at 6 years of age (although the children may have been exposed to a number of other organohalogen compounds) (Roze *et al.*, 2009). In the CHAMACOS study, maternal PBDE levels (BDE-47, BDE-99, BDE-100, and BDE-153) were associated with impaired attention in children at 5 years of age, lower scores on an IQ test at 5 and 7 years of age, and poorer fine motor coordination at 5 and 7 years of age (Eskenazi *et al.*, 2013). Another study reported that children prenatally exposed to PBDEs [mother's cord blood levels of BDE-47, median 11.2 ng/g lipid (maximum 613), BDE-99, median 3.2 ng/g lipid (maximum 20), or BDE-100, median 1.4 ng/g lipid (maximum 72)] scored lower on tests of mental and physical development at 12 through 48 and 72 months of age when including endpoints for verbal and performance IQ (Herbstman *et al.*, 2010). Postnatal exposure to BDE-47 was related to an increased risk of symptoms on the attention deficit subscale of ADHD symptoms, but not to hyperactivity symptoms (Gascon *et al.*, 2011). The odds ratio for a low cognitive score was observed among children in Taiwan (8 to 12 months of age) from mothers with a higher PBDE exposure (as measured in cord blood) (Shy *et al.*, 2011). Newborns body mass index was lower in mothers with higher levels of PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, and BDE-153) in a study conducted in Shanghai (Zhang *et al.*, 2011). Birth weights were lower for infants whose mothers had higher PBDE (BDE-47, BDE-99, BDE-100) serum levels (measured at the 26th week of pregnancy) in the CHAMACOS study (Harley *et al.*, 2011).

In a prospective birth cohort study, BDE-47 maternal serum concentration was measured at 16 weeks of gestation (Chen *et al.*, 2014). There was an association between a 4.5 point decrease in IQ and hyperactivity scores in 5-year-olds (but not in children 1 to 3 years of age) whose mothers had BDE-47 levels 10-fold higher than the geometric mean of 20.1 ng/g lipid.

The concentration of PBDE (sum of BDE-47, BDE-153, BDE-99, BDE-100, BDE-28, BDE-66, and BDE-154) in breast milk was 4.16 ng/g fat in mothers of boys with cryptorchidism compared with 3.16 ng/g fat in mothers of boys without cryptorchidism (Main *et al.*, 2007). In this study, the concentrations of BDE-47, BDE-100, and BDE-154 were positively correlated with serum LH values. BDE-154 exposure as measured in maternal blood correlated with decreased FSH levels in boys at 3 months of age (Meijer *et al.*, 2012), and BDE-47 and BDE-100 exposures have been correlated with decreases in sperm quality (Abdelouahab *et al.*, 2011).

Data on the effects of PBDE exposure in men are limited. In one group of men in Massachusetts, an exposure to pentaBDEs (BDE-47, BDE-99, BDE-100) in dust above a mean level of approximately 2,000 ng/g was associated with a 3.6% increase in T_4, a 5.4% increase in T_3, a 17% increase in estradiol, a 16.8% increase in sex hormone binding globulin, and a 20% decrease in follicle stimulating hormone serum levels (Johnson *et al.*, 2013). In a study in men between the ages of 18 and 54, there was an association between increased PBDE (BDE-47, BDE-99, BDE-100) levels in house dust and a decrease in testosterone levels (Meeker *et al.*, 2009).

CARCINOGENICITY

Experimental Animals

No studies were found in the literature that evaluated the carcinogenic potential of DE-71 in rodent models.

Humans

No epidemiology studies reported in the literature provided a definitive understanding of the carcinogenic potential of PBDE exposures in humans. One Swedish case-control study found higher values of PBDEs (sum of BDE-47, BDE-99, and BDE-153) in blood samples from mothers of young men with testicular cancer than in age-matched controls (Hardell *et al.*, 2006). Study subjects were also exposed to other persistent organic chemicals and a definitive link between the cancer and PBDE exposure could not be made.

GENETIC TOXICITY

There is little published genotoxicity data for DE-71. DE-71 (technical grade) was tested for mutagenicity in several strains of *Salmonella typhimurium* (TA100, TA98, TA1535, and TA1537) with and without hamster or rat liver S9 mix up to a high concentration of 10,000 µg/plate, and no mutagenic activity was observed in any strain (Zeiger *et al.*, 1987). DE-71 (312 to 1,250 mg/kg per day) was administered to male B6C3F1 mice by gavage once daily for 3 days, and 24 hours after the third treatment, the frequency of micronucleated immature erythrocytes was determined in peripheral blood using flow cytometry and in bone marrow using slide-based data acquisition methods (Witt *et al.*, 2008). No increases in the frequencies of micronucleated cells were seen in these mice in either bone marrow or peripheral blood samples. The only other information on the genotoxicity of DE-71 is from an industry study cited by the European Chemicals Bureau (ECB, 2001) that reported negative results with DE-71 in a cytogenetic assay conducted in human lymphocytes (Existing Substances Regulation 793/93/EEC, 2000).

Three related bromodiphenyl ethers, BDE-47, BDE-99, and BDE-153, all of which are components of DE-71, have undergone *in vitro* testing in the comet assay and the micronucleus assay in human cell lines. Overall, the results of these assays in a variety of different cell lines indicate that BDE-47, in the absence of S9 metabolic activation enzymes, is capable of inducing DNA damage at non-cytotoxic doses, possibly by increasing the formation of reactive oxygen species.

In one study, human SK-N-MC neuroblastoma cells were exposed to BDE-47 at concentrations of 0, 5, 10, or 20 µM for 4 or 24 hours and DNA damage was assessed using the comet assay without S9 mix (Pellacani *et al.*, 2012). A significant, dose-dependent increase in DNA damage was observed after 4 and 24 hours of exposure to BDE-47, with an approximately fivefold increase in DNA damage seen at the highest concentration in the 4-hour exposure study. Cell viability was approximately 90% or greater for all exposures.

In another study, human SH-SY5Y neuroblastoma cells were exposed to BDE-47 at concentrations of 1, 2, 4, or 8 µg/mL for 24 hours and assessed for DNA damage in the comet assay without S9 mix (He *et al.*, 2008). A significant increase in DNA damage was detected only in cells exposed to 8 µg/mL BDE-47, a concentration that was cytotoxic to SH-SY5Y cells, with approximately 60% of cells surviving at that dose. In addition, increased production of reactive oxygen species was detected in cells exposed to 2, 4, or 8 µg/mL BDE-47, detected by the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. A subsequent study by this same group reported exposing SH-SY5Y cells to 2, 4, or 8 µM BDE-47 or 5 µM BDE-153 for 24 hours and assessing them for DNA damage in the comet assay without S9 mix (He *et al.*, 2010). BDE-153 produced a small but significant increase in DNA damage. BDE-47 (4 or 8 µM) produced small but significant increases in DNA damage. Coexposure of BDE-47 (8 µM) with BDE-153 (5 µM) was suggestive of an additive increase in DNA damage.

One additional report of comet assay results with SH-SY5Y cells exposed to BDE-47 (1, 5, or 10 µM for 24 hours) showed a significant increase in DNA damage at the high dose of 10 µM along with significant increases in 8-oxo-7,8-dihydroguanine, which were reduced by coexposure with 100 µM N-acetylcysteine, an antioxidant (Gao *et al.*, 2009). Cell viability was not evaluated in these experiments. The experiments by Gao *et al.* (2009) were performed without S9 mix.

BDE-153, at a concentration of 5 µM for 24 hours, did not induce DNA damage in SH-SY5Y cells as measured by the comet assay and no additive or synergistic increases in DNA damage were seen with coexposure of SH-SY5Y cells to 5 µM BDE-153 and 10 µM BDE-47 compared to 10 µM BDE-47 alone (Gao *et al.*, 2009), in contrast to the results reported by He *et al.* (2010).

Human SH-SY5Y neuroblastoma cells were exposed to BDE-47 at concentrations of 1, 2, 4, or 8 µg/mL for 24 hours and assessed for chromosomal damage using the cytokinesis block micronucleus (CBMN) assay (He *et al.*, 2008). Cells were exposed to 1, 2, or 4 µg/mL BDE-47 for 24 hours and 1,000 binucleated cells were scored per treatment for micronuclei. A small but statistically significant increase in the frequency of micronuclei was detected in cells exposed to 2 or 4 µg/mL BDE-47. In a second study, SH-SY5Y cells were exposed to 2, 4, or 8 µM BDE-47 for 24 hours and a small but significant increase in micronucleus frequency was reported for cells exposed to 4 or 8 µM BDE-47 (He *et al.*, 2010). A slight but significant increase in micronucleus frequency was reported for cells exposed to 5 µM BDE-153. Cells coexposed to 2, 4, or 8 µM BDE-47 and 5 µM BDE-153 exhibited greater frequencies of micronuclei than either BDE alone, but the increased micronucleus frequencies were not additive or synergistic.

Human MCF-7 breast carcinoma cells were exposed to BDE-47, BDE-99, or BDE-153 at concentrations of 0.01, 0.1, or 1 nM for 24 hours without S9 mix and were assessed for frequency of micronuclei using the CBMN assay (Barber *et al.*, 2006). Small but significant increases in the frequencies of micronuclei were detected in cells exposed to 0.1 nM or 1 nM BDE-47 or 1 nM BDE-99. In cells exposed to 1 nM BDE-153, the micronucleus frequency was increased in one set of experiments but not in another (Barber *et al.*, 2006).

6-hydroxylated-BDE-47 and 6-methoxylated-BDE-47 are metabolites of BDE-47. The ability of these compounds to induce DNA damage was tested in human HepG2 hepatoma cells using the comet assay and the micronucleus assay (An *et al.*, 2011). Cells were exposed for 24 hours to either compound at concentrations of 0.1, 0.2, 0.5, 1, 2, or 5 µM. Small but statistically significant increases in DNA damage were reported in the comet assay at concentrations of 1 µM and greater for both compounds. Both compounds also produced small but significant increases in the number of micronucleated cells per 1,000 cells. The formation of reactive oxygen species, as detected by the DCFH-DA assay, increased with exposure to 6-hydroxylated-BDE-47 (0.1, 0.5, and 2 µM) and 6-methoxylated-BDE-47 (2 µM).

BDE-47, 6-hydroxylated BDE-47, and BDE-99 were tested over a concentration range of 25 to 400 µg/L for ability to induce DNA damage in a differential cytotoxicity assay using wild-type chicken B-lymphoma DT40 cells and isogenic mutant clones deficient for various DNA repair genes (*POLβ, XPA*, *RAD54*, *KU70*, or *REV3*) (Ji *et al.*, 2011). The authors reported that BDE-47 demonstrated enhanced cytotoxicity in *POLβ* –/– and *REV3* –/– clones, and this effect was more pronounced with 6-hydroxylated-BDE47. BDE-99 showed little evidence of cytotoxicity in DNA-repair deficient clones or wild-type DT40 cells. *POLβ* is a polymerase that is recruited to complete the base excision repair pathway, which is the primary pathway for removing oxidized bases from DNA, and *REV3* is a translesion polymerase that allows bypass of chemically modified DNA bases, including oxidized bases. Ji *et al.* (2011) also showed that coexposing cells to BDE-47 or 6-OH-BDE-47 with N-acetylcarnitine, a scavenger of reactive oxygen species, reduced the cytotoxicity of both chemicals in wild-type and in *POLβ* –/– and *REV3* –/– DT40 cells. These results suggest that BDE-47 and its metabolite, 6-hydroxy-BDE-47, induce DNA damage, possibly by generating reactive oxygen species.

Results of an *in vivo* comet assay in male rats revealed that the DNA of sperm was not damaged after dietary exposure for 70 days to a mixture of brominated flame retardants that contained DE-71 in addition to DE-79, decaBDE-209, and hexabromocyclododecane (0.02, 0.2, 2.0, or 20 mg/kg/day) (Ernest *et al.*, 2012).

STUDY RATIONALE

The California Office of Environmental Health Hazard Assessment nominated individual PBDE congeners for toxicity and carcinogenicity study (e.g., BDE-47, BDE-99, and BDE-153) because they were considered a health risk and have been found in human and animal tissue in the United States. Because the individual PBDE congeners were not available in sufficient amounts, the NTP conducted toxicity and carcinogenicity studies of DE-71 (a technical grade mixture that contained BDE-47, BDE-99, and BDE-153) in rats and mice to investigate the toxic and carcinogenic potential of the pentaPBDE formulation (DE-71).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

DE-71

DE-71 was obtained from Great Lakes Chemical Corporation (El Dorado, AR) in two lots (2550OA30A and 1550OK07A). Lot 2550OA30A was used during the 3-month and 2-year studies; lot 1550OK07A was used for dose formulation development studies performed by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) and was not used in any of the animal studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and by the study laboratory at Southern Research Institute (Birmingham, AL) (Appendix J). Karl Fischer titration was performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the DE-71 studies are on file at the National Institute of Environmental Health Sciences.

Lot 2550OA30A of the test chemical, a viscous, sticky brown liquid, was identified as DE-71 by the analytical chemistry laboratory using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR spectroscopy. IR spectra were consistent with the literature spectra (Bio-Rad Sadtler, 2003) and for the structures for a polybrominated diphenyl ether (PBDE) mixture. Proton and carbon-13 NMR spectra were consistent with computer-calculated spectra and the structures for a PBDE mixture.

For lot 2550OA30A, the moisture content was determined by Karl Fischer titration and the purity profile was determined by the analytical chemistry laboratory using gas chromatography (GC) with flame ionization detection (FID). The purity profile of the bulk chemical was also determined by the study laboratory using GC/FID analysis. In further analyses of the bulk chemical using GC coupled with mass spectrometry (MS) detection, the analytical chemistry laboratory confirmed the identity of the peaks observed in the purity profiles, and screened for the presence of polychlorinated and polybrominated dibenzodioxins and furans.

Karl Fischer titration indicated less than 0.1% water. GC/FID yielded a purity profile containing 16 reportable peaks, 11 of which were PBDEs tentatively identified by retention time matching to standards of PBDEs prepared in chloroform (Table J2). Six peaks in this profile contained areas exceeding 2% of the total peak area; 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99; 41.67%), 2,2',4,4'-tetrabromodiphenyl ether (BDE-47; 35.68%), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100; 10.44%), 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154; 3.63%), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153; 3.33%), and 2,2′,3,4,4′-pentabromodiphenyl ether (BDE-85; 2.03%) (Table 2). The identities of peaks in the GC/FID purity profile were confirmed by GC/MS using authentic PBDE standards for 11 peaks. The specific identity of an individual PBDE was based on the retention time and the mass spectrum of the standard to a peak in DE-71. It should be noted that other positional isomers with the same number of bromines might elute at the same retention time and would give the same mass spectrum. Therefore, the identity of the specific isomer should be considered tentative. Using polychlorinated analytical standards and high resolution GC/MS, samples of the bulk chemical were found to contain no polychlorinated dibenzodioxins or furans (Table J3). Polybrominated analytical standards and a second high resolution GC/MS system were used to determine that polybrominated dibenzodioxins and furans were present in the test article; concentrations of 2,3,7,8-tetrabromodibenzofuran (2,3,7,8-TBDF), 1,2,3,7,8-pentabromodibenzofuran (1,2,3,7,8-PeBDF), 2,3,4,7,8-pentabromodibenzofuran (2,3,4,7,8-PeBDF), and coeluting 1,2,3,4,7,8-hexabromodibenzofuran (1,2,3,4,7,8-HxBDF) and 1,2,3,6,7,8-hexabromodibenzofuran (1,2,3,6,7,8-HxBDF) were quantifiable (Tables 2 and J4). Taken together, these analyses indicated that the test article consisted of a mixture of approximately 54% pentabromodiphenyl ethers, 36% tetrabromodiphenyl ethers, 7% hexabromodiphenyl ethers, and low levels of a few polybrominated dibenzodioxins and furans (Table 2).

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC/FID and indicated that DE-71 was stable as a bulk chemical for 15 days when stored in sealed amber glass bottles at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in sealed glass containers. Periodic reanalyses of the bulk chemical were performed by the study laboratory during

TABLE 2
Composition of the DE-71 Lot Used in the Current Studies

Constituent	Name	CAS Number	% in DE71[a]	Concentration in DE-71 (pg/g)[b]
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	35.68	-
BDE-100	2,2′,4,4′,6-Pentabromodiphenyl ether	189084-64-8	10.44	-
BDE-99	2,2′,4,4′,5-Pentabromodiphenyl ether	60348-60-9	41.67	-
BDE-85	2,2′,3,4,4′-Pentabromodiphenyl ether	182346-21-0	2.03	-
BDE-154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether	207122-15-4	3.63	-
BDE-153	2,2′,4,4′,5,5′-Hexabromodiphenyl ether	68631-49-2	3.33	-
2,3,7,8-TBDF	2,3,7,8-Tetrabromodibenzofuran	67733-57-7	-	3,680
1,2,3,7,8-PeBDF	1,2,3,7,8-Pentabromodibenzofuran	107555-93-1	-	19,790
2,3,4,7,8-PeBDF	2,3,4,7,8-Pentabromodibenzofuran	131166-92-2	-	5,381
1,2,3,4,7,8-HxBDF[c]	1,2,3,4,7,8,Hexabromodibenzofuran	129880-08-6		
1,2,3,6,7,8-HxBDF[c]	1,2,3,6,7,8,Hexabromodibenzofuran	107555-94-2	-	43,088

[a] BDE congeners above 2% are shown. Other congeners detected are given in Table J2.
[b] Constituents detected above the limits of quantitation from duplicate analyses are reported.
[c] Quantified together due to coelution in chromatography.

the 3-month and 2-year studies with GC/FID and no degradation of the bulk chemical was detected.

Corn Oil

Mazola corn oil was obtained in multiple lots from Red Diamond Foodservice, Inc. (Birmingham, AL), and Sam's Club (Birmingham, AL) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared four times during the 3-month studies and approximately every 4 weeks during the 2-year studies by mixing DE-71 with corn oil to give the required concentrations (Table J5). Dose formulations were stored at approximately 5° C in amber glass containers sealed with Teflon®-lined lids for up to 46 days.

Stability studies of 0.05 mg/mL formulations were performed by the analytical chemistry laboratory using GC with electron capture detection (ECD). Stability was confirmed for at least 46 days for dose formulations stored in amber glass containers sealed with Teflon®-lined lids at temperatures up to 25° C and for 3 hours under simulated animal room conditions. An additional stability study was performed by the study laboratory on the 0.001 mg/mL dose formulation using a similar GC/ECD system, and stability was confirmed for at least 55 days for dose formulations stored in amber glass containers sealed with Teflon®-lined lids at 5° C and for 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of DE-71 were conducted by the study laboratory using GC/ECD. Determinations of the concentrations of DE-71 in corn oil were based on quantification of peak areas produced by the marker compound BDE-99. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations for rats and 14 of 15 for mice were within 10% of the target concentrations (Tables J6 and J7). Animal room samples of these dose formulations were also analyzed; 11 of 15 for rats and 12 of 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months (Tables J8 and J9). Of the dose formulations analyzed and used during the studies, 38 of 39 for rats and all 36 for mice were within 10% of the target concentrations; 23 of 24 animal room samples for rats and 13 of 14 for mice were within 10% of the target concentrations.

ANIMAL SOURCE

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

ANIMAL WELFARE

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

3-MONTH STUDIES

The doses for the 3-month studies were set at 0, 0.01, 5, 50, 100, and 500 mg/kg in order to examine the toxic effects in rats and mice at doses expected to cause liver toxicity (100 to 500 mg/kg) based on a previous 3-month rodent study where at 100 mg/kg there was no effect on survival although hepatomegaly, focal liver necrosis, and thyroid gland hyperplasia occurred (ATSDR, 2004). The oral LD_{50} for DE-71 was reported as greater than 5,000 mg/kg (ATSDR, 2004). The lower doses were added to expand the range of doses. The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to DE-71 and to determine the appropriate doses to be used in the 2-year studies.

On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 to 14 days and were 5 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program and there were no relevant findings (Appendix L). All tests results were negative.

Groups of 10 male and 10 female rats and mice were administered DE-71 in corn oil by gavage at doses of 0.01, 5, 50, 100, or 500 mg/kg body weight 5 days per week for 14 weeks. Additional groups of 10 male and 10 female special study rats were administered the same doses for 25 days. Vehicle control animals received the corn oil vehicle alone. Dosing volumes were 5 mL/kg body weight for rats and 10 mL/kg for mice. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage and male mice were housed singly. Clinical findings were recorded weekly for core study rats and mice. The animals were weighed initially, on day 2 (female mice), day 3 (male rats and mice), day 4 (female rats), then weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 3.

On days 4 and 25 (special study rats) and at the end of the 3-month studies (core groups), blood was collected from the retroorbital plexus under CO_2/O_2 anesthesia for hematology analyses in rats and mice as well as for clinical chemistry and thyroid hormone analyses in rats. Blood for hematology analyses was collected into tubes containing EDTA as an anticoagulant. Erythrocyte, reticulocyte, and platelet counts, automated hematocrit values, hemoglobin concentration, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were analyzed on the day of collection using an ADVIA 120 Hematology System (Bayer, Inc.; Tarrytown, NY) using reagents supplied by Bayer or Fisher Scientific (Norcross, GA). Manual hematocrit was determined using a Micro-MB microcentrifuge (Thermo Scientific, Waltham, MA). Blood smears were prepared within 3 hours of collection and stained with modified Wright's stain using an Ames HEMATEK slide stainer for evaluation of platelet and erythrocyte morphology by light microscopy. Blood for clinical chemistry and thyroid hormone analyses was collected into tubes with no anticoagulant and centrifuged. Clinical chemistry analyses were conducted using a Hitachi 911 Clinical Chemistry Analyzer (Roche Diagnostics Corporation; Indianapolis, IN) and thyroid hormone analyses were conducted by radioimmunoassay using a Packard Cobra Quantum 5005 Gamma Counter (Packard Instrument Company, Meriden, CT). The parameters measured are listed in Table 3.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats in the vehicle control, 50, 100, and 500 mg/kg groups and mice in the vehicle control, 5, 50, and 100 mg/kg groups. The parameters evaluated are listed in Table 3. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

On day 25 for special study rats and at the end of the studies for core study rats and mice, samples of liver were taken from the median and lateral lobes for determination of enzyme activities including expression of cytochrome P450 1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) activity, CYP1A2-associated acetanilide-4-hydroxylase (A4H) activity (known to be associated with dioxin-like activity), and CYP2B-associated pentoxyresorufin-*O*-dealkylase (PROD) activity. The samples from each lobe were minced, combined, frozen in liquid nitrogen, and then stored at approximately –70º C. Microsomes were prepared by the $CaCl_2$ aggregation method (Schenkman and Cinti, 1978). Microsome protein concentration was determined using the Lowry method (Lowry *et al.*, 1951). The enzymes measured were EROD (7-ethoxyresorufin as substrate), A4H (acetanilide as substrate), PROD (7-pentoxyresorufin as substrate), and uridine diphosphate glucuronosyl transferase (UDPGT; 4-nitrophenol as a substrate). CYP1A1 and CYP2B activities were determined by spectrofluorometric methods described by Chang and Waxman (1998) and Lubet *et al.* (1985), respectively. CYP1A2 was determined using HPLC as described by Hamm *et al.* (1998). UDPGT was determined by a spectrophotometric method described by Winsnes (1969). Adipose and liver samples were collected for analysis of concentrations BDE-47, BDE-99, and BDE-153. Samples of adipose and liver were collected from up to 10 male and 10 female special study F344/N rats on day 25 and from 10 male and 10 female core study rats at week 14. Adipose samples were collected from up to 10 male and 10 female mice at week 14. All samples were frozen at –70º C and shipped to the analytical chemistry laboratory. Details of analysis may be found in Appendix I.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on 0 and 500 mg/kg core study rats and mice as well as 100 mg/kg mice. The liver, lung, glandular stomach, testis, and thymus of rats and mice; the epididymis, mesenteric lymph node, ovary, thyroid gland (except 0.01 mg/kg females), and uterus of rats; and the adrenal gland, esophagus, heart (females), pleura (females), spleen (males), and forestomach of mice were examined in the remaining dose groups. Table 3 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathologist's Peer Review (PPR) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PPR or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PPR coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Rat Study Design

In order to evaluate potential toxicity that arises from *in utero* and early postnatal exposure, an exposure for these developmental windows was included in the rat study. Time-mated Wistar Han female rats, 12 to 13 weeks old, were received on the same day from Charles River Laboratories (Raleigh, NC) on gestational day 2 (GD 2). GD 1 was defined as the day females were determined to have evidence of mating. Upon receipt, time-mated female rats were quarantined, which continued throughout the perinatal period. Five non-mated female rats (from the same shipment) were used for parasite evaluation and gross observation for disease. The health of the animals was monitored during the studies according to the NTP Sentinel Animal Program (Appendix L). All test results were negative.

Time-mated female rats were housed individually during gestation; dams were housed with pups during lactation. F_1 offspring designated for the 2-year studies were initially housed 3 (males) or 5 (females) per cage after weaning, then separated as animals became larger according to the space requirements in the *Guide for the Care and Use of Laboratory Animals* (2011). Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details on animal maintenance are given in Table 3. Information on feed composition and contaminants is provided in Appendix K.

Groups of 62, 52, 52, and 62 time-mated female rats were administered DE-71 daily by gavage at doses of 0, 3, 15, and 50 mg DE-71/kg body weight, respectively, from GD 6 to weaning on PND 20. The vehicle was corn oil and control animals received the vehicle only. Body weights were taken daily and body weights from the previous day were used to calculate dosing volume (5 mL/kg).

The day of delivery was defined as postnatal day (PND) 0. Female rats that did not deliver had a gross examination for evidence of pregnancy (e.g., presence of resorptions or fetuses). On PND 1 the number, sex distribution, and viability of pups were evaluated and pup body weights were recorded through lactation and at weaning. Body weight of pups on PND 1 was calculated from litter weights divided by number of pups. After PND 1, pup body weights were measured individually.

On PND 4, each litter was standardized to a maximum of eight pups, including four males and four females when possible. Litters with less than eight pups per litter or without at least two pups per sex were removed from the study, with one exception of a litter of seven in the 3 mg/kg group. Eight pups per litter were chosen to equalize lactational demands on dams.

Beginning on PND 12, each pup was dosed by oral gavage daily at the same dose level administered to its respective dam until weaning and dosed 5 days per week for the remainder of the study. Pup body weights were recorded on PNDs 1, 4, 7, 12, 15, 18, and 21. Dose volumes administered to pups were calculated using the most recent body weight (5 mL/kg). All offspring were weaned on a single day, when animals were between the ages of PND 21 to 23. The day of weaning was considered study day one for retained animals. At weaning, up to two male and two female offspring were randomly selected from each litter and allocated to the 2-year study. Groups of 60 males and 60 females (0 and 50 mg/kg) or 50 males and 50 females (3 and 15 mg/kg) were assigned to the 2-year study. Ten males and

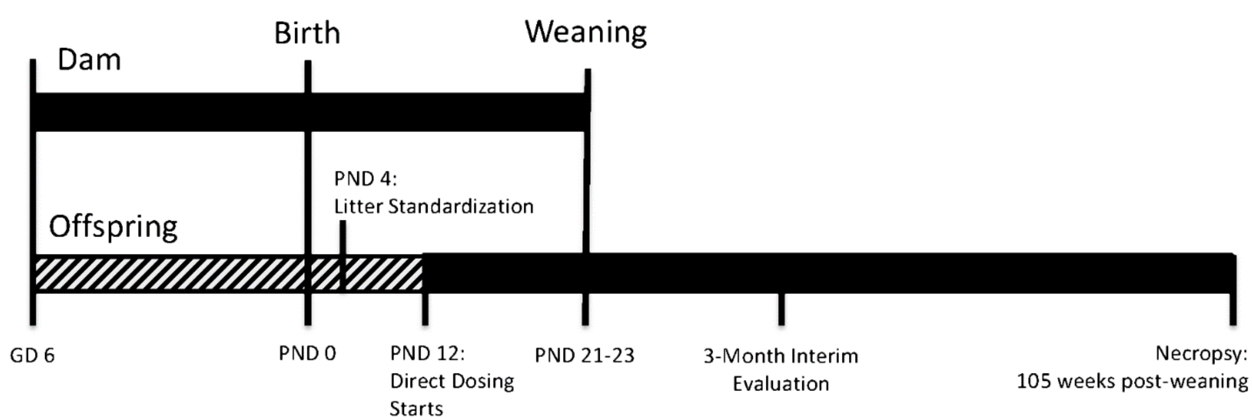


FIGURE 3
Study Design in the Perinatal and Postnatal Gavage Study of DE-71 in Wistar Han Rats
GD = gestational day, PND = postnatal day, solid shading = direct exposure,
hatched shading = indirect exposure

10 females were randomly selected from litters of the 0 and 50 mg/kg groups for a 3-month interim evaluation. The study design is illustrated in Figure 3.

Mouse Study Design

Groups of 50 male and 50 female mice were administered DE-71 in corn oil by gavage at doses of 0, 3, 30, or 100 mg DE-71/kg body weight 5 days per week for up to 105 weeks. Vehicle control animals received the corn oil vehicle alone. The dosing volume was 10 mL/kg.

Mice were quarantined for 12 days before the beginning of the studies. Five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. Mice were approximately 4 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L). All test results were negative.

Male mice were housed individually and females were housed five per cage. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 3. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All rats were observed twice daily. For F_0 rat dams, body weights were recorded on GD 5 through PND 20 and clinical observations were recorded daily on GD 6 through PND 21. For F_1 rat offspring in the 2-year study, body weights were recorded on days 1 (first day after weaning), 4 (males), 5 (females), then weekly for the first 13 weeks, at 4-week intervals thereafter, and again at necropsy. Clinical findings were recorded at 4-week intervals.

Mice were observed twice daily. Body weights were recorded on days 1, 4 (males), 5 (females), then weekly for the first 13 weeks, at 4-week intervals thereafter until week 77, then at 2-week intervals and again at necropsy. Clinical findings were recorded at 4-week intervals until week 77 and at 2-week intervals thereafter.

Adipose, liver, plasma, and carcasses were collected for analysis of concentrations of BDE-47, BDE-99, and BDE-153. In rats, livers and carcasses from six male and six or seven female F_1 offspring per dose group were collected after litter standardization on PND 4 following decapitation and exsanguination. Groups of six dams were randomly assigned to the tissue concentration study; on PND 21, adipose and livers from each dam and 1 pup/sex from their litters were collected per dose group. Samples of adipose, liver, and plasma (rats only) were collected at termination from up to 16 male and 16 female rats and mice per dose group. All samples were frozen at –70° C and shipped to the analytical chemistry laboratory. Details of analysis may be found in Appendix I.

Complete necropsies and microscopic examinations were performed on all 2-year rats and mice. At the 3-month interim evaluation in rats, the heart, right kidney, liver, lung, right testis, and thymus were weighed in the vehicle control and 50 mg/kg groups. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution, and testes and epididymis were fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. In the original evaluation of the uterus in rats, a cross section through each uterine horn, approximately 0.5 cm cranial to the cervix, was collected for histopathology review. For the residual evaluation, all remaining cervical, vaginal, and uterine tissue remnants were stored in 10% neutral buffered formalin, processed, and sectioned longitudinally. These evaluations were conducted for the 3-month interim and terminal kill groups of F_1 female Wistar Han animals. Tissues examined microscopically are listed in Table 3.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent QA laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a QA pathologist evaluated slides from all tumors and all potential target organs, which included the adrenal gland, kidney, liver, mammary gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, forestomach, thymus, thyroid gland, and uterus of rats and the adrenal gland, Harderian gland, small intestine, kidney, liver, mandibular lymph node, pancreas, pituitary gland, spleen, forestomach, testes, thymus, thyroid gland, and uterus of mice.

The QA report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the QA pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of Brix *et al.* (2010).

Study on the Relationship of the AhR to DE-71 Liver Tumor Formation

Formalin-fixed paraffin-embedded blocks of liver and kidney tissue from vehicle control and 50 mg/kg female rats were prepared at necropsy. Fresh-frozen control liver tissue was collected from five additional female rats and from one Sprague Dawley rat. Samples were shipped to ILS, Inc. (Research Triangle Park, NC), for DNA extraction and analyses of the aryl hydrocarbon receptor (AhR) genotypes. Further details may be found in Appendix M.

Evaluation of *Hras* and *Ctnnb1* Mutations in Hepatocellular Tumors

At necropsy, normal liver samples and hepatocellular tumors from vehicle control and DE-71-treated rats and mice were fixed in 10% neutral buffered formalin, transferred to 70% ethanol, and processed into paraffin blocks. The formalin-fixed paraffin-embedded normal liver tissue and liver tumors representative of spontaneous and DE-71-induced hepatocellular tumors were used for mutation analyses. Hepatocellular adenomas and carcinomas (n=40) and hepatocellular carcinomas (n=79) were used for mutation analyses in rats and mice, respectively. Further details may be found in Appendix N.

TABLE 3
Experimental Design and Materials and Methods in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71

3-Month Studies	2-Year Studies
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species	
F344/N rats B6C3F1/N mice	Wistar Han rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (females) or 12 (males) days Mice: 13 (males) or 14 (females) days	Rats: 4 days (F_0 females) Mice: 12 days
Average Age When Studies Began	
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 12 to 13 weeks (F_0 females) or gestational day 6 (F_1 offspring) Mice: 4 to 7 weeks
Date of First Dose	
Rats (core and special study): July 19 (females) or 20 (males), 2004 Mice: July 21 (males) or 22 (females), 2004	Rats: July 18, 2008 Mice: February 25, 2008
Duration of Dosing	
Rats (core) and mice: 5 days/week for 14-weeks (gavage) Rats (special study): 5 days/week for 25 days (gavage)	Rats: F_0 females from gestational day 6 to postnatal day 20; F_1 offspring from gestation day 6 to 105 weeks after weaning, Mice: 105 weeks
Date of Last Dose	
Rats (core): October 18 (females) or 19 (males), 2004 Rats (special study): August 12 (females) or 13 (males), 2004 Mice: October 20 (males) or 21 (females), 2004	Rats: August 25, 2008 (F_0 females); November 25, 2008 (F_1 offspring, 3-month interim evaluation); August 26 to 30, 2010 (F_1 offspring, 2-year study) Mice: February 25, 2010
Necropsy Dates	
Rats: October 19 (females) or 20 (males), 2004 Mice: October 21 (males) or 22 (females), 2004	Rats: November 26, 2008 (F_1 offspring, 3-month interim evaluation); August 24 to 31, 2010 (F_1 offspring, 2-year study) Mice: February 22 to 26, 2010
Average Age at Necropsy	
Female rats 19 weeks, male rats and male and female mice 19 to 20 weeks	Rats: 17 weeks (3-month interim evaluation) or 107 to 109 weeks Mice: 108 to 112 weeks
Size of Study Groups	
10 males and 10 females	Rats: F_0 females: 52 (3 and 15 mg/kg groups), or 62 (vehicle control and 50 mg/kg groups) F_1 offspring: 50 males and 50 females (3 and 15 mg/kg groups) or 60 males and 60 females (vehicle control and 50 mg/kg groups) Mice: 50 males and 50 females

TABLE 3
Experimental Design and Materials and Methods in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71

3-Month Studies	2-Year Studies
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: pregnant F_0 females housed individually, nursing F_0 females housed with pups, and F_1 offspring housed 3 (males) or 5 (females) per cage after postnatal day 20 Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Tail tattoo	Rats: F_0 females: tail tattoo F_1 offspring: paw tattoo on postnatal day 4 and then tail tattoo on postnatal day 20 Mice: tail tattoo
Diet	
Irradiated NTP-2000 open formula wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Rats: F_0 females and F_1 pups, irradiated NIH-07 open formula wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly F_1 Rats (after postnatal day 20) and mice; same as 3-month studies.
Water	
Tap water (Birmingham, AL municipal supply) via automatic watering system (Edstrom Industries, Inc. Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages	
Polycarbonate solid-bottom (Lab Products, Inc., Maywood, NJ), changed twice weekly (rats and female mice) or once weekly (male mice).	Same as 3-month studies, except changed weekly during gestation (rats) and rotated every 2 weeks
Bedding	
Irradiated hardwood bedding chips (P.J. Murphy Forest Products Corporation, Montville, NJ), changed twice weekly (rats and female mice) or once weekly (male mice).	Same as 3-month studies
Rack Filters	
Reemay® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks.	Same as 3-month studies
Racks	
Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks	Same as 3-month studies, except rotated every 2 weeks
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses	
0, 0.01, 5, 50, 100, or 500 mg/kg in corn oil; dosing volumes of 5 mL/kg (rats) or 10 mL/kg (mice)	Rats: 0, 3, 15, or 50 mg/kg in corn oil; dosing volume of 5 mL/kg Mice: 0, 3, 30, or 100 mg/kg in corn oil; dosing volume of 10 mL/kg

TABLE 3
Experimental Design and Materials and Methods in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71

3-Month Studies	2-Year Studies
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, on day 2 (female mice), day 3 (male rats and mice), day 4 (female rats), then weekly, and at the end of the studies; clinical findings were recorded weekly for core study rats and mice.	Rats: Observed twice daily F_0 females: Body weights recorded on gestational day 5 through postnatal day 20. Clinical findings recorded on gestational day 6 through postnatal day 21. F_1 offspring (perinatal): Number, sex, and viability status of pups determined on postnatal day 1. Body weights recorded on postnatal days 1 (litter weights by sex), 4, 7, 12, 15, 18, and 21. F_1 offspring (2-year study): Body weights recorded on days 1, 4 (males), 5 (females), weekly for the first 13 weeks, at 4-week intervals thereafter, and at necropsy. Clinical findings recorded at 4-week intervals. Mice: Observed twice daily. Body weights recorded on days 1, 4 (males); 5 (females); weekly for the first 13 weeks, at 4-week intervals thereafter until week 77, at 2-week intervals beginning week 77, and at necropsy. Clinical findings recorded at 4-week intervals until week 77 and at 2-week intervals thereafter.
Method of Kill	
Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all 2- year rats and mice. At the 3-month interim evaluation in rats the heart, right kidney, liver, lung, right testis, and thymus were weighed in the 0 and 50 mg/kg groups.
Clinical Pathology	
Blood was collected via the retroorbital sinus on days 4 and 25 (special study rats) and from all animals surviving to the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, nucleated erythrocytes, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, bile acids, total thyroxine, total triiodothyronine, and thyroid stimulating hormone	None

TABLE 3
Experimental Design and Materials and Methods in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71

3-Month Studies	2-Year Studies
Histopathology	
Complete histopathology was performed on 0 and 500 mg/kg core study rats and mice as well as 100 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, pleura (female mice) preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. In the remaining groups of rats and mice, the liver, lung, stomach (glandular), testis and thymus were examined. In the remaining groups of rats, the epididymis, lymph node (mesenteric), ovary, thyroid gland (except 0.01 mg/kg females), and uterus were examined. In the remaining groups of mice, the adrenal gland, esophagus, heart (females), pleura (females), spleen (males), and stomach (forestomach) were examined.	Complete histopathology was performed on 2-year rats and all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, cervix (rats), clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (rats).
Sperm Motility and Vaginal Cytology	
At the end of the studies, spermatid and sperm samples were collected from male rats in the vehicle control, 50, 100, and 500 mg/kg groups and male mice in the vehicle control, 5, 50, and 100 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats in the vehicle control, 50, 100, and 500 mg/kg groups and female mice in the vehicle control, 5, 50, and 100 mg/kg groups.	None
Liver Enzyme Activities	
Liver samples were collected on day 25 (special study rats) and at the end of the studies (rats and mice) 7-ethoxy-*O*-deethylase, acetanilide-4-hydroxylase, 7-pentoxy-*O*-dealkylase, and uridine diphosphate glucuronosyl transferase activities.	None
Tissue Concentration Studies	
Adipose and liver samples were collected from rats on day 25 (special study) and at the end of the study (core study), and adipose samples were collected from mice at the end of the study for analysis of concentrations of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153).	Adipose, liver, plasma, and carcasses were collected for analysis of concentrations of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). Lipid content was determined for all adipose and liver samples. Rats (F_0 and F_1): Carcasses and whole livers were collected from six male and six or seven female F_1 offspring per dose group at the time of litter adjustment on postnatal day 4. Adipose and whole liver samples were collected on postnatal day 21 from six F_0 females per dose group and one male and one female F_1 offspring from each of their litters. Rats (F_1) and Mice: Adipose, liver, and plasma (rats only) samples were collected from up to 16 males and 16 females per dose group at the end of the studies.

TABLE 3
Experimental Design and Materials and Methods in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71

3-Month Studies	2-Year Studies
Study on the Relationship of the AhR to DE-71 Liver Tumor Formation	
None	DNA was extracted from formalin-fixed paraffin-embedded blocks of liver (n=118) and kidney (n=122) tissues obtained at necropsy from vehicle control and 50 mg/kg female rats and analyzed for AhR genotype. DNA was also extracted from fresh-frozen liver samples from the control female Wistar Han (n=5) and Sprague Dawley (n=1) rats and analyzed for AhR genotype.
Evaluation of *Hras* and *Ctnnb1* Mutations in Hepatocellular Tumors	
None	At necropsy, male and female rat and mouse hepatocellular tumor tissues and normal liver tissue were obtained as formalin-fixed paraffin-embedded blocks. Hot-spot mutations were evaluated in the *Hras* and *Ctnnb1* genes in hepatocellular tumors representing all groups dosed with DE-71 (35 from rats and 62 from mice) and in spontaneous hepatocellular tumors from vehicle controls (5 from rats and 17 from mice). In addition, age-matched non-tumor livers from rats (n=10) and mice (n=8) were analyzed.

STATISTICAL METHODS

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. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight.

This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344/N rats and B6C3F1/N mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N). For neoplasms and nonneoplastic lesions detected at the 3-month interim evaluation, the Fisher exact test (Gart *et al.*, 1979), a procedure based on the overall proportion of affected animals, was used.

In a second set of analyses for the rat study, mixed effects logistic regression was also used to account for potential litter effects (McCullagh and Nelder, 1989). These models also incorporated the Poly-3 risk weights for each animal to adjust for survival. The primary tests in these models were for dose-related trends and pairwise comparisons of each dose group with the control group.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972), or a *t*-test (3-month interim evaluation in the 2-year rat study). Pups per litter, pup survival during lactation, hematology, clinical chemistry, percent lipid, liver enzymes, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the chi-square test (Conover, 1971). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period (Haseman, 1992, 1995; Haseman and Rao, 1992). In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current mouse study. The historical control database includes six studies in Wistar Han rats, and only two of these (including the current study) are corn oil gavage studies. The study presented in this Technical Report is the only one that has an *in utero* and perinatal component.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of DE-71 and three polybrominated diphenyl ether congeners, BDE-47, BDE-99, and BDE-153 were assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*. DE-71 was also assessed for its ability to induce mutations in *Escherichia coli*, micronucleated erythrocytes in mouse bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

3-MONTH STUDY IN F344/N RATS

All rats survived to the end of the study (Table 4). Final mean body weights and mean body weight gains were less than those of the vehicle controls in 500 mg/kg males by approximately 14% and 23%, respectively (Table 4 and Figure 4). In female rats, final mean body weights were decreased approximately 8% in the 100 mg/kg group and 15% in the 500 mg/kg group, while mean body weight gains were less than that of the vehicle controls by approximately 16% and 28% in these two groups. There were no clinical findings related to administration of DE-71.

TABLE 4
Survival and Body Weights of F344/N Rats in the 3-Month Gavage Study of DE-71[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	110 ± 2	316 ± 6	206 ± 5	
0.01	10/10	110 ± 2	335 ± 5	224 ± 6	106
5	10/10	109 ± 2	327 ± 6	218 ± 4	103
50	10/10	111 ± 2	330 ± 6	219 ± 6	104
100	10/10	110 ± 2	318 ± 8	208 ± 8	101
500	10/10	113 ± 1	272 ± 5**	159 ± 5**	86
Female					
0	10/10	91 ± 1	197 ± 3	106 ± 3	
0.01	10/10	90 ± 1	191 ± 2	101 ± 3	97
5	10/10	90 ± 1	203 ± 4	113 ± 4	103
50	10/10	92 ± 1	189 ± 2	97 ± 3	96
100	10/10	92 ± 1	181 ± 3**	89 ± 3**	92
500	10/10	92 ± 1	169 ± 4**	76 ± 3**	85

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group

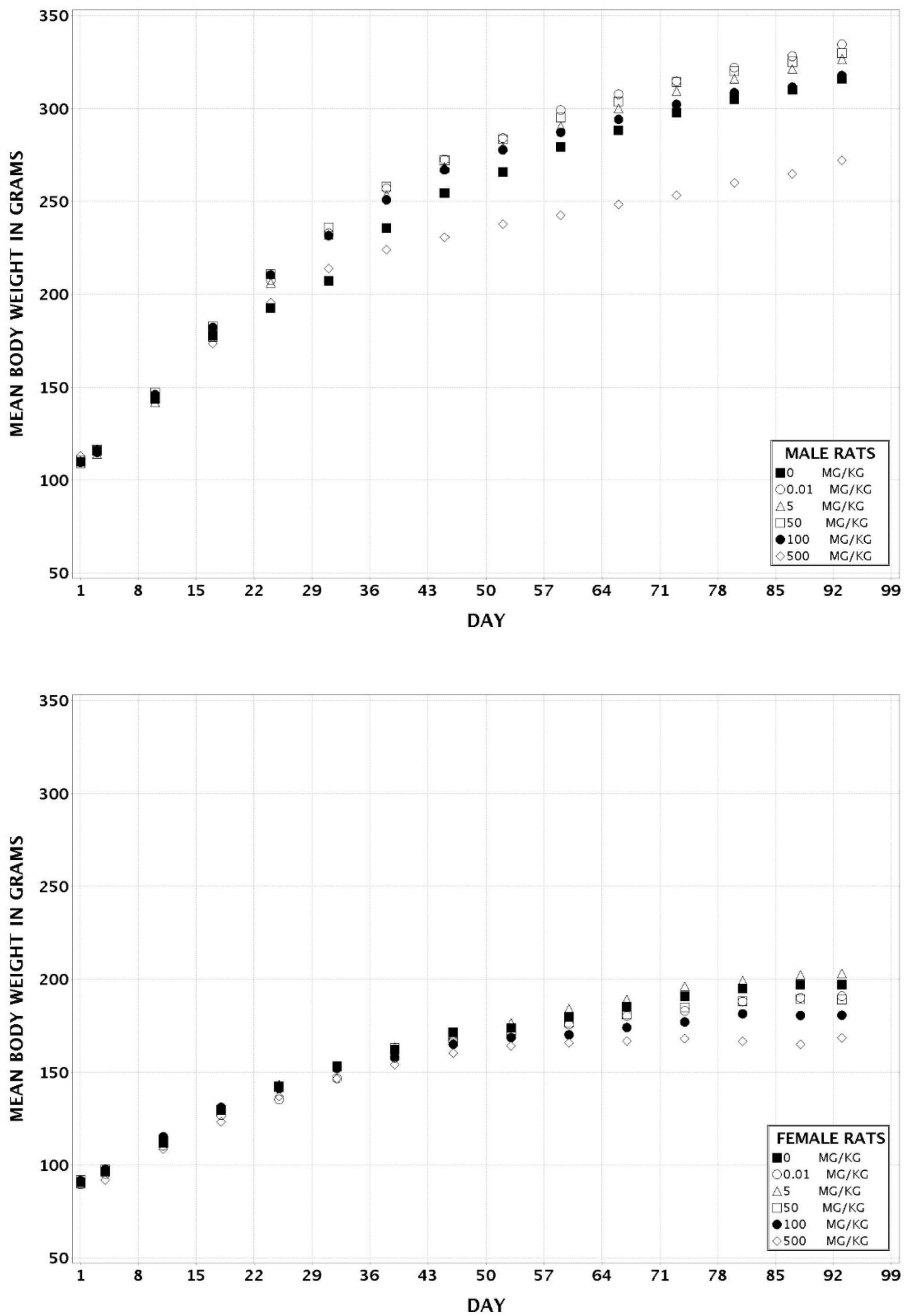


FIGURE 4
Growth Curves for F344/N Rats Administered DE-71 by Gavage for 3 Months

Consistent, dose-related decreases in thyroxine (T_4) concentration occurred at all time points in males and females administered 5 mg/kg or greater (Tables 5 and F1). In the 100 and 500 mg/kg groups, the T_4 concentrations were less than or equal to 15% of that of the vehicle control on day 4 and week 14. For the 5 and 50 mg/kg groups, the decrease appeared progressive with the strongest effect detected at week 14 (approximately 50% and less than or equal to 15% of the vehicle control concentration for the 5 and 50 mg/kg groups, respectively). The decreases in T_4 concentrations were accompanied by increases in thyroid stimulating hormone (TSH) concentrations. TSH increases were first apparent on day 25 and persisted to week 14. While strong decreases in T_4 occurred in males and females administered 5 mg/kg or greater, increases in TSH were most consistently detected in the 100 and 500 mg/kg groups, and at week 14 demonstrated a 60% to 70% increase compared to that of the vehicle control group. The decreases in T_4 were not accompanied by decreases in triiodothyronine (T_3) concentrations.

At all time points, the serum concentrations of cholesterol were consistently increased in males and females administered 50 mg/kg or greater (Tables 5 and F1). The increases demonstrated a dose relationship and progressed in severity with time (e.g., an approximate 60% increase in the 500 mg/kg females on day 4 increased to an approximate fourfold increase at week 14). Serum concentrations of bile salts, a marker of hepatic function/injury and cholestasis, also demonstrated consistent, dose-related increases in males and females administered 50 mg/kg or greater at essentially all time points. For bile salts, the absolute increases remained consistent across time and appeared to be of minimal (less than or equal to twofold) severity. Another marker of cholestasis, alkaline phosphatase activity, however, demonstrated no increases. Thus, it would appear the increases in bile salt

TABLE 5
Selected Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n						
Day 4	9	9	9	9	9	9
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Cholesterol (mg/dL)						
Day 4	105 ± 4	101 ± 3	106 ± 2	135 ± 3**	148 ± 4**	185 ± 6**
Day 25	77 ± 2	89 ± 2**	89 ± 2**	101 ± 1**	112 ± 2**	217 ± 3**
Week 14	88 ± 1	87 ± 2	83 ± 2	106 ± 3**	117 ± 3**	235 ± 5**
Bile salts (µmol/L)						
Day 4	20.3 ± 2.0	18.6 ± 1.4	21.5 ± 2.0	27.1 ± 1.2*	31.9 ± 2.1**	33.8 ± 1.6**
Day 25	21.1 ± 2.3	16.4 ± 0.7	22.8 ± 1.8	25.5 ± 1.5*	32.7 ± 1.4**	39.1 ± 2.2**
Week 14	15.5 ± 0.9	20.8 ± 2.2	22.3 ± 1.9*	20.8 ± 0.9**	27.0 ± 1.6**	32.9 ± 1.6**
Total thyroxine (µg/dL)						
Day 4	5.97 ± 0.34[b]	5.72 ± 0.12[b]	5.67 ± 0.29[b]	1.35 ± 0.10**[b]	0.87 ± 0.13**[b]	0.62 ± 0.11**[b]
Day 25	6.55 ± 0.26	6.54 ± 0.48	5.02 ± 0.31**	1.33 ± 0.16**	0.72 ± 0.10**	0.48 ± 0.07**
Week 14	4.25 ± 0.20	4.53 ± 0.18	2.29 ± 0.16**	0.50 ± 0.11**	0.10 ± 0.05**	0.46 ± 0.09**
Total triiodothyronine (ng/dL)						
Day 25	100.9 ± 3.1	113.1 ± 7.6	90.8 ± 6.5	79.4 ± 4.1*	80.0 ± 3.9	108.6 ± 3.9
Week 14	81.1 ± 4.5	75.7 ± 3.7	63.7 ± 5.6	77.9 ± 5.8	73.4 ± 5.3	120.7 ± 5.6
Thyroid stimulating hormone (ng/mL)						
Day 4	5.70 ± 0.41[b]	5.20 ± 0.40[b]	5.04 ± 0.47[b]	5.82 ± 0.55	5.10 ± 0.39[b]	4.42 ± 0.39[b]
Day 25	3.66 ± 0.15	4.69 ± 0.38	5.16 ± 0.64	5.57 ± 0.66	6.55 ± 0.84**	4.63 ± 0.60
Week 14	3.75 ± 0.33	3.61 ± 0.47	3.74 ± 0.46	4.62 ± 0.48	4.69 ± 0.57	6.19 ± 0.84*

TABLE 5
Selected Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Female						
n						
Day 4	3	3	3	4	6	2
Day 25	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Cholesterol (mg/dL)						
Day 4	112 ± 6[c]	108 ± 4[c]	113 ± 3[c]	136 ± 3**[b]	147 ± 7**[d]	176 ± 4**[b]
Day 25	75 ± 2	82 ± 3*	87 ± 3**	117 ± 4**	144 ± 4**[c]	244 ± 5**
Week 14	72 ± 2	74 ± 2	94 ± 3**	145 ± 4**	183 ± 9**	310 ± 9**
Bile acids (µmol/L)						
Day 4	16.2 ± 3.7	19.9 ± 2.8	16.4 ± 3.9	27.1 ± 4.9	26.4 ± 2.1	23.4 ± 0.2
Day 25	19.3 ± 1.9	25.4 ± 5.4	18.1 ± 1.9	25.0 ± 1.4*	31.6 ± 1.8**	32.3 ± 1.7**
Week 14	20.2 ± 6.0	16.8 ± 1.5	17.3 ± 0.6*	20.9 ± 1.1**	24.3 ± 0.9**	32.2 ± 2.5**
Total thyroxine (µg/dL)						
Day 4	4.88 ± 0.22[b]	4.90 ± 0.13[b]	4.12 ± 0.20*[b]	0.95 ± 0.12**[b]	0.57 ± 0.07**[b]	0.41 ± 0.08**[b]
Day 25	5.09 ± 0.17	4.89 ± 0.26	4.13 ± 0.25*	1.02 ± 0.11**	0.56 ± 0.14**	0.30 ± 0.07**
Week 14	3.19 ± 0.24	3.36 ± 0.16	1.68 ± 0.12**	0.41 ± 0.06**	0.48 ± 0.09**	0.50 ± 0.07**
Total triiodothyronine (ng/dL)						
Day 25	94.1 ± 5.1	98.1 ± 3.4	91.5 ± 4.5	95.7 ± 4.1	98.7 ± 4.0	120.4 ± 4.6**
Week 14	79.0 ± 5.8	75.2 ± 4.1	62.6 ± 2.0	74.9 ± 4.1	83.6 ± 6.2	137.3 ± 5.7**
Thyroid stimulating hormone (ng/mL)						
Day 4	4.57 ± 0.46[b]	4.08 ± 0.42[b]	5.80 ± 0.47[b]	4.51 ± 0.44[b]	4.55 ± 0.38[b]	3.61 ± 0.35[b]
Day 25	3.99 ± 0.26	3.96 ± 0.18	4.84 ± 0.32	5.27 ± 0.20**	4.86 ± 0.43*	5.56 ± 0.52*
Week 14	2.69 ± 0.20	2.95 ± 0.29	2.83 ± 0.28	3.40 ± 0.36	4.66 ± 0.72**	4.32 ± 0.34**

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

concentration were probably not related to a cholestatic event, but rather were an effect of hepatic function. Markers of hepatocellular leakage/injury, serum activities of alanine aminotransferase and sorbitol dehydrogenase, generally demonstrated minimal increases in the 100 and 500 mg/kg groups (most consistently in 500 mg/kg males and females). On day 25 and at week 14, small (less than 20%) increases occurred in serum albumin concentrations, and by extension, total protein concentrations in the 50 mg/kg or greater treatment groups, which would be suggestive of a physiological decrease in plasma volume (i.e., dehydration). Minimal increases in serum urea nitrogen concentration, but not creatinine concentration, in the 500 mg/kg groups at these time points would support the physiological nature of the protein increase.

At week 14, the hematology findings suggested small (less than or equal to 12%), dose-related decreases in the estimators of the circulating red cell mass in the 100 and 500 mg/kg males and females. The erythron decreases were evidenced by decreases in hematocrit values and hemoglobin concentrations, but not erythrocyte counts (Table F1). The erythron decreases were accompanied by dose-related decreases in erythrocyte size (i.e., mean cell volume) and mass of hemoglobin (i.e., mean cell hemoglobin). However, there was no change in the erythrocyte concentration of hemoglobin (i.e., mean cell hemoglobin concentration), and the statistical identification of minimally increased reticulocyte numbers in the males, but not females (which had the slightly bigger percentage erythron decreases), were of questionable importance.

The absolute and relative liver weights of male and female rats administered 5 mg/kg or greater were significantly greater than those of the vehicle controls (Tables 6 and G1). The absolute liver weight of 500 mg/kg males was approximately double that of the vehicle control group, while in females, the absolute liver weight of the 500 mg/kg group was approximately 220% that of the vehicle controls. The changes in liver weights correlated with hepatocyte hypertrophy observed histologically in both male and female rats.

Absolute kidney weights were significantly greater than that of the vehicle controls by approximately 15% to 16% in the 50, 100, and 500 mg/kg male groups; these groups also had increased relative kidney weights (Tables 6 and G1). In females, absolute kidney weights were significantly increased in the groups administered 5 mg/kg or greater; the greatest increase (approximately 27%) occurred in the 500 mg/kg group. Relative kidney weights were significantly greater than that of the vehicle controls in all dosed groups of females, with the largest increase in the 500 mg/kg group. No histological lesions were observed in either male or female rats that correlated with the changes in kidney weights.

The absolute thymus weight in 500 mg/kg male rats and absolute and relative thymus weights in female rats administered 50 mg/kg or greater were significantly decreased compared to those of the vehicle controls (Tables 6 and G1). In 500 mg/kg males, the decreased absolute thymus weight was consistent with decreased body weight. In female rats administered 50, 100, or 500 mg/kg, the decreased absolute (23%, 33%, and 56%, respectively) and relative thymus weights could not be

TABLE 6
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	316 ± 6	335 ± 5	327 ± 6	330 ± 6	318 ± 8	272 ± 5**
R. Kidney						
Absolute	0.93 ± 0.02	0.99 ± 0.03	1.00 ± 0.03	1.07 ± 0.03**	1.07 ± 0.03**	1.08 ± 0.02**
Relative	2.932 ± 0.023	2.942 ± 0.056	3.050 ± 0.054	3.240 ± 0.036**	3.349 ± 0.027**	3.958 ± 0.035**
Liver						
Absolute	10.09 ± 0.17	11.22 ± 0.33	12.13 ± 0.44**	16.04 ± 0.52**	17.42 ± 0.46**	20.01 ± 0.58**
Relative	31.940 ± 0.252	33.482 ± 0.536	37.037 ± 0.774**	48.628 ± 1.130**	54.787 ± 0.524**	73.381 ± 1.224**
Thymus						
Absolute	0.230 ± 0.012	0.243 ± 0.014	0.241 ± 0.012	0.221 ± 0.011	0.245 ± 0.020	0.163 ± 0.014**
Relative	0.727 ± 0.038	0.727 ± 0.041	0.739 ± 0.037	0.672 ± 0.038	0.772 ± 0.059	0.598 ± 0.048
Female						
Necropsy body wt	197 ± 3	191 ± 2	203 ± 4	189 ± 2	181 ± 3**	169 ± 4**
R. Kidney						
Absolute	0.62 ± 0.01	0.65 ± 0.01	0.68 ± 0.01**	0.68 ± 0.01**	0.68 ± 0.02**	0.79 ± 0.01**
Relative	3.132 ± 0.047	3.378 ± 0.063*	3.333 ± 0.050*	3.617 ± 0.055**	3.737 ± 0.048**	4.716 ± 0.105**
Liver						
Absolute	5.56 ± 0.16	5.92 ± 0.10	6.47 ± 0.13**	8.73 ± 0.16**	9.85 ± 0.27**	12.16 ± 0.35**
Relative	28.191 ± 0.616	31.009 ± 0.599*	31.891 ± 0.490**	46.139 ± 0.590**	54.511 ± 1.135**	72.195 ± 1.448**
Thymus						
Absolute	0.226 ± 0.011	0.212 ± 0.009	0.209 ± 0.007	0.174 ± 0.009**	0.152 ± 0.011**	0.099 ± 0.009**
Relative	1.149 ± 0.055	1.114 ± 0.051	1.032 ± 0.035	0.922 ± 0.051**	0.836 ± 0.055**	0.587 ± 0.050**

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

explained simply by decreased body weights. The decreased thymic weights in 500 mg/kg females correlated with thymic atrophy observed histologically, but this lesion was not observed in the 50 or 100 mg/kg groups.

In the male rats, relative heart weights of the 50, 100, and 500 mg/kg groups were significantly greater than that of the vehicle controls (Table G1). The relative weight increase of the 500 mg/kg group was considered secondary to decreased mean body weight compared to the vehicle control group; the increases in the other dose groups were considered biological variation. In female rats, relative heart weights of the 100 and 500 mg/kg groups were significantly greater than that of the vehicle controls and were attributed to decreased mean body weights in those groups. Significantly decreased absolute lung weights of 500 mg/kg males and females were also attributed to decreases in mean body weights.

Compared to the vehicle controls, uridine diphosphate glucuronosyl transferase (UDPGT) activities were significantly increased in male rats administered 0.01 mg/kg on day 25 and in male and female rats administered 5 mg/kg or greater on day 25 and at week 14 (Table 7). UDPGT activity at week 14 reached a peak induction of approximately 12.5-fold and 26-fold in 500 mg/kg males and females, respectively.

Hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activities on day 25 displayed generally dose-related increases with approximately 148-fold and 100-fold increases in 500 mg/kg males and females, respectively (Table 7). Significant increases were observed in males and females administered 5 mg/kg or greater. By week 14, EROD activity in 500 mg/kg males was induced approximately 105-fold, while in 500 mg/kg females, it was induced approximately 209-fold. Significant but smaller increases were observed in 50 and 100 mg/kg males and females administered 5 mg/kg or greater.

On day 25, hepatic acetanilide-4-hydroxylase (A4H) activities were significantly increased in male rats administered 50 mg/kg or greater and in female rats administered 5 mg/kg or greater, with maximal induction increased approximately 35-fold for males and females administered 500 mg/kg (Table 7). At week 14, maximal A4H induction was approximately 11-fold for male rats and 10-fold for female rats in the 500 mg/kg groups, and significant dose-related increases were observed in both male and female rats administered 5 mg/kg or greater.

Hepatic 7-pentoxyresorufin-*O*-dealkylase (PROD) activities were increased in male and female rats administered 5 mg/kg or greater on day 25 and at week 14 (Table 7). The greatest increase in PROD activity was seen at week 14 in males administered 500 mg/kg (approximately a 141-fold increase) and females administered 50 mg/kg (approximately a 233-fold increase).

Concentrations of BDE-47, BDE-99 and BDE-153 were determined in adipose and liver in special study males and females on day 25 and core study rats at the end of the study (Appendix I). In males and females administered 5 mg/kg or greater, the concentrations of all three congeners in adipose and liver increased with increasing dose and were higher than those of the respective vehicle controls at both time points (Table I1). The concentrations of congeners in adipose were higher than in liver suggesting preferential accumulation in the adipose. BDE-47 and BDE-99 concentrations in adipose were similar and were higher than the BDE-153 concentrations in both sexes; however, BDE-47, BDE-99, and BDE-153 concentrations were similar in the liver. In general, the congener concentration in adipose was higher in females compared to males; however, there was no sex difference in congener concentration in the liver. In the adipose, levels of congeners were higher at the end of the study (week 14) compared to day 25, supporting accumulation. Although there was no difference in BDE-153 concentrations on day 25 and at week 14 in the liver, BDE-47 and BDE-99 concentrations at week 14 were lower than on day 25, suggesting that BDE-47 and BDE-99 induce their own metabolism.

TABLE 7
Liver Enzyme Activities for F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n						
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Uridine diphosphate glucuronosyl transferase (UDPGT) (nmol/minute per mg microsomal protein)						
Day 25	2.9 ± 0.1	4.0 ± 0.2**	4.5 ± 0.2**	12.9 ± 0.4**	15.6 ± 0.8**	28.4 ± 0.8**
Week 14	4.2 ± 0.4	3.7 ± 0.3	5.9 ± 0.4*	21.4 ± 1.1**	35.8 ± 1.8**	52.6 ± 2.6**
7-Ethoxyresorufin-*O*-deethylase (EROD) (nmol/minute per mg microsomal protein)						
Day 25	0.007 ± 0.000	0.008 ± 0.001	0.037 ± 0.003**	0.386 ± 0.025**	0.444 ± 0.056**	1.034 ± 0.096**
Week 14	0.008 ± 0.001	0.006 ± 0.000	0.012 ± 0.001	0.282 ± 0.019**	0.358 ± 0.030**	0.843 ± 0.053**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 25	0.020 ± 0.002	0.009 ± 0.002	0.034 ± 0.004[b]	0.420 ± 0.045**[c]	0.441 ± 0.032**	0.704 ± 0.038**
Week 14	0.255 ± 0.020	0.185 ± 0.011	0.355 ± 0.019*	0.923 ± 0.041**	1.455 ± 0.050**	2.903 ± 0.071**
7-Pentoxyresorufin-*O*-dealkylase (PROD) (nmol/minute per mg microsomal protein)						
Day 25	0.001 ± 0.000	0.001 ± 0.000	0.022 ± 0.002**	0.133 ± 0.006**	0.124 ± 0.010**	0.077 ± 0.005**
Week 14	0.002 ± 0.000	0.001 ± 0.000	0.099 ± 0.006**	0.262 ± 0.016**	0.218 ± 0.014**	0.281 ± 0.013**
Female						
n						
Day 25	10	10	10	10	9	10
Week 14	9	10	10	10	10	10
Uridine diphosphate glucuronosyl transferase (UDPGT) (nmol/minute per mg microsomal protein)						
Day 25	3.2 ± 0.2	2.8 ± 0.2	6.4 ± 0.8*	12.4 ± 0.5**	15.0 ± 0.7**	50.8 ± 1.5**
Week 14	2.9 ± 0.3	3.2 ± 0.4	8.0 ± 0.2**	32.1 ± 0.9**	53.5 ± 2.0**	75.5 ± 3.2**
7-Ethoxyresorufin-*O*-deethylase (EROD) (nmol/minute per mg microsomal protein)						
Day 25	0.014 ± 0.001	0.017 ± 0.001	0.081 ± 0.004**	1.023 ± 0.044**	0.958 ± 0.052**	1.402 ± 0.079**
Week 14	0.004 ± 0.001	0.003 ± 0.000	0.075 ± 0.008**	0.648 ± 0.053**	0.650 ± 0.067**	0.836 ± 0.073**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 25	0.023 ± 0.002	0.023 ± 0.004	0.036 ± 0.005*	0.589 ± 0.050**	0.599 ± 0.110**	0.802 ± 0.040**[b]
Week 14	0.231 ± 0.013	0.205 ± 0.015	0.490 ± 0.022**[b]	1.400 ± 0.065**	1.723 ± 0.069**	2.384 ± 0.109**
7-Pentoxyresorufin-*O*-dealkylase (PROD) (nmol/minute per mg microsomal protein)						
Day 25	0.001 ± 0.000	0.001 ± 0.000	0.011 ± 0.001**	0.105 ± 0.011**	0.099 ± 0.008**	0.122 ± 0.011**
Week 14	0.001 ± 0.000	0.001 ± 0.000	0.054 ± 0.006**	0.233 ± 0.016**	0.112 ± 0.005**	0.086 ± 0.005**

* Significantly different (P≤0.05) from the vehicle control group by Shirley's test
** P≤0.01
[a] Enzyme activities are given as mean ± standard error.
[b] n=9
[c] n=8

Epididymis and cauda epididymis weights were significantly decreased in 500 mg/kg males (Tables 8 and H1). The 500 mg/kg group also exhibited significantly decreased sperm per cauda and sperm per gram of cauda. Histologically, this correlated with hypospermia of the epididymis. In general, dosed males exhibited fewer total spermatids per testis, and sperm per gram of testis were significantly decreased in the 100 and 500 mg/kg groups; however, no histologic alterations were observed in testes. Sperm motility was significantly decreased in the 500 mg/kg group. All 500 mg/kg females failed to cycle and remained in persistent diestrus throughout the examination period (Tables 9, H2, and H3; Figure H1). Based on these findings, DE-71 exhibits the potential to be a reproductive toxicant in both male and female rats.

Relevant gross findings included liver enlargement in both male and female rats, as well as small thymus and thin carcass in female rats. Statistically significant histologic changes occurred in the liver and thyroid gland of male and female rats, the epididymis and glandular stomach of male rats and the thymus of female rats.

TABLE 8
Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	50 mg/kg	100 mg/kg	500 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	316 ± 6	335 ± 7.9	318 ± 8	282 ± 12*
L. Cauda epididymis	0.1289 ± 0.0050	0.1385 ± 0.0119[b]	0.1328 ± 0.0087	0.0724 ± 0.0047**
L. Epididymis	0.4284 ± 0.0102	0.4485 ± 0.0168	0.4184 ± 0.0141	0.3135 ± 0.0128**
L. Testis	1.4061 ± 0.0343	1.5028 ± 0.0337	1.4981 ± 0.0279	1.4818 ± 0.0291
Spermatid measurements				
Spermatid heads (10^6/testis)	181.38 ± 3.90	186.38 ± 7.34	170.50 ± 5.90	164.88 ± 9.49
Spermatid heads (10^6/g testis)	152.48 ± 4.13	151.01 ± 6.13	137.20 ± 3.96*	130.36 ± 6.20**
Epididymal spermatozoal measurements				
Sperm motility (%)	86.6 ± 0.7	86.5 ± 0.9	87.0 ± 0.6	82.7 ± 0.8**
Sperm (10^6/cauda epididymis)	78.3 ± 4.2	63.2 ± 8.9	81.3 ± 4.9	9.9 ± 1.1**
Sperm (10^6/g cauda epididymis)	608.5 ± 25.8	457.2 ± 77.4	591.2 ± 44.2	137.1 ± 14.6**

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's (body weights) or Shirley's (spermatid heads/g testis) test

** Significantly different (P≤0.01) from the vehicle control group by Williams' (cauda epididymis and epididymis weights) or Shirley's (spermatid heads/g testis and epididymal spermatozoal measurements) test

a Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's (testis weights) or Dunn's (spermatid heads/testis) test.

b n=9

TABLE 9
Estrous Cycle Characterization for Female F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	50 mg/kg	100 mg/kg	500 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	197 ± 3	189 ± 2	181 ± 3**	169 ± 4**
Proportion of regular cycling females[b]	7/10	8/10	10/10*	0/10*
Estrous cycle length (days)	5.8 ± 0.40	5.8 ± 0.29	5.3 ± 0.15	—[c]
Estrous stages (% of cycle)				
Diestrus	61.7	60.0	56.7	100.0
Proestrus	13.3	12.5	18.3	0.0
Estrus	20.0	20.0	18.3	0.0
Metestrus	5.0	7.5	6.7	0.0

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Chi-square test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated a significantly higher probability of extended diestrus in the 500 mg/kg group compared to the vehicle control group.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 10 of 10 animals.

In the liver, there were significantly increased incidences of hepatocyte hypertrophy in males and females administered 5 mg/kg or greater (Table 10). The incidences of cytoplasmic vacuolization of the hepatocytes were significantly increased in 50 mg/kg males and 100 and 500 mg/kg males and females. The severity of hepatocyte hypertrophy also increased with increasing dose. Hepatocyte hypertrophy was characterized by enlarged hepatocytes, which often contained larger than average nuclei (Plate 1). Hepatocyte hypertrophy appeared to affect the centrilobular hepatocytes first, and as the severity of the lesion increased, the zonal specificity of the lesion decreased. Cytoplasmic vacuolization was represented by enlarged cells with discrete cytoplasmic vacuoles that varied in size (Plate 1). In some cells, the vacuoles were so small they appeared indistinguishable, giving the cytoplasm a pale, eosinophilic, almost granular appearance. In other cells, the vacuoles were distinct and recognizable as discrete vacuoles of lipid. Cytoplasmic vacuolization had a centrilobular distribution and tended to occur within hypertrophied areas of the liver. This change was characteristically similar to that of hepatocellular fatty change seen in the 2-year study.

There were significantly increased incidences of thyroid gland follicle hypertrophy in females administered 50 mg/kg or greater and in 500 mg/kg males (Table 10). In females, there was a concomitant increase in the average severity grade. The lesion was characterized by an increase in the number of small follicles lined by cuboidal to low columnar epithelial cells (Plates 2 and 3). Some of the follicles contained pale, often vacuolated colloid. Severity grading was based on the subjective number of thyroid follicles involved compared to the number of normal appearing follicles.

In the epididymis, there was a significantly increased incidence of hypospermia in 500 mg/kg males (Table 10). Histologically, the overall area of the cauda epididymis was smaller in affected animals and there were fewer, smaller, tubule cross sections. Tubules in these animals contained spermatids, but they were lined by tall cuboidal to columnar epithelial cells, compared to the flattened to cuboidal epithelium in vehicle control animals. While the smaller amount of tissue present might have been due to artifact or plane of sectioning differences, the sizes of the epididymides were consistent among animals within dose groups. Histology is not a sensitive indicator of decreased spermatid numbers, but the histologic observations and interpretations were confirmed by decreased sperm counts.

Erosion of the glandular stomach occurred only in dosed animals, and the incidence was significantly increased in

TABLE 10
Incidences of Selected Nonneoplastic Lesions in F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Hepatocyte, Hypertrophy[b]	0	0	9** (1.0)	10** (2.7)	10** (3.4)	10** (3.7)[c]
Hepatocyte, Cytoplasmic Vacuolization	0	0	0	10** (1.2)	10** (2.0)	10** (1.7)
Thyroid Gland	10	9	10	10	10	10
Follicle, Hypertrophy	0	0	0	0	1 (1.0)	9** (1.0)
Epididymis	10	10	10	10	10	10
Hypospermia	0	0	0	0	0	9** (1.9)
Stomach, Glandular	10	10	10	10	10	10
Erosion	0	0	1 (1.0)	2 (1.5)	3 (1.7)	4* (1.5)
Female						
Liver	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	2 (1.0)	5* (1.4)	10** (2.2)	10** (3.1)	10** (4.0)
Hepatocyte, Cytoplasmic Vacuolization	0	0	0	3 (1.0)	10** (1.1)	10** (1.0)
Thyroid Gland	10	0	10	10	10	10
Follicle, Hypertrophy	0		0	8** (1.0)	9** (1.4)	10** (2.9)
Stomach, Glandular	10	10	10	10	10	10
Erosion	0	0	0	0	0	3 (1.0)
Thymus	10	10	10	10	9	10
Atrophy	0	0	0	0	0	4* (1.3)

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

500 mg/kg males (Table 10). This lesion occurred with a positive trend in both males and females. Erosion of the glandular stomach was recorded when there was necrosis of the mucosa that did not extend below the basement membrane into the underlying lamina propria.

In the thymus of 500 mg/kg females, there was a significantly increased incidence of atrophy that was characterized by a small thymus with a thin cortex (Table 10).

Dose Selection Rationale: Due to reduced body weights observed in 100 mg/kg females and 500 mg/kg males and females, increased absolute and relative liver weights, and increased incidences and severities of hepatocyte hypertrophy and hepatocyte cytoplasmic vacuolization in males and females, the high dose selected for the 2-year gavage study in Wistar Han rats was 50 mg/kg. The low dose (3 mg/kg) and mid dose (15 mg/kg) were selected to give a broad range of exposure.

2-Year Study in Wistar Han Rats

Litter Effects Through Postnatal Day 21

Administration of DE-71 had no biologically relevant effect on survival or body weights of pups or dams, and no apparent effects on the percentage of mated females producing pups, litter size, pup sex distribution, weights of dams, or numbers of male or female pups (Tables 11, 12, 13, and 14; Figures 5 and 6). There were no clinical findings associated with exposure to DE-71 in the dams before or after parturition. Pups born to dams administered DE-71 during gestation were weaned on PND 21, and this was considered day 1 of the 2-year perinatal and postnatal study. There was no effect on the growth of the pups.

Table 11
Summary of Disposition During Perinatal Exposure and F_1 Allocation in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Time-Mated Females (GD 6)	62	52	52	62
Females Pregnant (%)	54 (87%)	42 (81%)	43 (83%)	51 (82%)
Females Not Pregnant (%)	8 (13%)	10 (19%)	9 (17%)	11 (18%)
Dams Not Delivering with Evidence of Pregnancy (%)	2 (4%)	1 (2%)	4 (9%)	2 (4%)
Dams with Litters on PND 0 (%)	52 (96%)	41 (98%)	39 (91%)	49 (96%)
Dams, Moribund	0	0	0	0
Dams, Natural Deaths	0	0	0	0
Litters Post-Standardization (PND 4)	36	29	28	37
Post-Weaning Allocation				
F_1 Males – Interim[a] (litters)	10 (9)			10 (9)
F_1 Females – Interim[a] (litters)	10 (10)			10 (8)
F_1 Males – Core[b] (litters)	50 (29)	50 (25)	50 (25)	50 (29)
F_1 Females – Core[b] (litters)	50 (30)	50 (25)	50 (25)	50 (28)

[a] 3-month interim evaluation
[b] 105-week evaluation

TABLE 12
Mean Body Weights of F_0 Female Wistar Han Rats During Gestation and Lactation in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Gestation Day				
5	209.0 ± 2.6 [52]	209.0 ± 3.3 [41]	207.4 ± 2.5 [39]	210.8 ± 2.9 [49]
6	208.8 ± 2.6 [52]	209.4 ± 3.2 [41]	209.7 ± 2.5 [39]	211.7 ± 2.9 [49]
7	213.4 ± 2.5 [52]	211.6 ± 3.2 [41]	212.2 ± 2.5 [39]	217.8 ± 3.0 [49]
8	216.1 ± 2.5 [52]	219.3 ± 3.2 [41]	214.8 ± 2.5 [39]	219.8 ± 2.9 [49]
9	221.1 ± 2.7 [52]	218.2 ± 3.3 [41]	218.9 ± 2.6 [39]	220.5 ± 3.0 [49]
10	224.7 ± 2.7 [52]	224.7 ± 3.2 [41]	221.2 ± 2.6 [39]	226.1 ± 3.1 [49]
11	231.6 ± 2.7 [52]	227.3 ± 3.2 [41]	227.3 ± 2.7 [39]	230.8 ± 3.1 [49]
12	235.4 ± 2.8 [52]	233.0 ± 3.3 [41]	230.6 ± 2.8 [39]	234.8 ± 3.2 [49]
13	238.7 ± 2.7 [52]	235.7 ± 3.2 [41]	233.8 ± 2.8 [39]	239.6 ± 3.3 [49]
14	242.4 ± 2.8 [52]	244.0 ± 3.5 [41]	240.8 ± 2.8 [39]	243.2 ± 3.3 [49]
15	250.4 ± 2.9 [52]	245.9 ± 3.6 [41]	243.7 ± 2.9 [39]	247.9 ± 3.4 [49]
16	253.9 ± 2.9 [52]	251.3 ± 3.6 [41]	249.9 ± 2.9 [39]	256.6 ± 3.4 [49]
17	261.4 ± 3.1 [52]	257.8 ± 3.7 [41]	258.1 ± 3.0 [39]	263.4 ± 3.7 [49]
18	271.4 ± 3.3 [52]	266.7 ± 3.9 [41]	268.5 ± 3.1 [39]	272.9 ± 3.7 [49]
19	279.9 ± 3.5 [52]	273.8 ± 4.3 [41]	275.4 ± 3.4 [39]	280.5 ± 3.8 [49]
20	290.2 ± 3.6 [52]	283.3 ± 4.3 [41]	285.5 ± 3.7 [39]	291.7 ± 4.0 [49]
21	301.6 ± 4.0 [52]	293.1 ± 4.7 [41]	295.0 ± 3.9 [39]	302.4 ± 4.3 [49]
Lactation Day				
0	247.1 ± 3.2 [47]	248.5 ± 4.9 [37]	255.2 ± 5.5 [36]	249.1 ± 4.5 [44]
1	244.8 ± 2.9 [52]	242.4 ± 3.7 [41]	243.6 ± 3.1 [39]	245.4 ± 3.3 [49]
2	246.6 ± 2.8 [51]	243.8 ± 3.9 [41]	247.3 ± 3.1 [39]	247.1 ± 3.3 [49]
3	250.9 ± 2.9 [48]	248.0 ± 4.1 [37]	249.4 ± 3.4 [36]	248.2 ± 3.6 [41]
4	254.0 ± 3.3 [38]	250.8 ± 4.4 [32]	250.6 ± 3.0 [31]	253.8 ± 4.1 [37]
5	257.3 ± 3.4 [36]	252.1 ± 4.6 [29]	251.9 ± 2.9 [28]	255.7 ± 4.3 [37]
6	259.9 ± 3.2 [36]	256.7 ± 4.7 [29]	255.8 ± 3.1 [28]	261.1 ± 3.9 [37]
7	263.5 ± 3.2 [36]	260.6 ± 4.4 [29]	259.8 ± 3.1 [28]	264.7 ± 4.0 [37]
8	266.1 ± 3.2 [36]	260.0 ± 4.3 [29]	261.8 ± 3.1 [28]	266.9 ± 4.0 [37]
9	269.8 ± 3.2 [36]	265.6 ± 4.4 [29]	266.8 ± 3.3 [28]	271.0 ± 4.2 [37]
10	272.7 ± 3.5 [36]	270.8 ± 5.0 [29]	270.2 ± 3.4 [28]	273.3 ± 4.4 [37]
11	274.2 ± 3.8 [36]	274.7 ± 5.0 [29]	274.3 ± 3.4 [28]	279.1 ± 4.2 [37]
12	276.1 ± 4.0 [36]	278.2 ± 5.0 [29]	280.9 ± 3.6 [28]	282.9 ± 4.4 [36]
13	277.5 ± 4.2 [36]	278.3 ± 5.0 [29]	277.6 ± 3.6 [28]	282.2 ± 4.4 [37]
14	279.4 ± 4.6 [36]	279.4 ± 5.0 [29]	277.0 ± 3.5 [28]	284.1 ± 4.4 [36]
15	275.5 ± 3.9 [36]	264.6 ± 3.5 [29]	265.7 ± 3.5 [28]	278.1 ± 4.2 [37]
16	276.5 ± 3.5 [36]	275.2 ± 5.2 [29]	276.9 ± 3.6 [28]	280.5 ± 4.3 [36]
17	278.4 ± 3.3 [36]	277.7 ± 4.2 [29]	278.5 ± 3.2 [28]	278.8 ± 4.2 [36]
18	274.5 ± 3.1 [36]	275.9 ± 4.2 [29]	272.7 ± 2.9 [28]	277.6 ± 4.1 [35]
19	274.2 ± 3.5 [36]	273.5 ± 4.5 [29]	270.1 ± 3.9 [27]	273.9 ± 4.1 [36]
20	275.0 ± 3.4 [36]	273.8 ± 4.3 [29]	269.6 ± 3.2 [28]	272.5 ± 4.0 [36]
21	276.8 ± 3.5 [34]	270.2 ± 4.8 [27]	267.3 ± 3.1 [26]	272.1 ± 5.0 [33]

[a] Data are presented as mean ± standard error [number of dams]. Differences from the vehicle control group are not significant by Dunnett's test.

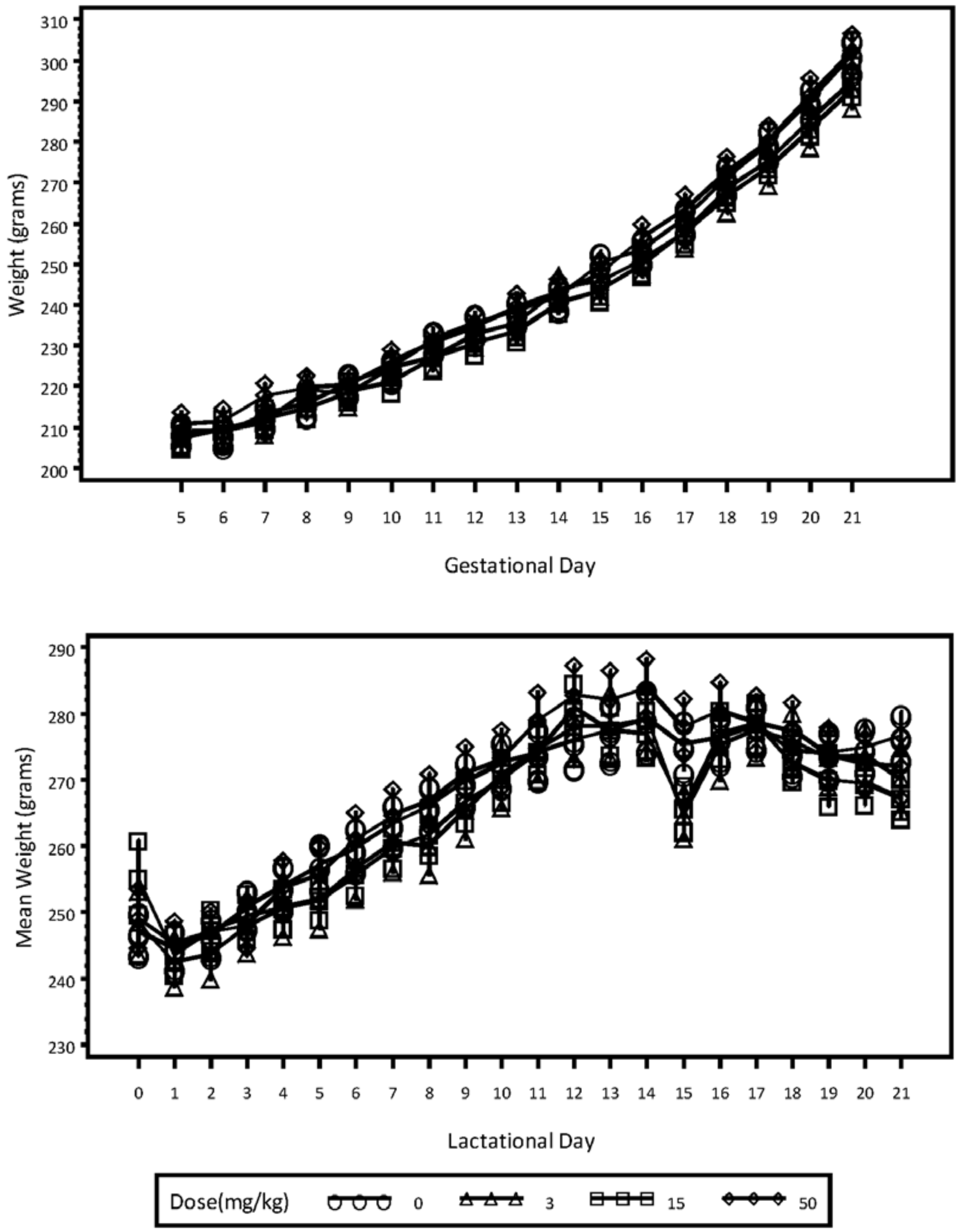


FIGURE 5
Mean Body Weights of F_0 Female Wistar Han Rats During Gestation and Lactation in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

TABLE 13
Mean Number of Surviving F_1 Male and Female Wistar Han Rats During Lactation in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

Postnatal Day	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Males				
1	4.00 ± 0.26 [52]	3.76 ± 0.31 [41]	4.18 ± 0.30 [38]	4.22 ± 0.28 [49]
4[b]	3.96 ± 0.27 [52]	3.76 ± 0.31 [41]	4.18 ± 0.30 [38]	4.16 ± 0.28 [49]
4[c]	4.57 ± 0.29 [35]	4.48 ± 0.29 [29]	4.69 ± 0.32 [29]	4.65 ± 0.29 [37]
7	3.89 ± 0.16 [35]	3.79 ± 0.19 [29]	4.07 ± 0.24 [29]	3.83 ± 0.19 [36]
12	3.89 ± 0.16 [35]	3.79 ± 0.19 [29]	4.07 ± 0.25 [28]	3.83 ± 0.19 [36]
15	3.86 ± 0.15 [35]	3.79 ± 0.19 [29]	4.07 ± 0.25 [28]	3.83 ± 0.19 [36]
18	3.83 ± 0.16 [35]	3.79 ± 0.19 [29]	4.07 ± 0.25 [28]	3.83 ± 0.19 [36]
21	3.80 ± 0.16 [35]	3.72 ± 0.20 [29]	4.00 ± 0.27 [28]	3.69 ± 0.22 [36]
Females				
1	4.52 ± 0.29 [52]	4.02 ± 0.31 [41]	3.74 ± 0.34 [38]	4.41 ± 0.32 [49]
4[b]	4.48 ± 0.30 [52]	4.00 ± 0.31 [41]	3.74 ± 0.34 [38]	4.35 ± 0.32 [49]
4[c]	5.33 ± 0.28 [36]	4.83 ± 0.32 [29]	4.34 ± 0.34 [29]	5.05 ± 0.33 [37]
7	4.22 ± 0.19 [36]	4.07 ± 0.19 [29]	3.83 ± 0.26 [29]	4.03 ± 0.19 [36]
12	4.22 ± 0.19 [36]	4.03 ± 0.19 [29]	3.93 ± 0.25 [28]	4.03 ± 0.19 [36]
15	4.22 ± 0.19 [36]	4.03 ± 0.19 [29]	3.86 ± 0.26 [28]	4.03 ± 0.19 [36]
18	4.17 ± 0.20 [36]	4.03 ± 0.19 [29]	3.86 ± 0.26 [28]	4.03 ± 0.19 [36]
21	4.11 ± 0.21 [36]	3.90 ± 0.19 [29]	3.68 ± 0.26 [28]	3.89 ± 0.19 [36]
Combined				
1	8.52 ± 0.35 [52]	7.78 ± 0.45 [41]	7.92 ± 0.41 [38]	8.63 ± 0.38 [49]
4[b]	8.44 ± 0.36 [52]	7.76 ± 0.45 [41]	7.92 ± 0.41 [38]	8.51 ± 0.39 [49]
4[c]	9.78 ± 0.25 [36]	9.31 ± 0.29 [29]	9.03 ± 0.24 [29]	9.70 ± 0.29 [37]
7	8.00 ± 0.00 [36]	7.86 ± 0.07 [29]*	7.90 ± 0.10 [29]	7.86 ± 0.11 [36]
12	8.00 ± 0.00 [36]	7.83 ± 0.07 [29]**	8.00 ± 0.00 [28]	7.86 ± 0.11 [36]
15	7.97 ± 0.03 [36]	7.83 ± 0.07 [29]	7.93 ± 0.05 [28]	7.86 ± 0.11 [36]
18	7.89 ± 0.07 [36]	7.83 ± 0.07 [29]	7.93 ± 0.05 [28]	7.86 ± 0.11 [36]
21	7.81 ± 0.10 [36]	7.62 ± 0.16 [29]	7.68 ± 0.20 [28]	7.58 ± 0.19 [36]

* Significantly different (P≤0.05) from the vehicle control group by Dunn's test
** P≤0.01
a Data are presented as mean number of surviving pups ± standard error [number of dams].
b Pre-standardization of litters
c Post-standardization of litters

TABLE 14
Mean Body Weights of F_1 Male and Female Wistar Han Rats During Lactation in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

Postnatal Day	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Males				
1	7.54 ± 0.12 [51]	7.36 ± 0.13 [39]	7.89 ± 0.20 [38]	7.48 ± 0.14 [48]
4[b]	11.60 ± 0.20 [35]	11.49 ± 0.24 [29]	12.19 ± 0.20 [28]	11.56 ± 0.25 [37]
7	17.58 ± 0.28 [35]	17.22 ± 0.35 [29]	17.86 ± 0.28 [29]	17.34 ± 0.37 [36]
12	29.20 ± 0.44 [35]	28.79 ± 0.48 [29]	29.64 ± 0.49 [28]	29.24 ± 0.60 [36]
15	36.40 ± 0.63 [35]	35.86 ± 0.58 [29]	36.62 ± 0.62 [28]	36.31 ± 0.72 [36]
18	43.65 ± 0.80 [35]	42.57 ± 0.66 [28]	44.28 ± 0.75 [28]	43.35 ± 0.89 [36]
21	55.03 ± 1.04 [34]	54.68 ± 0.87 [28]	55.19 ± 1.16 [28]	54.48 ± 1.17 [36]
Females				
1	7.06 ± 0.16 [52]	7.26 ± 0.12 [40]	7.51 ± 0.19 [36]	7.32 ± 0.13 [48]
4[b]	11.27 ± 0.20 [36]	11.26 ± 0.23 [29]	11.74 ± 0.21 [28]	11.30 ± 0.25 [37]
7	17.05 ± 0.29 [36]	16.78 ± 0.32 [29]	17.32 ± 0.27 [29]	16.85 ± 0.33 [36]
12	28.35 ± 0.46 [36]	28.20 ± 0.44 [29]	28.82 ± 0.44 [28]	28.52 ± 0.53 [36]
15	35.49 ± 0.66 [36]	35.15 ± 0.50 [29]	35.69 ± 0.55 [28]	35.30 ± 0.66 [36]
18	42.19 ± 0.79 [36]	41.55 ± 0.57 [28]	43.10 ± 0.68 [28]	42.09 ± 0.82 [36]
21	52.92 ± 0.95 [35]	52.64 ± 0.80 [28]	54.04 ± 0.87 [28]	52.64 ± 1.02 [36]
Combined				
1	7.30 ± 0.11 [52]	7.33 ± 0.11 [41]	7.68 ± 0.13 [38]	7.38 ± 0.12 [49]
4[b]	11.41 ± 0.20 [36]	11.34 ± 0.23 [29]	11.95 ± 0.20 [28]	11.43 ± 0.25 [37]
7	17.31 ± 0.28 [36]	16.95 ± 0.33 [29]	17.56 ± 0.27 [29]	17.09 ± 0.35 [36]
12	28.76 ± 0.44 [36]	28.43 ± 0.46 [29]	29.20 ± 0.45 [28]	28.87 ± 0.56 [36]
15	35.93 ± 0.63 [36]	35.43 ± 0.53 [29]	36.16 ± 0.57 [28]	35.78 ± 0.68 [36]
18	42.89 ± 0.78 [36]	41.97 ± 0.61 [28]	43.75 ± 0.69 [28]	42.71 ± 0.85 [36]
21	53.95 ± 0.98 [35]	53.53 ± 0.82 [28]	54.77 ± 0.93 [28]	53.53 ± 1.07 [36]

[a] Data are presented as mean ± standard error [number of dams]. Weights were calculated on postnatal day 1 by collecting total weights and dividing by number of pups; weights after postnatal day 1 are based on individual pup weights. Differences from the vehicle control group are not significant by Dunnett's test.

[b] Post-standardization of litters

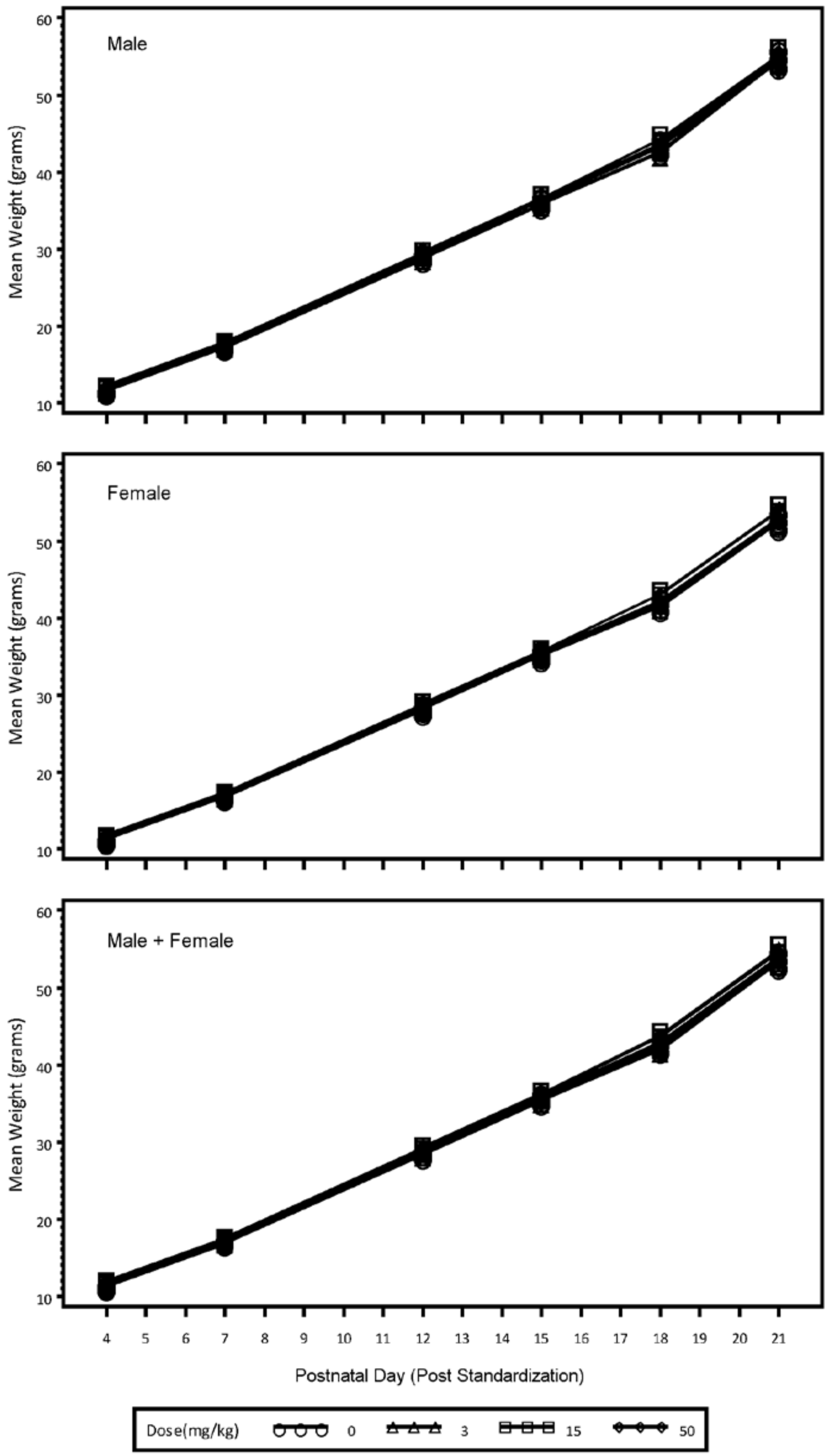


FIGURE 6
Mean Body Weights of F_1 Male and Female Wistar Han Rats During Lactation in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

Survival

Estimates of 2-year survival probabilities for F_1 male and female rats are shown in Table 15 and in the Kaplan-Meier survival curves (Figure 7). Survival of 50 mg/kg males was significantly less than that of the vehicle controls. Decreased survival in the 50 mg/kg males was due to a higher number of adenomas of the pituitary gland pars distalis compared to the vehicle control group. There was a significant trend of decreased survival in dosed groups of females, but the survival of individual dosed groups was not significantly different from that of the vehicle control group.

Body Weights and Clinical Findings

Mean body weights of dosed groups of males were similar to those of the vehicle controls throughout the study (Figure 8 and Table 16). In 50 mg/kg females, mean body weights were at least 10% less than those of the vehicle controls after week 37, and the incidence of thinness was increased (Figure 8 and Table 17).

.

TABLE 15
Survival of F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Number of litters contributing to groups	52	41	39	49
Male				
Animals initially in study	60	50	50	60
3-Month interim evaluation[a]	10			10
Accidental deaths[b]	1	1	0	1
Other[b,c]	1	0	0	0
Moribund	8	7	10	12
Natural deaths	4	7	2	12
Animals surviving to study termination	36	35	38	25
Percent probability of survival at end of study[d]	75	72	76	51
Mean survival (days)[e]	671	664	683	657
Survival analysis[f]	P=0.011	P=0.814	P=1.000	P=0.030
Female				
Animals initially in study	60	50	50	60
3-Month interim evaluation[a]	10			10
Accidental deaths[b]	2	1	0	0
Other[b,c]	0	0	0	1
Moribund	8	10	13	11
Natural deaths	3	0	4	10
Animals surviving to study termination	37[g]	39	33	28
Percent probability of survival at end of study	77	80	66	57
Mean survival (days)	676	705	689	640
Survival analysis	P=0.007	P=0.852N	P=0.350	P=0.054

a Excluded from survival analysis
b Censored from survival analysis
c Animals not necropsied.
d Kaplan-Meier determinations
e Mean of all deaths (uncensored, censored, and terminal kill).
f The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.
g Includes one animal that died during the last week of the study

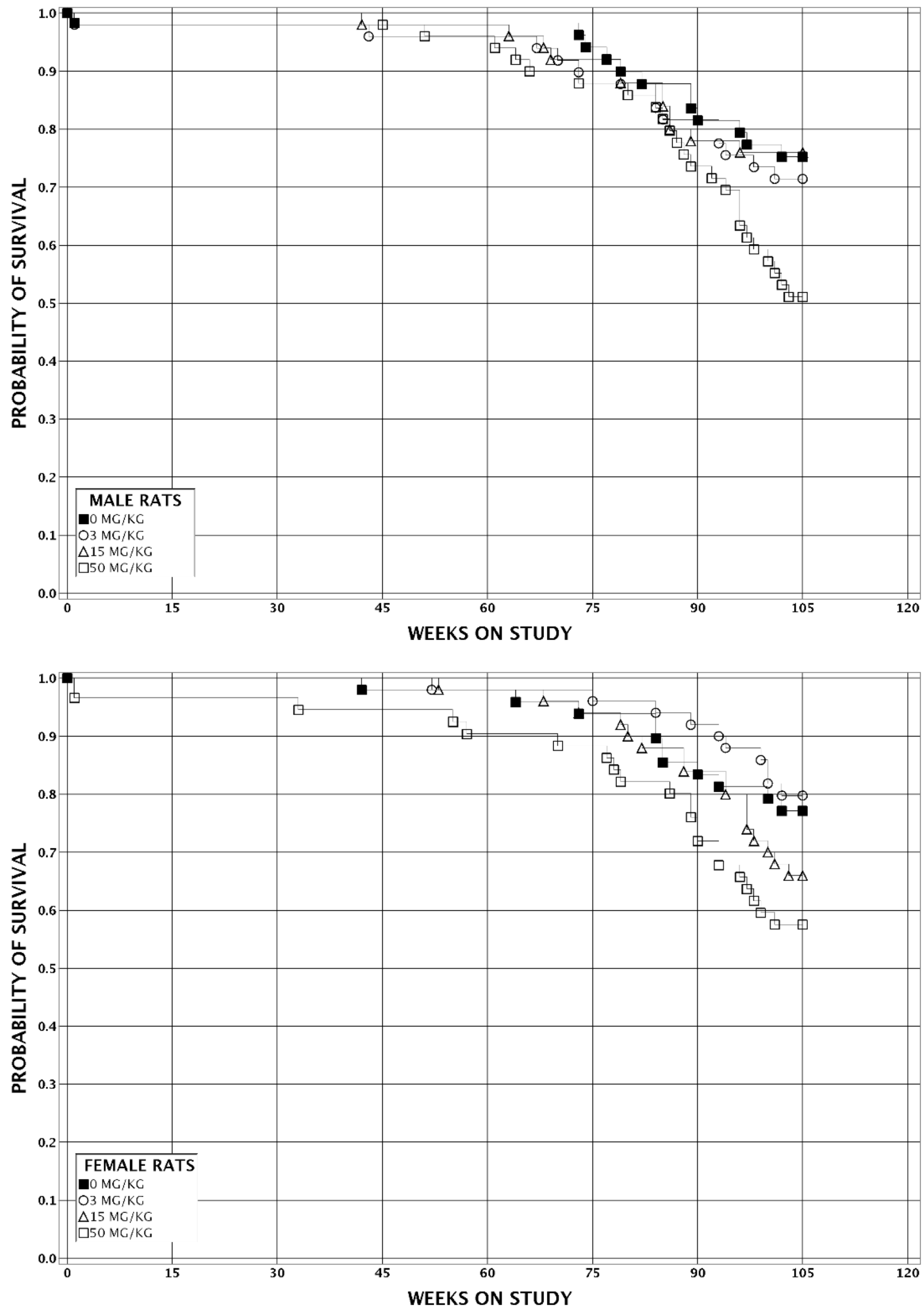
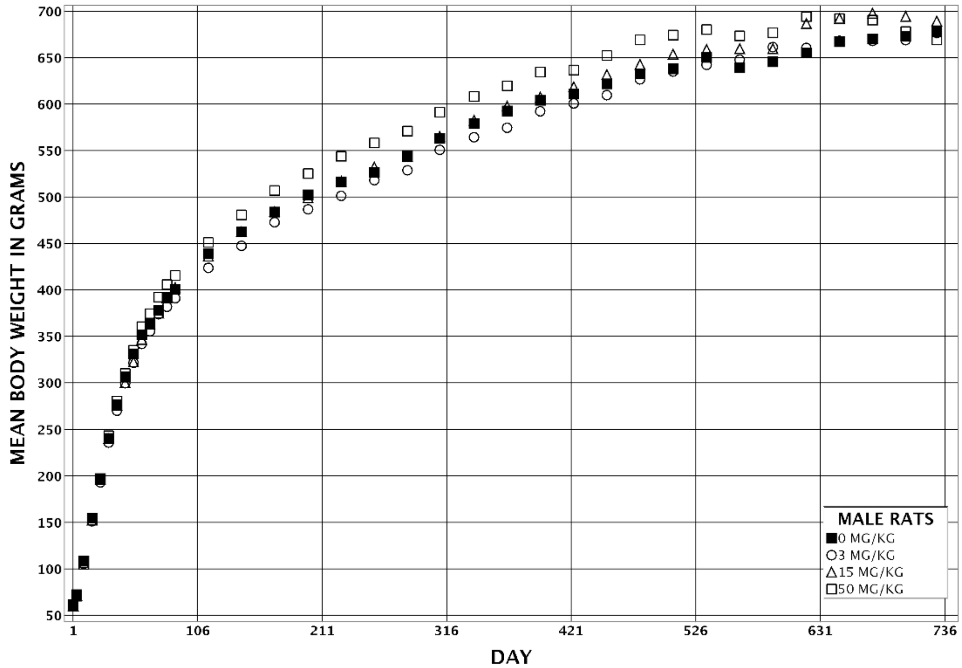


FIGURE 7
Kaplan-Meier Survival Curves for F_1 Wistar Han Rats Administered DE-71 by Gavage for 2 Years



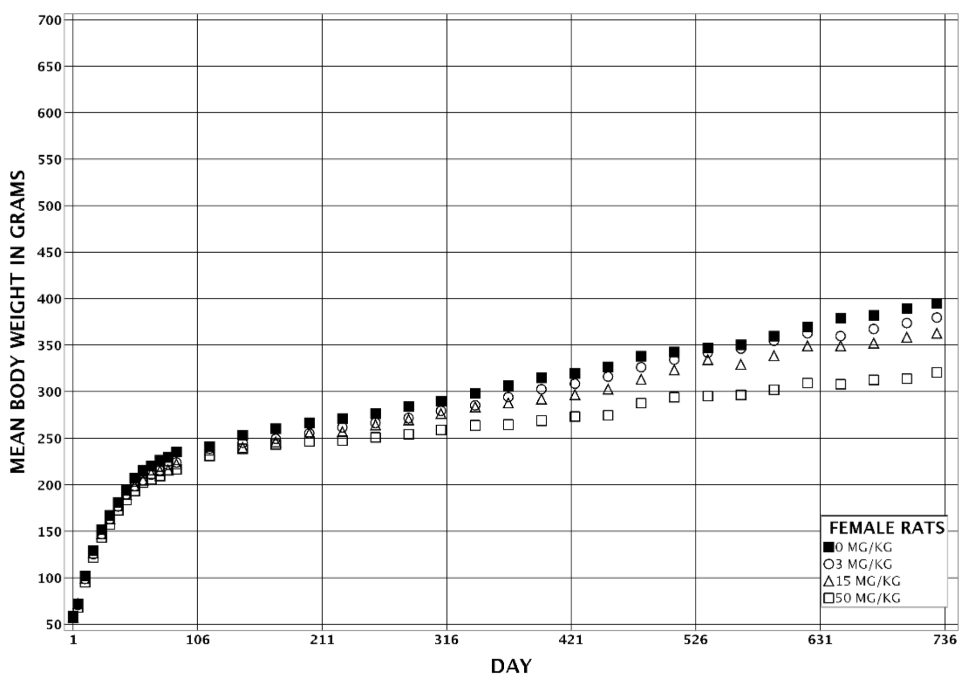


FIGURE 8
Growth Curves for F_1 Wistar Han Rats Administered DE-71 by Gavage for 2 Years

TABLE 16
Mean Body Weights and Survival of F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control		3 mg/kg			15 mg/kg			50 mg/kg		
Day	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	62	60	60	97	50	60	97	50	61	98	60
4	73	58	72	99	49	72	98	50	71	97	60
10	109	58	106	97	49	107	99	50	105	97	60
17	155	58	151	98	49	153	99	50	153	99	60
24	197	58	193	98	49	196	99	50	197	100	60
31	241	58	236	98	49	240	100	50	244	101	60
38	276	58	271	98	49	276	100	50	281	102	60
45	307	57	300	98	49	300	98	50	310	101	60
52	331	57	322	97	49	323	97	50	335	101	60
59	352	57	342	97	49	347	99	50	361	102	60
66	364	57	356	98	49	363	100	50	375	103	60
73	378	57	374	99	49	375	99	50	393	104	60
80	392	57	382	98	49	392	100	50	406	104	60
87	401	57	391	98	49	403	100	50	416	104	60
115	439	47[a]	424	97	49	437	99	50	451	103	50[a]
143	463	47	448	97	49	463	100	50	481	104	50
171	484	47	473	98	49	484	100	50	507	105	50
199	503	47	487	97	49	499	99	50	526	105	50
227	517	47	501	97	49	517	100	50	544	105	50
255	527	47	518	98	49	532	101	50	558	106	50
283	544	47	529	97	48	544	100	50	571	105	50
310	563	47	551	98	47	565	100	49	591	105	49
339	579	47	564	97	47	583	101	49	608	105	49
367	593	47	575	97	47	598	101	49	620	105	48
395	604	47	592	98	47	608	101	49	635	105	48
423	611	47	601	98	47	619	101	49	637	104	48
451	622	47	610	98	47	632	102	48	653	105	46
479	633	47	627	99	46	643	102	47	669	106	45
507	638	47	635	100	44	654	103	46	675	106	43
535	651	45	643	99	44	659	101	46	681	105	43
563	639	43	648	101	43	660	103	44	674	105	42
591	646	42	661	102	41	660	102	44	677	105	41
619	656	41	661	101	40	687	105	39	695	106	36
647	667	39	668	100	38	693	104	39	693	104	35
675	671	38	668	100	37	698	104	38	691	103	31
703	673	37	669	99	35	695	103	38	678	101	27
Mean for Weeks											
1-13	260		254	98		258	99		265	102	
14-52	521		507	97		522	100		546	105	
53-100	643		640	100		659	102		672	105	

[a] Interim evaluation occurred during week 14

TABLE 17
Mean Body Weights and Survival of F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control		3 mg/kg			15 mg/kg			50 mg/kg		
Day	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	59	60	57	97	50	58	98	50	57	97	60
5	72	60	71	99	50	72	100	50	68	95	57
11	102	60	99	97	50	99	97	50	95	93	57
18	129	60	126	97	50	127	98	50	122	95	57
25	152	60	147	97	50	148	97	50	144	94	57
32	168	60	163	97	50	164	98	50	158	94	57
39	181	59	177	97	50	177	98	50	173	95	57
46	195	59	190	97	50	190	97	50	184	94	57
53	207	59	199	96	50	199	96	50	194	93	57
60	216	59	205	95	50	205	95	50	203	94	57
67	221	59	211	96	50	212	96	50	206	93	57
74	227	59	216	95	50	215	95	50	210	93	57
81	230	59	222	97	50	216	94	50	216	94	57
88	236	59	224	95	50	223	95	50	217	92	57
116	241	49[a]	239	99	50	237	98	50	231	96	47[a]
144	254	49	245	97	50	240	95	50	239	94	47
172	261	49	250	96	50	246	95	50	244	94	47
200	267	49	256	96	50	256	96	50	247	93	47
228	271	49	262	97	50	257	95	50	248	91	46
256	276	49	267	97	50	265	96	50	251	91	46
284	284	49	272	96	50	270	95	50	255	90	46
311	290	48	279	96	50	276	95	50	259	90	46
340	298	48	285	96	50	284	95	50	264	89	46
368	307	48	294	96	49	288	94	49	265	86	46
396	315	48	303	96	49	292	93	49	269	86	44
424	320	48	308	97	49	297	93	49	274	86	44
452	327	47	316	97	49	303	93	49	275	84	44
480	338	47	327	97	49	313	93	48	288	85	44
508	343	46	335	98	49	323	94	47	294	86	43
536	347	45	342	99	48	334	96	47	295	85	43
564	351	45	346	99	48	329	94	45	297	85	40
592	360	41	355	99	47	339	94	44	302	84	40
620	370	41	363	98	46	349	94	42	309	84	37
648	379	39	360	95	46	349	92	42	308	81	33
676	382	39	368	96	43	352	92	37	313	82	32
704	390	38	374	96	40	358	92	34	314	81	28
Mean for Weeks											
1-13	171		165	96		165	96		161	94	
14-52	275		265	96		262	95		250	91	
53-100	352		341	97		328	93		295	84	

[a] Interim evaluation occurred during week 14

3-Month Interim Evaluation Organ Weights

At the 3-month interim evaluation, organ weights were measured in vehicle controls and 50 mg/kg rats. The absolute and relative liver weights of 50 mg/kg males and females were significantly increased compared to those of the vehicle controls at the 3-month interim evaluation (Tables 18 and G2). In 50 mg/kg males, the mean absolute liver weight was approximately 43% greater than that of the vehicle controls and in 50 mg/kg females, the absolute liver weight was 17% greater than that of the vehicle controls. The increased liver weights correlated with hepatocellular hypertrophy in the liver, and reflected what was observed in the 3-month study in F344/N rats (Tables 6 and G1).

The absolute and relative kidney weights of 50 mg/kg males were significantly increased (approximately 22% for the absolute weight; Tables 18 and G2). Similar increases in kidney weights were observed in the 3-month study in male F344/N rats (Tables 6 and G1). In contrast to the 3-month study in F344/N rats, there were minimal changes in kidney weights in the female rats at the 3-month interim evaluation.

The absolute testis weight of 50 mg/kg males was significantly increased (Tables 18 and G2). The absolute testis weight was 18% greater than that of the vehicle control group; however, no histologic changes were observed in the testes that correlated with this weight difference.

The absolute thymus weight of 50 mg/kg females was significantly decreased by approximately 27% (Tables 18 and G2). This degree of difference was considered greater than that expected from the difference in body weights, but the toxicologic significance of this change is unknown. Similar changes were seen in the

TABLE 18
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1 Wistar Han Rats at the 3-Month Interim Evaluation in the 2-Year Perinatal and Postnatal Gavage Study[a]

	Vehicle Control	50 mg/kg
n	10	10
Male		
Necropsy body wt	403 ± 10	433 ± 16
R. Kidney		
Absolute	1.29 ± 0.04	1.57 ± 0.08**
Relative	3.198 ± 0.102	3.618 ± 0.113*
Liver		
Absolute	13.68 ± 0.39	19.53 ± 0.76**
Relative	33.938 ± 0.702	45.180 ± 1.191**
R. Testis		
Absolute	1.836 ± 0.069	2.168 ± 0.075**
Female		
Necropsy body wt	246 ± 4	213 ± 7**
Liver		
Absolute	7.94 ± 0.18	9.28 ± 0.43*
Relative	32.350 ± 0.579	43.369 ± 0.745**
Thymus		
Absolute	0.362 ± 0.020	0.264 ± 0.016**
Relative	1.473 ± 0.071	1.239 ± 0.070*

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

thymus weights of female F344/N rats in the 3-month study (Tables 6 and G1). In that study, thymic atrophy was seen in 500 mg/kg females but not in 50 or 100 mg/kg females (Table 10). Thymic atrophy was not observed in 50 mg/kg females in this 3-month interim evaluation (Table B4).

In 50 mg/kg female rats, increased relative heart and kidney weights and a decreased absolute lung weight were considered secondary to the decrease in mean body weight when compared to the vehicle control group (Table G2).

Tissue Concentrations

Concentrations of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153) were determined in the following tissues following perinatal exposure of dams to DE-71; liver and carcass from PND 4 pups at litter standardization; adipose and liver from dams assigned to the tissue distribution study and their pups at PND 21; adipose, liver, and plasma in F_1 rats at the end of the study (Tables I2, I3, and I4). In PND 4 and PND 21 pup liver, the tissue concentrations of all congeners measured increased with increasing dose and were higher than corresponding vehicle control values. The concentrations in PND 4 pup liver were higher than those in the PND 21 pup liver, which is likely due to the increased metabolic capacity at PND 21 compared to PND 4. In PND 21 dam liver, the concentrations of all congeners were below the limit of quantitation except at 50 mg/kg; at 50 mg/kg, the dam liver values were lower than the corresponding pup liver values. The concentrations of all congeners in PND 21 pup and dam adipose was higher than the corresponding concentrations in liver, suggesting preferential accumulation in the adipose. The concentrations of BDE-99 and BDE-47 were similar in adipose from both dams and pups and were higher than BDE-153 concentrations. There were no sex differences in congener concentrations. In all matrices at the end of the study, concentrations of congeners increased with the dose and were higher than the corresponding vehicle control values (Figure 9). The concentrations were lowest in plasma and highest in adipose. In a given matrix, the concentrations of BDE-47, BDE-99, and BDE-153 were similar, regardless of the different percent of these congeners in DE-71. This suggests a higher rate of accumulation of BDE-153 regardless of the lower percentage of BDE-153 in DE-71. In general, there were no sex differences except plasma concentrations in 3 mg/kg (BDE-153 only) and 15 mg/kg (all three congeners) females were higher than concentrations in males.

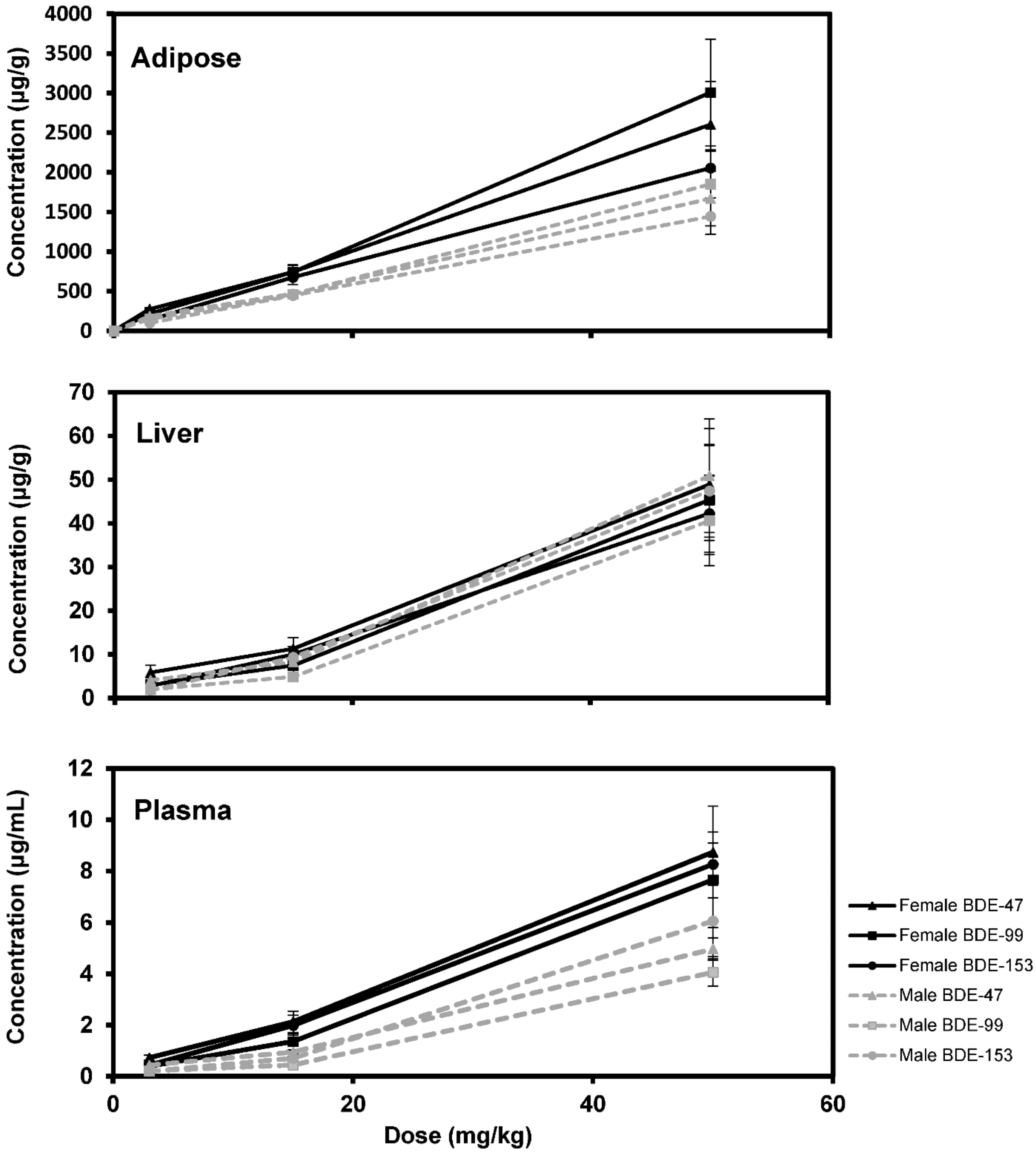


FIGURE 9
Concentrations of BDE-47, BDE-99, and BDE-153 in Adipose, Liver, and Plasma in F_1 Male and Female Wistar Han Rats Administered DE-71 by Gavage for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the liver, thyroid gland, pituitary gland, uterus, vagina, cervix, kidney, parotid salivary gland, prostate gland, epididymis, preputial gland, thymus, spleen, forestomach, adrenal cortex, and mammary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Liver: At the 3-month interim evaluation, the incidences of hepatocyte hypertrophy were significantly increased in 50 mg/kg males and females (Tables 19, A4, and B4). The incidence of fatty change of the hepatocytes was also significantly increased in 50 mg/kg male rats. Hepatocyte hypertrophy was characterized by enlarged hepatocytes with granular eosinophilic cytoplasm and variably enlarged nuclei. Hepatocyte hypertrophy involved primarily the centrilobular area, with the midzonal region being affected in more severe cases. The grading criteria for the diagnosis of hepatocyte hypertrophy were as follows: if less than 10% of the hepatocytes in the section were affected, it was recorded as minimal severity; if 10% or more but less than 50% of the hepatocytes in the section were affected, it was recorded as mild severity; if 50% or more but less than 75% of the hepatocytes in the section were affected, it was recorded as moderate severity; if more than 75% of the hepatocytes in the section were affected, it was recorded as marked severity. Fatty change of the hepatocytes consisted of a centrally located nucleus in cytoplasm that contained small discrete vacuoles. This change was characteristically the same as that observed as cytoplasmic vacuolization in the 3-month study in F344/N rats, but was recorded as fatty change.

At 2 years, there were positive trends in the incidences of hepatocellular adenoma or carcinoma (combined) and hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (combined) in males and females, and the incidences of these combined lesions were significantly increased in the 50 mg/kg groups (Tables 19, A1, A2, B1, and B2). The incidences of hepatocholangioma, hepatocellular adenoma, and hepatocellular carcinoma were also significantly increased in 50 mg/kg females. Hepatocellular adenomas typically consisted of well-circumscribed masses that caused compression of the surrounding hepatic parenchyma. These neoplasms were composed of a uniform population of hepatocytes and lacked the normal lobular architecture. Some adenomas displayed a little cellular atypia, but it was less common and less pronounced than that seen in the hepatocellular carcinomas. Hepatocellular carcinomas were also invasive and less well-demarcated than adenomas, and frequently contained areas of necrosis and blood-filled spaces (Plate 4). Their growth pattern was characterized by thickened hepatic trabeculae at least three cell layers wide compared with single-cell width trabeculae found in normal liver. Hepatocholangiomas are thought to arise from cells that can differentiate into either hepatocytes or biliary cells. They were nodular proliferative lesions similar to hepatocellular adenomas but also contained proliferations of single-layered, well-differentiated biliary epithelium, which formed cystic acini with occasional papillary infoldings (Plate 5). Hepatocholangiomas were distinguished from hepatocellular adenomas with dilated nonneoplastic bile ducts by the increased number of bile ducts within hepatocholangiomas and the fact that hepatocellular adenomas typically lack bile ducts. The epithelium of the biliary component of the hepatocholangiomas was cuboidal in contrast to the typically flattened epithelium found in biliary cysts.

Cholangiocarcinoma occurred in two 50 mg/kg females and cholangiofibrosis occurred in three different 50 mg/kg female rats (Tables 19 and B1). Cholangiofibrosis is believed to be a precursor lesion to cholangiocarcinoma (Thoolen *et al.,* 2010). Cholangiocarcinoma consisted of an irregular, relatively large, noncircumscribed lesion that effaced and invaded normal liver parenchyma (Plates 6 and 7). The lesion consisted of fibrous connective tissue stroma containing numerous atypical bile ducts, which frequently contained mucinous material and cellular debris. The epithelium forming the atypical bile ducts was often discontinuous, consisted of large atypical cells and intestinal goblet cells, and displayed degenerative changes. Cholangiofibrosis was smaller in size than cholangiocarcinoma, was well demarcated, and did not show evidence of localized invasion. Distinction between cholangiofibrosis and cholangiocarcinoma was primarily based upon liver invasion. Cholangiocarcinoma and cholangiofibrosis are uncommon in control rats but have been observed in previous NTP studies of rats exposed to hepatic carcinogens. Consequently, the observations of these neoplasms in the livers of rats exposed to DE-71 were considered related to exposure.

The incidence of nodular hyperplasia was significantly increased in 50 mg/kg females and slightly increased in 15 mg/kg males (Tables 19, A4, and B4). Nodular hyperplasia did not occur in the vehicle control groups.

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Male				
3-Month Interim Evaluation				
Number Examined Microscopically	10			10
Fatty Change[a]	2 (1.0)[b]			8* (1.5)
Hepatocyte, Hypertrophy	0			10** (3.1)
2-Year Study				
Number Examined Microscopically	49	50	50	50
Eosinophilic Focus	3	3	12*	15**
Fatty Change	32 (1.5)	37 (1.5)	48** (1.8)	48** (2.1)
Hyperplasia, Nodular	0	0	3 (1.7)	0
Inflammation, Chronic	1 (1.0)	2 (1.0)	0	5 (1.2)
Pigmentation	0	0	1 (2.0)	6* (1.0)
Hepatocyte, Hypertrophy	1 (1.0)	44** (2.0)	50** (3.0)	50** (3.8)
Oval Cell, Hyperplasia	0	0	2 (1.0)	3 (1.0)
Hepatocholangioma[c]	0	0	0	2
Hepatocellular Adenoma, Multiple	0	0	0	1
Hepatocellular Adenoma (includes multiple)[d]				
Number of litters with at least one neoplasm/total number of litters	3/29	2/25	4/25	8/29
Overall rate[e]	3/49 (6%)	2/50 (4%)	4/50 (8%)	8/50 (16%)
Adjusted rate[f]	7.1%	4.8%	9.2%	19.8%
Terminal rate[g]	3/36 (8%)	1/35 (3%)	4/38 (11%)	3/25 (12%)
First incidence (days)	729 (T)	658	729 (T)	595
Poly-3 test[h]	P=0.016	P=0.503N	P=0.512	P=0.081
Litter-adjusted Poly-3 test	—[i]	—	—	—
Hepatocellular Carcinoma[c]	0	0	0	2
Hepatocellular Adenoma or Carcinoma[d]				
Number of litters with at least one neoplasm/total number of litters	3/29	2/25	4/25	9/29
Overall rate	3/49 (6%)	2/50 (4%)	4/50 (8%)	9/50 (18%)
Adjusted rate	7.1%	4.8%	9.2%	22.3%
Terminal rate	3/36 (8%)	1/35 (3%)	4/38 (11%)	4/25 (16%)
First incidence (days)	729 (T)	658	729 (T)	595
Poly-3 test	P=0.006	P=0.503N	P=0.512	P=0.047
Litter-adjusted Poly-3 test	—	—	—	—
Hepatocholangioma, Hepatocellular Adenoma, or Hepatocellular Carcinoma[d]				
Number of litters with at least one neoplasm/total number of litters	3/29	2/25	4/25	11/29
Overall rate	3/49 (6%)	2/50 (4%)	4/50 (8%)	11/50 (22%)
Adjusted rate	7.1%	4.8%	9.2%	27.2%
Terminal rate	3/36 (8%)	1/35 (3%)	4/38 (11%)	5/25 (20%)
First incidence (days)	729 (T)	658	729 (T)	595
Poly-3 test	P<0.001	P=0.503N	P=0.512	P=0.014
Litter-adjusted Poly-3 test	—	—	—	—

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Female				
3-Month Interim Evaluation				
Number Examined Microscopically	9			10
Fatty Change	0			3 (1.0)
Hepatocyte, Hypertrophy	0			10** (3.0)
2-Year Study				
Number Examined Microscopically	50	49	50	47
Cholangiofibrosis	0	0	0	3 (2.3)
Eosinophilic Focus	5	7	21**	31**
Fatty Change	15 (1.4)	12 (2.5)	28** (1.6)	39** (1.3)
Hyperplasia, Nodular	0	0	2 (2.5)	7** (2.6)
Bile Duct, Cyst	2 (1.0)	2 (1.5)	5 (1.4)	7* (2.0)
Hepatocyte, Hypertrophy	0	48** (1.9)	49** (3.0)	45** (3.9)
Hepatocyte, Necrosis	4 (1.3)	2 (1.0)	1 (1.0)	8 (1.4)
Oval Cell, Hyperplasia	1 (1.0)	3 (1.0)	3 (1.0)	10** (1.2)
Cholangiocarcinoma, Multiple	0	0	0	1
Cholangiocarcinoma (includes multiple)[j]				
Number of litters with at least one neoplasm/total number of litters	0/30	0/25	0/25	2/27
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	2/47 (4%)
Adjusted rate	0.0%	0.0%	0.0%	5.4%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	2/28 (7%)
First incidence (days)	—[k]	—	—	729 (T)
Poly-3 test	P=0.030	—[i]	—	P=0.203
Litter-adjusted Poly-3 test	—	—	—	—
Hepatocholangioma, Multiple	0	0	0	3
Hepatocholangioma (includes multiple)[j]				
Number of litters with at least one neoplasm/total number of litters	0/30	0/25	0/25	7/27
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	8/47 (17%)
Adjusted rate	0.0%	0.0%	0.0%	21.5%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	7/28 (25%)
First incidence (days)	—	—	—	619
Poly-3 test	P<0.001	—	—	P<0.001
Litter-adjusted Poly-3 test	—	—	—	—
Hepatocellular Adenoma, Multiple	1	0	2	8*
Hepatocellular Adenoma (includes multiple)[l]				
Number of litters with at least one neoplasm/total number of litters	3/30	2/25	8/25	12/27
Overall rate	3/50 (6%)	2/49 (4%)	8/50 (16%)	16/47 (34%)
Adjusted rate	6.9%	4.4%	18.2%	41.4%
Terminal rate	3/37 (8%)	2/39 (5%)	6/33 (18%)	11/28 (39%)
First incidence (days)	729 (T)	729 (T)	656	490
Poly-3 test	P<0.001	P=0.476N	P=0.103	P<0.001
Litter-adjusted Poly-3 test	P<0.001	P=0.878	P=0.151	P=0.003

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Female (continued)				
2-Year Study (continued)				
Number Examined Microscopically	50	49	50	47
Hepatocellular Carcinoma, Multiple	0	0	0	3
Hepatocellular Carcinoma (includes multiple)[j]				
Number of litters with at least one neoplasm/total number of litters	0/30	0/25	1/25	6/27
Overall rate	0/50 (0%)	0/49 (0%)	1/50 (2%)	6/47 (13%)
Adjusted rate	0.0%	0.0%	2.3%	16.2%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	5/28 (18%)
First incidence (days)	—	—	686	677
Poly-3 test	P<0.001	—	P=0.503	P=0.008
Litter-adjusted Poly-3 test	—	—	—	—
Hepatocellular Adenoma or Carcinoma[l]				
Number of litters with at least one neoplasm/total number of litters	3/30	2/25	8/25	13/27
Overall rate	3/50 (6%)	2/49 (4%)	8/50 (16%)	17/47 (36%)
Adjusted rate	6.9%	4.4%	18.2%	44.0%
Terminal rate	3/37 (8%)	2/39 (5%)	6/33 (18%)	12/28 (43%)
First incidence (days)	729 (T)	729 (T)	656	490
Poly-3 test	P<0.001	P=0.476N	P=0.103	P<0.001
Litter-adjusted Poly-3 test	P<0.001	P=0.877	P=0.146	P=0.002
Hepatocholangioma, Hepatocellular Adenoma, or Hepatocellular Carcinoma[l]				
Number of litters with at least one neoplasm/total number of litters	3/30	2/25	8/25	15/27
Overall rate	3/50 (6%)	2/49 (4%)	8/50 (16%)	21/47 (45%)
Adjusted rate	6.9%	4.4%	18.2%	53.8%
Terminal rate	3/37 (8%)	2/39 (5%)	6/33 (18%)	15/28 (54%)
First incidence (days)	729 (T)	729 (T)	656	490
Poly-3 test	P<0.001	P=0.476N	P=0.103	P<0.001
Litter-adjusted Poly-3 test	P<0.001	P=0.877	P=0.151	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test.
** Significantly different (P≤0.01) from the vehicle control group by the Fisher exact test (interim evaluation) or Poly-3 test (2-year study).
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 0/99; all routes: 0/299
[d] Historical incidence for corn oil gavage studies: 3/99 (3.1% ± 4.3%), range 0%-6%; all routes: 4/299 (1.4% ± 2.5%), range 0%-6%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by N.
[i] Value of statistic cannot be computed.
[j] Historical incidence for corn oil gavage studies: 0/100; all routes: 0/300
[k] Not applicable; no neoplasms in animal group
[l] Historical incidence for corn oil gavage studies: 4/100 (4.0% ± 2.8%), range 2%-6%; all routes: 6/300 (2.0% ± 2.2%), range 0%-6%

There were significantly increased incidences of eosinophilic focus in 15 and 50 mg/kg male and female rats. The increased incidences of eosinophilic foci correlated with an increase in the number of multiple foci; multiple foci were not recorded in vehicle control males or females. Nodular hyperplasia, which was often difficult to distinguish from eosinophilic foci, was characterized by nodular proliferations of hepatocytes that were distinct from the surrounding parenchyma (Plate 8). These lesions were composed primarily of large, eosinophilic hepatocytes, which sometimes contained lipid and/or glycogen. Scattered among the hepatocytes were bile ducts and oval cells which differentiated them from hepatocellular adenomas. They tended to be larger and more compressive than eosinophilic foci, which were also discrete lesions made up of enlarged, eosinophilic hepatocytes. Foci also tended to merge more with surrounding hepatocytes when compared with nodular hyperplasia.

There were significantly increased incidences of hepatocyte hypertrophy in all dosed groups of male and female rats (Tables 19, A4, and B4). The severities of this lesion were greater than those in the vehicle controls (males) and increased with increasing dose. Hepatocellular hypertrophy was characterized by hepatocytes that were enlarged due to increased amounts of cytoplasm (Plate 9). This change primarily affected hepatocytes in the centrilobular regions, with larger portions of the lobules being affected with increased severity. Severity grading was based on how much of the liver section was involved, how much of each individual hepatic lobule was involved, and how enlarged the individual hepatocytes were.

Incidences of fatty change were significantly increased in 15 and 50 mg/kg male and female rats (Tables 19, A4, and B4). Histologically, fatty change consisted of discrete vacuoles within the cytoplasm of hepatocytes (Plates 10 and 11). The fatty change observed in these livers included both macrovesicular and microvesicular vacuoles. Although cells containing a single large vacuole were more obvious, the majority of the cells actually contained small, discrete vacuoles, often with a centrally located nucleus. This change was characteristically similar to that observed as cytoplasmic vacuolization in the 3-month study in F344/N rats.

A significantly increased incidence of oval cell hyperplasia occurred in 50 mg/kg females (Tables 19 and B4). This lesion also occurred in 15 and 50 mg/kg males, and the incidences were associated with a positive trend (Tables 19 and A4). Oval cell hyperplasia was characterized by proliferations of single or double rows of oval to spindle-shaped cells typically in the periportal regions, but extending into the midzonal areas with increasing severity.

There were significantly increased incidences of pigmentation in 50 mg/kg males and bile duct cyst in 50 mg/kg females (Tables 19, A4, and B4). There were positive trends in the incidences of chronic inflammation in males and hepatocyte necrosis in females. Pigmentation was recorded when globular, golden-brown material was present within the cytoplasm of hepatocytes in the periportal region. Staining with Perls' Prussian Blue was positive, indicating the pigment was consistent with hemosiderin, although lipofuschin cannot be ruled out. Bile duct cysts were lined by a flattened epithelium and were often multilocular. Chronic inflammation was characterized by focal collections of mixed mononuclear cells; these foci lacked a particular distribution and could be found randomly throughout the hepatic parenchyma. Hepatocyte necrosis consisted of either randomly scattered individual shrunken eosinophilic cells or small clusters of such cells. Nuclei of affected cells were often pyknotic or karyorrhectic.

There were significantly decreased incidences of basophilic focus in 50 mg/kg females and clear cell focus in 3 and 15 mg/kg females and 50 mg/kg males (Tables A4 and B4). These foci are common background findings in older rats and the biological significance of these decreases is unknown. Basophilic foci were discrete areas of hepatocytes with a more basophilic cytoplasm than surrounding hepatocytes; the hepatocytes within the foci were frequently smaller than normal. Clear cell foci were focal areas of hepatocytes containing glycogen within their cytoplasm; depending on the amount of cytoplasmic glycogen, these cells could be larger than surrounding, uninvolved hepatocytes.

Thyroid Gland: At 3 months, there were significantly increased incidences of follicle hypertrophy in 50 mg/kg males and females (Tables 20, A4, and B4). This lesion was characterized by a preponderance of small follicles that contained little colloid and were lined by cuboidal to low columnar epithelial cells.

At 2 years, there were significantly increased incidences of follicular cell adenoma in 50 mg/kg males (Tables 20, A1, and A2). Follicular cell carcinoma occurred in two 3 mg/kg males and one 15 mg/kg male; although this neoplasm did not occur in the vehicle controls, these increased incidences were not statistically significant. In 50 mg/kg female rats, there was a significantly increased incidence of follicular cell hyperplasia (Tables 20 and B4). Follicular cell adenoma was a discrete, compressive mass composed of proliferations of follicular cells

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Male				
3-Month Interim Evaluation				
Number Examined Microscopically	10			10
Follicle, Hypertrophy[a]	0			4* (1.3)[b]
2-Year Study				
Number Examined Microscopically	45	45	48	46
Follicle, Hypertrophy	1 (2.0)	26** (1.3)	34** (1.1)	23** (1.4)
Follicular Cell Adenoma[c]				
Number of litters with at least one neoplasm/total number of litters	1/29	3/25	2/25	6/29
Overall rate[d]	1/45 (2%)	3/45 (7%)	2/48 (4%)	6/46 (13%)
Adjusted rate[e]	2.5%	7.6%	4.7%	16.1%
Terminal rate[f]	1/36 (3%)	2/35 (6%)	2/38 (5%)	4/25 (16%)
First incidence (days)	729 (T)	647	729 (T)	609
Poly-3 test[g]	P=0.028	P=0.297	P=0.518	P=0.042
Litter-adjusted Poly-3 test	—[h]	—	—	—
Follicular Cell Carcinoma[i]	0	2	1	0
Follicular Cell Adenoma or Carcinoma[c]				
Number of litters with at least one neoplasm/total number of litters	1/29	5/25	3/25	6/29
Overall rate	1/45 (2%)	5/45 (11%)	3/48 (6%)	6/46 (13%)
Adjusted rate	2.5%	12.6%	7.0%	16.1%
Terminal rate	1/36 (3%)	4/35 (11%)	3/38 (8%)	4/25 (16%)
First incidence (days)	729 (T)	647	729 (T)	609
Poly-3 test	P=0.089	P=0.095	P=0.324	P=0.042
Litter-adjusted Poly-3 test	—	—	—	—

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Female				
3-Month Interim Evaluation				
Number Examined Microscopically	10			10
Follicle, Hypertrophy	1 (1.0)			5* (1.2)
2-Year Study				
Number Examined Microscopically	45	49	47	42
Follicle, Hypertrophy	8 (1.1)	17 (1.3)	22** (1.4)	35** (1.9)
Follicular Cell Hyperplasia	1 (1.0)	5 (1.0)	4 (1.8)	6* (1.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study)
** $P \leq 0.01$
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 4/95 (4.1% ± 2.7%), range 2%-6%; all routes: 5/295 (1.7% ± 2.4%), range 0%-6%
[d] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h] Value of statistic cannot be computed.
[i] Historical incidence for corn oil gavage studies: 0/95; all routes: 0/295

forming complex papillary infoldings and irregular follicular structures. The cells were slightly pleomorphic and larger than normal follicular cells. Follicular cell carcinoma displayed more disorganized growth patterns and cellular pleomorphism and invaded through the thyroid gland capsule. Follicular cell hyperplasia, like follicular cell adenoma, was a focal lesion. However, unlike adenomas, follicular cell hyperplasia was associated with little to no compression. Follicular cell hyperplasia was composed of follicles that were enlarged and cystic and occasionally contained a few simple papillary infoldings.

There were significantly increased incidences of follicle hypertrophy in all dosed male groups and in 15 and 50 mg/kg females (Tables 20, A4, and B4). Hypertrophic thyroid follicles were small and lined by cuboidal to columnar epithelial cells with pale eosinophilic to light golden-brown cytoplasm (Plates 12 and 13). Hypertrophy of the thyroid follicle was recorded when at least 50% of the follicles in both lobes (combined) of the thyroid gland were affected. Involvement of less than 50% was not recorded because the thyroid glands from the vehicle control animals were frequently observed to have this change in up to 50% of the follicles.

Pituitary Gland: At 2 years, there was a significantly increased incidence of adenoma in the pars distalis of the pituitary gland in 50 mg/kg males (Tables 21, A1, and A2). Pars distalis adenomas were typically composed of sheets of chromophobes, although scattered acidophils and basophils could be found in some neoplasms. Variable-sized blood vessels, some angiectatic, as well as hemorrhage, were present in many of the neoplasms. The adenomas were usually well-demarcated masses that caused compression of the surrounding parenchyma, with larger neoplasms causing dorsal compression of the hypothalamic region of the brain.

TABLE 21
Incidences of Adenoma of the Pituitary Gland (Pars Distalis) in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Number Examined Microscopically	49	49	50	50
Adenoma, Multiple[a]	0	0	1	1
Adenoma (includes multiple)[b]				
Number of litters with at least one neoplasm/total number of litters	16/29	9/25	18/25	26/29
Overall rate[c]	19/49 (39%)	12/49 (24%)	22/50 (44%)	35/50 (70%)
Adjusted rate[d]	40.7%	28.1%	47.4%	71.7%
Terminal rate[e]	10/36 (28%)	7/35 (20%)	16/38 (42%)	13/25 (52%)
First incidence (days)	508	485	436	351
Poly-3 test[f]	P<0.001	P=0.152N	P=0.328	P<0.001
Litter-adjusted Poly-3 test	P<0.001	P=0.983	P=0.495	P=0.007

a Number of animals with neoplasm

b Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 40/99 (40.4% ± 2.3%), range 39%-42%; all routes: 101/298 (33.9% ± 5.7%), range 28%-42%

c Number of animals with neoplasm per number of animals with pituitary gland examined microscopically

d Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

e Observed incidence at terminal kill

f Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by an N.

Female Reproductive System: Statistical evaluation was done for the incidence of uterine and vaginal lesions for the original cross sectional evaluation, the additional residual longitudinal section evaluation, and for the combination of the original and longitudinal evaluations.

At 2 years, there were significantly increased incidences of uterine stromal polyp in 3 and 15 mg/kg females in the combined evaluations (Tables 22 and B2). The incidences of uterine stromal polyp or uterine stromal sarcoma (combined) were significantly increased in the 3 and 15 mg/kg groups in the combined evaluation. Two 50 mg/kg females had multiple vaginal polyp in the combined evaluations; vaginal polyps were not recorded in any other treatment group.

In the original evaluation of the uterus and cervical gross lesions, there was a significantly increased incidence of squamous metaplasia of the uterus in 50 mg/kg females and two animals in this group had squamous hyperplasia of the cervix (which is normally lined by squamous epithelium) (Tables 22 and B4). Additional occurrences of these lesions were recorded in the longitudinal evaluation of these tissues. When the incidences from the original and longitudinal evaluations were combined, the incidences of squamous metaplasia of the uterus were significantly increased in the 15 and 50 mg/kg groups, and the incidence of squamous hyperplasia of the cervix was significantly increased in the 50 mg/kg group.

Histologically, stromal polyps were solitary exophytic nodules that projected into the uterine lumen. They were covered by normal-appearing endometrial surface epithelium and supported by a broad stalk of endometrial stroma, blood vessels, and a few entrapped glands. Polyps in the vagina were similar to those found in the uterus. Stromal sarcomas were composed of spindle-shaped cells with indistinct cytoplasmic borders that invaded into the uterine wall. Squamous metaplasia was recorded in the uterus when the normal cuboidal to columnar epithelium lining the uterus or endometrial glands was replaced by stratified squamous epithelium. In the cervix, squamous hyperplasia was characterized by increased layers of the normally present squamous epithelium.

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus, Vagina, and Cervix in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Uterus[a]	50	50	50	49
Squamous Metaplasia				
Original Cross Sectional Evaluation[b]	0	0	1 (1.0)[c]	4* (1.3)
Residual Longitudinal Evaluation	0	2	5*	6*
Original and Residual Evaluations (combined)	0	2	5*	6*
Stromal Polyp				
Original Cross Sectional Evaluation[d]				
Number of litters with at least one neoplasm/total number of litters	3/30	6/25	6/25	5/28
Overall rate[e]	3/50 (6%)	6/50 (12%)	7/50 (14%)	5/49 (10%)
Adjusted rate[f]	6.9%	12.8%	15.9%	12.8%
Terminal rate[g]	2/37 (5%)	5/39 (13%)	6/33 (18%)	4/28 (14%)
First incidence (days)	592	585	655	553
Poly-3 test[h]	P=0.388	P=0.277	P=0.158	P=0.296
Litter-adjusted Poly-3 test	—[i]	—	—	—
Residual Longitudinal Evaluation[j]				
Overall rate	3/50 (6%)	10/50 (20%)	6/50 (12%)	7/49 (14%)
Adjusted rate	6.9%	21.5%	13.5%	17.8%
Terminal rate	2/37 (5%)	8/39 (21%)	4/33 (12%)	5/28 (18%)
First incidence (days)	592	694	614	553
Poly-3 test	P=0.351	P=0.045	P=0.249	P=0.117
Litter-adjusted Poly-3 test	—	—	—	—
Original and Residual Evaluations (combined)[k]				
Overall rate	4/50 (8%)	12/50 (24%)	11/50 (22%)	9/49 (18%)
Adjusted rate	9.2%	25.5%	24.8%	22.8%
Terminal rate	3/37 (8%)	9/39 (23%)	9/33 (27%)	7/28 (25%)
First incidence (days)	592	585	614	553
Poly-3 test	P=0.283	P=0.037	P=0.045	P=0.077
Litter-adjusted Poly-3 test	—	—	—	—
Stromal Sarcoma				
Original Cross Sectional Evaluation[l]	0	0	1	0
Residual Longitudinal Evaluation[m]	0	0	1	0
Original and Residual Evaluations (combined)[n]	0	0	1	0
Stromal Polyp or Stromal Sarcoma				
Original and Residual Evaluations (combined)[o]				
Overall rate	4/50 (8%)	12/50 (24%)	12/50 (24%)	9/49 (18%)
Adjusted rate	9.2%	25.5%	27.1%	22.8%
Terminal rate	3/37 (8%)	9/39 (23%)	10/33 (30%)	7/28 (25%)
First incidence (days)	592	585	614	553
Poly-3 test	P=0.284	P=0.037	P=0.026	P=0.077

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus, Vagina, and Cervix in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Vagina	50	50	50	49
Polyp, Multiple				
Original Cross Sectional Evaluation	0	0	0	1
Residual Longitudinal Evaluation	0	0	0	1
Original and Residual Evaluations (combined)	0	0	0	2
Polyp (includes multiple)				
Original and Residual Evaluations (combined)[p]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/49 (4%)
Adjusted rate	0%	0%	0%	5.2%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	2/28 (7%)
First incidence (days)	—[q]	—	—	729 (T)
Poly-3 test	P=0.033	—[i]	—	P=0.212
Cervix	50	50	50	49
Squamous Hyperplasia				
Original Cross Sectional Evaluation	0	0	0	2 (2.5)
Residual Longitudinal Evaluation	2	3	4	8*
Original and Residual Evaluations (combined)	2	3	4	8*

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
(T) Terminal kill
[a] Number necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 5/100 (5.0% ± 1.4%), range 4%-6%; all routes: 13/194 (6.7% ± 2.5%), range 4%-10%
[e] Number of animals with neoplasm per number of animals necropsied
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[i] Value of statistic cannot be computed.
[j] Historical incidence for all routes: 20/194 (10.3% ± 2.9%), range 6%-12%
[k] Historical incidence for all routes: 27/194 (14.0% ± 5.2%), range 8%-20%
[l] Historical incidence for corn oil gavage studies: 0/100; all routes: 3/194 (1.6% ± 1.9%), range 0%-4%
[m] Historical incidence for all routes: 2/194 (1.1% ± 1.2%), range 0%-2%
[n] Historical incidence for all routes: 3/194 (1.6% ± 1.9%), range 0%-4%
[o] Historical incidence for all routes: 29/194 (15.1% ± 6.3%), range 8%-22%
[p] Historical incidence for all routes: 1/194 (0.6% ± 1.1%), range 0%-2%
[q] Not applicable; no neoplasms in animal group

Kidney: At 3 months, there was a slightly increased incidence of hydronephrosis in 50 mg/kg male rats (Tables 23 and A4). At 2 years, there were significantly increased incidences of hydronephrosis in 15 mg/kg males and 50 mg/kg males and females and in the incidence of pigmentation in 50 mg/kg females (Tables 23, A4, and B4). In the renal pelvis, there were significantly decreased incidences of chronic active inflammation in 15 and 50 mg/kg males and females and mineralization in all dosed male groups. The incidence of pelvic mineralization was slightly decreased in 50 mg/kg females. Hydronephrosis was typically grossly observed as unilateral enlargement of the kidney, most often the right kidney. Microscopically, a dilated pelvis, with remaining cortical tissue compressed into a thin band, characterized hydronephrosis. Pigmentation was golden brown and found scattered within the epithelium of the renal tubules. It was similar in nature to the pigment observed in the liver and spleen, and stained positive with Perl's Prussian Blue, consistent with hemosiderin. Chronic active inflammation of the pelvis consisted of a mixed cell population within the urothelium and underlying lamina propria of the renal pelvis. Pelvic mineralization was characterized by dark basophilic material (consistent with mineral deposition) within the urothelium of the renal pelvis; mineralized debris was occasionally also found within the urinary space. The biological significance of the pelvic pigmentation or of the decreased pelvic inflammation and mineralization is unknown.

TABLE 23
Incidences of Nonneoplastic Lesions of the Kidney in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Male				
3-Month Interim Evaluation				
Number Examined Microscopically	10			10
Hydronephrosis[a]	1 (1.0)[b]			3 (2.7)
2-Year Study				
Number Examined Microscopically	49	46	50	50
Hydronephrosis	1 (3.0)	5 (1.4)	8* (2.9)	10** (2.7)
Pelvis, Inflammation, Chronic Active	22 (1.4)	14 (1.2)	8** (1.5)	2** (1.0)
Pelvis, Mineralization	18 (1.3)	5** (1.0)	5** (1.0)	3** (1.0)
Female				
2-Year Study				
Number Examined Microscopically	50	50	49	47
Hydronephrosis	1 (3.0)	1 (2.0)	1 (4.0)	6* (2.5)
Pelvis, Inflammation, Chronic Active	16 (1.2)	10 (1.1)	6* (1.0)	3** (1.0)
Pelvis, Mineralization	31 (1.2)	29 (1.1)	23 (1.0)	19 (1.1)
Pigmentation	0	1 (1.0)	3 (1.0)	4* (1.0)

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

Parotid Salivary Gland: At 2 years, there were significantly increased incidences of atrophy and cytoplasmic vacuolization in 50 mg/kg male rats (Tables 24 and A4). Atrophy was characterized by a decrease in the number and size of the acini, along with a prominence of the stroma, often including an increase in adipocytes and infiltrates of inflammatory cells. Cytoplasmic vacuolization consisted of multiple, discrete, clear vacuoles within the cytoplasm of the acinar cells (Plate 14).

Male Reproductive System: At 2 years, there were significantly increased incidences of chronic active inflammation of the prostate gland in 15 and 50 mg/kg males and ectasia of the preputial gland duct in 50 mg/kg males (Tables 24 and A4). In the epididymis, there was a positive trend in the incidences of chronic active inflammation, but the incidences in the individual dosed groups were not significantly different from that of the vehicle control group. Chronic active inflammation of the prostate gland and epididymis were similar in character, and consisted of focal to focally extensive accumulations of mononuclear cells with scattered neutrophils. Inflammatory cells could be found in the stroma as well as within acinar or tubular lumens of the prostate gland or epididymis, respectively. Ductal ectasia was characterized by a markedly enlarged duct lined by attenuated squamous epithelium and filled with keratin and cell debris.

Thymus: There was a significantly increased incidence of atrophy in 50 mg/kg males (Tables 24 and A4) at 2 years. Atrophy was characterized by an overall decrease in the size of the thymus, with a thin, indistinct cortex, a loss of lymphocytes, and an increase in adipocytes.

Spleen: At 2 years, there were significantly increased incidences of pigmentation and lymphoid follicle atrophy in 50 mg/kg males (Tables 24 and A4). There was a significantly decreased incidence of hematopoietic cell proliferation in 50 mg/kg males and a slightly decreased incidence of this lesion in females (Tables 24, A4, and B4). Pigmentation in the spleen was qualitatively similar to that seen in the liver and kidney and was characterized by globules of golden-brown pigment found scattered throughout the red pulp that stained positively with Perl's Prussian Blue, consistent with hemosiderin. Lymphoid follicle atrophy was evidenced by fewer, or smaller, lymphoid follicles within the spleen. Hematopoietic cell proliferation consisted of increased numbers of hematopoietic cells, including megakaryocytes, within the red pulp. The biological significance of the splenic changes is unknown. While the mechanism is not known, it is possible the changes were due to stress or erythrocyte breakdown.

Forestomach: At 2 years, there was a significantly increased incidence of epithelium hyperplasia in 50 mg/kg males and a positive trend in the incidences of hyperkeratosis in males (Tables 24 and A4). These lesions often occurred together and sometimes occurred in association with ulceration or inflammation of the forestomach. Hyperkeratosis was characterized by a thickened layer of keratin overlying the epithelium, while epithelium hyperplasia was diagnosed when there was an increase in the number of layers of squamous epithelium.

Adrenal Cortex: At 2 years, there was a significantly increased incidence of focal hyperplasia in 50 mg/kg females (Tables 24 and B4). Focal hyperplasia consisted of focal areas of increased numbers of cells, usually within the zona fasciculata. The cells within these lesions may have been smaller and more basophilic or larger with slightly vacuolated eosinophilic cytoplasm when compared to normal cortical cells, but there was no evidence of atypia.

Mammary Gland: In 50 mg/kg male rats, there was a significantly increased incidence of hemosiderin pigmentation at 2 years (Tables 24 and A4). This change was typically minimal and was characterized by clumps and granules of brown to golden-brown material (consistent with hemosiderin) in macrophages or in epithelial cells lining the ducts. The biological significance of this lesion is unknown. The pigment may represent erythrocyte breakdown with subsequent phagocytosis by macrophages in the mammary gland or surrounding connective tissue. It was not considered a primary effect of exposure to DE-71.

TABLE 24
Incidences of Selected Nonneoplastic Lesions in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Male				
Salivary Gland, Parotid Gland[a]	46	48	50	50
Atrophy[b]	2 (2.5)[c]	2 (1.0)	4 (1.8)	13** (1.4)
Cytoplasmic Vacuolization	4 (1.5)	4 (2.0)	7 (1.9)	17** (1.7)
Prostate Gland	49	50	50	50
Inflammation, Chronic Active	17 (1.2)	20 (1.3)	28* (1.4)	27* (1.3)
Epididymis	49	50	50	50
Inflammation, Chronic	0	0	0	3 (1.0)
Preputial Gland	49	49	50	50
Duct, Ectasia	2 (2.0)	2 (1.5)	5 (2.2)	15** (2.2)
Thymus	45	49	49	50
Atrophy	14 (2.2)	11 (2.5)	15 (1.9)	26* (2.5)
Spleen	47	46	50	49
Pigmentation	12 (1.3)	11 (1.1)	17 (1.1)	27** (1.4)
Lymphoid Follicle, Atrophy	0	0	1 (2.0)	5* (1.8)
Hematopoietic Cell Proliferation	23 (1.3)	30 (1.2)	22 (1.2)	13* (1.5)
Stomach, Forestomach	49	50	50	50
Epithelium Hyperplasia	8 (2.6)	6 (2.0)	5 (2.8)	17* (2.1)
Hyperkeratosis	9 (2.0)	5 (1.8)	5 (2.2)	17 (1.8)
Mammary Gland	33	38	39	41
Pigmentation, Hemosiderin	3 (1.0)	9 (1.0)	2 (1.5)	13** (1.0)
Female				
Spleen	50	49	50	45
Hematopoietic Cell Proliferation	27 (1.7)	24 (1.5)	19 (1.8)	17 (1.8)
Adrenal Cortex	50	49	50	46
Focal Hyperplasia	8 (1.1)	6 (1.0)	12 (1.3)	19** (1.2)

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

MICE

3-MONTH STUDY

Survival of the 500 mg/kg groups was decreased, with seven males removed from the study from weeks 4 to 12 and one female mouse removed during week 5 and one female mouse removed during week 12 (Table 25). Six female mice were removed from the study during week 1 due to gavage accidents; one mouse each in the vehicle control and 50 and 100 mg/kg groups and three mice in the 500 mg/kg group. Abnormal breathing, lethargy, and tremors, attributed to their moribund condition, were observed in two 500 mg/kg female mice. No clinical findings directly attributed to administration of DE-71 were observed.

Final mean body weights were significantly lower in the 100 and 500 mg/kg males and mean body weight gains were significantly lower in males administered 50 mg/kg or greater relative to the vehicle controls (Table 25 and Figure 10). In 500 mg/kg males, the final mean body weight was approximately 27% less than that of the vehicle controls, and the mean body weight gain was approximately 65% less than that of the vehicle controls. In female mice, final mean body weights were significantly lower in the 5 and 500 mg/kg groups, and mean body weight gains were significantly lower in the 0.01, 5, and 500 mg/kg groups. In 500 mg/kg females, the final mean body weight was approximately 17% less than that of the vehicle controls.

TABLE 25
Survival and Body Weights of Mice in the 3-Month Gavage Study of DE-71[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	22.3 ± 0.4	39.3 ± 0.8	17.0 ± 0.6	
0.01	10/10	22.3 ± 0.5	38.8 ± 0.7	16.5 ± 0.6	99
5	10/10	22.3 ± 0.3	39.3 ± 1.0	17.0 ± 0.8	100
50	10/10	22.4 ± 0.3	37.3 ± 1.1	14.9 ± 0.9*	95
100	10/10	22.3 ± 0.2	35.9 ± 0.7**	13.7 ± 0.6**	91
500	3/10[c]	22.5 ± 0.2	28.6 ± 0.9**	5.9 ± 0.9**	73
Female					
0	9/10[d]	18.8 ± 0.2	32.8 ± 0.5	13.9 ± 0.5	
0.01	10/10	18.8 ± 0.3	29.9 ± 0.6	11.1 ± 0.6*	91
5	10/10	18.8 ± 0.3	29.5 ± 1.1*	10.7 ± 0.8*	90
50	9/10[d]	18.7 ± 0.4	30.3 ± 1.0	11.7 ± 1.0	92
100	9/10[d]	18.7 ± 0.4	31.0 ± 1.0	12.3 ± 0.7	94
500	5/10[e]	18.7 ± 0.3	27.3 ± 0.3**	8.3 ± 0.6**	83

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤0.01
[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Weeks of deaths: 4, 4, 5, 5, 9, 10, 12
[d] Week of death: 1
[e] Weeks of deaths: 1, 1, 1, 5, 12

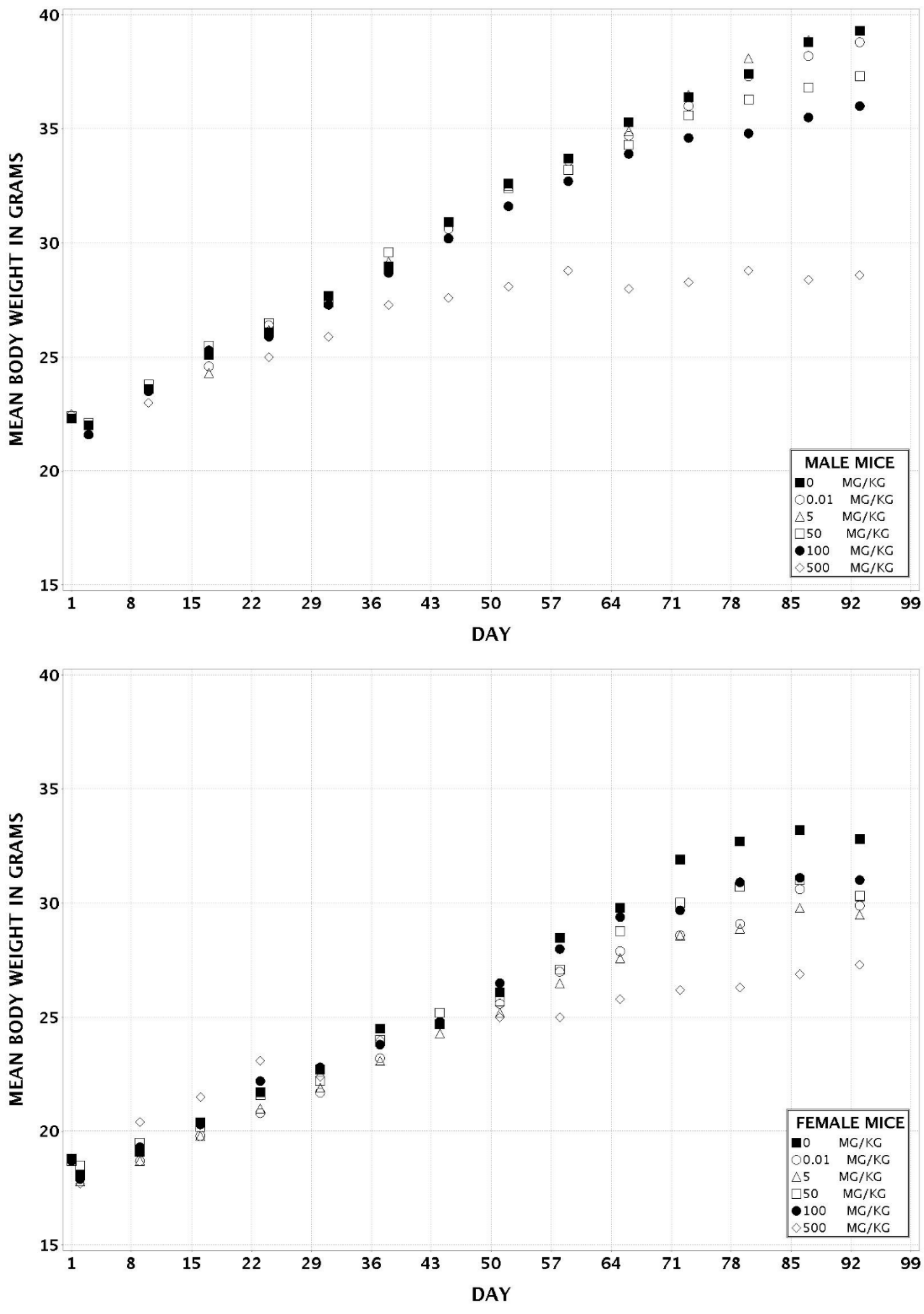


FIGURE 10
Growth Curves for Mice Administered DE-71 by Gavage for 3 Months

Similar to those of the 3-month rat study, the hematology findings suggested a small (approximately 10%) decrease in the erythron in the 500 mg/kg males and females (Table F2). In this study, the erythron decrease was evidenced by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts; there were no decreases in mean cell volume or mean cell hemoglobin. In general, reticulocyte counts were lower in males and females administered 50 mg/kg or greater, and the values for females demonstrated a dose-response relationship.

The absolute and relative liver weights of 50 mg/kg males and 100 and 500 mg/kg males and females were significantly greater than those of the vehicle controls; in 500 mg/kg males and females, the absolute liver weights were increased by approximately threefold (Tables 26 and G3). The absolute kidney weight of 500 mg/kg males was significantly less (26%) than that of the vehicle controls. The relative kidney weights of all dosed female groups were significantly greater than that of the vehicle controls. The absolute heart weights of 500 mg/kg males and females were significantly less (15% and 17%, respectively) than those of the vehicle controls. The absolute testis weight of 500 mg/kg males was significantly less than that of the vehicle controls. In males, the relative heart weights of the 100 and 500 mg/kg groups

TABLE 26
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n	10	10	10	10	10	3
Necropsy body wt	39.3 ± 0.8	38.8 ± 0.7	39.3 ± 1.0	37.3 ± 1.1	35.9 ± 0.7**	28.6 ± 0.9**
Heart						
Absolute	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.11 ± 0.00**
Relative	3.411 ± 0.090	3.562 ± 0.078	3.529 ± 0.093	3.582 ± 0.081	3.648 ± 0.055*	3.966 ± 0.091**
R. Kidney						
Absolute	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.20 ± 0.01**
Relative	6.784 ± 0.133	7.145 ± 0.175	7.067 ± 0.164	7.129 ± 0.148	7.245 ± 0.188	6.995 ± 0.056
Liver						
Absolute	1.38 ± 0.02	1.31 ± 0.05	1.50 ± 0.03	1.79 ± 0.08**	2.18 ± 0.07**	4.11 ± 0.02**
Relative	35.024 ± 0.417	33.701 ± 1.195	38.207 ± 0.870	48.005 ± 1.761**	60.684 ± 1.827**	144.118 ± 4.508**
R.Testis						
Absolute	0.115 ± 0.002	0.114 ± 0.002	0.116 ± 0.002	0.116 ± 0.002	0.112 ± 0.003	0.102 ± 0.007*
Female						
n	9	10	10	9	9	5
Necropsy body wt	32.8 ± 0.5	29.9 ± 0.6	29.5 ± 1.1*	30.3 ± 1.0	31.0 ± 1.0	27.3 ± 0.3**
Heart						
Absolute	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.10 ± 0.00**
Relative	3.596 ± 0.084	3.932 ± 0.103	3.849 ± 0.155	3.798 ± 0.083	3.813 ± 0.121	3.803 ± 0.072
R. Kidney						
Absolute	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Relative	4.954 ± 0.151	5.740 ± 0.162**	5.323 ± 0.157**	5.578 ± 0.111**	5.436 ± 0.115**	6.289 ± 0.190**
Liver						
Absolute	1.29 ± 0.20	1.10 ± 0.02	1.10 ± 0.03	1.51 ± 0.04	1.83 ± 0.05**	3.74 ± 0.10**
Relative	39.495 ± 6.272	36.887 ± 0.526	37.404 ± 0.887	50.224 ± 1.481*	59.150 ± 1.078**	137.002 ± 3.891**

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

and the relative thymus weight of the 500 mg/kg group were significantly greater than those of the vehicle controls and were consistent with decreased body weights.

UDPGT activities were significantly increased in all dosed groups of females, with a maximal induction of approximately 1.7-fold over the vehicle controls in the 500 mg/kg group (Table 27). Hepatic EROD activities were significantly increased in females administered 5 mg/kg or greater, with a maximal induction of approximately 5.3-fold in the 500 mg/kg group. Hepatic A4H activities were significantly increased in males administered 50 mg/kg or greater, and in females administered 5 mg/kg or greater. Maximal A4H induction occurred in the 500 mg/kg groups and was approximately twofold and threefold in males and females, respectively. Hepatic PROD activities were significantly increased in male and female mice administered 5 mg/kg or greater. Maximal PROD induction occurred in the 50 mg/kg males and females, with approximately 15-fold and sixfold increases, respectively.

TABLE 27
Liver Enzyme Activities for Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n	10	10	10	10	10	3
Uridine diphosphate glucuronosyl transferase (UDPGT) (nmol/minute per mg microsomal protein)						
	13.7 ± 0.2	11.8 ± 0.3	14.3 ± 0.3	14.6 ± 0.4	14.6 ± 0.4	14.1 ± 0.6
7-Ethoxyresorufin-*O*-deethylase (EROD) (nmol/minute per mg microsomal protein)						
	0.012 ± 0.001	0.013 ± 0.000	0.005 ± 0.001*	0.008 ± 0.002	0.016 ± 0.001	0.125 ± 0.006
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
	0.400 ± 0.028	0.439 ± 0.013	0.390 ± 0.035[b]	0.580 ± 0.030**	0.688 ± 0.035**	0.799 ± 0.061**
7-Pentoxyresorufin-*O*-dealkylase (PROD) (nmol/minute per mg microsomal protein)						
	0.002 ± 0.000	0.002 ± 0.000	0.014 ± 0.001**	0.029 ± 0.002**	0.011 ± 0.001**	0.006 ± 0.002**
Female						
n	9	10	10	9	9	5
Uridine diphosphate glucuronosyl transferase (UDPGT) (nmol/minute per mg microsomal protein)						
	8.2 ± 0.2	10.1 ± 0.3**	9.4 ± 0.5**	13.4 ± 0.9**	13.6 ± 0.9**	13.8 ± 0.9**
7-Ethoxyresorufin-*O*-deethylase (EROD) (nmol/minute per mg microsomal protein)						
	0.009 ± 0.001	0.008 ± 0.001	0.014 ± 0.001*	0.022 ± 0.003**	0.017 ± 0.003**	0.048 ± 0.004**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
	0.366 ± 0.030	0.355 ± 0.038	0.583 ± 0.050**	0.814 ± 0.066**	0.822 ± 0.081**	1.120 ± 0.155**
7-Pentoxyresorufin-*O*-dealkylase (PROD) (nmol/minute per mg microsomal protein)						
	0.005 ± 0.000	0.003 ± 0.000	0.014 ± 0.002**	0.028 ± 0.006**	0.013 ± 0.002**	0.009 ± 0.001*

* Significantly different (P≤0.05) from the vehicle control group by Shirley's or Dunn's test
** P≤0.01
[a] Enzyme activities are given as mean ± standard error.
[b] n=9

Concentrations of BDE-47, BDE-99, and BDE-153 were determined in adipose collected from mice at the end of the study (Table I5). In males, the concentrations of all three congeners increased linearly with dose up to 100 mg/kg, above which the concentrations increased more than proportional to the dose indicating saturation of metabolism at or above 500 mg/kg. The concentrations were higher than that in the vehicle control group except at 0.01 mg/kg. In females, the concentrations of all congeners increased proportionally to the dose and were higher than the vehicle control concentration except at 0.01 mg/kg. In general, the concentrations of BDE-99 were higher than those of the other two congeners; the concentrations of BDE-47 and BDE-153 were similar (except in 500 mg/kg males) suggesting a higher rate of accumulation of BDE-153 regardless of the lower percentage of BDE-153 in DE-71.

Due to early deaths in the 500 mg/kg groups, reproductive system evaluations including sperm and spermatid counts and vaginal cytology were performed on vehicle controls, 5, 50, and 100 mg/kg groups (Tables 28, H4, H5, and H6; Figure H2). Left cauda epididymis weight and sperm motility were significantly decreased in 100 mg/kg males. There were no histological correlates recorded in the testis or epididymis of 100 mg/kg males, but there were significantly increased incidences of abnormal residual bodies in the 500 mg/kg male group. Based on these findings, DE-71 exhibits the potential to be a reproductive toxicant in male mice. In female mice, there were no significant differences between the vehicle control and dosed groups in cycle length, number of cycling females, number of females with regular cycles, or relative amount of time spent in the estrous stages (Tables H5 an H6; Figure H2).

TABLE 28
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	5 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.3 ± 0.8	39.3 ± 1.0	37.3 ± 1.1	35.9 ± 0.7*
L. Cauda epididymis	0.0274 ± 0.0011	0.0246 ± 0.0015	0.0237 ± 0.0015	0.0214 ± 0.0010**
L. Epididymis	0.0560 ± 0.0019	0.0541 ± 0.0033	0.0554 ± 0.0028	0.0514 ± 0.0017
L. Testis	0.1143 ± 0.0024	0.1149 ± 0.0018	0.1188 ± 0.0028	0.1112 ± 0.0021
Spermatid measurements				
Spermatid heads (10^6/testis)	22.83 ± 0.77	23.39 ± 0.75	22.67 ± 0.58	23.10 ± 0.55
Spermatid heads (10^6/g testis)	221.67 ± 6.18	238.55 ± 9.18	218.16 ± 7.04	238.72 ± 4.68
Epididymal spermatozoal measurements				
Sperm motility (%)	88.5 ± 1.2	89.5 ± 0.2	88.7 ± 0.3	85.3 ± 0.8**
Sperm (10^6/cauda epididymis)	16.7 ± 0.8	15.8 ± 1.6	9.4 ± 2.4	12.1 ± 2.1
Sperm (10^6/g cauda epididymis)	614.1 ± 34.7	676.7 ± 86.4	425.9 ± 120.0	555.3 ± 92.1

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** Significantly different (P≤0.01) from the vehicle control group by Williams' (cauda epididymis weights) or Shirley's (sperm motility) test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (epididymis and testis weights) or Dunn's test (spermatid measurements, sperm/cauda epididymis, and sperm/g cauda epididymis)

.

Relevant gross lesions included liver enlargement, discoloration (mottling) of the glandular stomach wall, and thin carcass in male and female mice, and forestomach wall focus and liver focus in male mice.

In the liver, there were significantly increased incidences of hepatocyte hypertrophy in males administered 50 mg/kg or greater and in 100 and 500 mg/kg females (Table 29). There were also significantly increased incidences of hepatocyte necrosis in 500 mg/kg males and females and hepatocyte cytoplasmic vacuolization in 500 mg/kg males. There was a positive trend in the incidences of focal necrosis in males, but the incidences were not significantly increased in any dosed group. Hepatocyte hypertrophy was characterized by enlargement of hepatocytes up to two or three times normal size (Plate 15). The hypertrophic hepatocytes had increased amounts of cytoplasm and sometimes contained enlarged nuclei. In both male and female mice, the centrilobular hepatocytes were affected first and as severity increased, midzonal and periportal hepatocytes became affected.

Hepatocyte necrosis was characterized by a single hepatocyte, or clusters of two or three hepatocytes, having a shrunken, condensed appearance. This was in contrast to focal necrosis of the liver, which consisted of randomly located foci of coagulative necrosis. Hepatocyte cytoplasmic vacuolization consisted of very small discrete vacuoles that filled the cytoplasm, usually in enlarged hepatocytes.

In the adrenal cortex, there were significantly increased incidences of fatty degeneration and hypertrophy of the zona fasciculata in 500 mg/kg males (Table 29). In females, there was a positive trend in the incidences of fatty degeneration but the incidence in the 500 mg/kg group was not significantly increased. Fatty degeneration consisted of discrete, colorless vacuoles within cortical cells, consistent with fat accumulation. Hypertrophy was characterized by eosinophilic cells with increased amounts of cytoplasm within the zona fasciculata (Plate 16).

In the thymus, there was a significantly increased incidence of atrophy in 500 mg/kg males and a positive trend in the incidences of the lesion in females (Table 29). Atrophy was characterized by a reduction of the cortical region by thymocyte depletion.

In the testis of 500 mg/kg male mice, there was a significantly increased incidence of abnormal residual bodies (Table 29). This lesion was characterized by the presence of large, round to oval, amphophilic to eosinophilic bodies in the seminiferous tubules (Plate 17). These abnormal residual bodies were primarily seen at the luminal surface or in the lumen of the tubules.

Dose Selection Rationale: Due to reduced survival and increased incidences of hepatocyte necrosis of the liver in the 500 mg/kg groups, the high dose selected for the 2-year gavage study in mice was 100 mg/kg. The low dose (3 mg/kg) and mid dose (30 mg/kg) were selected to give a broad range of exposure.

TABLE 29
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Hepatocyte, Hypertrophy[b]	0	0	1 (1.0)[c]	10** (1.8)	10** (2.7)	10** (3.1)
Hepatocyte, Necrosis	0	0	0	0	1 (1.0)	10** (1.3)
Hepatocyte, Cytoplasmic Vacuolization	0	0	0	0	0	6** (1.2)
Necrosis, Focal	0	0	0	0	0	2 (2.0)
Adrenal Cortex	10	10	9	10	10	10
Degeneration, Fatty	0	0	0	0	0	4* (1.3)
Zona Fasciculata, Hypertrophy	0	0	0	0	0	5* (1.0)
Thymus	10	10	9	10	10	9
Atrophy	0	0	0	0	0	6** (2.5)
Testis	10	10	10	10	10	10
Abnormal Residual Bodies	0	0	1 (2.0)	0	1 (2.0)	5* (2.0)
Female						
Liver	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	0	0	0	9** (1.2)	6** (2.5)
Hepatocyte, Necrosis	0	0	0	0	0	6** (1.2)
Adrenal Cortex	10	10	10	10	10	10
Degeneration, Fatty	0	0	0	0	0	2 (2.0)
Thymus	9	10	10	10	9	8
Atrophy	0	0	0	1 (2.0)	0	3 (3.3)

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

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 30 and in the Kaplan-Meier survival curves (Figure 11). Survival of 100 mg/kg males and females was significantly less than that of the vehicle controls leading to these groups being removed from the study at 18 months. The cause of most early deaths in 100 mg/kg males and females was liver tumors. Survival of all other dosed groups was similar to that of the vehicle controls.

Body Weights and Clinical Findings

Mean body weights of 100 mg/kg males and females were at least 10% less than those of the vehicle control groups after weeks 17 and 21, respectively (Figure 12 and Tables 31 and 32). The mean body weights of 30 mg/kg males were at least 10% less than those of the vehicle controls after week 87. The mean body weight of the 30 mg/kg males was 84% that of the control group at terminal sacrifice. Clinical findings included increased occurrences of distended abdomen and thinness in 30 mg/kg males, and increased masses on appendages in all groups of males dosed with DE-71. Clinical findings of distended abdomen correlated with liver neoplasms. Clinical findings of masses on appendages were all related to lesions on the tail. Many of these did not have correlating lesions at necropsy or histologic examination, but several correlated to malformations of coccygeal vertebrae or associated skin lesions and were not considered related to DE-71 administration.

Table 30
Survival of Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	1	0	0	2
Moribund	15	7	14	36
Natural deaths	5	10	5	12
Animals surviving to study termination	29	33	31	0
Percent probability of survival at end of study[b]	59	66	62	0
Mean survival (days)[c]	657	689	691	505
Survival analysis[d]	P<0.001	P=0.520N	P=0.673N	P<0.001
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	1	0	1	0
Moribund	10	10	9	46
Natural deaths	6	5	3	4
Animals surviving to study termination	33	35	37	0
Percent probability of survival at end of study	67	70	76	0
Mean survival (days)	678	695	695	532
Survival analysis	P<0.001	P=0.932N	P=0.443N	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.

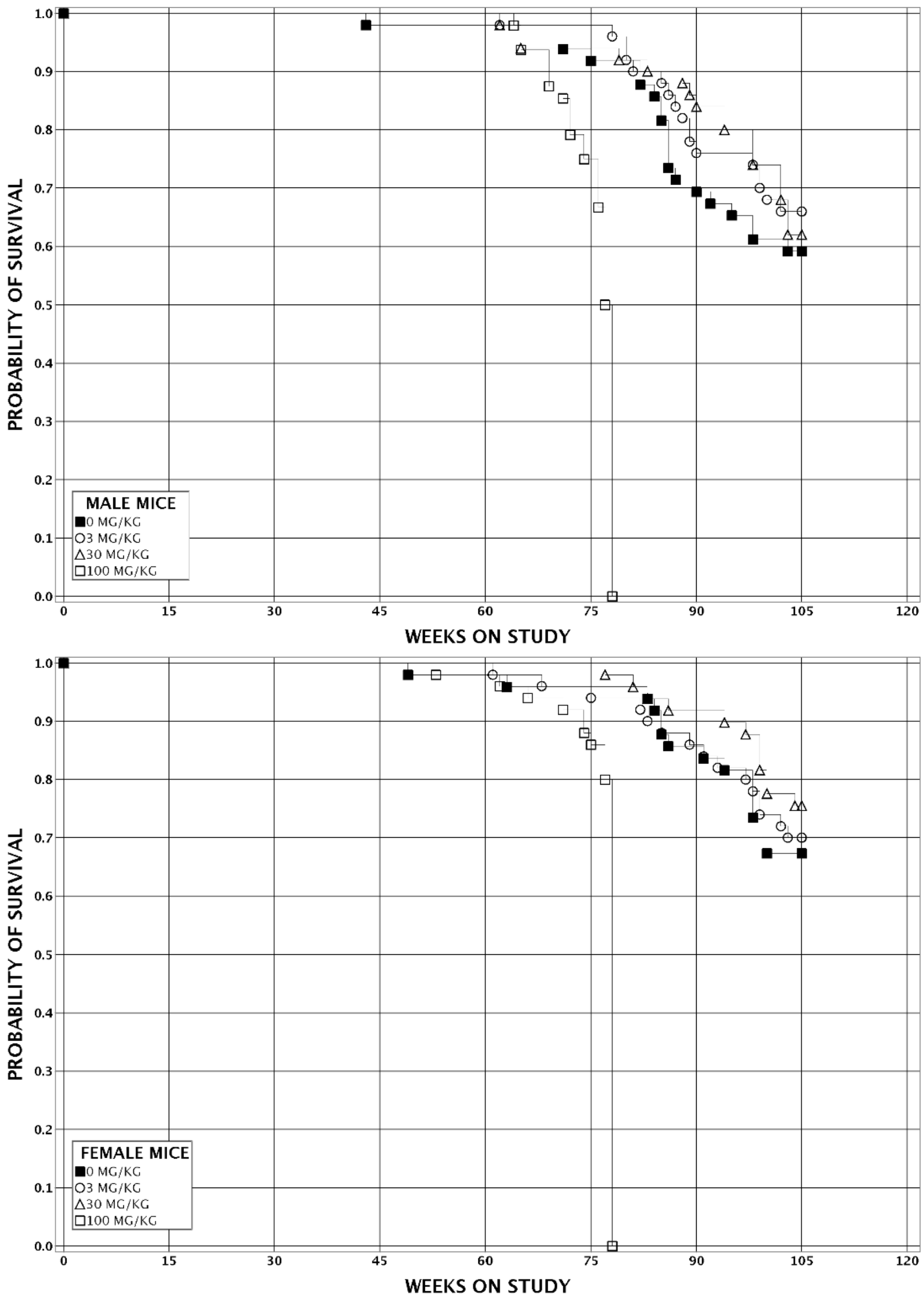


FIGURE 11
Kaplan-Meier Survival Curves for Mice Administered DE-71 by Gavage for 2 Years

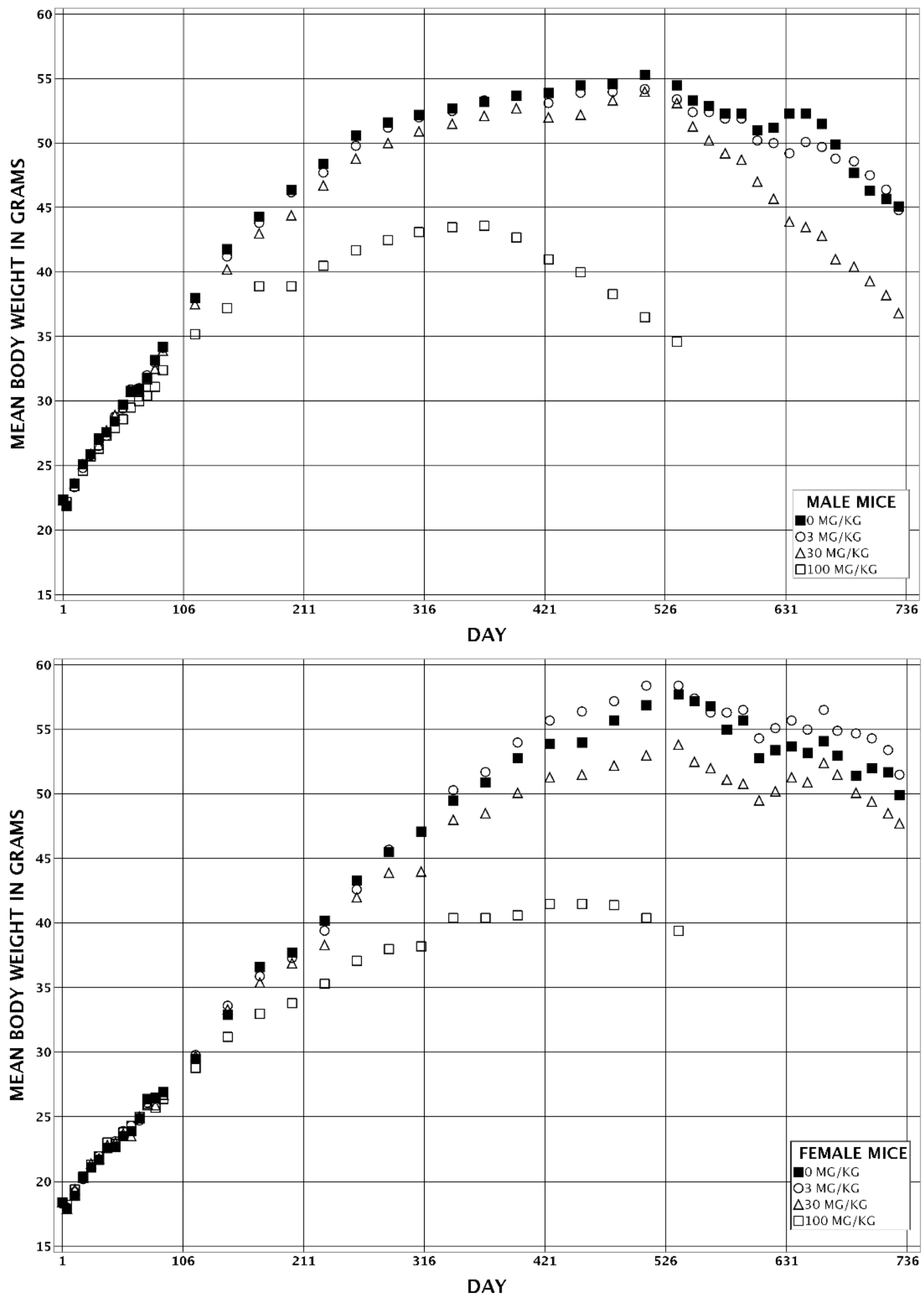


FIGURE 12
Growth Curves for Mice Administered DE-71 by Gavage for 2 Years

TABLE 31
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control		3 mg/kg			30 mg/kg			100 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	22.4	50	22.3	99	50	22.3	100	50	22.4	100	50
4	21.9	49	21.9	100	50	22.0	100	50	22.2	101	48
11	23.6	49	23.3	99	50	23.6	100	50	23.4	99	48
18	25.1	49	24.8	99	50	25.1	100	50	24.6	98	48
25	25.9	49	25.8	100	50	25.9	100	50	25.7	99	48
32	27.1	49	26.5	98	50	26.6	98	50	26.3	97	48
39	27.6	49	27.3	99	50	27.7	101	50	27.3	99	48
46	28.4	49	28.8	101	50	28.9	102	50	27.9	98	48
53	29.7	49	29.4	99	50	29.5	99	50	28.6	96	48
60	30.8	49	30.9	100	50	30.7	100	50	29.5	96	48
67	30.9	49	31.0	100	50	30.8	100	50	30.0	97	48
74	31.8	49	32.0	101	50	31.7	100	50	30.4	96	48
81	33.2	49	33.0	100	50	32.5	98	50	31.1	94	48
88	34.2	49	34.1	100	50	33.9	99	50	32.4	95	48
116	38.0	49	38.0	100	50	37.5	99	50	35.2	93	48
144	41.8	49	41.2	99	50	40.2	96	50	37.2	89	48
172	44.3	49	43.8	99	50	43.0	97	50	38.9	88	48
200	46.4	49	46.2	100	50	44.4	96	50	38.9	84	48
228	48.4	49	47.7	99	50	46.7	96	50	40.5	84	48
256	50.6	49	49.8	99	50	48.8	96	50	41.7	82	48
284	51.6	49	51.2	99	50	50.0	97	50	42.5	82	48
311	52.2	48	52.0	100	50	50.9	98	50	43.1	83	48
340	52.7	48	52.5	100	50	51.5	98	50	43.5	83	48
368	53.2	48	53.3	100	50	52.1	98	50	43.6	82	48
396	53.7	48	53.7	100	50	52.7	98	50	42.7	80	48
424	53.9	48	53.1	99	50	52.0	97	50	41.0	76	48
452	54.5	48	53.9	99	49	52.2	96	49	40.0	73	45
480	54.6	48	54.0	99	49	53.3	98	47	38.3	70	42
508	55.3	46	54.2	98	49	54.0	98	47	36.5	66	38
536	54.5	45	53.4	98	49	53.1	98	47	34.6	64	27
550	53.3	45	52.4	98	48	51.3	96	47			0
564	52.9	45	52.4	99	45	50.2	95	46			0
578	52.3	43	51.9	99	45	49.2	94	46			0
592	52.3	40	51.9	99	44	48.7	93	45			0
606	51.0	35	50.2	99	42	47.0	92	45			0
620	51.2	35	50.0	98	39	45.7	89	43			0
634	52.3	34	49.2	94	38	43.9	84	42			0
648	52.3	33	50.1	96	38	43.5	83	42			0
662	51.5	32	49.7	97	38	42.8	83	40			0
674	49.9	32	48.8	98	38	41.0	82	40			0
690	47.7	30	48.6	102	36	40.4	85	37			0
704	46.3	30	47.5	102	34	39.3	85	37			0
718	45.7	30	46.4	102	33	38.2	84	31			0
Mean for Weeks											
1-13	28.0		27.9	100		27.9	100		27.3	97	
14-52	47.3		46.9	99		45.9	97		40.2	85	
53-102	51.9		51.2	99		47.5	92		39.5	76	

TABLE 32
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control		3 mg/kg			30 mg/kg			100 mg/kg		
Day	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	18.4	50	18.3	100	50	18.4	100	50	18.4	101	50
5	17.9	49	18.0	100	50	17.9	100	49	18.2	102	50
12	18.9	49	19.2	102	50	19.4	102	49	19.4	102	50
19	20.3	49	20.2	100	50	20.3	100	49	20.4	100	50
26	21.1	49	21.1	100	50	21.4	101	49	21.3	101	50
33	21.7	49	22.0	101	50	21.8	100	49	22.0	101	50
40	22.6	49	22.6	100	50	22.8	101	49	23.0	102	50
47	22.7	49	23.1	102	50	23.0	101	49	22.9	101	50
54	23.5	49	23.9	102	50	23.7	101	49	23.8	101	50
61	23.9	49	24.3	102	50	23.5	99	49	24.3	102	50
68	24.9	49	24.7	99	50	25.0	101	49	25.0	100	50
75	26.4	49	26.1	99	50	26.0	99	49	25.9	98	50
82	26.5	49	26.4	100	50	25.9	98	49	25.7	97	50
89	26.9	49	26.9	100	50	26.7	99	49	26.4	98	50
117	29.5	49	29.8	101	50	29.7	101	49	28.8	98	50
145	32.9	49	33.6	102	50	33.3	101	49	31.2	95	50
173	36.6	49	35.9	98	50	35.4	97	49	33.0	90	50
201	37.7	49	37.3	99	50	36.9	98	49	33.8	90	50
229	40.2	49	39.4	98	50	38.3	95	49	35.3	88	50
257	43.3	49	42.6	98	50	42.0	97	49	37.1	86	50
285	45.5	49	45.7	100	50	43.9	97	49	38.0	84	50
313	47.1	49	47.1	100	50	44.0	94	49	38.2	81	50
341	49.5	48	50.3	102	50	48.0	97	49	40.4	82	50
369	50.9	48	51.7	102	50	48.5	95	49	40.4	79	49
397	52.8	48	54.0	102	50	50.1	95	49	40.6	77	49
425	53.9	48	55.7	103	49	51.3	95	49	41.5	77	49
453	54.0	47	56.4	105	49	51.5	95	49	41.5	77	48
481	55.7	47	57.2	103	48	52.2	94	49	41.4	74	47
509	56.9	47	58.4	103	48	53.0	93	49	40.4	71	46
537	57.7	47	58.4	101	47	53.8	93	48	39.4	68	40
551	57.2	47	57.4	101	47	52.5	92	48			0
565	56.8	47	56.3	99	47	52.0	92	47			0
579	55.0	46	56.3	102	45	51.1	93	46			0
593	55.7	43	56.5	101	44	50.8	91	46			0
607	52.8	42	54.3	103	44	49.5	94	45			0
621	53.4	42	55.1	103	43	50.2	94	45			0
635	53.7	41	55.7	104	42	51.3	96	45			0
649	53.2	41	55.0	103	41	50.9	96	45			0
663	54.1	40	56.5	104	41	52.4	97	44			0
675	53.0	40	54.9	104	40	51.5	97	44			0
691	51.4	36	54.7	106	37	50.1	98	40			0
705	52.0	33	54.3	104	37	49.4	95	38			0
719	51.7	33	53.4	103	35	48.5	94	38			0
Mean for Weeks											
1-13	22.6		22.6	100		22.6	100		22.6	100	
14-52	40.3		40.2	100		39.1	97		35.1	87	
53-103	54.1		55.6	103		51.0	94		40.7	75	

Tissue Concentrations

Concentrations of BDE-47, BDE-99, and BDE-153 were determined in adipose and liver of male and female mice at the end of the 2-year study, except for 30 mg/kg males. For the 30 mg/kg male group, samples were not collected due to insufficient normal tissue. The data are presented in Table I6 and Figure 13. In both males and females, the tissue concentrations of all three congeners in adipose and liver increased with increasing dose and were higher than those of the respective vehicle controls. The tissue concentrations of congeners in adipose were higher than in liver suggesting preferential accumulation in adipose. Regardless of the lower percentage of BDE-153 in DE-71 compared to the other two congeners, concentrations of BDE-153 were relatively higher in both adipose and liver suggesting a higher rate of accumulation of BDE-153.

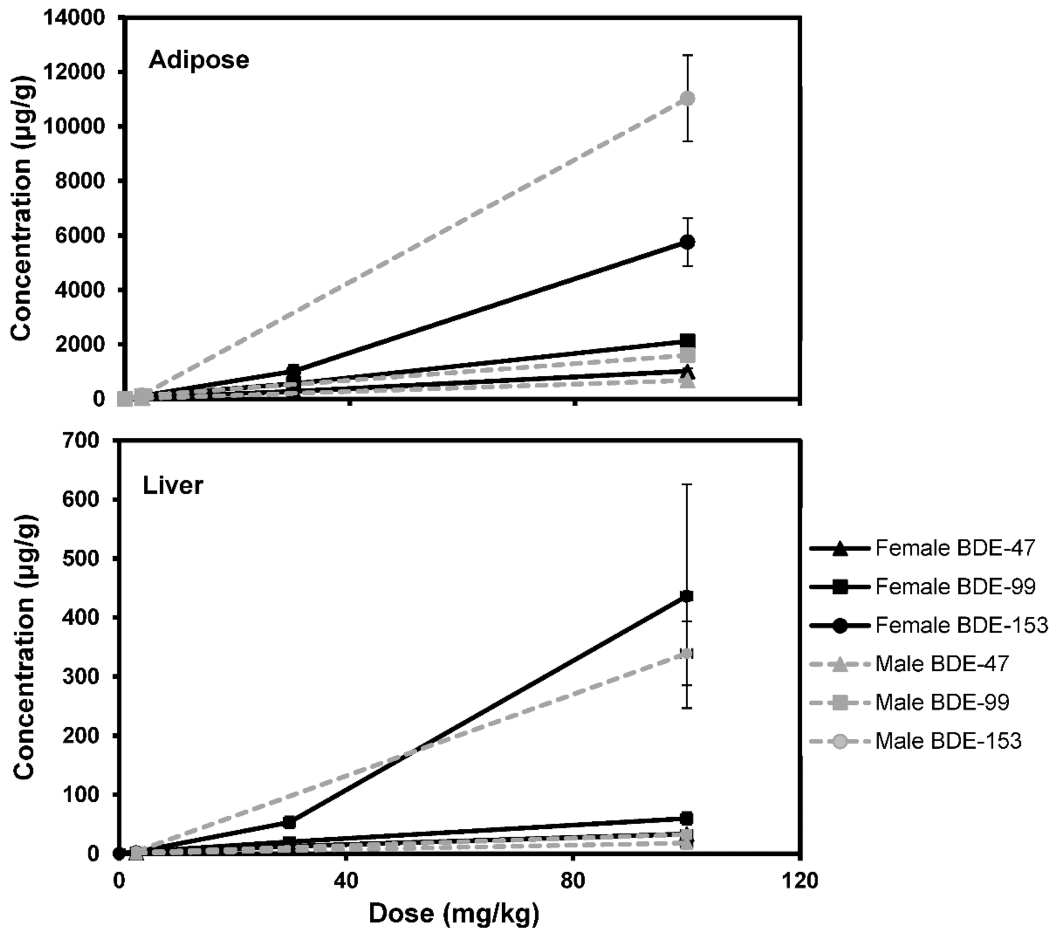


FIGURE 13
Concentrations of BDE-47, BDE-99, and BDE-153 in Adipose and Liver in Male and Female Mice Administered DE-71 by Gavage for 2 Years

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the liver, thyroid gland, forestomach, spleen, adrenal cortex, and testes. 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, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice. The 100 mg/kg males and females were terminated at 18 months. To adjust for differences in survival, statistical analyses were based on the poly-k test, as noted in the methods.

Liver: There were significantly increased incidences of hepatocellular adenoma in all dosed groups of male mice and in 30 and 100 mg/kg female mice (Tables 33, C1, C2, D1, and D2). There were significantly increased incidences of hepatocellular carcinoma in 30 mg/kg males and in 100 mg/kg males and females. Hepatoblastomas only occurred in male mice, with significantly increased incidences in the 30 and 100 mg/kg groups. There were also significantly increased incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in 30 and 100 mg/kg males and hepatocellular adenoma or carcinoma (combined) in 30 and 100 mg/kg males and females.

Hepatocellular adenomas were discrete, well-circumscribed lesions that compressed surrounding parenchyma (Plate 18). They were composed of irregular plates of hepatocytes, which were most commonly eosinophilic, but also basophilic or vacuolated. Central veins and portal areas were generally absent. Hepatocellular carcinomas were large lesions, frequently with areas of necrosis, which caused compression of, and invasion into, surrounding parenchyma. Typically, hepatocellular carcinomas were characterized by hepatocytes forming trabeculae that were at least three cells thick, although some of the areas of carcinomas were of a solid pattern of growth (Plate 19). Cells within the hepatocellular carcinomas ranged from eosinophilic to basophilic in staining, and displayed marked pleomorphism and an increased mitotic rate. Hepatoblastomas were composed of small cells with scant cytoplasm and hyperchromatic, oval nuclei (Plates 20 and 21). Cells were often arranged in rows around variably sized vascular spaces. Typically, hepatoblastomas arose from within a hepatocellular adenoma or carcinoma, and when this occurred only the hepatoblastoma was recorded.

There were significantly increased incidences of centrilobular hepatocyte hypertrophy in all dosed groups of male and female mice, and the severity of this lesion increased with increasing dose (Tables 33, C4, and D4). There were significantly increased incidences of eosinophilic focus in 30 and 100 mg/kg female mice. In 30 mg/kg males, there was a significantly increased incidence of clear cell focus and a significantly decreased incidence of basophilic focus. There were significantly increased incidences of fatty change in 30 and 100 mg/kg females. The incidence of focal necrosis was significantly increased in 30 mg/kg males, and there was a significant positive trend in the incidences of this lesion in male and female mice. There were significantly increased incidences of Kupffer cell pigmentation in all dosed groups of males and females.

Centrilobular hepatocyte hypertrophy was characterized by an accentuated lobular pattern due to the presence of very large, polygonal centrilobular hepatocytes with abundant granular eosinophilic cytoplasm containing clumped basophilic material. Nuclei were frequently enlarged and had stippled chromatin, prominent nucleoli, and occasional bright eosinophilic inclusions. Eosinophilic foci were discrete groups of enlarged hepatocytes with brightly eosinophilic cytoplasm (Plate 22). Some foci caused compression of some of the surrounding parenchyma, but not to the extent of hepatocellular adenomas. Hepatocytes within foci were generally aligned with hepatocytes in the normal liver, in contrast to those in hepatocellular adenomas. Foci typically lacked cellular atypia and mitotic figures. Clear cell foci consisted of small groups of cells with cytoplasm that was clear and vacuolated due to glycogen accumulation. Clear cell foci were found randomly scattered throughout the liver and were not associated with a particular zone. Basophilic foci were composed of clusters of hepatocytes that were smaller than normal hepatocytes, and whose cytoplasm was basophilic in color.

TABLE 33
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg[a]
Male				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hepatocyte, Hypertrophy[b]	0	28** (1.4)[c]	46** (3.7)	48** (3.9)
Clear Cell Focus	10	13	20*	7
Basophilic Focus	6	3	1*	5
Necrosis, Focal	2 (3.0)	2 (1.0)	16** (1.1)	2 (2.5)
Kupffer Cell, Pigmentation	5 (1.0)	15* (1.3)	33** (1.6)	25** (1.3)
Hepatocellular Adenoma, Multiple	10	23**	45**	33**
Hepatocellular Adenoma (includes multiple)[d]				
Overall rate[e]	23/50 (46%)	35/50 (70%)	49/50 (98%)	40/50 (80%)
Adjusted rate[f]	53.2%	72.9%	98.8%	93.5%
Terminal rate[g]	15/29 (52%)	25/33 (76%)	31/31 (100%)	0/0 (0%)
First incidence (days)	491	428	431	451
Poly-3 test[h]	P<0.001	P=0.034	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	4	2	17**	35**
Hepatocellular Carcinoma (includes multiple)[i]				
Overall rate	18/50 (36%)	15/50 (30%)	30/50 (60%)	45/50 (90%)
Adjusted rate	40.7%	33.0%	65.2%	97.7%
Terminal rate	8/29 (28%)	9/33 (27%)	21/31 (68%)	0/0 (0%)
First incidence (days)	491	540	453	451
Poly-3 test	P<0.001	P=0.293N	P=0.013	P<0.001
Hepatocellular Adenoma or Carcinoma[j]				
Overall rate	31/50 (62%)	40/50 (80%)[k]	49/50 (98%)	47/50 (94%)
Adjusted rate	68.1%	81.6%	98.8%	99.5%
Terminal rate	18/29 (62%)	26/33 (79%)	31/31 (100%)	0/0 (0%)
First incidence (days)	491	428	431	451
Poly-3 test	P<0.001	P=0.092	P<0.001	P<0.001
Hepatoblastoma, Multiple	0	0	4	0
Hepatoblastoma (includes multiple)[l]				
Overall rate	1/50 (2%)	1/50 (2%)	16/50 (32%)	5/50 (10%)
Adjusted rate	2.5%	2.3%	35.0%	23.4%
Terminal rate	1/29 (3%)	1/33 (3%)	9/31 (29%)	0/0 (0%)
First incidence (days)	729 (T)	729 (T)	453	477
Poly-3 test	P<0.001	P=0.743N	P<0.001	P=0.020
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[m]				
Overall rate	31/50 (62%)	40/50 (80%)[k]	49/50 (98%)	47/50 (94%)
Adjusted rate	68.1%	81.6%	98.8%	99.5%
Terminal rate	18/29 (62%)	26/33 (79%)	31/31 (100%)	0/0 (0%)
First incidence (days)	491	428	431	451
Poly-3 test	P<0.001	P=0.092	P<0.001	P<0.001

TABLE 33
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Female				
Number Examined Microscopically	50	49	50	49
Centrilobular, Hepatocyte, Hypertrophy	0	7** (1.0)	45** (2.2)	47** (2.9)
Eosinophilic Focus	3	2	16**	15**
Fatty Change	18 (1.4)	18 (1.4)	39** (1.6)	20* (1.2)
Necrosis, Focal	1 (1.0)	1 (2.0)	4 (1.8)	3 (2.7)
Kupffer Cell, Pigmentation	3 (1.3)	10* (1.1)	24** (1.2)	27** (1.4)
Hepatocellular Adenoma, Multiple	0	2	21**	42**
Hepatocellular Adenoma (includes multiple)[n]				
Overall rate	5/50 (10%)	7/49 (14%)	32/50 (64%)	46/49 (94%)
Adjusted rate	11.6%	16.0%	68.0%	97.9%
Terminal rate	5/33 (15%)	7/35 (20%)	26/37 (70%)	0/0 (0%)
First incidence (days)	729 (T)	729 (T)	563	432
Poly-3 test	P<0.001	P=0.385	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	0	1	1	8*
Hepatocellular Carcinoma (includes multiple)[o]				
Overall rate	4/50 (8%)	2/49 (4%)	6/50 (12%)	27/49 (55%)
Adjusted rate	9.2%	4.6%	13.0%	75.5%
Terminal rate	3/33 (9%)	1/35 (3%)	4/37 (11%)	0/0 (0%)
First incidence (days)	696	712	598	432
Poly-3 test	P<0.001	P=0.333N	P=0.411	P<0.001

TABLE 33
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Female (continued)				
Hepatocellular Adenoma or Carcinoma[p]				
Overall rate	8/50 (16%)	8/49 (16%)	33/50 (66%)	47/49 (96%)
Adjusted rate	18.4%	18.3%	69.5%	98.8%
Terminal rate	7/33 (21%)	7/35 (20%)	26/37 (70%)	0/0 (0%)
First incidence (days)	696	712	563	432
Poly-3 test	P<0.001	P=0.602N	P<0.001	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Groups terminated at 18 months
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 168/300 (56.0% ± 6.7%), range 46%-64%; all routes: 437/700 (62.4% ± 10.5%), range 46%-78%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by N.
[i] Historical incidence for corn oil gavage studies: 105/300 (35.0% ± 9.8%), range 22%-44%; all routes: 262/700 (37.4% ± 11.2%), range 22%-52%
[j] Historical incidence for corn oil gavage studies: 220/300 (73.3% ± 6.3%), range 62%-78%; all routes: 541/700 (77.3% ± 8.3%), range 62%-90%
[k] A single incidence of hepatocholangiocarcinoma occurred in an animal that also had an adenoma.
[l] Historical incidence for corn oil gavage studies: 10/300 (3.3% ± 2.4%), range 0%-6%; all routes: 34/700 (4.9% ± 3.7%), range 0%-12%
[m] Historical incidence for corn oil gavage studies: 221/300 (73.7% ± 6.1%), range 62%-78%; all routes: 545/700 (77.9% ± 8.3%), range 62%-90%
[n] Historical incidence for corn oil gavage studies: 67/300 (22.3% ± 10.5%), range 10%-34%; all routes: 272/698 (39.1% ± 21.9%), range 10%-78%
[o] Historical incidence for corn oil gavage studies: 30/300 (10.0% ± 5.1%), range 4%-18%; all routes: 112/698 (16.1% ± 8.1%), range 4%-34%
[p] Historical incidence for corn oil gavage studies: 85/300 (28.3% ± 10.2%), range 16%-40%; all routes: 320/698 (45.9% ± 21.9%), range 16%-82%

The large majority of hepatocytes with fatty change were characterized by a single, or a few, discrete vacuoles within the cytoplasm of the hepatocytes that displaced the nucleus peripherally, consistent with macrovesicular fatty change. Less commonly, microvesicular fatty change was also present and characterized by small, almost indistinct vacuoles filling the cytoplasm. Fatty change was most commonly found in the periportal regions. Focal necrosis was characterized by the loss of cellular detail and hypereosinophilia of small clusters of hepatocytes and was typically associated with a neutrophilic infiltrate. Kupffer cell pigmentation was a subtle change consisting of pale tan to brown pigment within the cytoplasm of Kupffer cells. The pigment appeared to be consistent with lipofuscin and may represent an increase in hepatocellular turnover.

Thyroid Gland: There were significantly increased incidences of follicle hypertrophy in all dosed groups of male mice and in 30 and 100 mg/kg female mice, and the severities were increased in the 100 mg/kg groups (Tables 34, C4, and D4). The incidences of follicle degeneration, an age-associated degenerative change in mice, were significantly decreased in 30 mg/kg males

TABLE 34
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg[a]
Male				
Thyroid Gland[b]	50	49	50	49
Follicle, Hypertrophy[c]	25 (1.2)[d]	35* (1.4)	41** (2.0)	45** (2.4)
Follicle, Degeneration	21 (1.6)	19 (1.6)	12* (1.4)	6 (1.3)
Stomach, Forestomach	50	50	50	50
Epithelium, Hyperplasia	26 (2.1)	19 (2.2)	40** (2.4)	29* (2.9)
Ulcer	9 (2.3)	8 (1.9)	14 (2.1)	11* (2.5)
Inflammation	18 (1.7)	18 (1.6)	34** (1.7)	19* (1.7)
Spleen	50	47	47	47
Hematopoietic Cell Proliferation	14 (2.1)	10 (2.2)	13 (2.2)	25** (1.8)
Adrenal Cortex	50	50	49	48
Hypertrophy, Diffuse	1 (1.0)	0	3 (1.3)	20** (1.4)
Testes	50	50	50	49
Germinal Epithelium, Atrophy	11 (1.5)	8 (1.4)	20 (1.4)	13* (1.5)
Female				
Thyroid Gland	50	49	48	47
Follicle, Hypertrophy	24 (1.3)	31 (1.5)	37** (1.5)	42** (2.4)
Follicle, Degeneration	34 (1.9)	28 (2.0)	26 (1.5)	11** (1.3)
Stomach, Forestomach	50	50	50	49
Epithelium, Hyperplasia	9 (1.9)	5 (1.6)	6 (2.7)	16** (2.6)
Spleen	50	47	48	48
Hematopoietic Cell Proliferation	15 (1.9)	10 (2.9)	11 (2.8)	24** (2.3)
Lymphoid Follicle, Hyperplasia	12 (1.7)	20 (1.7)	7 (2.3)	21** (1.6)
Adrenal Cortex	50	50	49	47
Hypertrophy, Diffuse	0	0	4 (1.0)	8** (1.4)

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Groups terminated at 18 months
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

and 100 mg/kg females. The decreases in the incidences of this degenerative change are most likely due to an increase in thyroid gland stimulation in the 30 mg/kg males, and an increase in early deaths in the 100 mg/kg females.

Follicle hypertrophy was recorded when more than 50% of the follicles were lined by cuboidal epithelial cells with round nuclei and cytoplasm containing hyaline droplets; the colloid was generally eosinophilic, but often contained clumps of dark eosinophilic to pale basophilic material. With increasing severity, an increasing percentage of follicles were involved. Epithelial cells progressed from cuboidal to columnar, and the cytoplasm was often vacuolated; colloid was generally basophilic and contained clear vacuoles, clumps of dark basophilic material, and occasionally mineralized material. Morphologically, follicle degeneration was characterized by a loss of stainable colloid with coalescence of contiguous follicles and formation of multilocular spaces lined by flattened epithelium. The colloid in affected follicles tended to have a pale blue hue and increased interfollicular connective tissue surrounding affected follicles was common.

Forestomach: There were significantly increased incidences of epithelium hyperplasia in 30 and 100 mg/kg males and in 100 mg/kg females (Tables 34, C4, and D4). In male mice, there were significantly increased incidences of ulcer in the 100 mg/kg group and inflammation in the 30 and 100 mg/kg groups. Epithelium hyperplasia, characterized by thickened squamous epithelium, usually lacked the solitary stalk of the papillomas; rather it had a broad base, and did not protrude as far into the lumen. Epithelium hyperplasia was sometimes associated with ulceration or erosion of the stomach epithelium but was often found in the absence of other lesions. Ulceration of the forestomach involved the loss of the entire thickness of the epithelium and generally extended through the basement membrane into the submucosa and muscularis mucosa. Ulcers were often associated with an inflammation, typically of mixed cell types, including neutrophils, macrophages, lymphocytes, and plasma cells. Eosinophilic cell debris, sloughed keratin, and bacteria could be found on the surface of some of the lesions. The biological significance of the forestomach ulcers is unknown.

Spleen: There were significantly increased incidences of hematopoietic cell proliferation in 100 mg/kg male and female mice (Tables 34, C4, and D4). In the 100 mg/kg females, there was a significantly increased incidence of lymphoid follicle hyperplasia. Hematopoietic cell proliferation was characterized by an increased number of hematopoietic and myeloid cell precursors and megakaryocytes at different stages of maturation within the red pulp of the spleen. Lymphoid follicle hyperplasia was characterized by follicles that were enlarged and almost coalescing with one another. The changes in the spleen were considered secondary, and not primary, effects of exposure to DE-71.

Adrenal Cortex: In 100 mg/kg males and females, there were significantly increased incidences of diffuse hypertrophy (Tables 34, C4, and D4). Diffuse cortical hypertrophy was characterized by enlargement of the majority of cortical epithelial cells, and was usually a bilateral finding.

Testes: There was a positive trend in the incidences of germinal epithelium atrophy in males, and the incidence in the 100 mg/kg group was significantly increased (Tables 34 and C4). Germinal epithelium atrophy was characterized by thinning of the germinal epithelium layer due to reduced numbers of germ cells.

GENETIC TOXICOLOGY

DE-71 was tested for mutagenic activity in bacteria in three independent studies at three separate laboratories using a total of six different bacterial tester strains (*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537 and *Escherichia coli* WP2 *uvrA*/pKM101) with and without 10% rat or hamster liver metabolic activation enzymes (S9). The study conducted by SITEK Research Laboratories used the same lot of DE-71 (2550OA30A) that was used in the 2-year gavage studies. No evidence of mutagenicity was observed (Zeiger *et al.*, 1987; Tables E1 and E2). In all three studies, dose levels ranged up to 10,000 µg/plate in the absence of observable toxicity, although precipitation occurred in one of the three studies at 1,000 µg/plate and above.

Three related test articles, BDE-47, BDE-99, and BDE-153 were tested for mutagenic activity in three bacterial tester strains (*S. typhimurium* TA98, TA100, and TA102) with and without rat liver S9 mix, and no evidence of mutagenicity was observed with any of the three test articles in any of the tests that were conducted (Tables E3, E4, and E5).

In vivo, no increases in the frequencies of micronucleated normochromatic erythrocytes (NCEs) were observed in peripheral blood samples from male or female mice in the 3-month gavage study of DE-71 (0.01 to 500 mg/kg; Table E6). Five mice were examined in each dose group except in the 500 mg/kg group only three male mice were available. In a second micronucleus study conducted in male B6C3F1/N mice, no increases in the frequencies of polychromatic erythrocytes (PCEs) or NCEs were seen in peripheral blood samples following administration of

DE-71 (312.5 to 1,250 mg/kg) by gavage once daily for 3 days; blood samples were evaluated using flow cytometric methods (Witt *et al.*, 2008; Table E7). In these same mice, slide-based data acquisition methods were used to evaluate bone marrow smears for induction of micronucleated PCEs and results were consistent with the results from blood samples (Table E8). In none of the micronucleus tests conducted with DE-71 were significant alterations in the percentage of PCEs seen over the dose range tested, suggesting that DE-71 did not induce toxicity in the bone marrow of treated mice. In the 3-day gavage study evaluated using flow cytometric methods, the trend test for percentage of PCEs gave a significant P value (0.023), but pairwise comparison of the top dose to the vehicle control group was not significant; thus, the small increase detected by flow cytometry (but not by slide scoring in the bone marrow) was not considered to be biologically significant.

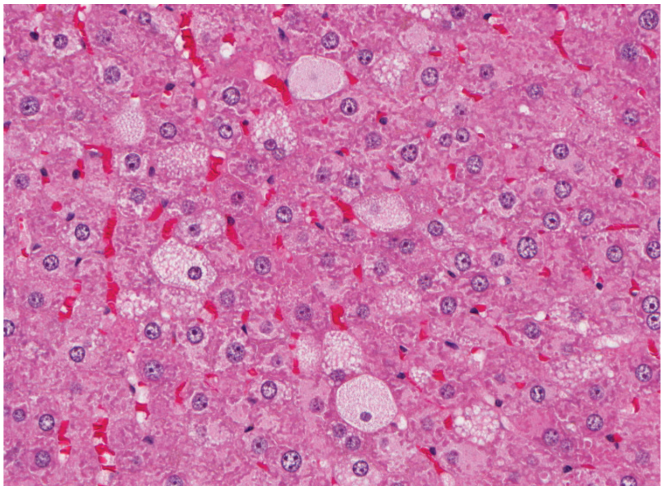


PLATE 1
Hepatocyte hypertrophy (enlarged hepatocytes) and cytoplasmic vacuolization (vacuolated hepatocytes) in the liver of a male F344/N rat administered 500 mg/kg DE-71 by gavage for 3 months. H&E

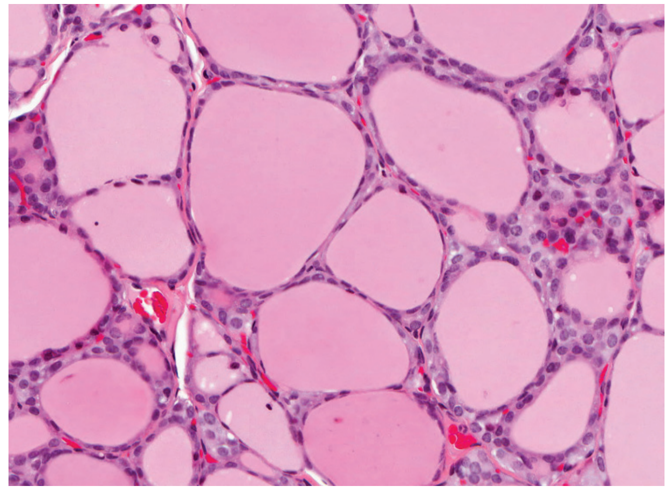


PLATE 2
Normal thyroid gland in a vehicle control female F344/N rat in the 3-month gavage study of DE-71. The follicles are lined by flattened epithelium and contain abundant amounts of brightly eosinophilic colloid. H&E

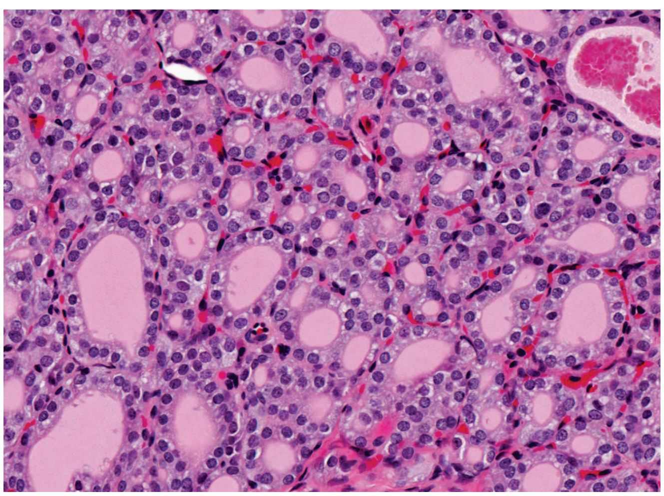


PLATE 3
Follicle hypertrophy in the thyroid gland of a female F344/N rat administered 500 mg/kg DE-71 by gavage for 3 months. Follicle hypertrophy is characterized by small follicles lined by cuboidal epithelial cells. H&E

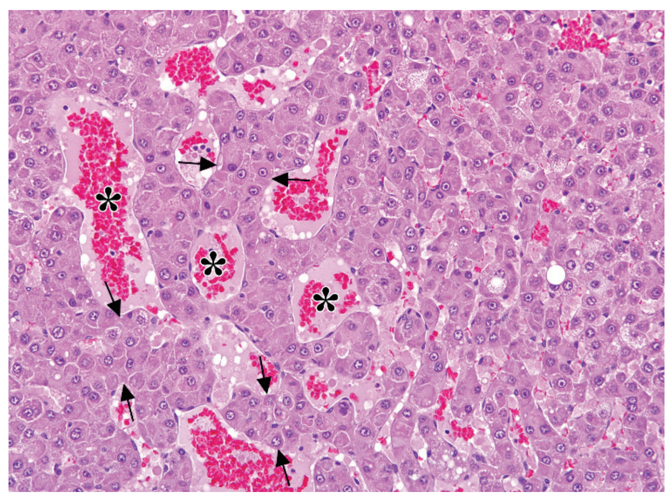


PLATE 4
Hepatocellular carcinoma in the liver of an F_1 female Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. There are thickened trabeculae of hepatocytes (arrows) separated by dilated spaces filled with blood (asterisks). H&E

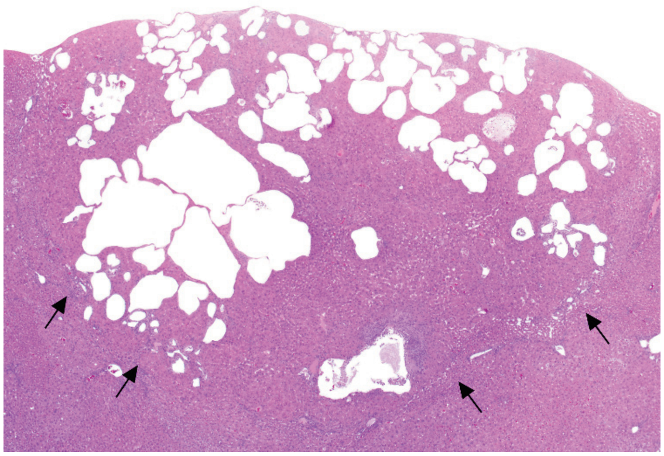


PLATE 5
Hepatocholangioma in the liver of an F_1 female Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. There is a well demarcated mass (arrows) composed of hepatocytes and proliferations of dilated bile ducts. H&E

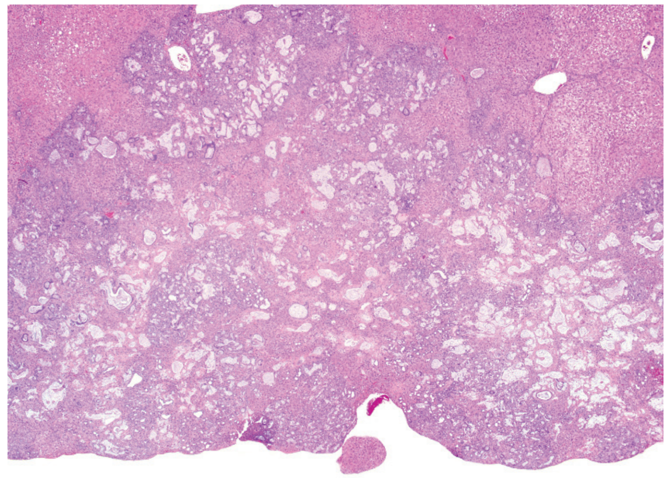


PLATE 6
Cholangiocarcinoma in the liver of an F_1 female Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. The neoplasm is large, effacing much of the lobe of the liver. H&E

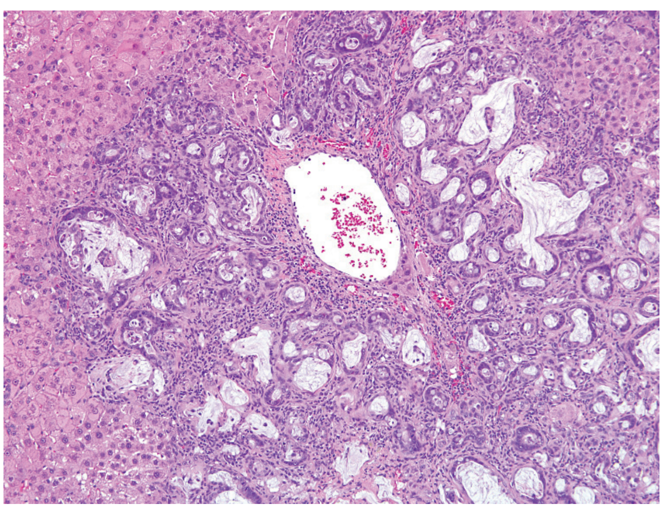


PLATE 7
Cholangiocarcinoma in the liver of an F_1 female Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. The neoplasm is characterized by invasive areas of atypical bile ducts and fibrous connective tissue. H&E

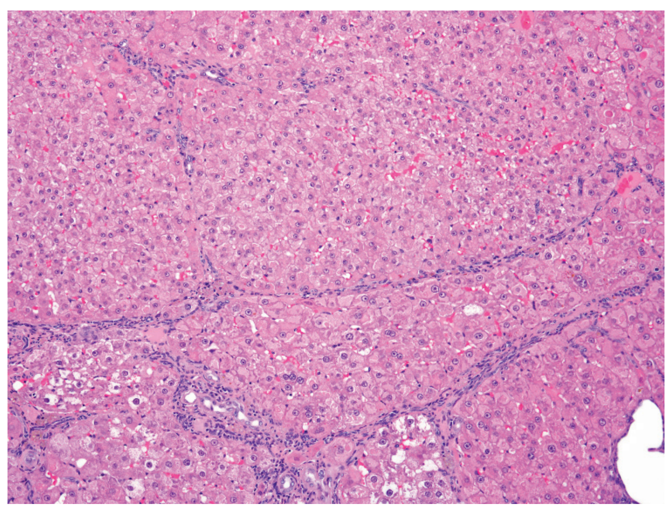


PLATE 8
Nodular hyperplasia in the liver of an F_1 female Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. The lesion is characterized by areas of large hepatocytes separated by thin bands of fibrous connective tissue, with bile duct and oval cell hyperplasia. H&E

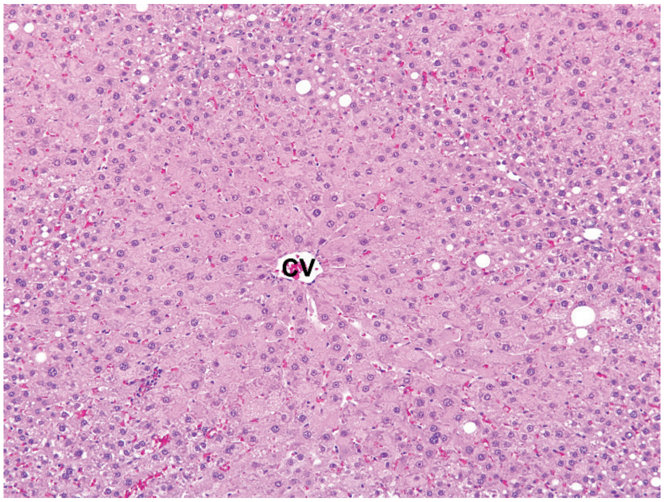


PLATE 9
Marked hypertrophy of the centrilobular hepatocytes in the liver of an F_1 female Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. CV=central vein. H&E

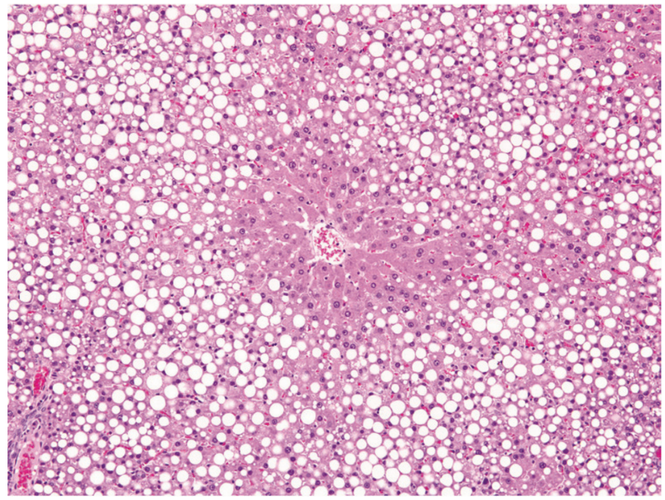


PLATE 10
Marked fatty change with discrete, large vacuoles filling the cytoplasm of the majority of hepatocytes in the liver of an F_1 female Wistar Han rat administered 15 mg/kg DE-71 by gavage for 2 years. H&E

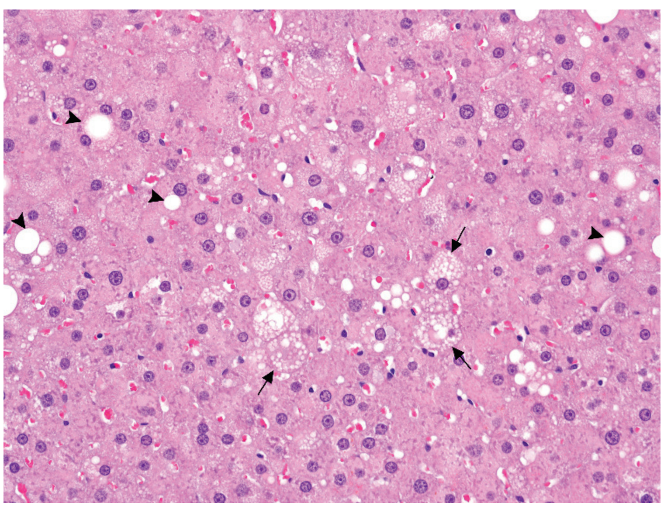


PLATE 11
High magnification of the liver of an F_1 male Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. There is both macrovesicular fatty change (arrowheads) indicated by single large vacuoles within the hepatocellular cytoplasm and microvesicular fatty change (arrows) evidenced by a lacey appearance of the cytoplasm due to many small vacuoles. Microvesicular and macrovesicular fatty change were not given separate diagnoses, but recorded under "liver - fatty change." H&E

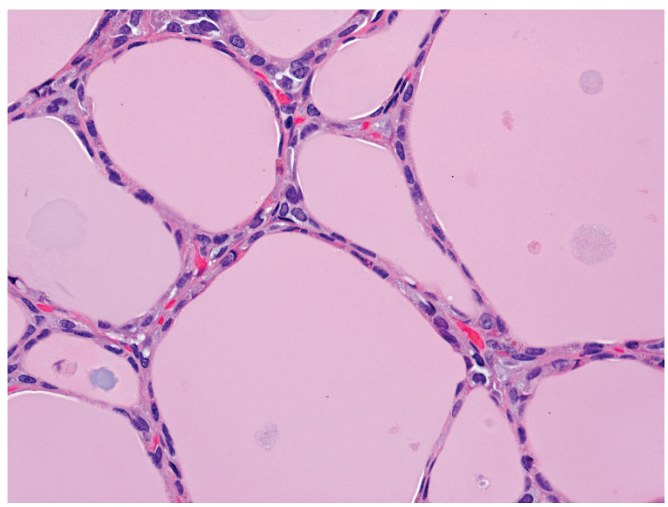


PLATE 12
Normal thyroid gland in a vehicle control F_1 male Wistar Han rat in the 2-year gavage study of DE-71. H&E

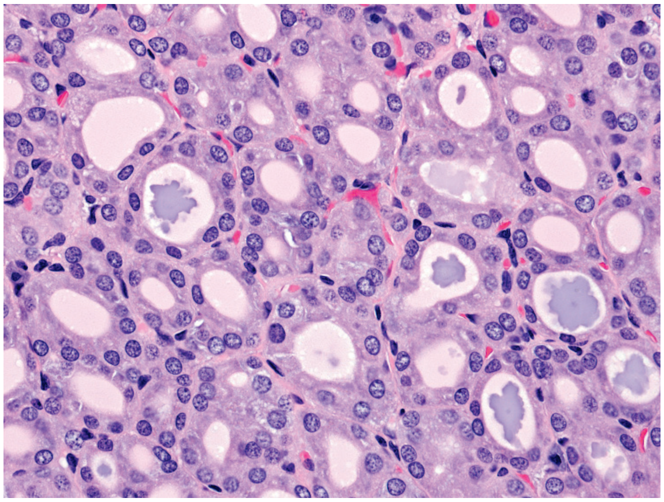


PLATE 13
Hypertrophy in the thyroid gland follicles of an F_1 male Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. The follicular epithelium is cuboidal, and the lumens are smaller with less colloid than seen in the vehicle control animal in Plate 12. Same magnification as Plate 12. H&E

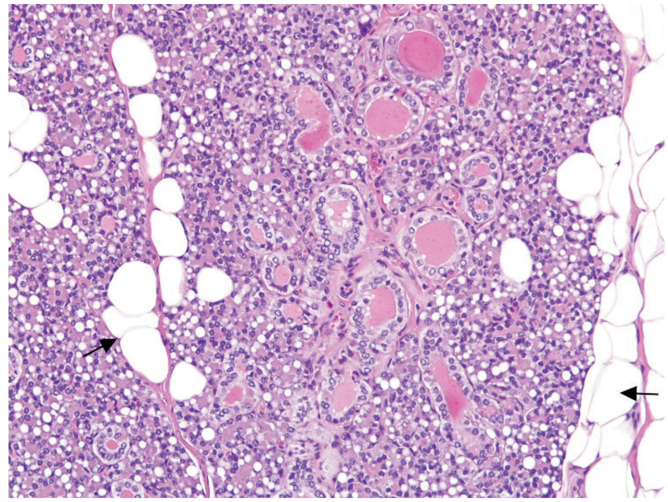


PLATE 14
Cytoplasmic vacuolization in the parotid salivary gland of an F_1 male Wistar Han rat administered 50 mg/kg DE-71 by gavage for 2 years. Most of the cells contain a single, large, discrete vacuole. There is also minimal atrophy of the gland, with infiltration of adipocytes (arrows). H&E.

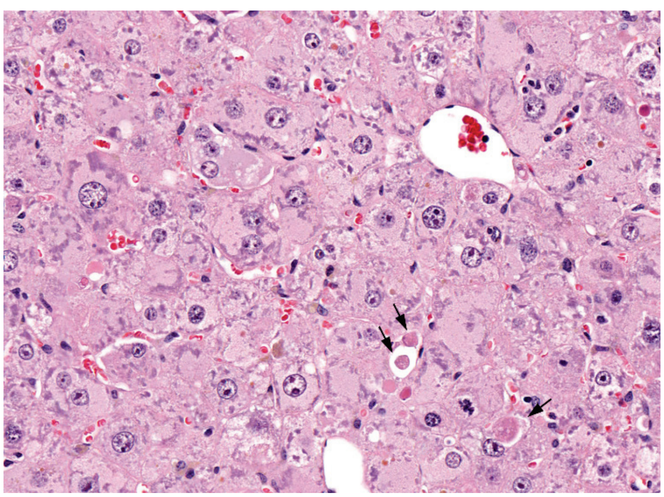


PLATE 15
Hepatocyte hypertrophy in the liver of a male B6C3F1/N mouse administered 500 mg/kg DE-71 by gavage for 3 months. The hepatocytes are larger than normal and there are scattered necrotic hepatocytes (arrows). H&E

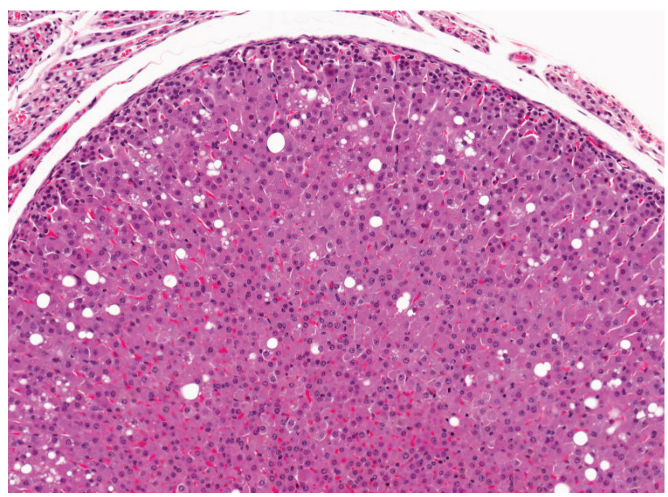


PLATE 16
Zona fasciculata hypertrophy and fatty degeneration in the adrenal gland of a male B6C3F1/N mouse administered 500 mg/kg DE-71 by gavage for 3 months. The cells are larger than normal and there are large discrete vacuoles consistent with fat accumulation. H&E

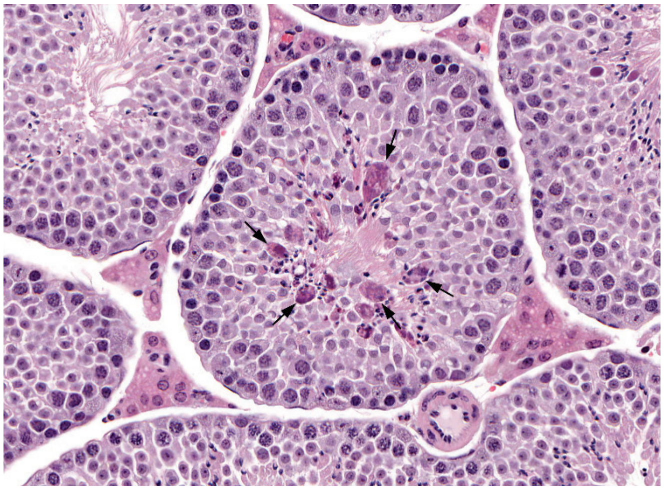


Plate 17
Abnormal residual bodies (arrows) in the testis of a male B6C3F1/N mouse administered 500 mg/kg DE-71 by gavage for 3 months. H&E

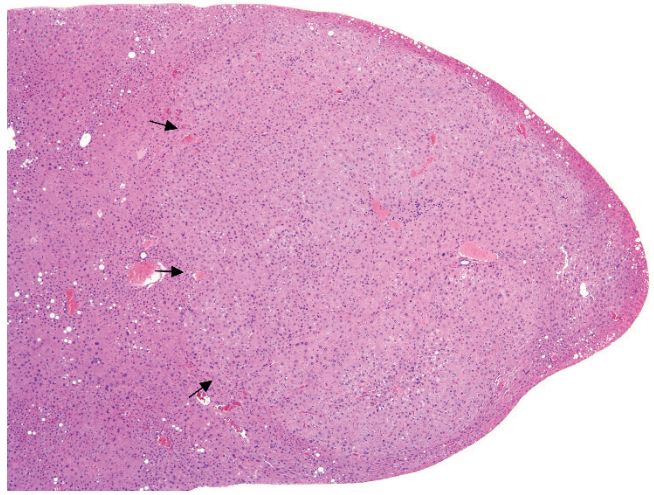


Plate 18
Hepatocellular adenoma in the liver of a male B6C3F1/N mouse administered 30 mg/kg DE-71 by gavage for 2 years. The large discrete mass (arrows) has a solid growth pattern that makes it distinct from the rest of the liver. H&E

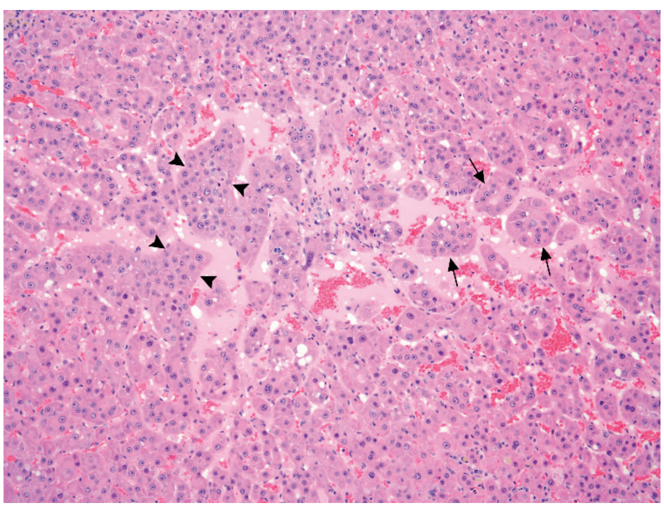


Plate 19
A large hepatocellular carcinoma in the liver of a male B6C3F1/N mouse administered 30 mg/kg DE-71 by gavage for 2 years. The neoplasm is characterized by trabeculae that are three or more cells wide (arrowheads) and by blunt-ended trabeculae (arrows). H&E

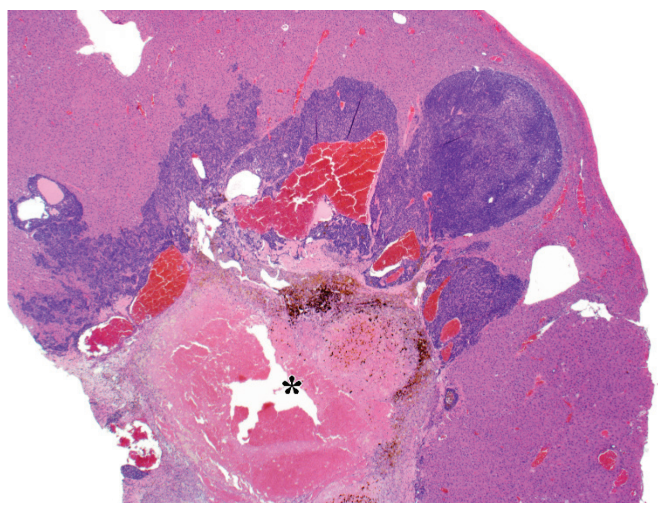


Plate 20
Hepatoblastoma in the liver of a male B6C3F1/N mouse administered 30 mg/kg DE-71 by gavage for 2 years. The neoplasm contains a large area of hemorrhage and necrosis (asterisk). H&E

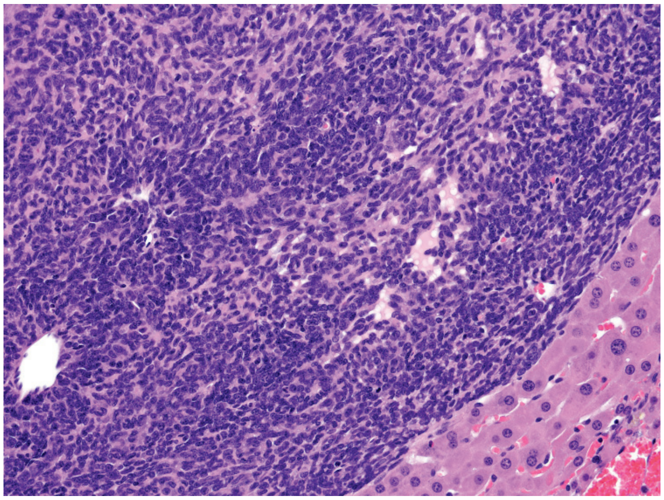


PLATE 21
Higher magnification of Plate 20. The cells are densely packed and small with oval, deeply basophilic nuclei. H&E

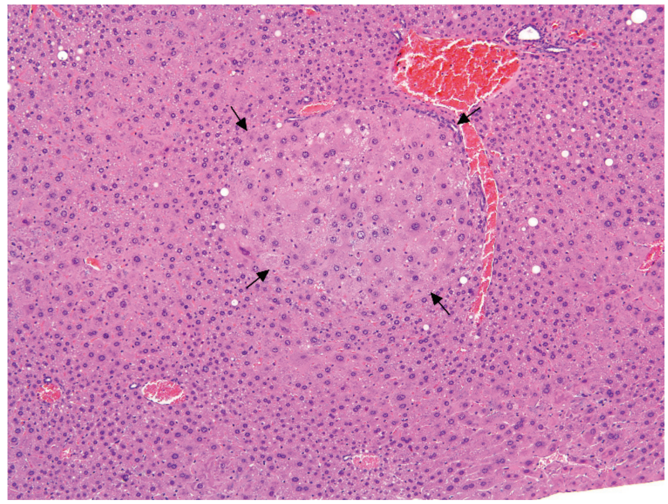


PLATE 22
Eosinophilic focus in the liver of a male B6C3F1/N mouse administered 30 mg/kg DE-71 by gavage for 2 years. The focal area of enlarged hepatocytes (arrows) is not causing compression of the surrounding liver parenchyma. The liver also has marked hepatocyte hypertrophy. H&E

DISCUSSION AND CONCLUSIONS

These NTP gavage studies evaluated the toxic and carcinogenic potential of a mixture of polybrominated diphenyl ethers (PBDEs) (DE-71, technical grade; Appendix J). Three-month studies were conducted in adult F344/N rats and B6C3F1/N mice at doses of 0, 0.01, 5, 50, 100, or 500 mg DE-71/kg body weight per day. Two-year studies were conducted in Wistar Han [Crl:WI(Han)] rats (referred to as Wistar Han rats below) at doses of 0, 3, 15, or 50 mg/kg, after *in utero*, postnatal, and adult exposure. This exposure paradigm was used in the 2-year rat study because of reported PBDE exposure to the human fetus and infant (USEPA, 2008a,b,c). Decreased survival in the 50 mg/kg male rats was due to adenomas of the pars distalis of the pituitary gland. Two-year studies were conducted in adult B6C3F1/N mice at doses of 0, 3, 30, or 100 mg/kg. In male and female mice, the 100 mg/kg groups were sacrificed at 18 months because of the moribund condition of the animals due to the development of liver neoplasms.

A major finding from these studies was the toxic effects of DE-71 administration in the liver of rats and mice. In the 3-month study, treatment-related liver lesions in male and female rats and mice included hepatocyte hypertrophy and cytoplasmic vacuolization (except female mice), with the incidences and severities increasing with increasing dose. Hepatocyte necrosis in 500 mg/kg mice was also treatment-related. Proposed mechanisms for hepatocyte necrosis included marked hypertrophy leading to reduced sinusoidal blood circulation, hypoxia, and necrosis (Slauson and Cooper, 2001) and/or metabolic activation forming more toxic active metabolites (Farber, 1980).

Liver toxicity at 3 months was also characterized by increases in liver enzyme levels and liver weights. Hepatic 7-pentoxyresorufin-*O*-dealkylase (PROD), 7-ethoxyresorufin-*O*-deethylase (EROD), acetanilide-4-hydroxylase (A4H), and uridine diphosphate glucuronosyl transferase (UDPGT) activities increased in these studies and the increases were generally greater in rats than mice. Liver weights were increased in male and female rats administered 5 mg/kg or greater. In mice, liver weights were increased in males administered 50 mg/kg or greater and in females administered 100 or 500 mg/kg.

In the 3-month study, dose-related decreases in serum thyroxine (T_4) concentrations occurred at all time points in male and female rats administered 5 mg/kg or greater. These findings are consistent with decreases in circulating T_4 that have been observed in other rat and mouse studies of PBDEs (Hallgren *et al.*, 2001; Zhou *et al.*, 2002; Richardson *et al.*, 2008; Blanco *et al.*, 2013). Mechanisms for the decrease in T_4 have been suggested and may involve interference by a PBDE congener with T_4 binding to the plasma transport protein transthyretin (Meerts *et al.*, 2000; Hamers *et al.*, 2006) and increased glucuronidation and excretion of T_4 after PBDE exposure (Richardson *et al.*, 2008). Decreases in serum T_4, and the observed concomitant increases in thyroid stimulating hormone (TSH) in response, may help explain, and would be consistent with, the increased incidences of thyroid gland follicular hypertrophy observed histologically in treated rats.

Dose-related increases in serum cholesterol concentrations occurred in male and female rats in the 3-month study. It is well known that, in humans, thyroid hormones regulate cholesterol and lipoprotein metabolism (Duntas and Brenta, 2012). Additionally, in rats it has been demonstrated that a hypothyroid state results in increased serum cholesterol (Dory and Roheim, 1981) and altered cholesterol and lipoprotein metabolism (Takeuchi *et al.*, 1975; Dory and Roheim, 1981; Apostolopoulos *et al.*, 1987). Thus, it seems that the increased serum cholesterol concentrations observed in this study can be explained by the hypothyroid state induced by DE-71 administration.

In both the rat and mouse 3-month studies, small decreases in the erythron were associated with DE-71 administration. In humans and mice, it has been demonstrated that a hypothyroid state resulted in a significant reduction in red blood cell mass and a decline of the erythropoietic activity of the bone marrow (Das *et al.*, 1975; Perrin *et al.*, 1997). Similar observations, which could be reversed with the administration of erythropoietin or thyroid hormone, have been reported in rats (Donati *et al.*, 1973). Further, it has been demonstrated that thyroid hormones have a direct stimulatory effect on bone marrow erythropoiesis in the rat (Malgor *et al.*, 1975). Thus, the decreased erythron observed in the rat and mouse studies would be consistent with the hypothyroid state induced by DE-71 administration. Because in mice this erythron effect only occurred in the 500 mg/kg groups, this may have also been secondary to the severe liver toxicity that occurred in these groups (Fruhman, 1966; Weiss and Goodnough, 2005).

Reproductive tract findings were observed in male rats in the 3-month study. Epididymis hypospermia and decreased epididymis weight were observed in the 500 mg/kg group, and decreased spermatid heads per gram testis were observed in the 100 and 500 mg/kg groups. Abnormal residual bodies were seen in half of the 500 mg/kg male mice. Abnormal residual bodies are generally larger than normal residual bodies, which represent remaining cytoplasm shed from elongating spermatids during their maturation and have the appearance of apoptotic bodies. Their significance is unclear, but they may represent a disruption of the spermiation process (Creasy *et al.*, 2012).

Disruption of the estrous cycle occurred in 500 mg/kg female rats. Liver toxicity in 500 mg/kg females may have impacted estrogen metabolism, causing reduced elimination of estrogen, because the liver is a major site for conjugation and elimination of estrogens (Tsuchiya *et al.*, 2005). Hypothyroidism and decreased thyroid hormone levels can also disrupt normal estrous cycling patterns (Ortega *et al.*, 1990).

In the 2-year rat study, after perinatal exposure to DE-71 there were no effects on littering parameters in Wistar Han rat dams or pups. At the 3-month interim evaluation of Wistar Han rats, which included the vehicle control and 50 mg/kg groups, liver and thyroid gland toxicity were observed in the 50 mg/kg group, as previously noted in the 3-month study in the F344/N rat. In 50 mg/kg male rats at the 3-month interim evaluation, there was an increase in testis weight (after *in utero*/postnatal/adult exposure to DE-71). This increase in testis weight at 3 months was not seen in F344/N male rats after adult-only DE-71 administration. This Wistar Han rat testicular effect may have been related to a decrease in T_4 levels during organ development, which has been previously reported to be associated with increased testis weight (Cooke *et al.*, 1993).

The occurrence of treatment-related benign and malignant liver neoplasms in male and female rats and mice was a major finding of these 2-year studies of DE-71. Some decreases in survival and/or decreases in mean body weights in dosed groups were attributed to the development of these liver neoplasms especially in the 100 mg/kg mouse groups that were terminated at 18 months.

In the 2-year male rat study, the combined incidences of hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma were considered to be clear evidence of carcinogenic activity based on the positive trend, and the combined incidence was significantly increased in the 50 mg/kg group. In female rats, the individual incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocholangioma were considered to be clear evidence of carcinogenic activity due to significantly increased incidences in the 50 mg/kg group. The combined incidence of these tumors was also significantly increased in the 50 mg/kg group. Liver neoplasm formation (first incidence) in 50 mg/kg male and female rats occurred earlier than in vehicle controls. In all dosed groups of male and female rats, the incidences of hepatocyte hypertrophy were significantly increased, and the severities of the lesion increased with increasing dose. The incidences of eosinophilic foci and fatty change were significantly increased in 15 and 50 mg/kg rats. There was a significant positive trend for the incidences of cholangiocarcinoma in female rats, an uncommon tumor in control rats (0/300 in the historical control database for Wistar Han female rats). This was considered to be related to treatment, and this was supported by the finding of cholangiofibrosis in a few 50 mg/kg female rats.

In the 2-year mouse study, there were treatment-related increases in the incidences of benign and malignant liver neoplasms in dosed groups of males and females. In male mice, the individual incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma were considered to be clear evidence of carcinogenic activity based on the significant pairwise comparisons in all the dosed groups (adenomas) or the 30 and 100 mg/kg dose groups (carcinomas and hepatoblastomas), in addition, these increases were generally above the NTP historical control ranges. In combination, incidences of these neoplasms were also significantly increased in the 30 and 100 mg/kg groups. In female mice, the individual increases in the incidences of hepatocellular adenoma (30 and 100 mg/kg groups) and hepatocellular carcinoma (100 mg/kg group) were determined to be clear evidence of carcinogenic activity. The combined incidences of hepatocellular adenoma or carcinoma in the 30 and 100 mg/kg groups were also significantly increased and occurred with a significant positive trend. Liver neoplasms in treated mice occurred earlier than those in vehicle controls; 100 mg/kg mice were euthanized at 18 months because of a moribund condition due to the occurrence of liver neoplasms. Liver toxicity was also seen in dosed groups of mice including centrilobular hepatocyte hypertrophy.

In addition to the liver tumor response in male rats, the incidences of thyroid gland follicular cell adenoma were also considered to be related to treatment because there was a significant increase in the incidence of follicular cell adenoma in 50 mg/kg males, and the incidences of this neoplasm occurred with a significant positive trend in dosed males. A few thyroid gland carcinomas occurred in the 3 and 15 mg/kg groups of males. Thyroid gland follicular cell adenomas are thought to be capable of progressing to thyroid gland follicular cell carcinomas (Hardisty and Boorman, 1990). It is possible that the increased number of early deaths in the 50 mg/kg males prevented the development of thyroid gland carcinomas in that group. This thyroid gland neoplasm response was supported by significantly increased incidences of follicle hypertrophy in all dosed groups of male rats. In addition, there was an increased incidence of follicular cell hyperplasia in 50 mg/kg females.

There is mechanistic support for the thyroid gland tumor response to DE-71 exposure based on the findings from the 3-month studies which showed that DE-71 can induce UDPGT and produce decreases in serum T_4 and increases in serum TSH, which can be associated with the development of thyroid gland cancer (Boelaert, 2009; Zabka *et al.*, 2011). Induction of hepatic UDP-GT activity increases the metabolic clearance of thyroid hormone and may act as a promoting stimulus for thyroid gland tumor growth in rats (Zabka *et al.*, 2011).

In addition to the liver and thyroid gland neoplasm responses in male rats at 2 years, there was an increase in the incidence of adenoma of the pars distalis of the pituitary gland in 50 mg/kg males. Because the incidence of pituitary gland adenoma was significantly increased in the 50 mg/kg group and the incidences of this neoplasm occurred with a significant positive trend, pituitary gland adenoma was considered to be related to DE-71 administration. The effect was not considered to be part of clear evidence because this pituitary gland neoplasm is a benign neoplasm that typically does not progress to carcinoma (Berry, 1986).

An extended evaluation of residual uterus, vagina, and cervix tissue was conducted due to concerns of toxicity in these target organs. When the original and residual evaluations were combined, there were significant increases in the incidences of stromal polyp or stromal sarcoma (combined) in the uterus in the 3 and 15 mg/kg groups. This combination consisted primarily of stromal polyps. In addition, two vaginal polyps occurred in the 50 mg/kg group. However, these uterine tumors were considered an equivocal effect because these neoplasms appear to be common, there was a lack of dose response, and the data from animals with both the original and residual evaluations indicates that the concurrent control value was at the low end of the range.

In addition to the neoplasms, there were increased incidences of nonneoplastic lesions in the liver, thyroid gland, and kidney (male and female rats); parotid salivary gland, prostate gland, preputial gland, thymus, and forestomach (male rats); uterus, cervix, and adrenal cortex (female rats); liver, thyroid gland, forestomach, and adrenal cortex (male and female mice); and testes (male mice). The liver and thyroid gland toxicity, as mentioned above, may be related to the increase in metabolic activation in the liver through interaction of PBDEs with nuclear receptors and/or decreases in thyroid hormones, which can alter liver metabolic activity resulting in accumulation of liver lipids. The thyroid gland lesions could be a result of decreased T_4 resulting in increased stimulation of the thyroid gland due to increased TSH levels. The forestomach toxicity and lesions in some of the other organs may have been related to the ability of PBDE metabolites to cause oxidative damage, and species differences in metabolism of PBDEs may have been the reason that the toxic forestomach lesions were seen only in mice.

Because 100 mg/kg male mice were euthanized at 18 months, a number of nonneoplastic lesions occurred with decreased incidences in this group, including epididymal inflammation, pancreatic islet hyperplasia, lung infiltration, pancreas atrophy, and spleen pigmentation. In 100 mg/kg male and female mice (also sacrificed early), the incidences of thyroid gland follicle degeneration were decreased. Some of these nonneoplastic lesions are late occurring lesions and, because of the early sacrifice time, did not have time to develop as normally occurs in aging mice.

In conjunction with the current 2-year DE-71 study, analysis of the aryl hydrocarbon receptor (AhR) genotype at exon 10 in vehicle control and 50 mg/kg female rats was also performed. The "wild" genotype at this locus characterizes an AhR receptor that can bind dioxin-like ligands; the mutant AhR genotype reduces ligand binding and some types of AhR downstream effects (Pohjanvirta *et al.*, 1993, 1998, 1999). The purpose of this study was to determine if the liver neoplasms in treated female rats were associated with a particular AhR genotype. Findings indicated that the 50 mg/kg female rat liver neoplasm response was independent of AhR genotype (Appendix M).

DE-71-related increases in liver EROD (CYP1A1) and A4H (CYP1A2) activities as seen in the current 3-month studies are characteristic of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and dioxin like chemicals (Waxman and Azaroff, 1992; Sanders *et al.*, 2005). In addition, hydronephrosis in rats has previously been seen after *in utero* exposure to both dioxin and polybrominated dibenzofurans (Couture *et al.*, 1990; Birnbaum *et al.*, 1991; Aragon *et al.*, 2008; Nishimura *et al.*, 2008).

The DE-71 used in these studies contained a mixture of lower molecular weight PBDEs (Appendix J). PBDEs have little or no ability to activate the AhR and are not assigned toxic equivalency factor (TEF) values (Peters *et al.*, 2004; Sanders *et al.*, 2005; van den Berg *et al.*, 2006). Chlorinated dioxins (e.g., TCDD) were below the limit of detection in the DE-71 mixture used in the current studies. Brominated dioxins and furans, which make up 7×10^{-6} % or approximately 70 ng/g of DE-71, were present in the mixture only at a low level (Appendix J). It is estimated that brominated dioxins and furans were delivered at approximately 3.5 ng/kg per day to rats at 50 mg/kg or 7 ng/kg per day to mice at 100 mg/kg. Generally, brominated dioxins and furans have lower TEFs than TCDD (Table J4), based on a range of *in vitro* and *in vivo* toxicity studies (van den Berg *et al.*, 2013; Frawley *et al.*, 2014; Venkatesan and Halden, 2014). When applying the TEF methodology (van den Berg *et al.*, 2013) this would translate to brominated dioxins and furans TEF delivery of approximately 0.35 ng/kg per day for high dose rats (50 mg/kg) and approximately 0.7 ng/kg per day for high dose mice (100 mg/kg). To put this in context, exposure to dioxin-equivalents from the brominated dioxins and dibenzofurans in the highest dose groups of DE-71 is lower than the lowest dose used in the NTP carcinogenicity studies of TCDD (NTP, 2006). In contrast, the level of CYP1A1 induction observed in the current DE-71 study is consistent with that observed in the highest dose group of the NTP carcinogenicity studies of TCDD (NTP, 2006). This suggests that using the present TEF methodology for brominated dioxins and dibenzofurans cannot explain the magnitude of the dioxin-like effect of DE-71. Since the major constituents of DE-71 are BDE-47 and BDE-99, there are several possible reasons for this difference. The TEF values for the brominated dioxins and dibenzofurans were based on acute exposure studies in mice or *in vitro* studies (van den Berg *et al.*, 2013) and may not accurately predict the relative potency of these chemicals for chronic exposures. Alternatively, or in combination, there may be components of DE-71 or their metabolites that are AhR ligands.

The observed DE-71 liver toxicity is consistent with activation of several receptor pathways including constitutive androstane receptor (CAR), pregnane X receptor (PXR), and the AhR. Activation of CAR and PXR results in induction of CYP2B and CYP3A, a phenobarbital-like effect (Elcombe *et al.*, 2014). Expression of CYP2B and CYP3A increased with an induction threshold between 1.5 and 15 mg/kg DE-71 in rats receiving three oral doses in corn oil by gavage for 3 consecutive days (Sanders *et al.*, 2005). These values are consistent with DE-71 effects on liver enzymes observed in the current 3-month studies. *In vitro* studies indicate that BDE-47 activation of CAR can also occur in human cells (Sueyoshi *et al.*, 2014). Activation of these nuclear hormone receptors is associated with increases in liver weights, hepatocellular hypertrophy, cell proliferation, and hepatocarcinogenesis (Hall *et al.*, 2012). Other mechanisms for PBDE carcinogenic activity in rats and mice may be related to oxidative stress and alterations in thyroid hormone homeostasis (Costa *et al.*, 2015; Usenko *et al.*, 2015). The hydroxylated metabolites are considered to be more toxic than the parent compounds (Su *et al.*, 2014). Oxidative damage from PBDEs and metabolites may be due to free radical formation. When rat pups were exposed *in utero* to BDE-99 there was an increase in the formation of reactive oxygen species in the liver (Blanco *et al.*, 2012, 2014). Production of reactive oxygen species may produce DNA damage (Finkel and Holbrook, 2000). PBDEs also affect levels of thyroid hormones which are critical regulators of hepatic lipid metabolism, and decreases in thyroid hormones may result in fatty livers (Sinha *et al.*, 2014) as were observed in the current DE-71 studies. Hypothyroidism may be a risk factor for hepatocellular carcinoma (Hassan *et al.*, 2009).

Significantly increased incidences of *Ctnnb1* mutations were noted in mouse hepatocellular carcinomas resulting from chronic exposure to DE-71 in the current study (Appendix N). Initiation and promotion experiments with a diethylnitrosamine (DEN) and phenobarbital protocol have demonstrated that neoplastic hepatocytes harboring *Ctnnb1* mutations have a selective growth advantage during the promotion stages of carcinogenesis (Aydinlik *et al.*, 2001). However, this effect was not noted in hepatocellular carcinomas of mice exposed to DEN alone suggesting that some of the phenobarbital promotion effects may be related to activation of CAR/PXR nuclear receptors. PBDE components within DE-71 can activate multiple nuclear receptors such as CAR, PXR, and AhR (Zhou *et al.*, 2001; Sanders *et al.*, 2005; Blanco *et al.*, 2012; Sueyoshi *et al.*, 2014) and may have contributed to the promotion effects of DE-71 (Pitot *et al.*, 1980; Schwarz *et al.*, 2000; Aydinlik *et al.*, 2001). DE-71 is nongenotoxic and may not directly cause somatic mutations and initiate carcinogenesis; however, metabolites of DE-71 including dihydroxylated PBDEs may cause oxidative stress (Lupton *et al.*, 2009; Blanco *et al.*, 2012) and subsequent DNA damage and somatic mutations in specific genes.

CONCLUSIONS

Under the conditions of these 2-year oral gavage studies, there was *clear evidence of carcinogenic activity** of DE-71 in male Wistar Han rats based on increased incidences of hepatocholangioma, hepatocellular adenoma, or hepatocellular carcinoma (combined). Increased incidences of thyroid gland follicular cell adenoma and increased incidences of pituitary gland (pars distalis) adenoma were also considered to be related to exposure. There was *clear evidence of carcinogenic activity* of DE-71 in female Wistar Han rats based on increased incidences of hepatocholangioma, hepatocellular adenoma, and hepatocellular carcinoma. The occurrence of cholangiocarcinoma of the liver was also considered related to treatment. The incidences of stromal polyp or stromal sarcoma (combined) of the uterus may have been related to treatment. There was clear *evidence of carcinogenic activity* of DE-71 in male B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma. There was *clear evidence of carcinogenic activity* of DE-71 in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma and hepatocellular carcinoma.

Administration of DE-71 resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, kidney, parotid salivary gland, prostate gland, preputial gland, thymus, and forestomach of male rats; liver, thyroid gland, uterus, cervix, kidney, and adrenal cortex of female rats; liver, thyroid gland, forestomach, adrenal cortex, and testes of male mice; and liver, thyroid gland, forestomach, and adrenal cortex of female mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Peer Review Panel comments and the public discussion on this Technical Report appears on page 16.

REFERENCES

Abdelouahab, N., AinMelk, Y., and Takser, L. (2011). Polybrominated diphenyl ethers and sperm quality. *Reprod. Toxicol*. **31**, 546-550.

Agency for Toxic Substances and Disease Registry (ATSDR) (2004). Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

An, J., Li, S., Zhong, Y., Wang, Y., Zhen, K., Zhang, X., Wang, Y., Wu, M., Yu, Z., Sheng, G., Fu, J., and Huang, Y. (2011). The cytotoxic effects of synthetic 6-hydroxylated and 6-methoxylated polybrominated diphenyl ether 47 (BDE47). *Environ. Toxicol.* **26**, 591-599.

Apostolopoulos, J.J., Howlett, G.J., and Fidge, N. (1987). Effects of dietary cholesterol and hypothyroidism on rat apolipoprotein mRNA metabolism. *J. Lipid Res.* **28**, 642-648.

Aragon, A.C., Kopf, P.G., Campen, M.J., Huwe, J.K., and Walker, M.K. (2008). In utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure: Effects on fetal and adult cardiac gene expression and adult cardiac and renal morphology. *Toxicol. Sci.* **101**, 321-330.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Athanasiadou, M., Cuadra, S.N., Marsh, G., Bergman, Å., and Jakobsson, K. (2008). Polybrominated diphenyl ethers (PBDEs) and bioaccumulative hydroxylated PBDE metabolites in young humans from Managua, Nicaragua. *Environ. Health Perspect.* **116**, 400-408.

Ausó, E., Lavado-Autric, R., Cuevas, E., Del Rey, F.E., De Escobar, G.M., and Berbel, P. (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. *Endocrinology* **145**, 4037-4047.

Aydinlik, H., Nguyen, T.D., Moennikes, O., Buchmann, A., and Schwarz, M. (2001). Selective pressure during tumor promotion by phenobarbital leads to clonal outgrowth of beta-catenin-mutated mouse liver tumors. *Oncogene* **20**, 7812-7816.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Barber, J.L., Walsh, M.J., Hewitt, R., Jones, K.C., and Martin, F.L. (2006). Low-dose treatment with polybrominated diphenyl ethers (PBDEs) induce altered characteristics in MCF-7 cells. *Mutagenesis* **21**, 351-360.

Berger, R.G., Lefèvre, P.L.C., Ernest, S.R., Wade, M.G., Ma, Y.-Q., Rawn, D.F.K, Gaertner, D.W., Robaire, B., and Hales, B.F. (2014). Exposure to an environmentally relevant mixture of brominated flame retardants affects fetal development in Sprague-Dawley rats. *Toxicology* **320**, 56-66.

Berry, P.H. (1986). Effect of diet or reproductive status on the histology of spontaneous pituitary tumors in female Wistar rats. *Vet. Pathol.* **23**, 606-618.

Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Bio-Rad Sadtler "KnowItAll"® Digital Infrared Libraries (2003): "IR-Polymers and Monomers (Basic) 1" (Unmodified Polymers and Monomers) library, spectrum BP: 962.

Birnbaum, L.S., Morrissey, R.E., and Harris, M.W. (1991). Teratogenic effects of 2,3,7,8-tetrabromodibenzo-p-dioxin and three polybrominated dibenzofurans in C57BL/6N mice. *Toxicol. Appl. Pharmacol.* **107**, 141-152.

Blanco, J., Mulero, M., Domingo, J.L., and Sánchez, D.J. (2012). Gestational exposure to BDE-99 produces toxicity through upregulation of CYP isoforms and ROS production in the fetal rat liver. *Toxicol. Sci.* **127**, 296-302.

Blanco, J., Mulero, M., Heredia, L., Pujol, A., Domingo, J.L., and Sánchez, D.J. (2013). Perinatal exposure to BDE-99 causes learning disorders and decreases serum thyroid hormone levels and BDNF gene expression in hippocampus in rat offspring. *Toxicology* **308**, 122-128.

Blanco, J., Mulero, M., Domingo, J.L., and Sanchez, D.J. (2014). Perinatal exposure to BDE-99 causes decreased protein levels of cyclin D1 via GSK3β activation and increased ROS production in rat pup livers. *Toxicol. Sci.* **137**, 491-498.

Boelaert, K. (2009). The association between serum TSH concentration and thyroid cancer. *Endocr. Relat. Cancer* **16**, 1065-1072.

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.

Bradner, J.M., Suragh, T.A., Wilson, W.W., Lazo, C.R., Stout, K.A., Kim, H.M., Wang, M.Z., Walker, D.I., Pennell, K.D., Richardson, J.R., Miller, G.W., and Caudle, W.M. (2013). Exposure to the polybrominated diphenyl ether mixture DE-71 damages the nigrostriatal dopamine system: Role of dopamine handling in neurotoxicity. *Exp. Neurol.* **241**, 138-147.

Braekevelt, E., Tittlemeir, S.A., and Tomy, G.T. (2003). Direct measurement of octanol-water partition coefficients of some environmentally relevant brominated diphenyl ether congeners. *Chemosphere* **51**, 563-567.

Branchi, I., Alleva, E., and Costa, L.G. (2002). Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. *Neurotoxicology* **23**, 375-384.

Branchi, I., Santucci, D., Puopolo, M., and Alleva, E. (2004). Neonatal behaviors associated with ultrasonic vocalizations in mice (Mus musculus): A slow-motion analysis. *Dev. Psychobiol.* **44**, 37-44.

Branchi, I., Capone, F., Vitalone, A., Madia, F., Santucci, D., Alleva, E., and Costa, L.G. (2005). Early developmental exposure to BDE 99 or Aroclor 1254 affects neurobehavioural profile: Interference from the administration route. *Neurotoxicology* **26**, 183-192.

Brix, A.E., Hardisty, J.F., and McConnell, E.E. (2010). Combining neoplasms for evaluation of rodent carcinogenesis studies. In *Cancer Risk Assessment: Chemical Carcinogenesis, Hazard Evaluation, and Risk Quantification* (C.-H. Hsu and T. Stedeford, Eds.), pp. 699-714. John Wiley & Sons, Inc., Hoboken, NJ.

Butt, C.M., Wang, D., and Stapleton, H.M. (2011). Halogenated phenolic contaminants inhibit the *in vitro* activity of the thyroid-regulating deiodinases in human liver. *Toxicol. Sci.* **124**, 339-347

California Department of Consumer Affairs (CDCA) (2013). Proposed regulations: New flammability standards for upholstered furniture and articles exempt from flammability standards. Bureau of Electronic and Appliance Repair, Home Furnishings and Thermal Insulation. <http://www.bhfti.ca.gov/about/laws/propregs.shtml>

Carignan, C.C., Heiger-Bernays, W., McClean, M.D., Roberts, S.C., Stapleton, H.M., Sjödin, A., and Webster, T.F. (2013). Flame retardant exposure among collegiate United States gymnasts. *Environ. Sci. Technol.* **47**, 13,848-13,856.

Ceccatelli, R., Faass, O., Schlumpf, M., and Lichtensteiger, W. (2006). Gene expression and estrogen sensitivity in rat uterus after developmental exposure to the polybrominated diphenylether PBDE 99 and PCB. *Toxicology* **220**, 104-116.

Chang, T.K.H., and Waxman, D.J. (1998). Enzymatic analysis of cDNA-expressed human CYP1A1, CYP1A2, and CYP1B1 with 7-ethoxyresorufin as substrate. *Methods Mol. Biol.* **107**, 103-110.

Chao, H.-R., Wang, S.-L., Lee, W.-J., Wang, Y.-F., and Päpke, O. (2007). Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. *Environ. Int.* **33**, 239-245.

Chen, A., Park, J.-S., Linderholm, L., Rhee, A., Petreas, M., DeFranco, E.A., Dietrich, K.N., and Ho, S.-M. (2013). Hydroxylated polybrominated diphenyl ethers in paired maternal and cord sera. *Environ. Sci. Technol.* **47**, 3902-3908.

Chen, A., Yolton, K., Rauch, S.A., Webster, G.M., Hornung, R., Sjödin, A., Dietrich, K.N., and Lanphear, B.P. (2014). Prenatal polybrominated diphenyl ether exposures and neurodevelopment in U.S. children through 5 years of age: The HOME study. *Environ. Health. Perspect.* **122**, 856-862.

Chen, D., and Hale, R.C. (2010). A global review of polybrominated diphenyl ether flame retardant contamination in birds. *Environ. Int.* **36**, 800-811.

Chen, G., Konstantinov, A.D., Chittim, B.G., Joyce, E.M., Bols, N.C., and Bunce, N.J. (2001). Synthesis of polybrominated diphenyl ethers and their capacity to induce CYP1A by the Ah receptor medidated pathway. *Environ. Sci. Technol.* **35**, 3749-3756.

Chen, L.-J., Lebetkin, E.H., Sanders, J.M., and Burka, L.T. (2006). Metabolism and disposition of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE99) following a single or repeated administration to rats or mice. *Xenobiotica* **36**, 515-534.

Chen, S.-J., Ma, Y.-J., Wang, J., Chen, D., Luo, X.-J., and Mai, B.-X. (2009). Brominated flame retardants in children’s toys: Concentration, composition, and children’s exposure and risk assessment. *Environ. Sci. Technol.* **43**, 4200-4206.

Cheng, J., Gu, J., Ma, J., Chen, X., Zhang, M., and Wang, W. (2009). Neurobehavioural effects, redox responses and tissue distribution in rat offspring developmental exposure to BDE-99. *Chemosphere* **75**, 963-968.

Chevrier, J., Harley, K.G., Bradman, A., Gharbi, M., Sjödin, A., and Eskenazi, B. (2010). Polybrominated diphenyl ether (PBDE) flame retardants and thyroid hormone during pregnancy. *Environ. Health Perspect.* **118**, 1444-1449.

Code of Federal Regulations (CFR) **21**, Part 58.

Conover, W.J. (1971). *Practical Nonparametric Statistics.* John Wiley & Sons, New York.

Cooke, P.S., Kirby, J.D., and Porcelli, J. (1993). Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: Optimization of the propylthiouracil dose and effects of methimazole. *J. Reprod. Fertil.* **97**, 493-499.

Costa, L.G., Pellacani, C., Dao, K., Kavanagh, T.J., and Roque, P.J. (2015). The brominated flame retardant BDE-47 causes oxidative stress and apoptotic cell death *in vitro* and *in vivo* in mice. *Neurotoxicology* **48**, 68-76.

Couture, L.A., Abbott, B.D., and Birnbaum, L.S. (1990). A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: Recent advances toward understanding the mechanism. *Teratology* **42**, 619-627.

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

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Creasy, D., Bube, A., de Rijk, E., Kandori, H., Kuwahara, M., Masson, R., Nolte, T., Reams, R., Regan, K., Rehm, S., Rogerson, P., and Whitney K. (2012). Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol. Pathol.* **40**, 40S-121S.

Dallaire, R., Ayotte, P., Pereg, D., Déry, S., Dumas, P., Langlois, E., and Dewailly, E. (2009). Determinants of plasma concentrations of perfluorooctanesulfonate and brominated organic compounds in Nunavik Inuit adults (Canada). *Environ. Sci. Technol.* **43**, 5130-5136.

Darnerud, P.O., and Risberg, S. (2006). Tissue localisation of tetra- and pentabromodiphenyl ether congeners (BDE-47, -85 and -99) in perinatal and adult C57BL mice. *Chemosphere* **62**, 485-493.

Darras, V.M. (2008). Endocrine disrupting polyhalogenated organic pollutants interfere with thyroid hormone signalling in the developing brain. *Cerebellum* **7**, 26-37.

Das, K.C., Mukherjee, M., Sarkar, T.K., Dash, R.J., and Rastogi, G.K. (1975). Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. *J. Clin. Endocrinol. Metab.* **40**, 211-220.

Dingemans, M.M., Ramakers, G.M., Gardoni, F., van Kleef, R.G., Bergman, A., Di Luca, M., van den Berg, M., Westerink, R.H., and Vijverberg, H.P. (2007). Neonatal exposure to brominated flame retardant BDE-47 reduces long-term potentiation and postsynaptic protein levels in mouse hippocampus. *Environ. Health Perspect.* **115**, 865-870.

Dingemans, M.M.L., van den Berg, M., and Westerink, R.H.S (2011). Neurotoxicity of brominated flame retardants: (In)direct effects of parent and hydroxylated polybrominated diphenyl ethers on the (developing) nervous system. *Environ. Health Perspect.* **119**, 900-907.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Donati, R.M., Fletcher, J.W., Warnecke, M.A., and Gallagher, N.I. (1973). Erythropoiesis in hypothyroidism. *Proc. Soc. Exp. Biol. Med.* **144**, 78-82.

Dory, L., and Roheim, P.S. (1981). Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. *J. Lipid Res.* **22**, 287-296.

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**, 1096-1121.

Duntas, L.H., and Brenta, G. (2012). The effect of thyroid disorders on lipid levels and metabolism. *Med. Clin. North Am.* **96**, 269-281.

Elcombe, C.R., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W., and Lake, B.G. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. *Crit. Rev. Toxicol.* **44**, 64-82

Emond, C., Sanders, J.M., Wikoff, D., and Birnbaum, L.S. (2013). Proposed mechanistic description of dose-dependent BDE-47 urinary elimination in mice using a physiologically based pharmacokinetic model. *Toxicol. Appl. Pharmacol.* **273**, 335-344.

Eriksson, P., Jakobsson, E., and Fredriksson, A. (2001). Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* **109**, 903-908.

Ernest, S.R., Wade, M.G., Lalancette, C., Ma, Y.Q., Berger, R.G., Robaire, B., and Hales, B.F. (2012). Effects of chronic exposure to an environmentally relevant mixture of brominated flame retardants on the reproductive and thyroid system in adult male rats. *Toxicol. Sci*, **127**, 496-507.

Erratico, C.A., Szeitz, A., and Bandiera, S.M. (2012). Oxidative metabolism of BDE-99 by human liver microsomes: Predominant role of CYP2B6. *Toxicol. Sci.* **129**, 280-292.

Erratico, C.A., Szeitz, A., and Bandiera, S.M. (2013). Biotransformation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) by human liver microsomes: Identification of cytochrome P450 2B6 as the major enzyme involved. *Chem. Res. Toxicol.* **26**, 721-731.

Eskenazi, B., Fenster, L., Castorina, R., Marks, A.R., Sjödin, A., Rosas, L.G., Holland, N., Guerra, A.G., Lopez-Carrillo, L., and Bradman, A. (2011). A comparison of PBDE serum concentrations in Mexican and Mexican-American children living in California. *Environ. Health Perspect.* **119**, 1442-1448.

Eskenazi, B., Chevrier, J., Rauch, S.A., Kogut, K., Harley, K.G., Johnson, C., Trujillo, C., Sjödin, A., and Bradman, A. (2013). *In utero* and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. *Environ. Health Perspect.* **121**, 257-262.

European Chemicals Bureau (ECB) (2001). European Union Risk Assessment Report. Diphenyl ether, pentabromo derivative. CAS No. 32534-81-9, ElNECS No. 251-084-2. Office for Official Publications of the European Communities.

European Food Safety Authority (EFSA) (2011). Scientific opinion on polybrominated diphenyl ethers (PBDEs) in food. *EFSA J.* **9**, 1-274.

European Parliament and the Council of the European Union (EPCEU) (2003). Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003, amending for the 24th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octobromodiphenyl ether). *Off. J. Eur. Union* **15.2.2003**, L42/45-L42/46.

Fair, P.A., Stavros, H.-C., Mollenhauer, M.A.M., Dewitt, J.C., Henry, N., Kannan, K., Yun, S.H., Bossart, G.D., Keil, D.E., and Peden-Adams, M.M. (2012). Immune function in female $B_6C_3F_1$ mice is modulated by DE-71, a commercial polybrominated diphenyl ether mixture. *J. Immunotoxicol.* **9**, 96-107.

Farber, E. (1980). Toxicological significance of liver hypertrophy produced by inducers of drug-metabolizing enzymes. *Ciba Found. Symp.* **76**, 261-274.

Federal Register (2012). Certain Polybrominated Diphenylethers; Significant New Use Rule and Test Rule. Vol. **77**, 19,862-19,899. U.S. Environmental Protection Agency, Washington, DC.

Feo, M.L., Gross, M.S., McGarrigle, B.P., Eljarrat, E., Barceló, D., Aga, D.S., and Olson, J.R. (2013). Biotransformation of BDE-47 to potentially toxic metabolites is predominantly mediated by human CYP2B6. *Environ. Health Perspect.* **121**, 440-446.

Finkel, T., and Holbrook, N.J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature,* **408**, 239-247.

Fischer, D., Hooper, K., Athanasiadou, M., Athanassiadis, I., and Bergman, Å. (2006). Children show highest levels of polybrominated diphenyl ethers in a California family of four: A case study. *Environ. Health Perspect.* **114**, 1581-1584.

Foster, W.G., Gregorovich, S., Morrison, K.M., Atkinson, S.A., Kubwabo, C., Stewart, B., and Teo, K. (2011). Human maternal and umbilical cord blood concentrations of polybrominated diphenyl ethers. *Chemosphere* **84**, 1301-1309.

Fowles, J.R., Fairbrother, A., Baecher-Steppan, L., and Kerkvliet, N.I. (1994). Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology* **86**, 49-61.

Frawley, R., DeVito, M., Walker, N.J., Birnbaum, L., White, K. Jr., Smith, M., Maynor, T., Recio, L., and Germolec, D. (2014). Relative potency for altered humoral immunity induced by polybrominated and polychlorinated dioxins/furans in female B6C3F1/N mice. *Toxicol Sci.* **139**, 488-500.

Frederiksen, M., Thomsen, M., Vorkamp, K., and Knudsen, L.E. (2009). Patterns and concentration levels of polybrominated diphenyl ethers (PBDEs) in placental tissue of women in Denmark. *Chemosphere* **76**, 1464-1469.

Frederiksen, M., Thomsen, C., Frøshaug, M., Vorkamp, K., Thomsen, M., Becher, G., and Knudsen, L.E. (2010a). Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a Danish cohort. *Int. J. Hyg. Environ. Health* **213**, 233-242.

Frederiksen, M., Vorkamp, K., Mathiesen, L., Mose, T., and Knudsen, L.E. (2010b). Placental transfer of the polybrominated diphenyl ethers BDE-47, BDE-99 and BDE-209 in a human placenta perfusion system: An experimental study. *Environ. Health* **9**, 1-10.

Fruhman, G.J. (1966). Effects of starvation and refeeding on erythropoiesis in mice. *Z. Zellforsch. Mikrosk. Anat.* **75**, 258-271.

Gao, P., He, P., Wang, A., Xia, T., Xu, B., Xu, Z., Niu, Q., Guo, L., and Chen, X. (2009). Influence of PCB153 on oxidative DNA damage and DNA repair-related gene expression induced by PBDE-47 in human neuroblastoma cells in vitro. *Toxicol. Sci.* **107**, 165-170.

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

Gascon, M., Vrijheid, M., Martínez, D., Forns, J., Grimalt, J.O., Torrent, M., and Sunyer, J. (2011). Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. *Environ. Int.* **37**, 605-611.

Gee, J.R., and Moser, V.C. (2008). Acute postnatal exposure to brominated diphenylether 47 delays neuromotor ontogeny and alters motor activity in mice. *Neurotoxicol. Teratol.* **30**, 79-87.

Geyer, H.J., Schramm, K.-W., Darnerud, P.O., Aune, M., Feicht, E.A., Fried, K.W., Henkelmann, B., Lenoir, D., Schmid, P., and McDonald, T.A. (2004). Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. *Organohalogen Compounds* **66**, 3820-3825.

Girard, D.M., and Sager, D.B. (1987). The use of Markov chains to detect subtle variation in reproductive cycling. *Biometrics* **43**, 225-234.

Gosavi, R.A., Knudsen, G.A., Birnbaum, L.S., and Pedersen, L.C. (2013). Mimicking of estradiol binding by flame retardants and their metabolites: A crystallographic analysis. *Environ. Health Perspect.* **121**, 1194-1199.

Guide for the Care and Use of Laboratory Animals (2011). 8th ed. Institute for Laboratory Animal Research, National Research Council. National Academies Press, Washington, DC.

Guvenius, D.M., Aronsson, A., Ekman-Ordeberg, G., Bergman, A., and Norén, K. (2003). Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol. *Environ. Health Perspect.* **111**, 1235-1241.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Faix, J.D., and Klein, R.Z. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N. Engl. J. Med.* **341**, 549-555.

Hakk, H., Larsen, G., and Klasson-Wehler, E. (2002). Tissue disposition, excretion and metabolism of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) in the male Sprague-Dawley rat. *Xenobiotica* **32**, 369-382.

Hakk, H., Huwe, J., Low, M., Rutherford, D., and Larsen, G. (2006). Tissue disposition, excretion and metabolism of 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100) in male Sprague-Dawley rats. *Xenobiotica* **36**, 79-94.

Hakk, H., Huwe, J.K., and Larsen, G.L. (2009). Absorption, distribution, metabolism and excretion (ADME) study with 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154) in male Sprague-Dawley rats. *Xenobiotica* **39**, 46-56.

Hale, R.C., La Guardia, M.J., Harvey, E.P., Gaylor, M.O., Mainor, T.M., and Duff, W.H. (2001). Flame retardents: Persistant pollutants in land-applied sludges. *Nature* **412**, 140-141.

Hale, R.C., La Guardia, M.J., Harvey, E., and Mainor, T.M. (2002). Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment. *Chemosphere* **46**, 729-735.

Hale, R.C., Alaee, M., Manchester-Neesvig, J.B., Stapleton, H.M., and Ikonomou, M.G. (2003). Polybrominated diphenyl ether flame retardants in the North American environment. *Environ. Int.* **29**, 771-779.

Hall, A.P., Elcombe, C.R., Foster, J.R., Harada, T., Kaufmann, W., Knippel, A., Küttler, K., Malarkey, D.E., Maronpot, R.R., Nishikawa, A., Nolte, T., Schulte, A., Strauss, V., and York, M.J. (2012). Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes - Conclusions from the 3rd International ESTP Expert Workshop. *Toxicol. Pathol.* **40**, 971-994.

Hallgren, S., Sinjari, T., Håkansson, H., and Darnerud, P.O. (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.* **75**, 200-208.

Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Kester, M.H.A, Andersson, P.L., Legler, J., and Brouwer, A. (2006). *In vitro* profiling of the endocrine-disrupting potency of brominated flame retardants. *Toxicol. Sci.* **92**, 157-173.

Hamm, J.T., Ross, D.G., Richardson, V.M., Diliberto, J.J., and Birnbaum, L.S. (1998). Methoxyresorufin: An inappropriate substrate for CYP1A2 in the mouse. *Biochem. Pharmacol.* **56**, 1657-1660.

Hardell, L., van Bavel, B., Lindström, G., Eriksson, M., and Carlberg, M. (2006). In utero exposure to persistent organic pollutants in relation to testicular cancer risk. *Int. J. Androl.* **29**, 228-234.

Hardisty, J.F., and Boorman, G.A. (1990). Thyroid Gland. 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. 519-536. Academic Press, Inc., San Diego.

Harley, K.G., Marks, A.R., Chevrier, J., Bradman, A., Sjödin, A., and Eskenazi, B. (2010). PBDE concentrations in women's serum and fecundability. *Environ. Health Perspect.* **118**, 699-704.

Harley, K.G., Chevrier, J., Aquilar Schall, R., Sjödin, A., Bradman, A., and Eskenazi, B. (2011). Association of prenatal exposure to polybrominated diphenyl ethers and infant birth weight. *Am. J. Epidemiol.* **174**, 885-892.

Harrad, S., Goosey, E., Desborough, J., Abdallah, M.A.-E, Roosens, L., and Covaci, A. (2010). Dust from U.K. primary school classrooms and daycare centers: The significance of dust as a pathway of exposure of young U.K. children to brominated flame retardants and polychlorinated biphenyls. *Environ. Sci. Technol.* **44**, 4198-4202.

Haseman, J.K. (1992). Value of historical controls in the interpretation of rodent tumor data. *Drug Inf. J.* **26**, 191-200.

Haseman, J.K. (1995). Data analysis: Statistical analysis and use of historical control data. *Regul. Toxicol. Pharmacol.* **21**, 52-59.

Haseman, J.K., and Rao, G.N. (1992). Effects of corn oil, time-related changes, and inter-laboratory variability on tumor occurrence in control Fischer 344 (F344/N) rats. *Toxicol. Pathol.* **20**, 52-60.

Hassan, M.M., Kaseb, A., Li, D., Part, Y.Z., Vauthey, J.-N., Thomas, M.B., Curley, S.A., Spitz, M.R., Sherman, S.I., Abdalla, E.K., Davila, M., Lozano, R.D., Hassan, D.M., Chan, W., Brown, T.D., and Abbruzzese, J.L. (2009). Association between hypothyroidism and hepatocellular carcinoma: A case-control study in the United States. *Hepatology* **49**, 1563-1570.

He, P., Wang, A.-G., Xia, T., Gao, P., Niu, Q., Guo, L.-J., and Chen, X.-M. (2009). Mechanisms underlying the developmental neurotoxic effect of PBDE-47 and the enhanced toxicity associated with its combination with PCB153 in rats. *Neurotoxicology* **30**, 1088-1095.

He, P., Wang, A., Niu, Q., Guo, L., Xia, T., and Chen, X. (2011). Toxic effect of PBDE-47 on thyroid development, learning, and memory, and the interaction between PBDE-47 and PCB153 that enhances toxicity in rats. *Toxicol. Ind. Health* **27**, 279-288.

He, W., He, P., Wang, A., Xia, T., Xu, B., and Chen, X. (2008). Effects of PBDE-47 on cytotoxicity and genotoxicity in human neuroblastoma cells in vitro. *Mutat. Res.* **649**, 62-70.

He, W., Wang, A., Xia, T., Gao, P., Xu, B., Xu, Z., He, P., and Chen, X. (2010). Cytogenotoxicity induced by PBDE-47 combined with PCB153 treatment in SH-SY5Y cells. *Environ. Toxicol.* **25** 564-572.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* **123**, 61-118.

Herbstman, J.B., Sjödin, A., Apelberg, B.J., Witter, F.R., Halden, R.U., Patterson, D.G., Jr., Panny, S.R., Needham, L.L., and Goldman, L.R. (2008). Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. *Environ. Health Perspect.* **116**, 1376-1382.

Herbstman, J.B., Sjödin, A., Kurzon, M., Lederman, S.A., Jones, R.S., Rauh, V., Needham, L.L., Tang, D., Niedzwiecki, M., Wang, R.Y., and Perera, F. (2010). Prenatal exposure to PBDEs and neurodevelopment. *Environ. Health Perspect.* **118**, 712-719.

Huang, H., Zhang, S., and Christie, P. (2011). Plant uptake and dissipation of PBDEs in the soils of electronic waste recycling sites. *Environ. Pollut.* **159**, 238-243.

Hurley, S., Reynolds, P., Goldberg, D., Nelson, D.O., Jeffrey, S.S., and Petreas, M. (2011). Adipose levels of polybrominated diphenyl ethers and risk of breast cancer. *Breast Cancer Res. Treat*. **129**, 505-511.

Huwe, J.K., and West, M. (2011). Polybrominated diphenyl ethers in U.S. meat and poultry from two statistically designed surveys showing trends and levels from 2002 to 2008. *J. Agric. Food Chem.* **59**, 5428-5434.

Ji, K., Choi, K., Giesy, J.P., Musarrat, J., and Takeda, S. (2011). Genotoxicity of several polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs, and their mechanisms of toxicity. *Environ. Sci. Technol.* **45**, 5003-5008.

Jiang, H., Lin, Z., Wu, Y., Chen, X., Hu, Y., Li, Y., Huang, C., and Dong, Q. (2014). Daily intake of polybrominated diphenyl ethers via dust and diet from an e-waste recycling area in China. *J. Hazard. Mater.* **276**, 35-42.

Johnson, P.I., Stapleton, H.M., Sjodin, A., and Meeker, J.D. (2010). Relationships between polybrominated diphenyl ether concentrations in house dust and serum. *Environ. Sci. Technol.* **44**, 5627-5632.

Johnson, P.I., Stapleton, H.M., Mukherjee, B., Hauser, R., and Meeker, J.D. (2013). Associations between brominated flame retardants in house dust and hormone levels in men. *Sci. Total Environ.* **445-446**, 177-184.

Johnson-Restrepo, B., Kannan, K., Rapaport, D.P., and Rodan, B.D. (2005). Polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue from New York. *Environ. Sci. Technol.* **39**, 5177-5182.

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.

King-Herbert, A., and Thayer, K. (2006). NTP Workshop: Animal models for the NTP rodent cancer bioassay: Stocks and strains – should we switch? *Toxicol. Pathol.* **34**, 802-805.

Kissling, G.E., Dertinger, S.D., Hayashi, M., and MacGregor, J.T. (2007). Sensitivity of the erythrocyte micronucleus assay: Dependence on number of cells scored and inter-animal variability. *Mutat. Res.* **634**, 235-240.

Kodavanti, P.R.S., Coburn, C.G., Moser, V.C., MacPhail, R.C., Fenton, S.E., Stoker, T.E., Rayner, J.L., Kannan, K., and Birnbaum, L.S. (2010). Developmental exposure to a commercial PBDE mixture, DE-71: Neurobehavioral, hormonal, and reproductive effects. *Toxicol. Sci.* **116**, 297-312.

Kuriyama, S.N., Talsness, C.E., Grote, K., and Chahoud, I. (2005). Developmental exposure to low-dose PBDE-99: Effects on male fertility and neurobehavior in rat offspring. *Environ. Health Perspect.* **113**, 149-154.

Kuriyama, S.N., Wanner, A., Fidalgo-Neto, A.A., Talsness, C.E., Koerner, W., and Chahoud, I. (2007). Developmental exposure to low-dose PBDE-99: Tissue distribution and thyroid hormone levels. *Toxicology* **242**, 80-90.

Labunska, I., Harrad, S., Wang, M., Santillo, D., and Johnston, P. (2014). Human dietary exposure to PBDEs around E-waste recycling sites in Eastern China. *Environ. Sci. Technol.* **48**, 5555-5564.

Lasky, R.E., Widholm, J.J., Crofton, K.M., and Schantz, S.L. (2002). Perinatal exposure to Aroclor 1254 impairs distortion product otoacoustic emissions (DPOAEs) in rats. *Toxicol. Sci.* **68**, 458-464.

Law, R.J., Covaci, A., Harrad, S., Herzke, D., Abdallah, M.A.-E., Fernie, K., Toms, L.-M.L., and Takigami, H. (2014). Levels and trends of PBDEs and HBCDs in the global environment: Status at the end of 2012. *Environ. Int.* **65**, 147-158.

Lazarus, J.H. (2005a). Thyroid disease in pregnancy and childhood. *Minerva Endocrinol.* **30**, 71-87.

Lazarus, J.H. (2005b). Thyroid disorders associated with pregnancy: Etiology, diagnosis, and management. *Treat. Endocrinol.* **4**, 31-41.

Lee, L.K., and He, J. (2010). Reductive debromination of polybrominated diphenyl ethers by anaerobic bacteria from soils and sediments. *Appl. Environ. Microbiol.* **76**, 794-802.

Lee, L.K., Ding, C., Yang, K.-L., and He, J. (2011). Complete debromination of tetra- and penta-brominated diphenyl ethers by a coculture consisting of *Dehalococcoides* and *Desulfovibrio* species. *Environ. Sci. Technol.* **45**, 8475-8482.

Lignell, S., Aune, M., Darnerud, P.O., Hanberg, A., Larsson, S.C., and Glynn, A. (2013). Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure: A cross-sectional study. *Environ. Health* **12**, 1-9.

Lilienthal, H., Hack, A., Roth-Härer, A., Grande, S.W., and Talsness, C.E. (2006). Effects of developmental exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. *Environ. Health Perspect*. **114**, 194-201.

Lorber, M. (2008). Exposure of Americans to polybrominated diphenyl ethers. *J. Expo. Sci. Environ. Epidemiol.* **18**, 2-19.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Lubet, R.A., Mayer, R.T., Cameron, J.W., Nims, R.W., Burke, M.D., Wolff, T., and Guengerich, F.P. (1985). Dealkylation of pentoxyresorufin: A rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys.* **238**, 43-48.

Lupton, S.J., McGarrigle, B.P., Olson, J.R., Wood, T.D., and Aga, D.S. (2009). Human liver microsome-mediated metabolism of brominated diphenyl ethers 47, 99, and 153 and identification of their major metabolites. *Chem. Res. Toxicol.* **22**, 1802-1809.

McCullagh, P., and Nelder, J.A. (1989). *Generalized Linear Models*, 2nd ed. Chapman and Hall, New York.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Mackay, D., Shiu, W.Y., Ma, K.-C., and Lee, S.C. (2006). *Physical-Chemical Properties and Environmental Fate for Organic Chemicals, Second Edition: Volume III, Oxygen Containing Compounds*, pp. 2436-2437, 2446-2447. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J., Vartiainen, T., Skakkebaek, N.E., and Toppari, J. (2007). Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. *Environ. Health Perspect.* **115**, 1519-1526.

Malgor, L.A., Blanc, C.C., Klainer, E., Irizar, S.E., Torales, P.R., and Barrios, L. (1975). Direct effects of thyroid hormones on bone marrow erythroid cells of rats. *Blood* **45**, 671-679.

Malmberg, T., Athanasiadou, M., Marsh, G., Brandt, I., and Bergman, Å. (2005). Identification of hydroxylated polybrominated diphenyl ether metabolites in blood plasma from polybrominated diphenyl ether exposed rats. *Environ. Sci. Technol.* **39**, 5342-5348.

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.

Marsh, G., Bergman, Å., Bladh, L-.G., Gillner, M., and Jakobsson, E. (1998). Synthesis of *p*-hydroxybromodiphenyl ethers and binding to thyroid receptor. *Organohalogen Compounds* **37**, 305-308.

Marsh, G., Athanasiadou, M., Athanassiadis, I., and Sandholm, A. (2006). Identification of hydroxylated metabolites in 2,2′,4,4′-tetrabromodiphenyl ether exposed rats. *Chemosphere* **63**, 690-697.

Mazdai, A., Dodder N.G., Abernathy, M.P., Hites, R.A., and Bigsby, R.M. (2003). Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environ. Health Perspect.* **111**, 1249-1252.

Meeker, J.D., Johnson, P.I., Camann, D., and Hauser, R. (2009). Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. *Sci. Total Environ.* **407**, 3425-3429.

Meerts, I.A.T.M., van Zanden, J.J., Luijks, E.A.C., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, Å., and Brouwer, A. (2000). Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin *in vitro*. *Toxicol. Sci.* **56**, 95-104.

Meerts, I.A.T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, Å., Lemmen, J.G., van der Burg, B., and Brouwer, A. (2001). *In vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.* **109**, 399-407.

Meijer, L., Martijn, A., Melessen, J., Brouwer, A., Weiss, J., de Jong, F.H., and Sauer, P.J.J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: Sex hormone levels, testes volume and penile length. *Hum. Reprod.* **27**, 867-872.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

National Toxicology Program (NTP) (2006). Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 521. NIH Publication No. 06-4455. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

Nishimura, N., Matsumura, F., Vogel, C.F., Nishimura, H., Yonemoto, J., Yoshioka, W., and Tohyama, C. (2008). Critical role of cyclooxygenase-2 activation in pathogenesis of hydronephrosis caused by lactational exposure of mice to dioxin. *Toxicol. Appl. Pharmacol.* **231**, 374-383.

Örn, U., and Klasson-Wehler, E. (1998). Metabolism of 2,2′,4,4′-tetrabromodiphenyl ether in rat and mouse. *Xenobiotica* **28**, 199-211.

Ortega, E., Rodriguez, E., Ruiz, E., and Osorio, C. (1990). Activity of the hypothalamo-pituitary ovarian axis in hypothyroid rats with or without triiodothyronine replacement. *Life Sci.* **46**, 391-395.

Park, J.-S., She, J., Holden, A., Sharp, M., Gephart, R., Souders-Mason, G., Zhang, V., Chow, J., Leslie, B., and Hooper, K. (2011). High postnatal exposures to polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) via breast milk in California: Does BDE-209 transfer to breast milk? *Environ. Sci. Technol.* **45**, 4579-4585.

Pellacani, C., Buschini, A., Galati, S., Mussi, F., Franzoni, S., and Costa, L.G. (2012). Evaluation of DNA damage induced by 2 polybrominated diphenyl ether flame retardants (BDE-47 and BDE-209) in SK-N-MC cells. *Int. J. Toxicol.* **31**, 372-379.

Perrin, M.C., Blanchet, J.P., and Mouchiroud, G. (1997). Modulation of human and mouse erythropoiesis by thyroid hormone and retinoic acid: Evidence for specific effects at different steps of the erythroid pathway. *Hematol. Cell Ther.* **39**, 19-26.

Peters, A.K., van Londen, K., Bergman, Å., Bohonowych, J., Denison, M.S., van den Berg, M., and Sanderson, J.T. (2004). Effects of polybrominated diphenyl ethers on basal and TCDD-induced ethoxyresorufin activity and cytochrome P450-1A1 expression in MCF-7, HepG2, and H4IIE cells. *Toxicol. Sci.* **82**, 488-496.

Peters, A.K, Nijmeijer, S., Gradin, K., Backlund, M., Bergman, Å, Poellinger, L., Denison, M.S., and Van den Berg, M. (2006a). Interactions of polybrominated diphenyl ethers with the aryl hydrocarbon receptor pathway. *Toxicol. Sci.* **92**, 133-142.

Peters, A.K, Sanderson, J.T., Bergman, Å, and van den Berg, M. (2006b). Antagonism of TCDD-induced ethoxyresorufin-*O*-deethylation activity by polybrominated diphenyl ethers (PBDEs) in primary cynomolgus monkey (*Macaca fascicularis*) hepatocytes. *Toxicol. Lett.* **164**, 123-132.

Petreas, M., She, J., Brown, F.R., Winkler, J., Windham, G., Rogers, E., Zhao, G., Bhatia, R., and Charles, M.J. (2003). High body burdens of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in California women. *Environ. Health Perspect.* **111**, 1175-1179.

Petreas, M., Nelson, D., Brown, F.R., Goldberg, D., Hurley, S., and Reynolds, P. (2011). High concentrations of polybrominated diphenylethers (PBDEs) in breast adipose tissue of California women. *Environ. Int.* **37**, 190-197.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology*, Section 6.3.2. Chapman and Hall, London.

Pitot, H.C., Goldsworthy, T., Campbell, H.A., and Poland, A. (1980). Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. *Cancer Res.* **40**, 3616-3620.

Pohjanvirta, R., Unkila, M., and Tuomisto, J. (1993). Comparative acute lethality of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin and 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin in the most TCDD-susceptible and the most TCDD-resistant rat strain. *Pharmacol. Toxicol.* **73**, 52-56.

Pohjanvirta, R., Wong, J.M., Li, W., Harper, P.A., Tuomisto, J., and Okey, A.B. (1998). Point mutation in intron sequence causes altered carboxyl-terminal structure in the aryl hydrocarbon receptor of the most 2,3,7,8-tetrachlorodibenzo-p-dioxin-resistant rat strain. *Mol. Pharmacol.* **54**, 86-93.

Pohjanvirta, R., Viluksela, M., Tuomisto, J.T., Unkila, M., Karasinska, J., Franc, M.A., Holowenko, M., Giannone, J.V., Harper, P.A., Tuomisto, J., and Okey, A.B. (1999). Physicochemical differences in the AH receptors of the most TCDD-susceptible and the most TCDD-resistant rat strains. *Toxicol. Appl. Pharmacol.* **155**, 82-95.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Qiu, X., Mercado-Feliciano, M., Bigsby, R.M., and Hites, R.A. (2007). Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. *Environ. Health Perspect.* **115**, 1052-1058.

Qiu, X., Bigsby, R.M., and Hites, R.A. (2009). Hydroxylated metabolites of polybrominated diphenyl ethers in human blood samples from the United States. *Environ. Health Perspect.* **117**, 93-98.

Richardson, V.M., Staskal, D.F., Ross, D.G., Diliberto, J.J., DeVito, M.J., and Birnbaum, L.S. (2008). Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. *Toxicol. Appl. Pharmacol.* **226**, 244-250.

Rodenburg, L.A., Meng, Q., Yee, D., and Greenfield, B.K. (2014). Evidence for photochemical and microbial debromination of polybrominated diphenyl ether flame retardants in San Francisco Bay sediment. *Chemosphere* **106**, 36-43.

Roze, E., Meijer, L., Bakker, A., Van Braeckel, K.N.J.A., Sauer, P.J.J., and Bos, A.F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. *Environ. Health Perspect.* **117**, 1953-1958.

Sanders, J.M., Burka, L.T., Smith, C.S., Black, W., James, R., and Cunningham, M.L. (2005). Differential expression of *CYP1A*, *2B*, and *3A* genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual components. *Toxicol. Sci.* **88**, 127-133.

Sanders J.M., Chen, L-.J., Lebetkin, E.H., and Burka, L.T. (2006a). Metabolism and disposition of 2,2′,4,4′-tetrabromodiphenyl ether following administration of single or multiple doses to rats and mice. *Xenobiotica* **36**, 103-117.

Sanders, J.M., Lebetkin, E.H., Chen, L.-J., and Burka, L.T. (2006b). Disposition of 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE153) and its interaction with other polybrominated diphenyl ethers (PBDEs) in rodents. *Xenobiotica* **36**, 824-837.

Schecter, A., Päpke, O., Harris, T.R., Tung, K.C., Musumba, A., Olson, J., and Birnbaum, L. (2006). Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S. food and estimated PBDE dietary intake by age and sex. *Environ. Health Perspect.* **114**, 1515-1520.

Schecter, A., Colacino, J., Sjödin, A., Needham, L., and Birnbaum, L. (2010a). Partitioning of polybrominated diphenyl ethers (PBDEs) in serum and milk from the same mothers. *Chemosphere* **78**, 1279-1284.

Schecter, A., Haffner, D., Colacino, J., Patel, K., Päpke, O., Opel, M., and Birnbaum, L. (2010b). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclodecane (HBCD) in composite U.S. food samples. *Environ. Health Perspect.* **118**, 357-362.

Schecter, A., Smith, S., Haffner, D., Colacino, J., Malik, N., Patel, K., Harris, T.R., Opel, M., and Paepke, O. (2010c). Does flying present a threat of polybrominated diphenyl ether exposure? *J. Occup. Environ. Med.* **52**, 1230-1235.

Schecter, A., Smith, S., Colacino, J., Malik, N., Opel, M., Paepke, O., and Birnbaum, L. (2011). Contamination of U.S. butter with polybrominated diphenyl ethers from wrapping paper. *Environ. Health Perspect.* **119**, 151-154.

Schenkman, J.B., and Cinti, D.L. (1978). Preparation of microsomes with calcium. *Methods Enzymol.* **52**, 83-89.

Schmid, W. (1975). The micronucleus test. *Mutat. Res.* **31**, 9-15.

Schwarz, M., Buchmann, A., Stinchcombe, S., Kalkuhl, A., and Bock, K. (2000). Ah receptor ligands and tumor promotion: Survival of neoplastic cells. *Toxicol. Lett.* **112-113**, 69-77.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Shy, C.-G., Huang, H.-L., Chang-Chien, G.-P., Chao, H.-R., and Tsou, T.-C. (2011). Neurodevelopment of infants with prenatal exposure to polybrominated diphenyl ethers. *Bull. Environ. Contam. Toxicol.* **87**, 643-648.

Sinha, R.A., Singh, B.K., and Yen, P.M. (2014). Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends Endocrinol. Metab.* **25**, 538-545.

Sjödin, A., Jones, R.S., Focant, J.F., Lapeza, C., Wang, R.Y., McGahee, E.E., III, Zhang, Y., Turner, W.E., Slazyk, B., Needham, L.L., and Patterson, D.G., Jr. (2004). Retrospective time-trend study of polybrominate diphenyl ether and polybrominated and polychlorineated biphenyl levels in human serum from the United States. *Environ. Health Perspect.* **112**, 654-658.

Sjödin, A., Jones, R.S., Caudill, S.P., Wong, L.-Y., Turner, W.E., and Calafat, A.M. (2014). Polybrominated diphenyl ethers, polychlorinated biphenyls, and persistent pesticides in serum from the National Health and Nutrition Examination Survey: 2003-2008. *Environ. Sci. Technol.* **48**, 753-760.

Slauson, D.O., and Cooper, B.J. (2001). *Mechanisms of Disease: A Textbook of Comparative General Pathology, Third Edition*, Mosby, Maryland Heights, MO.

Söderström, G., Sellström, U., De Wit, C.A., and Tysklind, M. (2004). Photolytic debromination of decabromodiphenyl ether (BDE 209). *Environ. Sci. Technol.* **38**, 127-132.

Stahl, L.L., Snyder, B.D., Olsen, A.R., and Walters, L.S. (2013). A national probabilistic study of polybrominated diphenyl ethers in fish from US lakes and reservoirs. *Environ. Monit. Assess.* **185**, 10,351-10,364.

Stapleton, H.M., Sjödin, A., Jones, R.S., Niehüser, S., Zhang, Y., and Patterson, D.G., Jr. (2008). Serum levels of polybrominated diphenyl ethers (PBDEs) in foam recyclers and carpet installers working in the United States. *Environ. Sci. Technol.* **42**, 3453-3458.

Stapleton, H.M., Kelly, S.M., Pei, R., Letcher, R.J., and Gunsch, C. (2009). Metabolism of polybrominated diphenyl ethers (PBDEs) by human hepatocytes *in vitro*. *Environ. Health Perspect.* **117**, 197-202.

Stapleton, H.M., Eagle, S., Anthopolos, R., Wolkin, A., and Miranda, M.L. (2011). Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. *Environ. Health Perspect.* **119**, 1454-1459.

Stapleton, H.M., Sharma, S., Getzinger, G., Ferguson, P.L., Gabriel, M., Webster, T.F., and Blum, A. (2012). Novel and high volume use flame retardants in US couches reflective of the 2005 pentaBDE phase out. *Environ. Sci. Technol.* **46**, 13,432-13,439.

Stapleton, H.M., Misenheimer, J., Hoffman, K., and Webster, T.F. (2014). Flame retardant associations between children's handwipes and house dust. *Chemosphere* **116**, 54-60.

Staskal, D.F., Diliberto, J.J., DeVito, M.J., and Birnbaum, L.S. (2005). Toxicokinetics of BDE 47 in female mice: Effect of dose, route of exposure, and time. *Toxicol. Sci.* **83**, 215-223.

Staskal, D.F., Diliberto, J.J., and Birnbaum, L.S. (2006a). Disposition of BDE 47 in developing mice. *Toxicol. Sci.* **90**, 309-316.

Staskal, D.F., Diliberto, J.J., and Birnbaum, L.S. (2006b). Impact of repeated exposure on the toxicokinetics of BDE 47 in mice. *Toxicol. Sci.* **89**, 380-385.

Staskal, D.F., Hakk, H., Bauer, D., Diliberto, J.J., and Birnbaum, L.S. (2006c). Toxicokinetics of polybrominated diphenyl ether congeners 47, 99, 100, and 153 in mice. *Toxicol. Sci.* **94**, 28-37.

Stoker, T.E., Laws, S.C., Crofton, K.M., Hedge, J.M., Ferrell, J.M., and Cooper, R.L. (2004). Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols. *Toxicol. Sci.* **78**, 144-155.

Stoker, T.E., Cooper, R.L., Lambright, C.S., Wilson, V.S., Furr, J., and Gray, L.E. (2005). In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. *Toxicol. Appl. Pharmacol.* **207**, 78-88.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Su, G., Yu, H., Lam, M.H., Giesy, J.P., and Zhang, X. (2014). Mechanisms of toxicity of hydroxylated polybrominated diphenyl ethers (HO-PBDEs) determined by toxicogenomic analysis with a live cell array coupled with mutagenesis in Escherichia coli. *Environ. Sci. Technol.* **48**, 5929-5937.

Sueyoshi, T., Li, L., Wang, H., Moore, R., Kodavanti, P.R.S., Lehmler, H.-J., Negishi, M., and Birnbaum, L.S. (2014). Flame retardant BDE-47 effectively activates nuclear receptor CAR in human primary hepatocytes. *Toxicol. Sci.* **137**, 292-302.

Suvorov, A., Battista, M.-C., and Takser, L. (2009a). Perinatal exposure to low-dose 2,2′,4,4′-tetrabromodiphenyl ether affects growth in rat offspring: What is the role of IGF-1? *Toxicology* **260**, 126-131.

Suvorov, A., Girard, S., Lachapelle, S., Abdelouahab, N., Sebire, G., and Takser, L. (2009b). Perinatal exposure to low-dose BDE-47, an emergent environmental contaminant, causes hyperactivity in rat offspring. *Neonatology* **95**, 203-209.

Suzuki, G., Tue, N.M., Malarvannan, G., Sudaryanto, A., Takahashi, S., Tanabe, S., Sakai, S., Brouwer, A., Uramaru, N., Kitamura, S., and Takigami, H. (2013). Similarities in the endocrine-disrupting potencies of indoor dust and flame retardants by using human osteosarcoma (U2OS) cell-based reporter gene assays. *Environ. Sci. Technol.* **47**, 2898-2908.

Szabo, D.T., Richardson, V.M., Ross, D.G., Diliberto, J.J., Kodavanti, P.R.S., and Birnbaum, L.S. (2009). Effects of perinatal PBDE exposure on hepatic phase I, phase II, phase III, and deiodinase 1 gene expression involved in thyroid hormone metabolism in male rat pups. *Toxicol. Sci.* **107**, 27-39.

Takeuchi, N., Ito, M., Uchida, K., and Yamamura, Y. (1975). Effect of modification of thyroid function on cholesterol 7α-hydroxylation in rat liver. *Biochem. J.* **148**, 499-503.

Talsness, C.E., Shakibaei, M., Kuriyama, S.N., Grande, S.W., Sterner-Kock, A., Schnitker, P., de Souza, C., Grote, K., and Chahoud, I. (2005). Ultrastructural changes observed in rat ovaries following in utero and lactational exposure to low doses of a polybrominated flame retardant. *Toxicol. Lett.* **157**, 189-202.

Talsness, C.E., Kuriyama, S.N., Sterner-Kock, A., Schnitker, P., Grande, S.W., Shakibaei, M., Andrade, A., Grote, K., and Chahoud, I. (2008). *In utero* and lactational exposures to low doses of polybrominated diphenyl ether-47 alter the reproductive system and thyroid gland of female rat offspring. *Environ. Health Perspect.* **116**, 308-314.

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

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* **236**, 933-941.

Thoolen, B., Maronpot, R.R., Harada, T., Nyska, A., Rousseaux, C., Nolte, T., Malarkey, D.E., Kaufmann, W., Küttler, K., Deschl, U., Nakae, D., Gregson, R., Vinlove, M.P., Brix, A.E., Singh, B., Belpoggi, F., and Ward, J.M. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol. Pathol.* **38**, 5S-81S.

Tsuchiya, Y., Nakajima, M., and Yokoi, T. (2005). Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett.* **227**, 115-124.

Turyk, M.E., Persky, V.W., Imm, P., Knobeloch, L., Chatterton, R., Jr., and Anderson, H.A. (2008). Hormone disruption by PBDEs in adult male sport fish consumers. *Environ. Health Perspect.* **116**, 1635-1641.

United Nations Environmental Programme (UNEP) (2008). What are POPs? Secretariat of the Stockholm Convention. <http://chm.pops.int/Convention/The POPs/tabid/673/language/en-US/Default.aspx>

United Nations Environmental Programme (UNEP) (2009a). The 9 new POPs under the Stockholm Convention. <http://chm.pops.int/Programmes/NewPOPs/The9newPOPs/tabid/672/language/en-US/Default.aspx>

United Nations Environmental Programme (UNEP) (2009b). Stockholm Convention On Persistent Organic Pollutants, Adoption of amendments to Annexes A, B and C. Reference: C.N.524.2009.TREATIES-4 (Depository Notification).

U.S. Environmental Protection Agency (USEPA) (2008a). Toxicological Review of 2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47) (CAS No. 5436-43-1). EPA Report No. EPA/635/R-07/005F. <http://www.epa.gov/iris/>

U.S. Environmental Protection Agency (USEPA) (2008b). Toxicological Review of 2,2′.4,4′,5-Pentabromodiphenyl Ether (BDE-99) (CAS No. 60348-60-9). EPA Report No. EPA/635/R-07/006F. <http://www.epa.gov/iris/>

U.S. Environmental Protection Agency (USEPA) (2008c). Toxicological Review of 2,2′,4,4′,5,5′-Hexabromodiphenyl Ether (BDE-153) (CAS No. 68631-49-2). EPA Report No. EPA/635/R-07/007F. <http://www.epa.gov/iris/>

U.S. Environmental Protection Agency (USEPA) (2010a). An Exposure Assessment of Polybrominated Diphenyl Ethers. EPA Report No. EPA/600/R-08/086F. <www.epa.gov/dfe>

U.S. Environmental Protection Agency (USEPA) (2010b). Design for the Environment, An EPA Partnership Program. <http://www.epa.gov/dfe/alternative_assessments.html>

Usenko, C.Y., Abel, E.L., Kudela, M., Janise, A., and Bruce, E.D. (2015). Comparison of PBDE congeners as inducers of oxidative stress in zebrafish. *Environ. Toxicol. Chem.* **34**, 1154-1160.

van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., and Peterson, R.E. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* **93**, 223-241.

van den Berg, M., Denison, M.S., Birnbaum, L.S., DeVito, M.J., Fiedler, H., Falandysz, J., Rose, M., Schrenk, D., Safe, S., Tohyama, C., Tritscher, A., Tysklind, M., and Peterson, R.E. (2013). Polybrominated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls: Inclusion in the toxicity equivalency factor concept for dioxin-like compounds. *Toxicol. Sci.* **133**, 197-208.

van der Ven, L.T., van de Kuil, T., Verhoef, A., Leonards, P.E., Slob, W., Cantón, R.F., Germer, S., Hamers, T., Visser, T.J., Litens, S., Håkansson, H., Fery, Y., Schrenk, D., van den Berg, M., Piersma, A.H., and Vos, J.G. (2008). A 28-day oral dose toxicity study enhanced to detect endocrine effects of a purified technical pentabromodiphenyl ether (pentaBDE) mixture in Wistar rats. *Toxicology* **245**, 109-122.

van Pée, K.H., and Unversucht, S. (2003). Biological dehalogenation and halogenation reactions. *Chemosphere* **52**, 299-312.

Venkatesan, A.K., and Halden, R.U. (2014). Contribution of polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) to the toxic equivalency of dioxin-like compounds in archived biosolids from the U.S. EPA's 2001 national sewage sludge survey. *Environ. Sci. Technol.* **48**, 10,843-10,849.

Viberg, H., and Eriksson, P. (2011). Differences in neonatal neurotoxicity of brominated flame retardants, PBDE 99 and TBBPA, in mice. *Toxicology* **289**, 59-65.

Viberg, H., Fredriksson, A., and Eriksson, P. (2003). Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol. Appl. Pharmacol.* **192**, 95-106.

Viberg, H., Fredriksson, A., and Eriksson, P. (2004a). Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl etheres in mice. *Toxicol. Sci.* **81**, 344-353.

Viberg, H., Fredriksson, A., and Eriksson, P. (2004b). Neonatal exposure to the brominated flame-retardant, 2,2′,4,4′,5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. *Environ. Toxicol. Pharmacol.* **17**, 61-65.

Viberg, H., Fredriksson, A., and Eriksson, P. (2005). Deranged spontaneous behaviour and decrease in cholinergic muscarinic receptors in hippocampus in the adult rat, after neonatal exposure to the brominated flame-retardant, 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE 99). *Environ. Toxicol. Pharmacol.* **20**, 283-288.

Waxman, D.J., and Azaroff, L. (1992). Phenobarbital induction of cytochrome P-450 gene expression. *Biochem. J.* **281**, 577-592.

Weiss, G., and Goodnough, L.T. (2005). Anemia of chronic disease. *N. Eng. J. Med.* **352**, 1011-1023.

Westerink, R.H.S. (2014). Modulation of cell viability, oxidative stress, calcium homeostasis, and voltage- and ligand-gated ion channels as common mechanisms of action of (mixtures of) non-dioxin-like polychlorinated biphenyls and polybrominated diphenyl ethers. *Environ. Sci. Pollut. Res. Int.* **21**, 6373-6383.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Winsnes, A. (1969). Studies on the activation in vitro of glucuronyltransferase. *Biochim. Biophys. Acta* **191**, 279-291.

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36**, 163-194.

Witt, K.L., Livanos, E., Kissling, G.E., Torous, D.K., Caspary, W., Tice, R.R., and Recio, L. (2008). Comparison of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. *Mutat. Res.* **649**, 101-113.

Woodruff, T.J., Zota, A.R., and Schwartz, J.M. (2011). Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ. Health Perspect.* **119**, 878-885.

World Health Organization (WHO) (1994). International Programme on Chemical Safety. Environmental Health Criteria 162. Brominated diphenyl ethers. WHO, Geneva.

World Health Organization (WHO) (2012). State of the science of endocrine disrupting chemicals - 2012. <http://www.who.int/ceh/publications/endocrine/en/index.html>

Wu, J.-P., Guan, Y.-T., Zhang, Y., Luo, X.-J., Zhi, H., Chen, S.-J., and Mai, B.-X. (2011). Several current-use, non-PBDE brominated flame retardants are highly bioaccumulative: Evidence from field determined bioaccumulation factors. *Environ. Int.* **37**, 210-215.

Wyrzykowska-Ceradini, B., Gullett, B.K., Tabor, D., and Touati, A. (2011). PBDDs/Fs and PCDDs/Fs in the raw and clean flue gas during steady state and transient operation of a municipal waste combustor. *Environ. Sci. Technol.* **45**, 5853-5860.

Yuan, J., Chen, L., Chen, D., Guo, H., Bi, X., Ju, Y., Jiang, P., Shi, J., Yu, Z., Yang, J., Li, L., Jiang, Q., Sheng, G., Fu, J., Wu, T., and Chen, X. (2008). Elevated serum polybrominated diphenyl ethers and thyroid-stimulating hormone associated with lymphocytic micronuclei in Chinese workers from an e-waste dismantling site. *Environ. Sci. Technol.* **42,** 2195-2200.

Zabka, T.S., Fielden, M.R., Garrido, R., Tao, J., Fretland, A.J., Fretland, J.L., Albassam, M.A., Singer, T., and Kolaja, K.L. (2011). Characterization of xenobiotic-induced hepatocellular enzyme induction in rats: Anticipated thyroid effects and unique pituitary gland findings. *Toxicol. Pathol.* **39**, 664-677.

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.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four *in vitro* genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

Zhang, L., Tian, Y., Yang, X.F., Cui, C., Gao, Y., Wang, X.J., Wang, P., Ding, W.W., Shi, R., Wang, Y., Jin, J., and Jin, P. (2011). Concentration of polybrominated diphenyl ethers in umbilical cord serum and the influence on newborns birth outcomes in Shanghai [in Chinese]. *Zhonghua Yu Fang Yi Xue Za Zhi* **45**, 490-493.

Zhou, T., Ross, D.G., DeVito, M.J., and Crofton, K.M. (2001). Effects of short-term *in vivo* exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.* **61**, 76-82.

Zhou, T., Taylor, M.M., DeVito, M.J., and Crofton, K.M. (2002). Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol. Sci.* **66**, 105-116.

Zota, A.R., Park, J.-S., Wang, Y., Petreas, M., Zoeller, R.T., and Woodruff, T.J. (2011). Polybrominated diphenyl ethers, hydroxylated polybrominated diphenyl ethers, and measures of thyroid function in second trimester pregnant women in California. *Environ. Sci. Technol.* **45**, 7896-7905.

APPENDIX A
SUMMARY OF LESIONS
IN F_1 MALE WISTAR HAN RATS
IN THE 2-YEAR PERINATAL
AND POSTNATAL GAVAGE STUDY OF DE-71

TABLE A1
Summary of the Incidence of Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
3-Month interim evaluation	10			10
Early deaths				
Accidental deaths	1	1		1
Moribund	8	7	10	12
Natural deaths	4	7	2	12
Survivors				
Terminal kill	36	35	38	25
Other	1			
Animals examined microscopically	59	50	50	60
Systems Examined at 3 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Intestine large, cecum	(46)	(43)	(49)	(43)
Intestine large, colon	(48)	(45)	(50)	(48)
Intestine large, rectum	(48)	(46)	(49)	(47)
Intestine small, duodenum	(46)	(45)	(49)	(46)
Fibroma	1 (2%)			
Intestine small, ileum	(45)	(43)	(49)	(42)
Intestine small, jejunum	(45)	(44)	(50)	(46)
Fibroma				1 (2%)
Liver	(49)	(50)	(50)	(50)
Hepatocellular adenoma	3 (6%)	2 (4%)	4 (8%)	7 (14%)
Hepatocellular adenoma, multiple				1 (2%)
Hepatocellular carcinoma				2 (4%)
Hepatocholangioma				2 (4%)
Mesentery	(12)	(6)	(13)	(10)
Lipoma	1 (8%)			
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(46)	(47)	(50)	(49)
Adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Adenoma, multiple			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Salivary glands	(46)	(48)	(50)	(50)
Parotid gland, adenoma	1 (2%)		1 (2%)	
Parotid gland, carcinoma			1 (2%)	
Stomach, forestomach	(49)	(50)	(50)	(50)
Fibrosarcoma	1 (2%)			
Leiomyosarcoma				1 (2%)
Squamous cell papilloma	1 (2%)			
Squamous cell papilloma, multiple			1 (2%)	
Stomach, glandular	(48)	(46)	(50)	(49)
Fibrosarcoma	1 (2%)			
Tongue	(0)	(1)	(0)	(0)
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(0)	(2)	(0)	(0)
Heart	(49)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(49)	(49)	(50)	(49)
Carcinoma	1 (2%)			1 (2%)
Adrenal medulla	(49)	(48)	(50)	(49)
Pheochromocytoma benign			1 (2%)	
Pheochromocytoma complex		1 (2%)		
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(49)	(49)	(50)	(50)
Adenoma	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Carcinoma	2 (4%)		1 (2%)	
Parathyroid gland	(47)	(49)	(50)	(50)
Adenoma	1 (2%)			
Adenoma, multiple	1 (2%)			
Pituitary gland	(49)	(49)	(50)	(50)
Craniopharyngioma		1 (2%)		
Ganglioneuroma		1 (2%)		
Glioma malignant, metastatic, brain	1 (2%)		1 (2%)	
Pars distalis, adenoma	19 (39%)	12 (24%)	21 (42%)	34 (68%)
Pars distalis, adenoma, multiple			1 (2%)	1 (2%)
Pars intermedia, adenoma		2 (4%)		
Thyroid gland	(45)	(45)	(48)	(46)
C-cell, adenoma	11 (24%)	12 (27%)	10 (21%)	6 (13%)
C-cell, adenoma, multiple			1 (2%)	
C-cell, carcinoma				1 (2%)
Follicular cell, adenoma	1 (2%)	3 (7%)	2 (4%)	6 (13%)
Follicular cell, carcinoma		2 (4%)	1 (2%)	
General Body System				
Tissue NOS	(3)	(3)	(2)	(1)
Schwannoma malignant		1 (33%)	1 (50%)	

TABLE A1
Summary of the Incidence of Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Preputial gland	(49)	(49)	(50)	(50)
Carcinoma	1 (2%)			
Prostate	(49)	(50)	(50)	(50)
Adenoma			1 (2%)	
Seminal vesicle	(49)	(46)	(50)	(49)
Testes	(49)	(49)	(50)	(50)
Interstitial cell, adenoma	2 (4%)	4 (8%)	2 (4%)	4 (8%)
Hematopoietic System				
Bone marrow	(49)	(48)	(50)	(50)
Lymph node	(2)	(6)	(5)	(6)
Lymph node, mandibular	(48)	(49)	(50)	(50)
Lymph node, mesenteric	(49)	(49)	(50)	(50)
Hemangioma	1 (2%)		2 (4%)	1 (2%)
Hemangiosarcoma	7 (14%)	2 (4%)	3 (6%)	4 (8%)
Spleen	(47)	(46)	(50)	(49)
Hemangiosarcoma	1 (2%)			1 (2%)
Thymus	(45)	(49)	(49)	(50)
Thymoma benign			1 (2%)	
Thymoma malignant			1 (2%)	
Integumentary System				
Mammary gland	(33)	(38)	(39)	(41)
Fibroadenoma			3 (8%)	
Fibroma		1 (3%)		
Skin	(49)	(49)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)	1 (2%)	
Fibroma	1 (2%)	3 (6%)	1 (2%)	
Fibrosarcoma		1 (2%)		1 (2%)
Hamartoma		1 (2%)		
Hemangiosarcoma		1 (2%)		
Keratoacanthoma	2 (4%)		1 (2%)	
Lipoma			1 (2%)	
Schwannoma malignant		1 (2%)	1 (2%)	2 (4%)
Squamous cell papilloma		1 (2%)		2 (4%)
Pinna, squamous cell papilloma			1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Skeletal muscle	(1)	(2)	(4)	(0)
Hemangiosarcoma	1 (100%)			

TABLE A1
Summary of the Incidence of Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Nervous System				
Brain	(49)	(50)	(50)	(50)
Glioma malignant	1 (2%)		2 (4%)	
Granular cell tumor benign	1 (2%)			1 (2%)
Meninges, granular cell tumor benign			1 (2%)	2 (4%)
Meninges, hemangioma		1 (2%)		
Peripheral nerve	(2)	(1)	(3)	(0)
Spinal cord	(2)	(1)	(3)	(0)
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		3 (6%)		
Carcinoma, metastatic, adrenal cortex				1 (2%)
Osteosarcoma, metastatic, lung			1 (2%)	
Schwannoma malignant, metastatic, skin		1 (2%)		
Thymoma malignant, metastatic, thymus			1 (2%)	
Nose	(49)	(49)	(50)	(50)
Fibrosarcoma				1 (2%)
Respiratory epithelium, adenoma		1 (2%)		
Trachea	(49)	(46)	(50)	(49)
Special Senses System				
Eye	(46)	(46)	(50)	(45)
Harderian gland	(49)	(49)	(50)	(50)
Lacrimal gland	(0)	(0)	(1)	(2)
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(49)	(46)	(50)	(50)
Lipoma				1 (2%)
Ureter	(1)	(0)	(0)	(0)
Urinary bladder	(49)	(48)	(50)	(50)
Leiomyoma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(49)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)	1 (2%)	
Leukemia	1 (2%)			
Lymphoma malignant		4 (8%)	1 (2%)	
Mesothelioma malignant		1 (2%)		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]				
2-Year study	36	39	44	47
Total primary neoplasms				
2-Year study	71	68	76	88
Total animals with benign neoplasms				
2-Year study	33	34	40	45
Total benign neoplasms				
2-Year study	53	52	63	72
Total animals with malignant neoplasms				
2-Year study	13	14	10	12
Total malignant neoplasms				
2-Year study	18	16	13	16
Total animals with metastatic neoplasms				
2-Year study	1	1	3	1
Total metastatic neoplasms				
2-Year study	1	1	3	1

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

TABLE A2
Statistical Analysis of Primary Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Brain: Granular Cell Tumor Benign				
Overall rate[a]	1/49 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate[b]	2.4%	0.0%	2.3%	7.5%
Terminal rate[c]	0/36 (0%)	0/35 (0%)	1/38 (3%)	1/25 (4%)
First incidence (days)	669	—[e]	729 (T)	558
Poly-3 test[d]	P=0.071	P=0.504N	P=0.757N	P=0.285
Liver: Hepatocellular Adenoma				
Overall rate	3/49 (6%)	2/50 (4%)	4/50 (8%)	8/50 (16%)
Adjusted rate	7.1%	4.8%	9.2%	19.8%
Terminal rate	3/36 (8%)	1/35 (3%)	4/38 (11%)	3/25 (12%)
First incidence (days)	729 (T)	658	729 (T)	595
Poly-3 test	P=0.016	P=0.503N	P=0.512	P=0.081
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/49 (6%)	2/50 (4%)	4/50 (8%)	9/50 (18%)
Adjusted rate	7.1%	4.8%	9.2%	22.3%
Terminal rate	3/36 (8%)	1/35 (3%)	4/38 (11%)	4/25 (16%)
First incidence (days)	729 (T)	658	729 (T)	595
Poly-3 test	P=0.006	P=0.503N	P=0.512	P=0.047
Liver: Hepatocholangioma, Hepatocellular Adenoma, or Hepatocellular Carcinoma				
Overall rate	3/49 (6%)	2/50 (4%)	4/50 (8%)	11/50 (22%)
Adjusted rate	7.1%	4.8%	9.2%	27.2%
Terminal rate	3/36 (8%)	1/35 (3%)	4/38 (11%)	5/25 (20%)
First incidence (days)	729 (T)	658	729 (T)	595
Poly-3 test	P<0.001	P=0.503N	P=0.512	P=0.014
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/49 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	0.0%	0.0%
Terminal rate	0/36 (0%)	3/35 (9%)	0/38 (0%)	0/25 (0%)
First incidence (days)	—	729 (T)	—	—
Poly-3 test	P=0.249N	P=0.116	—[f]	—
Mammary Gland: Fibroadenoma				
Overall rate	0/49 (0%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.9%	0.0%
Terminal rate	0/36 (0%)	0/35 (0%)	3/38 (8%)	0/25 (0%)
First incidence (days)	—	—	729 (T)	—
Poly-3 test	P=0.667N	—	P=0.122	—
Mammary Gland: Fibroma or Fibroadenoma				
Overall rate	0/49 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.4%	6.9%	0.0%
Terminal rate	0/36 (0%)	0/35 (0%)	3/38 (8%)	0/25 (0%)
First incidence (days)	—	592	729 (T)	—
Poly-3 test	P=0.515N	P=0.500	P=0.122	—
Mesenteric Lymph Node: Hemangiosarcoma				
Overall rate	7/49 (14%)	2/50 (4%)	3/50 (6%)	4/50 (8%)
Adjusted rate	16.1%	4.8%	6.9%	10.2%
Terminal rate	5/36 (14%)	2/35 (6%)	3/38 (8%)	3/25 (12%)
First incidence (days)	515	729 (T)	729 (T)	701
Poly-3 test	P=0.516N	P=0.087N	P=0.157N	P=0.318N

TABLE A2
Statistical Analysis of Primary Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Pancreas: Adenoma				
Overall rate	1/46 (2%)	1/47 (2%)	3/50 (6%)	1/49 (2%)
Adjusted rate	2.4%	2.5%	6.9%	2.6%
Terminal rate	0/36 (0%)	1/35 (3%)	3/38 (8%)	1/25 (4%)
First incidence (days)	620	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.636	P=0.754	P=0.325	P=0.746
Pancreatic Islets: Adenoma				
Overall rate	4/49 (8%)	2/49 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate	9.4%	4.9%	4.6%	2.5%
Terminal rate	3/36 (8%)	2/35 (6%)	2/38 (5%)	1/25 (4%)
First incidence (days)	630	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.215N	P=0.354N	P=0.331N	P=0.204N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	6/49 (12%)	2/49 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	14.0%	4.9%	6.9%	2.5%
Terminal rate	5/36 (14%)	2/35 (6%)	2/38 (5%)	1/25 (4%)
First incidence (days)	630	729 (T)	595	729 (T)
Poly-3 test	P=0.111N	P=0.144N	P=0.229N	P=0.069N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	19/49 (39%)	12/49 (24%)	22/50 (44%)	35/50 (70%)
Adjusted rate	40.7%	28.1%	47.4%	71.7%
Terminal rate	10/36 (28%)	7/35 (20%)	16/38 (42%)	13/25 (52%)
First incidence (days)	508	485	436	351
Poly-3 test	P<0.001	P=0.152N	P=0.328	P<0.001
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Basal Cell Adenoma				
Overall rate	3/49 (6%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	7.0%	4.8%	6.9%	5.1%
Terminal rate	2/36 (6%)	2/35 (6%)	3/38 (8%)	2/25 (8%)
First incidence (days)	574	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.528N	P=0.511N	P=0.658N	P=0.540N
Skin: Fibroma				
Overall rate	1/49 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.4%	7.2%	2.3%	0.0%
Terminal rate	1/36 (3%)	3/35 (9%)	1/38 (3%)	0/25 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.177N	P=0.300	P=0.756N	P=0.515N
Skin: Fibroma or Fibrosarcoma				
Overall rate	1/49 (2%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.4%	9.5%	2.3%	2.5%
Terminal rate	1/36 (3%)	3/35 (9%)	1/38 (3%)	1/25 (4%)
First incidence (days)	729 (T)	585	729 (T)	729 (T)
Poly-3 test	P=0.345N	P=0.178	P=0.756N	P=0.744
Testes: Adenoma				
Overall rate	2/49 (4%)	4/49 (8%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.7%	9.6%	4.6%	10.0%
Terminal rate	1/36 (3%)	3/35 (9%)	2/38 (5%)	2/25 (8%)
First incidence (days)	620	585	729 (T)	610
Poly-3 test	P=0.346	P=0.324	P=0.690N	P=0.305

TABLE A2
Statistical Analysis of Primary Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/45 (2%)	3/45 (7%)	2/48 (4%)	6/46 (13%)
Adjusted rate	2.5%	7.6%	4.7%	16.1%
Terminal rate	1/36 (3%)	2/35 (6%)	2/38 (5%)	4/25 (16%)
First incidence (days)	729 (T)	647	729 (T)	609
Poly-3 test	P=0.028	P=0.297	P=0.518	P=0.042
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/45 (2%)	5/45 (11%)	3/48 (6%)	6/46 (13%)
Adjusted rate	2.5%	12.6%	7.0%	16.1%
Terminal rate	1/36 (3%)	4/35 (11%)	3/38 (8%)	4/25 (16%)
First incidence (days)	729 (T)	647	729 (T)	609
Poly-3 test	P=0.089	P=0.095	P=0.324	P=0.042
Thyroid Gland (C-Cell): Adenoma				
Overall rate	11/45 (24%)	12/45 (27%)	11/48 (23%)	6/46 (13%)
Adjusted rate	27.1%	29.5%	25.2%	16.3%
Terminal rate	11/36 (31%)	9/35 (26%)	9/38 (24%)	5/25 (20%)
First incidence (days)	729 (T)	485	592	698
Poly-3 test	P=0.116N	P=0.503	P=0.521N	P=0.190N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/45 (24%)	12/45 (27%)	11/48 (23%)	7/46 (15%)
Adjusted rate	27.1%	29.5%	25.2%	19.0%
Terminal rate	11/36 (31%)	9/35 (26%)	9/38 (24%)	6/25 (24%)
First incidence (days)	729 (T)	485	592	698
Poly-3 test	P=0.190N	P=0.503	P=0.521N	P=0.282N
All Organs: Hemangiosarcoma				
Overall rate	8/49 (16%)	3/50 (6%)	3/50 (6%)	5/50 (10%)
Adjusted rate	18.4%	7.2%	6.9%	12.5%
Terminal rate	6/36 (17%)	3/35 (9%)	3/38 (8%)	3/25 (12%)
First incidence (days)	515	729 (T)	729 (T)	595
Poly-3 test	P=0.524N	P=0.109N	P=0.098N	P=0.332N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	9/49 (18%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	20.7%	9.6%	11.4%	15.1%
Terminal rate	7/36 (19%)	4/35 (11%)	4/38 (11%)	4/25 (16%)
First incidence (days)	515	729 (T)	476	595
Poly-3 test	P=0.528N	P=0.127N	P=0.183N	P=0.349N
All Organs: Malignant Lymphoma				
Overall rate	0/49 (0%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	9.4%	2.3%	0.0%
Terminal rate	0/36 (0%)	2/35 (6%)	1/38 (3%)	0/25 (0%)
First incidence (days)	—	549	729 (T)	—
Poly-3 test	P=0.195N	P=0.060	P=0.504	—
All Organs: Benign Neoplasms				
Overall rate	33/49 (67%)	34/50 (68%)	40/50 (80%)	45/50 (90%)
Adjusted rate	70.3%	74.8%	83.9%	91.7%
Terminal rate	23/36 (64%)	26/35 (74%)	32/38 (84%)	21/25 (84%)
First incidence (days)	508	301	436	351
Poly-3 test	P=0.004	P=0.404	P=0.088	P=0.006

TABLE A2
Statistical Analysis of Primary Neoplasms in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	13/49 (27%)	14/50 (28%)	11/50 (22%)	12/50 (24%)
Adjusted rate	29.8%	31.5%	23.8%	28.9%
Terminal rate	10/36 (28%)	8/35 (23%)	6/38 (16%)	6/25 (24%)
First incidence (days)	515	465	289	310
Poly-3 test	P=0.491N	P=0.522	P=0.344N	P=0.559N
All Organs: Benign or Malignant Neoplasms				
Overall rate	36/49 (73%)	39/50 (78%)	44/50 (88%)	47/50 (94%)
Adjusted rate	76.7%	82.3%	88%	94%
Terminal rate	26/36 (72%)	27/35 (77%)	32/38 (84%)	22/25 (88%)
First incidence (days)	508	301	289	310
Poly-3 test	P=0.015	P=0.339	P=0.116	P=0.015

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for brain, liver, lung, pancreas, pancreatic islets, pituitary gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Liver Neoplasms in Control Male Wistar Han Rats[a]

Study (Study Start)	Hepatocholangioma	Hepatocellular Adenoma	Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (August 2008)	0/49	3/49	0/49
Tetrabromobisphenol A (July 2007)	0/50	0/50	0/50
Total (%)	0/99	3/99 (3.0%)	0/99
Mean ± standard deviation		3.1% ± 4.3%	
Range		0%-6%	
Overall Historical Incidence: All Routes			
Total (%)	0/299	4/299 (1.3%)	0/299
Mean ± standard deviation		1.4% ± 2.5%	
Range		0%-6%	

	Hepatocellular Adenoma or Hepatocellular Carcinoma	Hepatocholangioma, Hepatocellular Adenoma, or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies		
DE-71 (August 2008)	3/49	3/49
Tetrabromobisphenol A (July 2007)	0/50	0/50
Total (%)	3/99 (3.0%)	3/99 (3.0%)
Mean ± standard deviation	3.1% ± 4.3%	3.1% ± 4.3%
Range	0%-6%	0%-6%
Overall Historical Incidence: All Routes		
Total (%)	4/299 (1.3%)	4/299 (1.3%)
Mean ± standard deviation	1.4% ± 2.5%	1.4% ± 2.5%
Range	0%-6%	0%-6%

[a] Data as of November 2014

TABLE A3b
Historical Incidence of Thyroid Gland Neoplasms in Control Male Wistar Han Rats[a]

Study (Study Start)	Follicular Cell Adenoma	Follicular Cell Carcinoma	Follicular Cell Adenoma or Follicular Cell Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (August 2008)	1/45	0/45	1/45
Tetrabromobisphenol A (July 2007)	3/50	0/50	3/50
Total (%)	4/95 (4.2%)	0/95	4/95 (4.2%)
Mean ± standard deviation	4.1% ± 2.7%		4.1% ± 2.7%
Range	2%-6%		2%-6%
Overall Historical Incidence: All Routes			
Total (%)	5/295 (1.7%)	0/295	5/295 (1.7%)
Mean ± standard deviation	1.7% ± 2.4%		1.7% ± 2.4%
Range	0%-6%		0%-6%

[a] Data as of November 2014

TABLE A3c
Historical Incidence of Pituitary Gland (Pars Distalis) Adenoma in Control Male Wistar Han Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
DE-71 (August 2008)	19/49
Tetrabromobisphenol A (July 2007)	21/50
Total (%)	40/99 (40.4%)
Mean ± standard deviation	40.4% ± 2.3%
Range	39%-42%
Overall Historical Incidence: All Routes	
Total (%)	101/298 (33.9%)
Mean ± standard deviation	33.9% ± 5.7%
Range	28%-42%

[a] Data as of November 2014

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
3-Month interim evaluation	10			10
Early deaths				
Accidental deaths	1	1		1
Moribund	8	7	10	12
Natural deaths	4	7	2	12
Survivors				
Terminal kill	36	35	38	25
Other	1			
Animals examined microscopically	59	50	50	60
3-Month Interim Evaluation				
Alimentary System				
Esophagus	(10)			(10)
Intestine large, cecum	(10)			(10)
Intestine large, colon	(10)			(10)
Intestine large, rectum	(10)			(10)
Intestine small, duodenum	(10)			(10)
Intestine small, ileum	(10)			(10)
Intestine small, jejunum	(10)			(10)
Liver	(10)			(10)
Fatty change	2 (20%)			8 (80%)
Hepatocyte, hypertrophy				10 (100%)
Mesentery	(0)			(1)
Fibrosis, focal				1 (100%)
Oral mucosa	(0)			(1)
Pancreas	(10)			(10)
Atrophy	1 (10%)			2 (20%)
Salivary glands	(10)			(10)
Stomach, forestomach	(10)			(10)
Stomach, glandular	(10)			(10)
Cardiovascular System				
Blood vessel	(0)			(1)
Heart	(10)			(10)
Cardiomyopathy	1 (10%)			
Endocrine System				
Adrenal cortex	(10)			(10)
Accessory adrenal cortical nodule	1 (10%)			2 (20%)
Vacuolization cytoplasmic	1 (10%)			1 (10%)
Adrenal medulla	(10)			(10)
Vacuolization cytoplasmic				1 (10%)
Islets, pancreatic	(10)			(10)
Parathyroid gland	(10)			(10)
Pituitary gland	(10)			(9)
Thyroid gland	(10)			(10)
Follicle, hypertrophy				4 (40%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
3-Month Interim Evaluation (continued)				
Genital System				
Epididymis	(10)			(10)
Preputial gland	(10)			(10)
Inflammation, chronic	9 (90%)			8 (80%)
Prostate	(10)			(10)
Inflammation, chronic	2 (20%)			3 (30%)
Seminal vesicle	(10)			(10)
Testes	(10)			(10)
Hematopoietic System				
Bone marrow	(10)			(10)
Lymph node	(1)			(1)
Pigmentation				1 (100%)
Lymph node, mandibular	(10)			(10)
Lymph node, mesenteric	(10)			(10)
Spleen	(10)			(10)
Thymus	(10)			(10)
Respiratory System				
Lung	(10)			(10)
Inflammation, chronic	2 (20%)			4 (40%)
Metaplasia, osseous				1 (10%)
Alveolus, infiltration cellular, histiocyte				1 (10%)
Nose	(10)			(10)
Trachea	(10)			(10)
Urinary System				
Kidney	(10)			(10)
Casts protein				1 (10%)
Hydronephrosis	1 (10%)			3 (30%)
Inflammation, chronic	1 (10%)			
Renal tubule, vacuolization cytoplasmic				1 (10%)
Urinary bladder	(10)			(10)

Systems Examined at 3 Months with No Lesions Observed
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study				
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Hyperkeratosis	1 (2%)	1 (2%)		
Inflammation, acute				1 (2%)
Ulcer				1 (2%)
Muscularis, degeneration		1 (2%)		
Muscularis, hemorrhage		1 (2%)		
Periesophageal tissue, inflammation, granulomatous, chronic	1 (2%)			
Intestine large, cecum	(46)	(43)	(49)	(43)
Inflammation, chronic		1 (2%)		
Intestine large, colon	(48)	(45)	(50)	(48)
Inflammation, chronic		1 (2%)		
Intestine large, rectum	(48)	(46)	(49)	(47)
Inflammation, acute				2 (4%)
Intestine small, duodenum	(46)	(45)	(49)	(46)
Inflammation, acute				1 (2%)
Epithelium, vacuolization cytoplasmic	1 (2%)			
Intestine small, ileum	(45)	(43)	(49)	(42)
Inflammation, focal, chronic active			1 (2%)	
Peyer's patch, hyperplasia			1 (2%)	
Intestine small, jejunum	(45)	(44)	(50)	(46)
Ulcer		1 (2%)		
Epithelium, vacuolization cytoplasmic	1 (2%)			
Peyer's patch, hyperplasia			1 (2%)	
Liver	(49)	(50)	(50)	(50)
Angiectasis			1 (2%)	1 (2%)
Basophilic focus	8 (16%)	4 (8%)	3 (6%)	7 (14%)
Basophilic focus, multiple	8 (16%)	17 (34%)	8 (16%)	4 (8%)
Cholangiofibrosis			1 (2%)	1 (2%)
Clear cell focus	1 (2%)	1 (2%)		2 (4%)
Clear cell focus, multiple	38 (78%)	36 (72%)	35 (70%)	27 (54%)
Congestion	4 (8%)	3 (6%)		2 (4%)
Degeneration, cystic			1 (2%)	1 (2%)
Eosinophilic focus	3 (6%)	2 (4%)	10 (20%)	7 (14%)
Eosinophilic focus, multiple		1 (2%)	2 (4%)	8 (16%)
Fatty change	32 (65%)	37 (74%)	48 (96%)	48 (96%)
Fibrosis	1 (2%)			1 (2%)
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage	2 (4%)		1 (2%)	
Hepatodiaphragmatic nodule		1 (2%)		1 (2%)
Hyperplasia, nodular			3 (6%)	
Inflammation, chronic	1 (2%)	2 (4%)		5 (10%)
Mixed cell focus	2 (4%)	2 (4%)		2 (4%)
Mixed cell focus, multiple		1 (2%)	1 (2%)	2 (4%)
Pigmentation			1 (2%)	6 (12%)
Thrombosis	1 (2%)			
Artery, degeneration			1 (2%)	
Artery, inflammation, chronic				1 (2%)
Bile duct, cyst				1 (2%)
Bile duct, hyperplasia	16 (33%)	17 (34%)	16 (32%)	16 (32%)
Hepatocyte, hypertrophy	1 (2%)	44 (88%)	50 (100%)	50 (100%)
Hepatocyte, necrosis	4 (8%)	2 (4%)	1 (2%)	5 (10%)
Oval cell, hyperplasia			2 (4%)	3 (6%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(12)	(6)	(13)	(10)
Hemorrhage	2 (17%)	1 (17%)		1 (10%)
Inflammation, chronic			1 (8%)	
Fat, necrosis	9 (75%)	5 (83%)	11 (85%)	9 (90%)
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(46)	(47)	(50)	(49)
Atrophy	3 (7%)	5 (11%)	7 (14%)	1 (2%)
Basophilic focus			1 (2%)	
Basophilic focus, multiple				1 (2%)
Hyperplasia		1 (2%)	2 (4%)	1 (2%)
Inflammation, acute		1 (2%)		
Inflammation, chronic	1 (2%)			
Pigmentation, hemosiderin	1 (2%)			
Duct, cyst		1 (2%)		
Duct, cyst, multiple			2 (4%)	
Salivary glands	(46)	(48)	(50)	(50)
Duct, parotid gland, cyst		1 (2%)	2 (4%)	
Duct, parotid gland, inflammation, acute	1 (2%)	1 (2%)		4 (8%)
Duct, submandibular gland, inflammation, acute				1 (2%)
Parotid gland, atrophy	2 (4%)	2 (4%)	4 (8%)	13 (26%)
Parotid gland, basophilic focus				1 (2%)
Parotid gland, hyperplasia, focal	1 (2%)			
Parotid gland, inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Parotid gland, vacuolization cytoplasmic	4 (9%)	4 (8%)	7 (14%)	17 (34%)
Sublingual gland, atrophy				1 (2%)
Sublingual gland, vacuolization cytoplasmic				1 (2%)
Submandibular gland, inflammation, acute				1 (2%)
Submandibular gland, inflammation, chronic		1 (2%)		
Submandibular gland, vacuolization cytoplasmic				1 (2%)
Stomach, forestomach	(49)	(50)	(50)	(50)
Edema		1 (2%)		2 (4%)
Erosion	1 (2%)	1 (2%)		
Hyperkeratosis	9 (18%)	5 (10%)	5 (10%)	17 (34%)
Inflammation, acute	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Inflammation, chronic	3 (6%)	1 (2%)	2 (4%)	4 (8%)
Inflammation, chronic active	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Ulcer	3 (6%)	1 (2%)	3 (6%)	5 (10%)
Epithelium, hyperplasia	8 (16%)	6 (12%)	5 (10%)	17 (34%)
Stomach, glandular	(48)	(46)	(50)	(49)
Cyst			1 (2%)	
Fibrosis		1 (2%)		
Inflammation, multifocal, chronic		1 (2%)		
Inflammation, acute			1 (2%)	2 (4%)
Inflammation, chronic				1 (2%)
Mineralization	7 (15%)	3 (7%)	5 (10%)	2 (4%)
Tongue	(0)	(1)	(0)	(0)
Infiltration cellular		1 (100%)		
Tooth	(1)	(0)	(0)	(0)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Cardiovascular System				
Blood vessel	(0)	(2)	(0)	(0)
Angiectasis		1 (50%)		
Heart	(49)	(50)	(50)	(50)
Cardiomyopathy	33 (67%)	32 (64%)	34 (68%)	29 (58%)
Inflammation, acute				1 (2%)
Necrosis, multifocal				1 (2%)
Pigmentation, hemosiderin				1 (2%)
Thrombosis				1 (2%)
Endocardium, hyperplasia		1 (2%)		
Epicardium, inflammation, granulomatous	1 (2%)			
Epicardium, inflammation, chronic				1 (2%)
Pericardium, inflammation, granulomatous				1 (2%)
Pericardium, necrosis		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(49)	(50)	(49)
Accessory adrenal cortical nodule				1 (2%)
Angiectasis	13 (27%)	17 (35%)	15 (30%)	18 (37%)
Degeneration, cystic	1 (2%)			
Hyperplasia, focal	13 (27%)	10 (20%)	18 (36%)	16 (33%)
Hypertrophy, focal	9 (18%)	11 (22%)	7 (14%)	8 (16%)
Necrosis, focal				1 (2%)
Vacuolization cytoplasmic	12 (24%)	9 (18%)	10 (20%)	17 (35%)
Adrenal medulla	(49)	(48)	(50)	(49)
Infiltration cellular, eosinophil		1 (2%)		
Islets, pancreatic	(49)	(49)	(50)	(50)
Hyperplasia	1 (2%)			1 (2%)
Parathyroid gland	(47)	(49)	(50)	(50)
Cyst		2 (4%)		1 (2%)
Cyst, multiple	1 (2%)			
Hyperplasia, focal		1 (2%)	2 (4%)	
Pituitary gland	(49)	(49)	(50)	(50)
Pars distalis, cyst	3 (6%)	3 (6%)	4 (8%)	4 (8%)
Pars distalis, cyst, multiple	1 (2%)		1 (2%)	
Pars distalis, hyperplasia, focal	15 (31%)	11 (22%)	13 (26%)	8 (16%)
Pars intermedia, cyst			1 (2%)	
Pars intermedia, hemorrhage	1 (2%)			
Pars intermedia, hyperplasia, focal	2 (4%)		1 (2%)	
Pars nervosa, inflammation, chronic	2 (4%)			2 (4%)
Thyroid gland	(45)	(45)	(48)	(46)
Cyst	1 (2%)	1 (2%)		
Mineralization			1 (2%)	
C-cell, hyperplasia	44 (98%)	41 (91%)	47 (98%)	44 (96%)
Follicle, hypertrophy	1 (2%)	26 (58%)	34 (71%)	23 (50%)
Follicular cell, hyperplasia	8 (18%)	5 (11%)	5 (10%)	7 (15%)
General Body System				
Tissue NOS	(3)	(3)	(2)	(1)
Fibrosis	1 (33%)		1 (50%)	1 (100%)
Inflammation, chronic active	1 (33%)			
Fat, necrosis		1 (33%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Inflammation, chronic				3 (6%)
Vacuolization cytoplasmic	1 (2%)			
Bilateral, granuloma sperm		1 (2%)		
Preputial gland	(49)	(49)	(50)	(50)
Inflammation, granulomatous, chronic	1 (2%)			
Inflammation, chronic	3 (6%)	2 (4%)		2 (4%)
Inflammation, chronic active		4 (8%)	6 (12%)	2 (4%)
Mineralization	1 (2%)			
Duct, ectasia	2 (4%)	2 (4%)	5 (10%)	15 (30%)
Prostate	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic active	17 (35%)	20 (40%)	28 (56%)	27 (54%)
Mineralization			1 (2%)	
Vacuolization cytoplasmic				1 (2%)
Epithelium, hyperplasia		1 (2%)		
Seminal vesicle	(49)	(46)	(50)	(49)
Hyperplasia				1 (2%)
Inflammation, acute	1 (2%)	1 (2%)		2 (4%)
Inflammation, chronic active		1 (2%)	1 (2%)	1 (2%)
Testes	(49)	(49)	(50)	(50)
Cyst				1 (2%)
Degeneration	14 (29%)	11 (22%)	12 (24%)	6 (12%)
Inflammation, acute			1 (2%)	
Mineralization			1 (2%)	
Interstitial cell, hyperplasia, focal			1 (2%)	
Interstitial cell, hyperplasia, multifocal		1 (2%)		
Hematopoietic System				
Bone marrow	(49)	(48)	(50)	(50)
Hyperplasia		1 (2%)		
Lymph node	(2)	(6)	(5)	(6)
Ectasia		1 (17%)		
Mediastinal, congestion		1 (17%)		
Mediastinal, ectasia			2 (40%)	2 (33%)
Mediastinal, hemorrhage	1 (50%)	2 (33%)	1 (20%)	2 (33%)
Mediastinal, hyperplasia, plasma cell	1 (50%)			
Mediastinal, pigmentation, hemosiderin		1 (17%)	1 (20%)	1 (17%)
Pancreatic, ectasia			1 (20%)	
Pancreatic, inflammation, chronic				1 (17%)
Renal, ectasia		1 (17%)		
Lymph node, mandibular	(48)	(49)	(50)	(50)
Angiectasis		1 (2%)		
Ectasia	2 (4%)	7 (14%)	8 (16%)	7 (14%)
Hemorrhage	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Hyperplasia, plasma cell			1 (2%)	
Pigmentation, hemosiderin	1 (2%)			
Lymph node, mesenteric	(49)	(49)	(50)	(50)
Ectasia		2 (4%)	2 (4%)	1 (2%)
Hemorrhage	2 (4%)	1 (2%)	1 (2%)	4 (8%)
Pigmentation, hemosiderin			2 (4%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Hematopoietic System (continued)				
Spleen	(47)	(46)	(50)	(49)
Accessory spleen			1 (2%)	
Fibrosis, focal	1 (2%)		1 (2%)	
Hematopoietic cell proliferation	23 (49%)	30 (65%)	22 (44%)	13 (27%)
Hemorrhage, focal	1 (2%)			1 (2%)
Pigmentation	12 (26%)	11 (24%)	17 (34%)	27 (55%)
Lymphoid follicle, atrophy			1 (2%)	5 (10%)
Thymus	(45)	(49)	(49)	(50)
Atrophy	14 (31%)	11 (22%)	15 (31%)	26 (52%)
Ectopic parathyroid gland			3 (6%)	2 (4%)
Fibrosis				1 (2%)
Hemorrhage	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Integumentary System				
Mammary gland	(33)	(38)	(39)	(41)
Cyst			1 (3%)	
Galactocele		1 (3%)		1 (2%)
Hyperplasia			3 (8%)	
Pigmentation, hemosiderin	3 (9%)	9 (24%)	2 (5%)	13 (32%)
Duct, dilatation	4 (12%)	1 (3%)	1 (3%)	1 (2%)
Skin	(49)	(49)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	3 (6%)	1 (2%)	
Fibrosis		1 (2%)		
Hyperkeratosis		3 (6%)		
Inflammation, granulomatous			1 (2%)	
Inflammation, acute	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)			
Inflammation, chronic active	1 (2%)	3 (6%)		
Pigmentation				1 (2%)
Ulcer	1 (2%)	2 (4%)		
Epidermis, hyperplasia		4 (8%)		
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Skeletal muscle	(1)	(2)	(4)	(0)
Fibrosis			1 (25%)	
Inflammation, chronic active			1 (25%)	
Nervous System				
Brain	(49)	(50)	(50)	(50)
Compression	10 (20%)	9 (18%)	10 (20%)	26 (52%)
Meninges, hyperplasia, granulocytic			1 (2%)	
Peripheral nerve	(2)	(1)	(3)	(0)
Spinal cord	(2)	(1)	(3)	(0)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Infiltration cellular, histiocyte	24 (49%)	24 (48%)	32 (64%)	30 (60%)
Inflammation, granulomatous, multifocal	1 (2%)			
Inflammation, acute	2 (4%)	1 (2%)		1 (2%)
Inflammation, chronic	4 (8%)			1 (2%)
Mineralization			2 (4%)	
Alveolar epithelium, hyperplasia	2 (4%)	3 (6%)	1 (2%)	5 (10%)
Artery, mineralization				1 (2%)
Bronchus, hyperplasia, lymphoid				2 (4%)
Mediastinum, inflammation, granulomatous	1 (2%)			
Serosa, fibrosis				1 (2%)
Vein, mineralization		1 (2%)		
Nose	(49)	(49)	(50)	(50)
Fungus	2 (4%)		1 (2%)	1 (2%)
Inflammation, acute	2 (4%)		2 (4%)	2 (4%)
Inflammation, chronic active			1 (2%)	1 (2%)
Ulcer, multifocal				1 (2%)
Squamous epithelium, cyst	1 (2%)			
Trachea	(49)	(46)	(50)	(49)
Inflammation, acute	1 (2%)			
Special Senses System				
Eye	(46)	(46)	(50)	(45)
Retina, atrophy	6 (13%)	8 (17%)	8 (16%)	3 (7%)
Harderian gland	(49)	(49)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia, focal	1 (2%)			
Lacrimal gland	(0)	(0)	(1)	(2)
Inflammation, chronic			1 (100%)	1 (50%)
Karyomegaly			1 (100%)	2 (100%)
Zymbal's gland	(0)	(0)	(0)	(1)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Urinary System				
Kidney	(49)	(46)	(50)	(50)
Bacterium			1 (2%)	
Casts protein				1 (2%)
Cyst	1 (2%)		3 (6%)	
Cyst, multiple			1 (2%)	
Hydronephrosis	1 (2%)	5 (11%)	8 (16%)	10 (20%)
Hyperplasia, oncocytic				1 (2%)
Inflammation, acute			1 (2%)	1 (2%)
Inflammation, chronic				1 (2%)
Inflammation, chronic active	1 (2%)			
Nephropathy	37 (76%)	35 (76%)	32 (64%)	37 (74%)
Vacuolization cytoplasmic				1 (2%)
Pelvis, inflammation, acute				1 (2%)
Pelvis, inflammation, chronic active	22 (45%)	14 (30%)	8 (16%)	2 (4%)
Pelvis, mineralization	18 (37%)	5 (11%)	5 (10%)	3 (6%)
Renal tubule, dilatation			1 (2%)	
Renal tubule, hyperplasia	1 (2%)			
Transitional epithelium, hyperplasia		1 (2%)		1 (2%)
Ureter	(1)	(0)	(0)	(0)
Cyst	1 (100%)			
Urinary bladder	(49)	(48)	(50)	(50)
Calculus gross observation				1 (2%)
Inflammation, chronic		1 (2%)		1 (2%)
Ulcer			1 (2%)	
Transitional epithelium, hyperplasia		1 (2%)	1 (2%)	1 (2%)

APPENDIX B SUMMARY OF LESIONS IN F_1 FEMALE WISTAR HAN RATS IN THE 2-YEAR PERINATAL AND POSTNATAL GAVAGE STUDY OF DE-71

TABLE B1
Summary of the Incidence of Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
3-Month interim evaluation	10			10
Early deaths				
Accidental deaths	2	1		
Moribund	8	10	13	11
Natural deaths	3		4	10
Survivors				
Died last week of study	1			
Terminal kill	36	39	33	28
Other				1
Animals examined microscopically	60	50	50	59
Systems Examined at 3 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Intestine large, cecum	(48)	(49)	(47)	(40)
Intestine large, colon	(48)	(49)	(50)	(46)
Carcinoma, metastatic, pancreas				1 (2%)
Intestine large, rectum	(49)	(49)	(49)	(45)
Intestine small, duodenum	(47)	(49)	(47)	(42)
Leiomyosarcoma		1 (2%)		
Intestine small, ileum	(47)	(49)	(48)	(41)
Intestine small, jejunum	(46)	(49)	(47)	(42)
Leiomyoma		1 (2%)	1 (2%)	
Liver	(50)	(49)	(50)	(47)
Adenocarcinoma, metastatic, uterus			2 (4%)	
Carcinoma, metastatic, pancreas				1 (2%)
Cholangiocarcinoma				1 (2%)
Cholangiocarcinoma, multiple				1 (2%)
Hepatocellular adenoma	2 (4%)	2 (4%)	6 (12%)	8 (17%)
Hepatocellular adenoma, multiple	1 (2%)		2 (4%)	8 (17%)
Hepatocellular carcinoma			1 (2%)	3 (6%)
Hepatocellular carcinoma, multiple				3 (6%)
Hepatocholangiocarcinoma				1 (2%)
Hepatocholangioma				5 (11%)
Hepatocholangioma, multiple				3 (6%)

TABLE B1
Summary of the Incidence of Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(10)	(7)	(9)	(6)
Adenocarcinoma, metastatic, uterus			3 (33%)	
Granulosa cell tumor malignant, metastatic, ovary	1 (10%)			
Schwannoma malignant			1 (11%)	
Oral mucosa	(0)	(0)	(1)	(1)
Squamous cell carcinoma			1 (100%)	
Pancreas	(50)	(49)	(49)	(47)
Adenocarcinoma, metastatic, uterus			3 (6%)	
Carcinoma				1 (2%)
Salivary glands	(50)	(50)	(49)	(45)
Parotid gland, adenocarcinoma				1 (2%)
Sublingual gland, adenocarcinoma	1 (2%)			
Stomach, forestomach	(50)	(49)	(50)	(48)
Adenocarcinoma, metastatic, uterus			2 (4%)	
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(49)	(49)	(50)	(46)
Adenocarcinoma, metastatic, uterus			1 (2%)	
Carcinoma, metastatic, pancreas				1 (2%)
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(1)	(0)	(3)	(3)
Heart	(50)	(50)	(50)	(48)
Endocardium, schwannoma benign		1 (2%)		
Endocrine system				
Adrenal cortex	(50)	(49)	(50)	(46)
Adenocarcinoma, metastatic, uterus			2 (4%)	
Adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(47)
Pheochromocytoma benign	1 (2%)	1 (2%)	1 (2%)	
Pheochromocytoma complex	1 (2%)		1 (2%)	
Pheochromocytoma malignant			1 (2%)	
Islets, pancreatic	(50)	(49)	(49)	(47)
Adenoma		1 (2%)		
Parathyroid gland	(49)	(47)	(49)	(46)
Adenoma	1 (2%)	1 (2%)		
Pituitary gland	(50)	(49)	(50)	(47)
Pars distalis, adenoma	21 (42%)	20 (41%)	23 (46%)	20 (43%)
Pars distalis, adenoma, multiple	2 (4%)			1 (2%)
Pars intermedia, adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Thyroid gland	(45)	(49)	(47)	(42)
C-cell, adenoma	6 (13%)	3 (6%)	7 (15%)	2 (5%)
C-cell, adenoma, multiple	1 (2%)	3 (6%)	3 (6%)	2 (5%)
Follicular cell, adenoma	1 (2%)	3 (6%)	3 (6%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
General Body System				
Tissue NOS	(3)	(2)	(4)	(2)
Adenocarcinoma, metastatic, uterus			2 (50%)	
Abdominal, carcinoma, metastatic, pancreas				1 (50%)
Genital System				
Clitoral gland	(49)	(49)	(50)	(47)
Ovary	(50)	(49)	(50)	(46)
Adenocarcinoma, metastatic, uterus			1 (2%)	
Carcinoma, metastatic, pancreas				1 (2%)
Cystadenoma		1 (2%)	1 (2%)	2 (4%)
Granulosa cell tumor benign	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Granulosa cell tumor malignant	1 (2%)			
Leiomyosarcoma		1 (2%)		
Luteoma		1 (2%)		
Schwannoma malignant, metastatic, mesentery			1 (2%)	
Uterus	(50)	(49)	(50)	(47)
Adenocarcinoma		1 (2%)	3 (6%)	2 (4%)
Adenocarcinoma, multiple	1 (2%)			
Adenoma	1 (2%)		1 (2%)	
Carcinoma, metastatic, pancreas				1 (2%)
Granular cell tumor benign			1 (2%)	
Leiomyoma		1 (2%)		
Malignant mixed Müllerian tumor	1 (2%)			
Polyp stromal	3 (6%)	5 (10%)	6 (12%)	5 (11%)
Polyp stromal, multiple		1 (2%)	1 (2%)	
Sarcoma stromal			1 (2%)	
Schwannoma malignant			2 (4%)	1 (2%)
Cervix, granular cell tumor benign	1 (2%)			
Cervix, polyp stromal				1 (2%)
Cervix, schwannoma malignant	1 (2%)			
Vagina	(1)	(1)	(2)	(2)
Granular cell tumor benign		1 (100%)		
Granular cell tumor benign, multiple				1 (50%)
Polyp, multiple				1 (50%)
Sarcoma stromal, metastatic, uterus			1 (50%)	
Schwannoma malignant, metastatic, uterus			1 (50%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(46)
Lymph node	(10)	(5)	(6)	(9)
Mediastinal, adenocarcinoma, metastatic, uterus			1 (17%)	
Lymph node, mandibular	(50)	(50)	(50)	(48)
Adenocarcinoma, metastatic, salivary glands				1 (2%)
Lymph node, mesenteric	(50)	(49)	(50)	(46)
Hemangiosarcoma	2 (4%)			
Spleen	(50)	(49)	(50)	(45)
Adenocarcinoma, metastatic, uterus			1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Hematopoietic System (continued)				
Thymus	(50)	(49)	(48)	(46)
Thymoma benign				1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(48)
Carcinoma	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Carcinoma, multiple				1 (2%)
Fibroadenoma	8 (16%)	7 (14%)	10 (20%)	6 (13%)
Fibroadenoma, multiple	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Skin	(50)	(50)	(50)	(49)
Basal cell adenoma	1 (2%)			
Osteosarcoma, metastatic, bone	1 (2%)			
Schwannoma malignant		1 (2%)		
Squamous cell papilloma			1 (2%)	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Femur, osteosarcoma	1 (2%)			
Skeletal muscle	(1)	(0)	(0)	(0)
Granulosa cell tumor malignant, metastatic, ovary	1 (100%)			
Nervous system				
Brain	(50)	(50)	(50)	(49)
Glioma malignant			1 (2%)	
Peripheral nerve	(0)	(0)	(1)	(0)
Respiratory system				
Lung	(50)	(50)	(50)	(49)
Adenocarcinoma, metastatic, uterus			2 (4%)	1 (2%)
Carcinoma, metastatic, pancreas				1 (2%)
Malignant mixed Müllerian tumor, metastatic, uterus	1 (2%)			
Schwannoma malignant, metastatic, uterus			1 (2%)	
Nose	(50)	(50)	(50)	(47)
Chondroma	1 (2%)			
Trachea	(47)	(50)	(50)	(47)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(49)	(47)	(45)
Harderian gland	(49)	(50)	(50)	(49)

TABLE B1
Summary of the Incidence of Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Urinary System				
Kidney	(50)	(50)	(49)	(47)
Ureter	(1)	(0)	(0)	(1)
Urinary bladder	(50)	(49)	(49)	(45)
Adenocarcinoma, metastatic, uterus			2 (4%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(49)
Histiocytic sarcoma			1 (2%)	1 (2%)
Leukemia granulocytic			2 (4%)	
Lymphoma malignant		3 (6%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]				
2-Year study	40	38	46	44
Total primary neoplasms				
2-Year study	65	68	90	94
Total animals with benign neoplasms				
2-Year study	37	35	41	43
Total benign neoplasms				
2-Year study	55	60	73	75
Total animals with malignant neoplasms				
2-Year study	10	8	13	16
Total malignant neoplasms				
2-Year study	10	8	17	19
Total animals with metastatic neoplasms				
2-Year study	3		7	3
Total metastatic neoplasms				
2-Year study	4		26	9

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

TABLE B2
Statistical Analysis of Primary Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Adrenal Medulla: Pheochromocytoma Benign, Complex, or Malignant				
Overall rate[a]	2/50 (4%)	1/50 (2%)	3/50 (6%)	0/47 (0%)
Adjusted rate[b]	4.6%	2.2%	6.8%	0.0%
Terminal rate[c]	2/37 (5%)	1/39 (3%)	1/33 (3%)	0/28 (0%)
First incidence (days)	729 (T)	729 (T)	614	—[e]
Poly-3 test[d]	P=0.291N	P=0.476N	P=0.510	P=0.274N
Liver: Cholangiocarcinoma				
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	2/47 (4%)
Adjusted rate	0.0%	0.0%	0.0%	5.4%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	2/28 (7%)
First incidence (days)	—	—	—	729 (T)
Poly-3 test	P=0.030	—[f]	—	P=0.203
Liver: Hepatocholangioma				
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	8/47 (17%)
Adjusted rate	0.0%	0.0%	0.0%	21.5%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	7/28 (25%)
First incidence (days)	—	—	—	619
Poly-3 test	P<0.001	—	—	P<0.001
Liver: Hepatocellular Adenoma				
Overall rate	3/50 (6%)	2/49 (4%)	8/50 (16%)	16/47 (34%)
Adjusted rate	6.9%	4.4%	18.2%	41.4%
Terminal rate	3/37 (8%)	2/39 (5%)	6/33 (18%)	11/28 (39%)
First incidence (days)	729 (T)	729 (T)	656	490
Poly-3 test	P<0.001	P=0.476N	P=0.103	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	0/50 (0%)	0/49 (0%)	1/50 (2%)	6/47 (13%)
Adjusted rate	0.0%	0.0%	2.3%	16.2%
Terminal rate	0/37 (0%)	0/39 (0%)	0/33 (0%)	5/28 (18%)
First incidence (days)	—	—	686	677
Poly-3 test	P<0.001	—	P=0.503	P=0.008
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	8/50 (16%)	17/47 (36%)
Adjusted rate	6.9%	4.4%	18.2%	44.0%
Terminal rate	3/37 (8%)	2/39 (5%)	6/33 (18%)	12/28 (43%)
First incidence (days)	729 (T)	729 (T)	656	490
Poly-3 test	P<0.001	P=0.476N	P=0.103	P<0.001
Liver: Hepatocholangioma, Hepatocellular Adenoma, or Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	8/50 (16%)	21/47 (45%)
Adjusted rate	6.9%	4.4%	18.2%	53.8%
Terminal rate	3/37 (8%)	2/39 (5%)	6/33 (18%)	15/28 (54%)
First incidence (days)	729 (T)	729 (T)	656	490
Poly-3 test	P<0.001	P=0.476N	P=0.103	P<0.001
Mammary Gland: Fibroadenoma				
Overall rate	9/50 (18%)	10/50 (20%)	12/50 (24%)	9/49 (18%)
Adjusted rate	20.5%	21.4%	27.1%	22.4%
Terminal rate	8/37 (22%)	9/39 (23%)	10/33 (30%)	4/28 (14%)
First incidence (days)	508	585	610	537
Poly-3 test	P=0.491	P=0.562	P=0.317	P=0.521

TABLE B2
Statistical Analysis of Primary Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Mammary Gland: Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	4/49 (8%)
Adjusted rate	2.3%	2.2%	4.6%	10.3%
Terminal rate	1/37 (3%)	0/39 (0%)	0/33 (0%)	2/28 (7%)
First incidence (days)	729 (T)	658	676	597
Poly-3 test	P=0.052	P=0.744N	P=0.506	P=0.148
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	10/50 (20%)	11/50 (22%)	14/50 (28%)	13/49 (27%)
Adjusted rate	22.8%	23.4%	31.4%	32.0%
Terminal rate	9/37 (24%)	9/39 (23%)	10/33 (30%)	6/28 (21%)
First incidence (days)	508	585	610	537
Poly-3 test	P=0.189	P=0.572	P=0.251	P=0.241
Ovary: Benign Granulosa Cell Tumor				
Overall rate	1/50 (2%)	3/49 (6%)	1/50 (2%)	2/46 (4%)
Adjusted rate	2.3%	6.6%	2.3%	5.5%
Terminal rate	1/37 (3%)	2/39 (5%)	0/33 (0%)	2/28 (7%)
First incidence (days)	729 (T)	651	676	729 (T)
Poly-3 test	P=0.524	P=0.327	P=0.757N	P=0.442
Ovary: Benign or Malignant Granulosa Cell Tumor				
Overall rate	2/50 (4%)	3/49 (6%)	1/50 (2%)	2/46 (4%)
Adjusted rate	4.6%	6.6%	2.3%	5.5%
Terminal rate	2/37 (5%)	2/39 (5%)	0/33 (0%)	2/28 (7%)
First incidence (days)	729 (T)	651	676	729 (T)
Poly-3 test	P=0.612N	P=0.527	P=0.495N	P=0.633
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	23/50 (46%)	20/49 (41%)	23/50 (46%)	21/47 (45%)
Adjusted rate	50.9%	41.8%	48.3%	50.5%
Terminal rate	18/37 (49%)	14/38 (37%)	13/33 (39%)	9/28 (32%)
First incidence (days)	445	358	368	396
Poly-3 test	P=0.389	P=0.250N	P=0.480N	P=0.568N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/45 (2%)	3/49 (6%)	3/47 (6%)	1/42 (2%)
Adjusted rate	2.5%	6.6%	7.3%	2.9%
Terminal rate	1/36 (3%)	3/39 (8%)	3/33 (9%)	0/28 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	553
Poly-3 test	P=0.494N	P=0.345	P=0.310	P=0.724
Thyroid Gland (C-Cell): Adenoma				
Overall rate	7/45 (16%)	6/49 (12%)	10/47 (21%)	4/42 (10%)
Adjusted rate	17.0%	13.1%	24.2%	11.7%
Terminal rate	6/36 (17%)	5/39 (13%)	10/33 (30%)	4/28 (14%)
First incidence (days)	592	694	729 (T)	729 (T)
Poly-3 test	P=0.411N	P=0.422N	P=0.295	P=0.378N
Uterus (Original and Residual Evaluations): Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	1/49 (2%)
Adjusted rate	2.3%	2.2%	6.8%	2.6%
Terminal rate	0/37 (0%)	1/39 (3%)	2/33 (6%)	0/28 (0%)
First incidence (days)	508	729 (T)	614	686
Poly-3 test	P=0.597	P=0.749N	P=0.306	P=0.732

TABLE B2
Statistical Analysis of Primary Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Uterus (Original Evaluation): Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/49 (4%)
Adjusted rate	2.3%	2.2%	6.8%	5.2%
Terminal rate	0/37 (0%)	1/39 (3%)	1/33 (3%)	2/28 (7%)
First incidence (days)	592	729 (T)	686	729 (T)
Poly-3 test	P=0.332	P=0.748N	P=0.307	P=0.456
Uterus (Residual Evaluation): Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	4/50 (8%)	3/49 (6%)
Adjusted rate	4.5%	0.0%	9.1%	7.8%
Terminal rate	0/37 (0%)	0/39 (0%)	1/33 (3%)	3/28 (11%)
First incidence (days)	508	—	676	729 (T)
Poly-3 test	P=0.172	P=0.228N	P=0.335	P=0.436
Uterus (Original and Residual Evaluations): Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	4/49 (8%)
Adjusted rate	4.5%	2.2%	9.1%	10.4%
Terminal rate	0/37 (0%)	1/39 (3%)	1/33 (3%)	4/28 (14%)
First incidence (days)	508	729 (T)	676	729 (T)
Poly-3 test	P=0.118	P=0.485N	P=0.335	P=0.274
Uterus (Original Evaluation): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	2/49 (4%)
Adjusted rate	4.5%	2.2%	9.0%	5.2%
Terminal rate	0/37 (0%)	1/39 (3%)	1/33 (3%)	2/28 (7%)
First incidence (days)	508	729 (T)	614	729 (T)
Poly-3 test	P=0.450	P=0.485N	P=0.337	P=0.642
Uterus (Residual Evaluation): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	6/50 (12%)	4/49 (8%)
Adjusted rate	4.5%	2.2%	13.6%	10.4%
Terminal rate	0/37 (0%)	1/39 (3%)	3/33 (9%)	3/28 (11%)
First incidence (days)	508	729 (T)	676	686
Poly-3 test	P=0.135	P=0.485N	P=0.131	P=0.275
Uterus (Original and Residual Evaluations): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	7/50 (14%)	5/49 (10%)
Adjusted rate	4.5%	4.3%	15.7%	12.9%
Terminal rate	0/37 (0%)	2/39 (5%)	3/33 (9%)	4/28 (14%)
First incidence (days)	508	729 (T)	614	686
Poly-3 test	P=0.100	P=0.678N	P=0.079	P=0.163
Uterus (Original Evaluation): Stromal Polyp				
Overall rate	3/50 (6%)	6/50 (12%)	7/50 (14%)	5/49 (10%)
Adjusted rate	6.9%	12.8%	15.9%	12.8%
Terminal rate	2/37 (5%)	5/39 (13%)	6/33 (18%)	4/28 (14%)
First incidence (days)	592	585	655	553
Poly-3 test	P=0.388	P=0.277	P=0.158	P=0.296
Uterus (Residual Evaluation): Stromal Polyp				
Overall rate	3/50 (6%)	10/50 (20%)	6/50 (12%)	7/49 (14%)
Adjusted rate	6.9%	21.5%	13.5%	17.8%
Terminal rate	2/37 (5%)	8/39 (21%)	4/33 (12%)	5/28 (18%)
First incidence (days)	592	694	614	553
Poly-3 test	P=0.351	P=0.045	P=0.249	P=0.117

TABLE B2
Statistical Analysis of Primary Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Uterus (Original and Residual Evaluations): Stromal Polyp				
Overall rate	4/50 (8%)	12/50 (24%)	11/50 (22%)	9/49 (18%)
Adjusted rate	9.2%	25.5%	24.8%	22.8%
Terminal rate	3/37 (8%)	9/39 (23%)	9/33 (27%)	7/28 (25%)
First incidence (days)	592	585	614	553
Poly-3 test	P=0.283	P=0.037	P=0.045	P=0.077
Uterus (Original Evaluation): Stromal Polyp or Stromal Sarcoma				
Overall rate	3/50 (6%)	6/50 (12%)	8/50 (16%)	5/49 (10%)
Adjusted rate	6.9%	12.8%	18.2%	12.8%
Terminal rate	2/37 (5%)	5/39 (13%)	7/33 (21%)	4/28 (14%)
First incidence (days)	592	585	655	553
Poly-3 test	P=0.390	P=0.277	P=0.099	P=0.296
Uterus (Residual Evaluation): Stromal Polyp or Stromal Sarcoma				
Overall rate	3/50 (6%)	10/50 (20%)	7/50 (14%)	7/49 (14%)
Adjusted rate	6.9%	21.5%	15.8%	17.8%
Terminal rate	2/37 (5%)	8/39 (21%)	5/33 (15%)	5/28 (18%)
First incidence (days)	592	694	614	553
Poly-3 test	P=0.354	P=0.045	P=0.162	P=0.117
Uterus (Original and Residual Evaluations): Stromal Polyp or Stromal Sarcoma				
Overall rate	4/50 (8%)	12/50 (24%)	12/50 (24%)	9/49 (18%)
Adjusted rate	9.2%	25.5%	27.1%	22.8%
Terminal rate	3/37 (8%)	9/39 (23%)	10/33 (30%)	7/28 (25%)
First incidence (days)	592	585	614	553
Poly-3 test	P=0.284	P=0.037	P=0.026	P=0.077
All Organs: Malignant Lymphoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/49 (0%)
Adjusted rate	0.0%	6.4%	0.0%	0.0%
Terminal rate	0/37 (0%)	1/39 (3%)	0/33 (0%)	0/28 (0%)
First incidence (days)	—	651	—	—
Poly-3 test	P=0.239N	P=0.133	—	—
All Organs: Benign Neoplasms				
Overall rate	37/50 (74%)	35/50 (70%)	41/50 (82%)	43/49 (88%)
Adjusted rate	78.9%	70.8%	84.3%	95.8%
Terminal rate	29/37 (78%)	27/39 (69%)	28/33 (85%)	27/28 (96%)
First incidence (days)	445	358	368	396
Poly-3 test	P=0.002	P=0.246N	P=0.334	P=0.012

TABLE B2
Statistical Analysis of Primary Neoplasms in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	10/50 (20%)	8/50 (16%)	13/50 (26%)	16/49 (33%)
Adjusted rate	22.5%	16.8%	27.8%	39.5%
Terminal rate	7/37 (19%)	3/39 (8%)	3/33 (9%)	10/28 (36%)
First incidence (days)	585	585	508	385
Poly-3 test	P=0.014	P=0.337N	P=0.365	P=0.069
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	38/50 (76%)	46/50 (92%)	44/49 (90%)
Adjusted rate	83.8%	76.4%	92.0%	96.2%
Terminal rate	30/37 (81%)	28/39 (72%)	29/33 (88%)	27/28 (96%)
First incidence (days)	445	358	368	385
Poly-3 test	P=0.007	P=0.255N	P=0.171	P=0.043

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

TABLE B3a
Historical Incidence of Liver Neoplasms in Control Female Wistar Han Rats[a]

Study (Study Start)	Cholangiocarcinoma	Hepatocholangioma	Hepatocellular Adenoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (August 2008)	0/50	0/50	3/50
Tetrabromobisphenol A (July 2007)	0/50	0/50	1/50
Total (%)	0/100	0/100	4/100 (4.0%)
Mean ± standard deviation			4.0% ± 2.8%
Range			2%-6%
Overall Historical Incidence: All Routes			
Total (%)	0/300	0/300	6/300
Mean ± standard deviation			2.0% ± 2.2%
Range			0%-6%

	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma	Hepatocholangioma, Hepatocellular Adenoma, or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (August 2008)	0/50	3/50	3/50
Tetrabromobisphenol A (July 2007)	0/50	1/50	1/50
Total (%)	0/100	4/100 (4.0%)	4/100 (4.0%)
Mean ± standard deviation		4.0% ± 2.8%	4.0% ± 2.8%
Range		2%-6%	2%-6%
Overall Historical Incidence: All Routes			
Total (%)	0/300	6/300	6/300
Mean ± standard deviation		2.0% ± 2.2%	2.0% ± 2.2%
Range		0%-6%	0%-6%

[a] Data as of November 2014

TABLE B3b
Historical Incidence of Uterus Neoplasms in Control Female Wistar Han Rats[a]

Study (Study Start)	Stromal Polyp	Stromal Sarcoma	Stromal Polyp or Stromal Sarcoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (August 2008)	3/50	0/50	3/50
Tetrabromobisphenol A (July 2007)	2/50	0/50	2/50
Total (%)	5/100 (5.0%)	0/100	5/100 (5.0%)
Mean ± standard deviation	5.0% ± 1.4%		5.0% ± 1.4%
Range	4%-6%		4%-6%
Overall Historical Incidence: All Routes (Original Evaluation)			
Total (%)	13/194	3/194	15/194
Mean ± standard deviation	6.7% ± 2.5%	1.6% ± 1.9%	7.8% ± 3.5%
Range	4%-10%	0%-4%	4%-12%
Overall Historical Incidence: All Routes (Residual Evaluation)			
Total (%)	20/194	2/194	22/194
Mean ± standard deviation	10.3% ± 2.9%	1.1% ± 1.2%	11.4% ± 3.7%
Range	6%-12%	0%-2%	6%-14%
Overall Historical Incidence: All Routes (Original and Residual Evaluations)			
Total (%)	27/194	3/194	29/194
Mean ± standard deviation	14.0% ± 5.2%	1.6% ± 1.9%	15.1% ± 6.3%
Range	8%-20%	0%-4%	8%-22%

[a] Data as of May 2015

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
3-Month interim evaluation	10			10
Early deaths				
Accidental deaths	2	1		
Moribund	8	10	13	11
Natural deaths	3		4	10
Survivors				
Died last week of study	1			
Terminal kill	36	39	33	28
Other				1
Animals examined microscopically	60	50	50	59
3-Month Interim Evaluation				
Alimentary System				
Esophagus	(10)			(10)
Intestine large, cecum	(10)			(10)
Intestine large, colon	(10)			(10)
Intestine large, rectum	(10)			(10)
Intestine small, duodenum	(10)			(10)
Intestine small, ileum	(10)			(10)
Intestine small, jejunum	(10)			(10)
Liver	(9)			(10)
Fatty change				3 (30%)
Hepatocyte, hypertrophy				10 (100%)
Oral mucosa	(1)			(0)
Pancreas	(10)			(10)
Atrophy	1 (10%)			
Salivary glands	(10)			(10)
Stomach, forestomach	(10)			(10)
Stomach, glandular	(10)			(10)
Endocrine System				
Adrenal cortex	(10)			(10)
Accessory adrenal cortical nodule	3 (30%)			2 (20%)
Adrenal medulla	(9)			(10)
Islets, pancreatic	(10)			(10)
Parathyroid gland	(10)			(10)
Pituitary gland	(10)			(10)
Thyroid gland	(10)			(10)
Follicle, hypertrophy	1 (10%)			5 (50%)
Genital System				
Clitoral gland	(10)			(10)
Inflammation, chronic	7 (70%)			6 (60%)
Inflammation, chronic active				1 (10%)
Ovary	(10)			(10)
Uterus	(10)			(10)

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
3-Month Interim Evaluation (continued)				
Hematopoietic System				
Bone marrow	(10)			(10)
Lymph node	(2)			(2)
Pigmentation	1 (50%)			
Popliteal, pigmentation				2 (100%)
Lymph node, mandibular	(10)			(10)
Hyperplasia, lymphoid	1 (10%)			
Lymph node, mesenteric	(10)			(10)
Spleen	(10)			(10)
Hematopoietic cell proliferation				1 (10%)
Pigmentation				1 (10%)
Thymus	(10)			(10)
Respiratory System				
Lung	(10)			(10)
Inflammation, chronic				2 (20%)
Metaplasia, osseous				1 (10%)
Nose	(10)			(10)
Trachea	(10)			(10)
Systems Examined at 3 Months with No Lesions Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Hyperkeratosis	1 (2%)			
Inflammation, chronic active	1 (2%)			
Intestine large, cecum	(48)	(49)	(47)	(40)
Intestine large, colon	(48)	(49)	(50)	(46)
Intestine large, rectum	(49)	(49)	(49)	(45)
Degeneration, fatty, focal		1 (2%)		
Intestine small, duodenum	(47)	(49)	(47)	(42)
Epithelium, vacuolization cytoplasmic		1 (2%)		
Intestine small, ileum	(47)	(49)	(48)	(41)
Intestine small, jejunum	(46)	(49)	(47)	(42)
Liver	(50)	(49)	(50)	(47)
Angiectasis	1 (2%)		4 (8%)	2 (4%)
Basophilic focus	2 (4%)	4 (8%)	7 (14%)	5 (11%)
Basophilic focus, multiple	42 (84%)	39 (80%)	33 (66%)	28 (60%)
Cholangiofibrosis				3 (6%)
Clear cell focus	2 (4%)	3 (6%)	1 (2%)	
Clear cell focus, multiple	33 (66%)	18 (37%)	25 (50%)	31 (66%)
Congestion	3 (6%)			1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Liver (continued)	(50)	(49)	(50)	(47)
Cyst			1 (2%)	
Eosinophilic focus	5 (10%)	5 (10%)	10 (20%)	12 (26%)
Eosinophilic focus, multiple		2 (4%)	11 (22%)	19 (40%)
Fatty change	15 (30%)	12 (24%)	28 (56%)	39 (83%)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	4 (8%)	1 (2%)		
Hepatodiaphragmatic nodule	4 (8%)	5 (10%)		1 (2%)
Hyperplasia, nodular			2 (4%)	7 (15%)
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic	1 (2%)			
Mixed cell focus		1 (2%)		
Pigmentation	1 (2%)	2 (4%)	1 (2%)	
Bile duct, cyst	1 (2%)	2 (4%)	4 (8%)	6 (13%)
Bile duct, cyst, multiple	1 (2%)		1 (2%)	1 (2%)
Bile duct, fibrosis	1 (2%)			
Bile duct, hyperplasia	16 (32%)	20 (41%)	16 (32%)	14 (30%)
Bile duct, inflammation, chronic active				1 (2%)
Hepatocyte, degeneration			1 (2%)	
Hepatocyte, hypertrophy		48 (98%)	49 (98%)	45 (96%)
Hepatocyte, mitosis			1 (2%)	
Hepatocyte, necrosis	4 (8%)	2 (4%)	1 (2%)	8 (17%)
Oval cell, hyperplasia	1 (2%)	3 (6%)	3 (6%)	10 (21%)
Serosa, inflammation, acute	1 (2%)			
Mesentery	(10)	(7)	(9)	(6)
Congestion	1 (10%)			
Inflammation, granulomatous, chronic active		1 (14%)		
Inflammation, chronic	1 (10%)			
Fat, necrosis	8 (80%)	6 (86%)	5 (56%)	6 (100%)
Oral mucosa	(0)	(0)	(1)	(1)
Pancreas	(50)	(49)	(49)	(47)
Atrophy	3 (6%)	3 (6%)	3 (6%)	5 (11%)
Inflammation, granulomatous, chronic		1 (2%)		
Inflammation, chronic	3 (6%)		1 (2%)	
Salivary glands	(50)	(50)	(49)	(45)
Cyst	1 (2%)			
Inflammation, chronic	1 (2%)		1 (2%)	
Duct, degeneration, hyaline		1 (2%)		
Duct, parotid gland, inflammation, acute	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Duct, submandibular gland, inflammation, acute	1 (2%)			
Parotid gland, atrophy	4 (8%)	4 (8%)	4 (8%)	5 (11%)
Parotid gland, basophilic focus				1 (2%)
Parotid gland, inflammation		1 (2%)		
Parotid gland, inflammation, acute			1 (2%)	
Parotid gland, inflammation, chronic	1 (2%)			
Parotid gland, necrosis				1 (2%)
Parotid gland, vacuolization cytoplasmic	6 (12%)	9 (18%)	7 (14%)	2 (4%)
Sublingual gland, ectopic tissue			1 (2%)	1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Alimentary System (continued)				
Salivary glands (continued)	(50)	(50)	(49)	(45)
Sublingual gland, vacuolization cytoplasmic		1 (2%)		
Submandibular gland, ectopic tissue				1 (2%)
Submandibular gland, inflammation, acute		1 (2%)		
Submandibular gland, inflammation, chronic				1 (2%)
Submandibular gland, necrosis		1 (2%)		
Stomach, forestomach	(50)	(49)	(50)	(48)
Edema	1 (2%)	1 (2%)	2 (4%)	
Foreign body	1 (2%)			
Hyperkeratosis	4 (8%)	6 (12%)	7 (14%)	4 (8%)
Inflammation, acute		3 (6%)	1 (2%)	
Inflammation, chronic	1 (2%)		3 (6%)	2 (4%)
Inflammation, chronic active	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Mineralization		2 (4%)	3 (6%)	1 (2%)
Ulcer	2 (4%)	4 (8%)	3 (6%)	3 (6%)
Epithelium, hyperplasia	5 (10%)	6 (12%)	6 (12%)	4 (8%)
Stomach, glandular	(49)	(49)	(50)	(46)
Erosion			1 (2%)	
Inflammation, acute		1 (2%)	2 (4%)	
Inflammation, chronic				1 (2%)
Mineralization	9 (18%)	11 (22%)	14 (28%)	7 (15%)
Necrosis			1 (2%)	
Ulcer		1 (2%)	1 (2%)	1 (2%)
Tooth	(1)	(0)	(0)	(0)
Inflammation, chronic	1 (100%)			
Cardiovascular System				
Blood vessel	(1)	(0)	(3)	(3)
Inflammation, acute	1 (100%)			
Heart	(50)	(50)	(50)	(48)
Cardiomyopathy	12 (24%)	8 (16%)	10 (20%)	4 (8%)
Inflammation, chronic	1 (2%)			
Epicardium, inflammation, chronic		1 (2%)		
Epicardium, inflammation, chronic active	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(46)
Accessory adrenal cortical nodule, multifocal		1 (2%)		
Angiectasis	45 (90%)	44 (90%)	44 (88%)	34 (74%)
Hematopoietic cell proliferation	1 (2%)		3 (6%)	1 (2%)
Hemorrhage	1 (2%)			
Hyperplasia, focal	8 (16%)	6 (12%)	12 (24%)	19 (41%)
Hypertrophy, focal	13 (26%)	9 (18%)	12 (24%)	14 (30%)
Necrosis		1 (2%)		
Vacuolization cytoplasmic	5 (10%)	5 (10%)	7 (14%)	9 (20%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(47)
Hyperplasia, focal			1 (2%)	
Islets, pancreatic	(50)	(49)	(49)	(47)
Hyperplasia	1 (2%)			
Hypertrophy	1 (2%)			
Pigmentation, hemosiderin	1 (2%)			
Parathyroid gland	(49)	(47)	(49)	(46)
Pituitary gland	(50)	(49)	(50)	(47)
Pigmentation, hemosiderin			2 (4%)	
Pars distalis, cyst	3 (6%)			1 (2%)
Pars distalis, cyst, multiple		1 (2%)	3 (6%)	
Pars distalis, hyperplasia, focal	14 (28%)	9 (18%)	17 (34%)	9 (19%)
Pars distalis, vacuolization cytoplasmic				1 (2%)
Pars intermedia, hyperplasia, focal		1 (2%)		1 (2%)
Pars intermedia, hypertrophy		1 (2%)		
Pars nervosa, cyst, multiple			1 (2%)	
Pars nervosa, inflammation, chronic	1 (2%)		1 (2%)	1 (2%)
Thyroid gland	(45)	(49)	(47)	(42)
Mineralization				1 (2%)
C-cell, hyperplasia	45 (100%)	48 (98%)	46 (98%)	38 (90%)
Follicle, cyst	2 (4%)		2 (4%)	
Follicle, cyst, multiple	1 (2%)			
Follicle, hypertrophy	8 (18%)	17 (35%)	22 (47%)	35 (83%)
Follicular cell, hyperplasia	1 (2%)	5 (10%)	4 (9%)	6 (14%)
Follicular cell, hypertrophy			1 (2%)	
General Body System				
Tissue NOS	(3)	(2)	(4)	(2)
Abscess	1 (33%)			
Fibrosis			1 (25%)	1 (50%)
Inflammation, suppurative, chronic active		1 (50%)		
Inflammation, acute	1 (33%)			
Inflammation, chronic active	1 (33%)			
Genital System				
Clitoral gland	(49)	(49)	(50)	(47)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Inflammation, chronic active			1 (2%)	2 (4%)
Duct, cyst	2 (4%)	4 (8%)	3 (6%)	3 (6%)
Duct, cyst, multiple		1 (2%)		
Ovary	(50)	(49)	(50)	(46)
Atrophy	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Cyst	5 (10%)	5 (10%)	8 (16%)	8 (17%)
Cyst, multiple			1 (2%)	
Hyperplasia, tubulostromal		4 (8%)	3 (6%)	1 (2%)
Follicle, cyst	4 (8%)	2 (4%)	3 (6%)	5 (11%)
Follicle, cyst, multiple	2 (4%)	1 (2%)		1 (2%)
Granulosa cell, hyperplasia, multifocal				1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Genital System (continued)				
Uterus	(50)	(49)	(50)	(47)
Adenomyosis		1 (2%)		
Angiectasis	1 (2%)			
Cyst		1 (2%)		
Cyst, squamous				1 (2%)
Decidual reaction			1 (2%)	1 (2%)
Dilatation			1 (2%)	1 (2%)
Hemorrhage	1 (2%)		1 (2%)	
Hyperplasia, atypical	3 (6%)	1 (2%)	2 (4%)	
Inflammation, chronic	1 (2%)			
Inflammation, chronic active		1 (2%)		
Metaplasia, squamous			1 (2%)	4 (9%)
Cervix, hyperkeratosis			1 (2%)	
Cervix, hyperplasia, squamous				2 (4%)
Endometrium, hyperplasia, cystic	15 (30%)	9 (18%)	17 (34%)	14 (30%)
Myometrium, degeneration, mucoid			1 (2%)	
Serosa, cyst			1 (2%)	
Serosa, inflammation, acute	1 (2%)			
Vagina	(1)	(1)	(2)	(2)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(46)
Fibrosis				1 (2%)
Myeloid cell, hyperplasia	6 (12%)	4 (8%)	7 (14%)	11 (24%)
Lymph node	(10)	(5)	(6)	(9)
Pigmentation, hemosiderin	1 (10%)			
Axillary, ectasia	1 (10%)			
Axillary, hyperplasia, lymphoid	1 (10%)			
Axillary, pigmentation	1 (10%)			
Iliac, hyperplasia, lymphoid				1 (11%)
Inguinal, pigmentation	2 (20%)		2 (33%)	
Mediastinal, ectasia		1 (20%)	1 (17%)	
Mediastinal, hemorrhage	2 (20%)	1 (20%)		1 (11%)
Mediastinal, hyperplasia, lymphoid				1 (11%)
Mediastinal, hyperplasia, plasma cell			1 (17%)	
Mediastinal, inflammation, granulomatous, chronic active		1 (20%)		
Mediastinal, pigmentation			1 (17%)	
Mediastinal, pigmentation, hemosiderin	3 (30%)	2 (40%)	1 (17%)	4 (44%)
Pancreatic, hemorrhage	1 (10%)			
Pancreatic, pigmentation, hemosiderin	1 (10%)			1 (11%)
Popliteal, hemorrhage				1 (11%)
Popliteal, hyperplasia, lymphoid				1 (11%)
Popliteal, pigmentation		1 (20%)		3 (33%)
Lymph node, mandibular	(50)	(50)	(50)	(48)
Ectasia	4 (8%)	2 (4%)	4 (8%)	1 (2%)
Hemorrhage	2 (4%)			
Hyperplasia, lymphoid				2 (4%)
Hyperplasia, plasma cell				1 (2%)
Necrosis	1 (2%)			
Pigmentation, hemosiderin		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(49)	(50)	(46)
Ectasia	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)			
Infiltration cellular, histiocyte	1 (2%)			
Inflammation, acute	1 (2%)			
Pigmentation, hemosiderin	1 (2%)	1 (2%)	1 (2%)	
Spleen	(50)	(49)	(50)	(45)
Accessory spleen	1 (2%)	1 (2%)		
Angiectasis				1 (2%)
Hematopoietic cell proliferation	27 (54%)	24 (49%)	19 (38%)	17 (38%)
Hemorrhage		1 (2%)	1 (2%)	
Pigmentation	31 (62%)	31 (63%)	32 (64%)	27 (60%)
Capsule, fibrosis, focal	1 (2%)			
Lymphoid follicle, atrophy		2 (4%)	3 (6%)	1 (2%)
Thymus	(50)	(49)	(48)	(46)
Atrophy	10 (20%)	7 (14%)	18 (38%)	9 (20%)
Cyst				1 (2%)
Hemorrhage	4 (8%)	6 (12%)	5 (10%)	1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(48)
Degeneration, fatty	1 (2%)			
Fibrosis			2 (4%)	
Galactocele	2 (4%)		3 (6%)	3 (6%)
Hyperplasia	26 (52%)	28 (57%)	24 (48%)	19 (40%)
Inflammation, granulomatous			1 (2%)	
Inflammation, chronic active				1 (2%)
Duct, cyst	1 (2%)		1 (2%)	
Duct, dilatation	16 (32%)	19 (39%)	13 (26%)	6 (13%)
Duct, inflammation, acute				1 (2%)
Skin	(50)	(50)	(50)	(49)
Fibrosis	1 (2%)			
Hyperkeratosis	1 (2%)		2 (4%)	
Inflammation, acute			2 (4%)	
Inflammation, chronic		1 (2%)	1 (2%)	
Inflammation, chronic active		1 (2%)		1 (2%)
Ulcer	1 (2%)		2 (4%)	1 (2%)
Epidermis, hyperplasia		1 (2%)	1 (2%)	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Skeletal muscle	(1)	(0)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(49)
Compression	8 (16%)	9 (18%)	11 (22%)	13 (27%)
Cyst	1 (2%)			
Hemorrhage, multifocal			1 (2%)	
Peripheral nerve	(0)	(0)	(1)	(0)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
2-Year Study (continued)				
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Infiltration cellular, histiocyte	25 (50%)	23 (46%)	22 (44%)	30 (61%)
Inflammation, acute				1 (2%)
Inflammation, chronic	1 (2%)			1 (2%)
Mineralization			1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	3 (6%)	1 (2%)	
Serosa, inflammation, acute	1 (2%)			
Nose	(50)	(50)	(50)	(47)
Inflammation, acute		1 (2%)	1 (2%)	1 (2%)
Trachea	(47)	(50)	(50)	(47)
Inflammation, chronic		1 (2%)	1 (2%)	
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(49)	(47)	(45)
Developmental malformation		1 (2%)		
Mineralization	1 (2%)			
Retina, atrophy	9 (18%)	3 (6%)	9 (19%)	10 (22%)
Harderian gland	(49)	(50)	(50)	(49)
Urinary System				
Kidney	(50)	(50)	(49)	(47)
Calculus gross observation	1 (2%)			
Casts protein	2 (4%)			2 (4%)
Cyst	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Cyst, multiple	1 (2%)			1 (2%)
Hydronephrosis	1 (2%)	1 (2%)	1 (2%)	6 (13%)
Inflammation, chronic	2 (4%)		1 (2%)	
Inflammation, chronic active			1 (2%)	
Nephropathy	13 (26%)	8 (16%)	17 (35%)	15 (32%)
Pigmentation		1 (2%)	3 (6%)	4 (9%)
Pelvis, inflammation, acute	1 (2%)			
Pelvis, inflammation, chronic active	16 (32%)	10 (20%)	6 (12%)	3 (6%)
Pelvis, mineralization	31 (62%)	29 (58%)	23 (47%)	19 (40%)
Renal tubule, dilatation			1 (2%)	
Transitional epithelium, hyperplasia	3 (6%)	4 (8%)	1 (2%)	2 (4%)
Ureter	(1)	(0)	(0)	(1)
Inflammation, chronic				1 (100%)
Mineralization				1 (100%)
Transitional epithelium, hyperplasia	1 (100%)			
Urinary bladder	(50)	(49)	(49)	(45)
Inflammation, chronic				1 (2%)
Inflammation, chronic active	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF DE-71

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1			2
Moribund	15	7	14	36
Natural deaths	5	10	5	12
Survivors				
Terminal kill	29	33	31	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(43)	(42)	(41)	(31)
Intestine large, cecum	(46)	(43)	(45)	(45)
Intestine large, colon	(48)	(44)	(46)	(46)
Intestine large, rectum	(48)	(46)	(46)	(46)
Intestine small, duodenum	(46)	(43)	(47)	(44)
Intestine small, ileum	(45)	(41)	(44)	(43)
Intestine small, jejunum	(46)	(42)	(44)	(46)
Adenoma				1 (2%)
Carcinoma				1 (2%)
Liver	(50)	(50)	(50)	(50)
Hepatoblastoma	1 (2%)	1 (2%)	12 (24%)	5 (10%)
Hepatoblastoma, multiple			4 (8%)	
Hepatocellular adenoma	13 (26%)	12 (24%)	4 (8%)	7 (14%)
Hepatocellular adenoma, multiple	10 (20%)	23 (46%)	45 (90%)	33 (66%)
Hepatocellular carcinoma	14 (28%)	13 (26%)	13 (26%)	10 (20%)
Hepatocellular carcinoma, multiple	4 (8%)	2 (4%)	17 (34%)	35 (70%)
Hepatocholangiocarcinoma		1 (2%)		
Mesentery	(12)	(3)	(9)	(5)
Hepatoblastoma, metastatic, liver			2 (22%)	
Hepatocellular carcinoma, metastatic, liver	1 (8%)		1 (11%)	
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Stomach, glandular	(50)	(48)	(48)	(50)
Tongue	(0)	(0)	(0)	(1)
Tooth	(2)	(1)	(0)	(1)
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(48)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Capsule, adenoma		1 (2%)	1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(48)
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Parathyroid gland	(48)	(43)	(49)	(44)
Adenoma		1 (2%)		
Pituitary gland	(47)	(43)	(43)	(44)
Pars distalis, adenoma		1 (2%)		
Thyroid gland	(50)	(49)	(50)	(49)
Follicular cell, adenoma	1 (2%)	1 (2%)	2 (4%)	
General Body System				
Tissue NOS	(2)	(1)	(0)	(1)
Genital System				
Coagulating gland	(1)	(0)	(1)	(0)
Hepatoblastoma, metastatic, liver			1 (100%)	
Epididymis	(50)	(50)	(50)	(50)
Granular cell tumor benign			1 (2%)	
Hepatoblastoma, metastatic, liver			1 (2%)	
Penis	(1)	(0)	(1)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(49)	(49)
Testes	(50)	(50)	(50)	(49)
Interstitial cell, adenoma	1 (2%)		1 (2%)	
Rete testes, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Cranium, carcinoma, metastatic, Zymbal's gland	1 (2%)			
Lymph node	(5)	(4)	(4)	(1)
Fat, hemangiosarcoma		1 (25%)		
Mediastinal, hepatocholangiocarcinoma, metastatic, liver		1 (25%)		
Thoracic, hepatocholangiocarcinoma, metastatic, liver		1 (25%)		
Lymph node, mandibular	(50)	(49)	(49)	(46)
Lymph node, mesenteric	(49)	(47)	(46)	(47)
Hepatocellular carcinoma, metastatic, liver	1 (2%)		1 (2%)	
Spleen	(50)	(47)	(47)	(47)
Hemangiosarcoma	1 (2%)	1 (2%)		
Thymus	(40)	(41)	(40)	(39)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Integumentary System				
Mammary gland	(2)	(2)	(1)	(4)
Skin	(50)	(50)	(50)	(50)
Lipoma	1 (2%)			
Schwannoma malignant			1 (2%)	
Lip, mast cell tumor benign			1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)			
Musculoskeletal System				
Bone	(49)	(50)	(50)	(49)
Skeletal muscle	(2)	(2)	(0)	(0)
Hepatocholangiocarcinoma, metastatic, liver		1 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(2)	(1)	(1)	(0)
Spinal cord	(1)	(1)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	4 (8%)	3 (6%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple		2 (4%)		
Alveolar/bronchiolar carcinoma	4 (8%)	3 (6%)	1 (2%)	
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	3 (6%)		
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Hepatoblastoma, metastatic, liver			2 (4%)	
Hepatocellular carcinoma, metastatic, liver	6 (12%)	2 (4%)	4 (8%)	4 (8%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Nose	(50)	(48)	(50)	(50)
Pleura	(0)	(1)	(0)	(0)
Hepatocholangiocarcinoma, metastatic, liver		1 (100%)		
Trachea	(47)	(48)	(49)	(47)
Special Senses System				
Eye	(49)	(47)	(47)	(46)
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	5 (10%)	6 (12%)	3 (6%)	
Carcinoma	1 (2%)			
Zymbal's gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Renal tubule, adenoma		1 (2%)		
Renal tubule, carcinoma		1 (2%)	1 (2%)	
Urethra	(0)	(4)	(3)	(0)
Urinary bladder	(50)	(49)	(48)	(48)
Hepatoblastoma, metastatic, liver			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Lymphoma malignant	5 (10%)	7 (14%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	46	49	48
Total primary neoplasms	75	89	113	96
Total animals with benign neoplasms	30	39	49	41
Total benign neoplasms	42	53	63	45
Total animals with malignant neoplasms	27	25	37	45
Total malignant neoplasms	33	36	50	51
Total animals with metastatic neoplasms	7	3	7	4
Total metastatic neoplasms	11	9	13	4

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	6/50 (12%)	3/50 (6%)	0/50 (0%)
Adjusted rate[b]	12.4%	13.8%	6.7%	0.0%
Terminal rate[c]	4/29 (14%)	6/33 (18%)	1/31 (3%)	0/0 (0%)
First incidence (days)	684	729 (T)	618	—[e]
Poly-3 test[d]	P=0.078N	P=0.554	P=0.300N	P=0.175N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	6/50 (12%)	3/50 (6%)	0/50 (0%)
Adjusted rate	14.9%	13.8%	6.7%	0.0%
Terminal rate	5/29 (17%)	6/33 (18%)	1/31 (3%)	0/0 (0%)
First incidence (days)	684	729 (T)	618	—
Poly-3 test	P=0.057N	P=0.567N	P=0.192N	P=0.131N
Liver: Hepatocellular Adenoma				
Overall rate	23/50 (46%)	35/50 (70%)	49/50 (98%)	40/50 (80%)
Adjusted rate	53.2%	72.9%	98.8%	93.5%
Terminal rate	15/29 (52%)	25/33 (76%)	31/31 (100%)	0/0 (0%)
First incidence (days)	491	428	431	451
Poly-3 test	P<0.001	P=0.034	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	18/50 (36%)	15/50 (30%)	30/50 (60%)	45/50 (90%)
Adjusted rate	40.7%	33.0%	65.2%	97.7%
Terminal rate	8/29 (28%)	9/33 (27%)	21/31 (68%)	0/0 (0%)
First incidence (days)	491	540	453	451
Poly-3 test	P<0.001	P=0.293N	P=0.013	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	31/50 (62%)	40/50 (80%)[f]	49/50 (98%)	47/50 (94%)
Adjusted rate	68.1%	81.6%	98.8%	99.5%
Terminal rate	18/29 (62%)	26/33 (79%)	31/31 (100%)	0/0 (0%)
First incidence (days)	491	428	431	451
Poly-3 test	P<0.001	P=0.092	P<0.001	P<0.001
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	1/50 (2%)	16/50 (32%)	5/50 (10%)
Adjusted rate	2.5%	2.3%	35.0%	23.4%
Terminal rate	1/29 (3%)	1/33 (3%)	9/31 (29%)	0/0 (0%)
First incidence (days)	729 (T)	729 (T)	453	477
Poly-3 test	P<0.001	P=0.743N	P<0.001	P=0.020
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	18/50 (36%)	15/50 (30%)	36/50 (72%)	45/50 (90%)
Adjusted rate	40.7%	33.0%	76.8%	97.7%
Terminal rate	8/29 (28%)	9/33 (27%)	25/31 (81%)	0/0 (0%)
First incidence (days)	491	540	453	451
Poly-3 test	P<0.001	P=0.293N	P<0.001	P<0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	31/50 (62%)	40/50 (80%)[f]	49/50 (98%)	47/50 (94%)
Adjusted rate	68.1%	81.6%	98.8%	99.5%
Terminal rate	18/29 (62%)	26/33 (79%)	31/31 (100%)	0/0 (0%)
First incidence (days)	491	428	431	451
Poly-3 test	P<0.001	P=0.092	P<0.001	P<0.001

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	5/50 (10%)	6/50 (12%)	3/50 (6%)	1/50 (2%)
Adjusted rate	12.4%	13.7%	6.8%	5.3%
Terminal rate	4/29 (14%)	5/33 (15%)	3/31 (10%)	0/0 (0%)
First incidence (days)	639	557	729 (T)	543
Poly-3 test	P=0.177N	P=0.560	P=0.309N	P=0.371N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	1/50 (2%)	0/50 (0%)
Adjusted rate	12.3%	13.8%	2.3%	0.0%
Terminal rate	4/29 (14%)	6/33 (18%)	1/31 (3%)	0/0 (0%)
First incidence (days)	568	729 (T)	729 (T)	—
Poly-3 test	P=0.029N	P=0.548	P=0.083N	P=0.177N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	10/50 (20%)	12/50 (24%)	4/50 (8%)	1/50 (2%)
Adjusted rate	24.5%	27.3%	9.1%	5.3%
Terminal rate	8/29 (28%)	11/33 (33%)	4/31 (13%)	0/0 (0%)
First incidence (days)	568	557	729 (T)	543
Poly-3 test	P=0.013N	P=0.480	P=0.051N	P=0.103N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	2/50 (4%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	5.0%	2.3%	4.6%	15.0%
Terminal rate	2/29 (7%)	0/33 (0%)	2/31 (7%)	0/0 (0%)
First incidence (days)	729 (T)	692	729 (T)	492
Poly-3 test	P=0.109	P=0.471N	P=0.660N	P=0.229
All Organs: Malignant Lymphoma				
Overall rate	5/50 (10%)	7/50 (14%)	1/50 (2%)	0/50 (0%)
Adjusted rate	12.4%	16.1%	2.2%	0.0%
Terminal rate	4/29 (14%)	7/33 (21%)	0/31 (0%)	0/0 (0%)
First incidence (days)	680	729 (T)	431	—
Poly-3 test	P=0.020N	P=0.433	P=0.078N	P=0.175N
All Organs: Benign Neoplasms				
Overall rate	30/50 (60%)	39/50 (78%)	49/50 (98%)	41/50 (82%)
Adjusted rate	67.9%	81.2%	98.8%	94.1%
Terminal rate	21/29 (72%)	29/33 (88%)	31/31 (100%)	0/0 (0%)
First incidence (days)	298	428	431	442
Poly-3 test	P<0.001	P=0.094	P<0.001	P<0.001

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	25/50 (50%)	37/50 (74%)	45/50 (90%)
Adjusted rate	59.5%	54.7%	77.6%	97.7%
Terminal rate	14/29 (48%)	18/33 (55%)	25/31 (81%)	0/0 (0%)
First incidence (days)	491	540	431	451
Poly-3 test	P<0.001	P=0.400N	P=0.041	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	46/50 (92%)	49/50 (98%)	48/50 (96%)
Adjusted rate	84.0%	93.9%	98.8%	100.0%
Terminal rate	23/29 (79%)	32/33 (97%)	31/31 (100%)	0/0 (0%)
First incidence (days)	298	428	431	442
Poly-3 test	P=0.005	P=0.096	P=0.007	P=0.003

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

TABLE C3
Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (February 2008)	23/50	18/50	31/50
Ginkgo biloba extract (March 2005)	31/50	22/50	39/50
Indole-3-carbinol (April 2007)	26/50	12/50	35/50
Kava kava extract (August 2004)	27/50	20/50	38/50
N,*N*-dimethyl-*p*-toluidine (October 2004)	29/50	22/50	38/50
Tetrabromobisphenol A (August 2007)	32/50	11/50	39/50
Total (%)	168/300 (56%)	105/300 (35.0%)	220/300 (73.3%)
Mean ± standard deviation	56.0% ± 6.7%	35.0% ± 9.8%	73.3% ± 6.3%
Range	46%-64%	22%-44%	62%-78%
Overall Historical Incidence: All Routes			
Total (%)	437/700 (62.4%)	262/700 (37.4%)	541/700 (77.3%)
Mean ± standard deviation	62.4% ± 10.5%	37.4% ± 11.2%	77.3% ± 8.3%
Range	46%-78%	22%-52%	62%-90%

	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma	Hepatocholangio-carcinoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (February 2008)	1/50	31/50	0/50
Ginkgo biloba extract (March 2005)	3/50	39/50	0/50
Indole-3-carbinol (April 2007)	3/50	36/50	0/50
Kava kava extract (August 2004)	0/50	38/50	4/50
N,*N*-dimethyl-*p*-toluidine (October 2004)	1/50	38/50	0/50
Tetrabromobisphenol A (August 2007)	2/50	39/50	0/50
Total (%)	10/300 (3.3%)	221/300 (73.7%)	4/300 (1.3%)
Mean ± standard deviation	3.3% ± 2.4%	73.7% ± 6.1%	1.3 ± 3.3%
Range	0%-6%	62%-78%	0%-8%
Overall Historical Incidence: All Routes			
Total (%)	34/700 (4.9%)	545/700 (77.9%)	9/700 (1.3%)
Mean ± standard deviation	4.9% ± 3.7%	77.9% ± 8.3%	1.3% ± 2.4%
Range	0%-12%	62%-90%	0%-8%

[a] Data as of November 2014

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1			2
Moribund	15	7	14	36
Natural deaths	5	10	5	12
Survivors				
Terminal kill	29	33	31	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Foreign body				1 (2%)
Inflammation, acute	1 (2%)			2 (4%)
Inflammation, chronic		1 (2%)		
Mineralization	1 (2%)			
Necrosis	1 (2%)			2 (4%)
Muscularis, degeneration		2 (4%)	2 (4%)	
Gallbladder	(43)	(42)	(41)	(31)
Cyst			1 (2%)	
Intestine large, cecum	(46)	(43)	(45)	(45)
Lymphoid tissue, necrosis	1 (2%)			
Intestine large, colon	(48)	(44)	(46)	(46)
Intestine large, rectum	(48)	(46)	(46)	(46)
Serosa, fibrosis		1 (2%)		
Intestine small, duodenum	(46)	(43)	(47)	(44)
Infiltration cellular, plasma cell			1 (2%)	
Inflammation			1 (2%)	
Intestine small, ileum	(45)	(41)	(44)	(43)
Intestine small, jejunum	(46)	(42)	(44)	(46)
Peyer’s patch, hyperplasia		1 (2%)		
Serosa, fibrosis				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	6 (12%)	2 (4%)		5 (10%)
Basophilic focus, multiple		1 (2%)	1 (2%)	
Clear cell focus	10 (20%)	6 (12%)	17 (34%)	7 (14%)
Clear cell focus, multiple		7 (14%)	3 (6%)	
Congestion		1 (2%)		
Depletion glycogen	1 (2%)	1 (2%)		
Eosinophilic focus	14 (28%)	12 (24%)	4 (8%)	10 (20%)
Eosinophilic focus, multiple	1 (2%)	10 (20%)	6 (12%)	1 (2%)
Fatty change	17 (34%)	25 (50%)	17 (34%)	5 (10%)
Hematopoietic cell proliferation	10 (20%)	5 (10%)	1 (2%)	3 (6%)
Hemorrhage	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic	13 (26%)	19 (38%)	22 (44%)	12 (24%)
Mineralization	1 (2%)			
Mixed cell focus	2 (4%)	4 (8%)		1 (2%)
Mixed cell focus, multiple		1 (2%)		
Necrosis, focal	2 (4%)	2 (4%)	16 (32%)	2 (4%)

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Tension lipidosis	1 (2%)			
Bile duct, cyst	1 (2%)			
Centrilobular, hepatocyte, hypertrophy		28 (56%)	46 (92%)	48 (96%)
Hepatocyte, mitotic alteration		1 (2%)		
Hepatocyte, necrosis				1 (2%)
Kupffer cell, pigmentation	5 (10%)	15 (30%)	33 (66%)	25 (50%)
Mesentery	(12)	(3)	(9)	(5)
Hemorrhage	1 (8%)			
Inflammation, chronic	1 (8%)			1 (20%)
Artery, inflammation, chronic active			2 (22%)	2 (40%)
Artery, thrombosis	1 (8%)			
Fat, necrosis	8 (67%)	3 (100%)	6 (67%)	2 (40%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	10 (20%)	14 (28%)	7 (14%)	
Cyst	1 (2%)	4 (8%)		
Degeneration				1 (2%)
Hemorrhage				1 (2%)
Hypertrophy, focal	1 (2%)			
Inflammation, granulomatous, focal		1 (2%)		
Inflammation, acute		1 (2%)		
Inflammation, chronic	12 (24%)	17 (34%)	19 (38%)	8 (16%)
Inflammation, chronic active	2 (4%)			
Mineralization		2 (4%)		
Necrosis		2 (4%)		
Acinus, hyperplasia, focal			1 (2%)	
Artery, inflammation, chronic active		1 (2%)		
Artery, mineralization		1 (2%)		
Artery, necrosis		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	2 (4%)			
Cyst	1 (2%)			
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, mononuclear cell	31 (62%)	38 (76%)	30 (60%)	21 (42%)
Inflammation, granulomatous	1 (2%)			
Inflammation, acute		1 (2%)		
Mineralization		5 (10%)	4 (8%)	1 (2%)
Necrosis		1 (2%)		
Vacuolization cytoplasmic, macrovesicular		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Edema			2 (4%)	
Erosion	4 (8%)	5 (10%)	9 (18%)	3 (6%)
Fibrosis	2 (4%)			
Foreign body			2 (4%)	
Inflammation	18 (36%)	18 (36%)	34 (68%)	19 (38%)
Mineralization	1 (2%)			1 (2%)
Necrosis	1 (2%)		1 (2%)	
Ulcer	9 (18%)	8 (16%)	14 (28%)	11 (22%)
Epithelium, hyperplasia	26 (52%)	19 (38%)	40 (80%)	29 (58%)
Serosa, fibrosis			1 (2%)	

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Alimentary System (continued)				
Stomach, glandular	(50)	(48)	(48)	(50)
Dilatation			1 (2%)	
Edema			1 (2%)	
Erosion			1 (2%)	2 (4%)
Fibrosis		1 (2%)		
Hemorrhage				1 (2%)
Inflammation	1 (2%)	1 (2%)	2 (4%)	
Inflammation, acute	1 (2%)	1 (2%)		
Mineralization	4 (8%)	5 (10%)	6 (13%)	2 (4%)
Necrosis			1 (2%)	
Ulcer	1 (2%)		2 (4%)	1 (2%)
Glands, ectasia, focal		1 (2%)	2 (4%)	
Serosa, fibrosis			1 (2%)	
Tongue	(0)	(0)	(0)	(1)
Angiectasis				1 (100%)
Tooth	(2)	(1)	(0)	(1)
Inflammation, acute	1 (50%)			1 (100%)
Inflammation, chronic active	1 (50%)			
Malformation		1 (100%)		
Necrosis	1 (50%)			
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	8 (16%)	10 (20%)	7 (14%)	1 (2%)
Inflammation, acute		1 (2%)		
Mineralization	1 (2%)		5 (10%)	5 (10%)
Thrombosis		1 (2%)		
Artery, inflammation, chronic active	1 (2%)			
Artery, mineralization			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(48)
Accessory adrenal cortical nodule	2 (4%)	2 (4%)	7 (14%)	1 (2%)
Degeneration, fatty	1 (2%)		1 (2%)	2 (4%)
Hyperplasia			1 (2%)	
Hypertrophy, focal	10 (20%)	10 (20%)	5 (10%)	3 (6%)
Hypertrophy, diffuse	1 (2%)		3 (6%)	20 (42%)
Vacuolization cytoplasmic	1 (2%)		1 (2%)	1 (2%)
Capsule, fibrosis				1 (2%)
Capsule, hemorrhage				1 (2%)
Capsule, hyperplasia	42 (84%)	41 (82%)	47 (96%)	41 (85%)
Adrenal medulla	(50)	(50)	(50)	(48)
Hyperplasia		1 (2%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	32 (64%)	25 (50%)	21 (42%)	6 (12%)
Parathyroid gland	(48)	(43)	(49)	(44)
Cyst	3 (6%)	1 (2%)	5 (10%)	
Pituitary gland	(47)	(43)	(43)	(44)
Pars distalis, angiectasis	1 (2%)			
Pars distalis, cyst	2 (4%)	2 (5%)		1 (2%)
Pars distalis, hyperplasia, focal		1 (2%)	4 (9%)	1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Alimentary System (continued)				
Thyroid gland	(50)	(49)	(50)	(49)
Hypertrophy		1 (2%)		
Mineralization	1 (2%)			
C-cell, hyperplasia			1 (2%)	
Follicle, cyst			1 (2%)	1 (2%)
Follicle, degeneration	21 (42%)	19 (39%)	12 (24%)	6 (12%)
Follicle, degeneration, focal		1 (2%)		
Follicle, hypertrophy	25 (50%)	35 (71%)	41 (82%)	45 (92%)
General Body System				
Tissue NOS	(2)	(1)	(0)	(1)
Abdominal, fibrosis		1 (100%)		
Fat, necrosis		1 (100%)		
Genital System				
Coagulating gland	(1)	(0)	(1)	(0)
Cyst	1 (100%)			
Epididymis	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Granuloma sperm	1 (2%)		1 (2%)	
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic	21 (42%)	28 (56%)	24 (48%)	7 (14%)
Inflammation, chronic active	1 (2%)			
Necrosis	1 (2%)			
Artery, inflammation	1 (2%)			
Penis	(1)	(0)	(1)	(0)
Concretion	1 (100%)			
Inflammation, acute	1 (100%)			
Preputial gland	(50)	(50)	(50)	(50)
Cyst	9 (18%)	16 (32%)	14 (28%)	3 (6%)
Ectasia		1 (2%)		
Fibrosis		1 (2%)		
Inflammation, acute	2 (4%)			1 (2%)
Inflammation, chronic	24 (48%)	28 (56%)	25 (50%)	5 (10%)
Inflammation, chronic active	1 (2%)	1 (2%)	3 (6%)	
Necrosis			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Fibrosis		1 (2%)		
Inflammation, granulomatous				1 (2%)
Inflammation, acute	4 (8%)	2 (4%)	1 (2%)	
Inflammation, chronic	27 (54%)	33 (66%)	30 (60%)	15 (30%)
Inflammation, chronic active		1 (2%)		
Necrosis		1 (2%)		
Epithelium, hyperplasia	3 (6%)		1 (2%)	
Seminal vesicle	(50)	(50)	(49)	(49)
Atrophy			1 (2%)	
Dilatation		1 (2%)		
Hemorrhage		1 (2%)	1 (2%)	
Inflammation, acute	2 (4%)	1 (2%)		
Inflammation, chronic	3 (6%)	7 (14%)	8 (16%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Genital System (continued)				
Testes	(50)	(50)	(50)	(49)
Abnormal residual body		1 (2%)	1 (2%)	1 (2%)
Angiectasis				1 (2%)
Giant cell	2 (4%)		4 (8%)	2 (4%)
Germinal epithelium, atrophy	11 (22%)	8 (16%)	20 (40%)	13 (27%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myeloid cell, hyperplasia	2 (4%)			
Lymph node	(5)	(4)	(4)	(1)
Hyperplasia, lymphoid	1 (20%)			
Pigmentation	2 (40%)		2 (50%)	
Iliac, hyperplasia, lymphoid		1 (25%)		
Inguinal, hyperplasia, lymphoid				1 (100%)
Inguinal, pigmentation			1 (25%)	
Lymph node, mandibular	(50)	(49)	(49)	(46)
Atrophy	1 (2%)		1 (2%)	
Hemorrhage	2 (4%)	3 (6%)		
Hyperplasia, lymphoid		1 (2%)		
Hyperplasia, plasma cell	1 (2%)			
Infiltration cellular, polymorphonuclear	1 (2%)	1 (2%)		
Necrosis, lymphoid	1 (2%)			
Pigmentation	45 (90%)	48 (98%)	46 (94%)	44 (96%)
Lymph node, mesenteric	(49)	(47)	(46)	(47)
Angiectasis			1 (2%)	
Atrophy				3 (6%)
Congestion	1 (2%)		3 (7%)	
Ectasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	1 (2%)		1 (2%)	
Hemorrhage	3 (6%)	7 (15%)	7 (15%)	8 (17%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, plasma cell			1 (2%)	
Infiltration cellular, polymorphonuclear	1 (2%)	1 (2%)		1 (2%)
Necrosis, lymphoid		1 (2%)		
Pigmentation	1 (2%)		2 (4%)	4 (9%)
Spleen	(50)	(47)	(47)	(47)
Atrophy	1 (2%)			
Hematopoietic cell proliferation	14 (28%)	10 (21%)	13 (28%)	25 (53%)
Infiltration cellular, eosinophil				1 (2%)
Pigmentation	13 (26%)	12 (26%)	2 (4%)	2 (4%)
Capsule, fibrosis, focal		1 (2%)		
Capsule, inflammation, granulomatous, focal		1 (2%)		
Lymphoid follicle, atrophy	1 (2%)	2 (4%)	3 (6%)	4 (9%)
Lymphoid follicle, hyperplasia	11 (22%)	7 (15%)	9 (19%)	5 (11%)
Thymus	(40)	(41)	(40)	(39)
Atrophy	26 (65%)	28 (68%)	23 (58%)	23 (59%)
Cyst	14 (35%)	12 (29%)	13 (33%)	3 (8%)
Hemorrhage				1 (3%)
Hyperplasia, lymphoid	1 (3%)	2 (5%)	3 (8%)	1 (3%)
Infiltration cellular, histiocyte			1 (3%)	1 (3%)
Inflammation, granulomatous, focal			1 (3%)	
Necrosis, lymphoid		6 (15%)	3 (8%)	3 (8%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Integumentary System				
Mammary gland	(2)	(2)	(1)	(4)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Fibrosis		1 (2%)		1 (2%)
Fibrosis, focal			1 (2%)	
Foreign body				1 (2%)
Hemorrhage				1 (2%)
Hyperkeratosis	1 (2%)	3 (6%)		1 (2%)
Inflammation, acute	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic	2 (4%)	3 (6%)		2 (4%)
Inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)
Mineralization			2 (4%)	
Thrombosis				1 (2%)
Ulcer	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Epidermis, hyperplasia	1 (2%)	1 (2%)		
Epidermis, tail, hyperplasia				2 (4%)
Lip, inflammation, acute	2 (4%)	1 (2%)		
Prepuce, inflammation, acute			2 (4%)	
Subcutaneous tissue, angiectasis, focal		1 (2%)		
Subcutaneous tissue, cyst			1 (2%)	
Subcutaneous tissue, inflammation, chronic	1 (2%)			
Subcutaneous tissue, necrosis	1 (2%)			
Musculoskeletal System				
Bone	(49)	(50)	(50)	(49)
Fibro-osseous lesion	2 (4%)	1 (2%)		
Tail, callus		2 (4%)		1 (2%)
Tail, developmental malformation	1 (2%)	3 (6%)		
Vertebra, callus	2 (4%)			
Skeletal muscle	(2)	(2)	(0)	(0)
Fibrosis	2 (100%)			
Hemorrhage	2 (100%)			
Inflammation, chronic	2 (100%)			
Regeneration	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cyst epithelial inclusion				1 (2%)
Hemorrhage	6 (12%)	2 (4%)	2 (4%)	5 (10%)
Infiltration cellular, mononuclear cell		2 (4%)		
Inflammation, acute				1 (2%)
Metaplasia, osseous		1 (2%)		
Necrosis			1 (2%)	
Meninges, inflammation, acute		1 (2%)		
Meninges, inflammation, chronic	1 (2%)			
Meninges, thrombosis		1 (2%)		
Peripheral nerve	(2)	(1)	(1)	(0)
Degeneration	2 (100%)	1 (100%)	1 (100%)	
Hemorrhage	1 (50%)			
Spinal cord	(1)	(1)	(1)	(0)
Degeneration			1 (100%)	
Hemorrhage			1 (100%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)			2 (4%)
Fibrosis			1 (2%)	
Foreign body				1 (2%)
Hemorrhage	10 (20%)	6 (12%)	6 (12%)	3 (6%)
Hyperplasia				1 (2%)
Infiltration cellular, histiocyte	8 (16%)	11 (22%)	5 (10%)	1 (2%)
Inflammation, acute	2 (4%)	1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)			1 (2%)
Metaplasia, osseous				1 (2%)
Mineralization	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Thrombosis	3 (6%)			
Alveolar epithelium, hyperplasia	2 (4%)	6 (12%)	1 (2%)	
Alveolar epithelium, hypertrophy				1 (2%)
Alveolus, infiltration cellular, histiocyte			1 (2%)	
Nose	(50)	(48)	(50)	(50)
Foreign body	4 (8%)	7 (15%)	7 (14%)	5 (10%)
Fungus	1 (2%)		2 (4%)	
Hemorrhage			1 (2%)	
Inflammation, acute	9 (18%)	12 (25%)	19 (38%)	6 (12%)
Mineralization	2 (4%)	2 (4%)	2 (4%)	
Glands, fibrosis	1 (2%)			
Pleura	(0)	(1)	(0)	(0)
Trachea	(47)	(48)	(49)	(47)
Hemorrhage		1 (2%)		
Special Senses System				
Eye	(49)	(47)	(47)	(46)
Atrophy	1 (2%)			
Cataract			1 (2%)	
Anterior chamber, edema	1 (2%)			
Anterior chamber, infiltration cellular, polymorphonuclear	1 (2%)			
Anterior chamber, necrosis	1 (2%)			
Cornea, fibrosis	1 (2%)			
Cornea, inflammation	1 (2%)			
Cornea, inflammation, acute	1 (2%)		1 (2%)	
Cornea, inflammation, chronic active	1 (2%)	1 (2%)		
Cornea, necrosis	1 (2%)		1 (2%)	
Cornea, epithelium, hyperplasia	1 (2%)			
Nerve, degeneration	1 (2%)			
Nerve, inflammation, acute	1 (2%)			
Retrobulbar, inflammation, acute			1 (2%)	
Harderian gland	(50)	(49)	(50)	(50)
Atrophy	1 (2%)			
Fibrosis	1 (2%)			
Hemorrhage	1 (2%)			
Hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Hyperplasia, focal	1 (2%)			
Inflammation, acute	1 (2%)			
Inflammation, chronic	1 (2%)			
Necrosis	1 (2%)			
Zymbal's gland	(1)	(0)	(0)	(0)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Casts protein		1 (2%)		
Congestion	1 (2%)			
Hydronephrosis		1 (2%)		
Hyperplasia, lymphoid		1 (2%)	1 (2%)	
Infarct	2 (4%)	1 (2%)	5 (10%)	
Infarct, multiple	1 (2%)			
Infiltration cellular, mononuclear cell	35 (70%)	41 (82%)	39 (80%)	26 (52%)
Inflammation			1 (2%)	
Inflammation, acute	1 (2%)	1 (2%)		
Metaplasia, osseous	2 (4%)	2 (4%)	1 (2%)	
Mineralization	36 (72%)	32 (64%)	24 (49%)	6 (12%)
Nephropathy	38 (76%)	45 (90%)	37 (76%)	9 (18%)
Artery, perirenal tissue, inflammation	1 (2%)			
Interstitium, inflammation	1 (2%)			
Interstitium, inflammation, chronic	2 (4%)	1 (2%)	1 (2%)	
Papilla, inflammation, acute	1 (2%)	2 (4%)		
Papilla, necrosis	4 (8%)	6 (12%)	2 (4%)	
Papilla, pelvis, inflammation, acute			1 (2%)	
Papilla, renal tubule, necrosis		1 (2%)		
Pelvis, inflammation, acute	1 (2%)			
Pelvis, inflammation, chronic	1 (2%)			
Renal tubule, cyst	1 (2%)	2 (4%)	1 (2%)	
Renal tubule, cyst, multiple	1 (2%)			
Renal tubule, degeneration	3 (6%)		2 (4%)	
Renal tubule, dilatation	14 (28%)	14 (28%)	15 (31%)	9 (18%)
Renal tubule, hyperplasia		1 (2%)		
Renal tubule, pigmentation	1 (2%)		3 (6%)	
Transitional epithelium, hyperplasia	1 (2%)			
Urethra	(0)	(4)	(3)	(0)
Angiectasis		1 (25%)	1 (33%)	
Hemorrhage			1 (33%)	
Inflammation, acute		2 (50%)	2 (67%)	
Necrosis		1 (25%)	1 (33%)	
Bulbourethral gland, cyst		1 (25%)		
Bulbourethral gland, hemorrhage			1 (33%)	
Bulbourethral gland, inflammation		1 (25%)		
Bulbourethral gland, necrosis		2 (50%)	1 (33%)	
Urinary bladder	(50)	(49)	(48)	(48)
Fibrosis		1 (2%)		
Hemorrhage	2 (4%)	1 (2%)	1 (2%)	
Hyperplasia, lymphoid			1 (2%)	
Inflammation, acute	2 (4%)	1 (2%)	1 (2%)	
Inflammation, chronic active		1 (2%)	1 (2%)	
Necrosis	1 (2%)	3 (6%)	3 (6%)	

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF DE-71

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1		1	
Moribund	10	10	9	46
Natural deaths	6	5	3	4
Survivors				
Terminal kill	33	35	37	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Gallbladder	(44)	(44)	(47)	(45)
Intestine large, cecum	(46)	(45)	(47)	(47)
Intestine large, colon	(47)	(45)	(47)	(47)
Intestine large, rectum	(47)	(46)	(47)	(47)
Rhabdomyosarcoma, metastatic, skeletal muscle			1 (2%)	
Intestine small, duodenum	(46)	(45)	(47)	(47)
Intestine small, ileum	(46)	(45)	(47)	(47)
Intestine small, jejunum	(46)	(45)	(47)	(47)
Carcinoma			1 (2%)	
Liver	(50)	(49)	(50)	(49)
Hemangioma		1 (2%)		
Hemangiosarcoma			1 (2%)	
Hepatocellular adenoma	5 (10%)	5 (10%)	11 (22%)	4 (8%)
Hepatocellular adenoma, multiple		2 (4%)	21 (42%)	42 (86%)
Hepatocellular carcinoma	4 (8%)	1 (2%)	5 (10%)	19 (39%)
Hepatocellular carcinoma, multiple		1 (2%)	1 (2%)	8 (16%)
Serosa, fibrosarcoma, metastatic, skin	1 (2%)			
Mesentery	(11)	(26)	(12)	(5)
Fibrosarcoma, metastatic, skin	1 (9%)			
Oral mucosa	(0)	(1)	(0)	(0)
Pancreas	(50)	(48)	(50)	(50)
Fibrosarcoma, metastatic, skin	1 (2%)			
Salivary glands	(50)	(50)	(50)	(48)
Fibrosarcoma, metastatic, skin	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(49)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Squamous cell papilloma		3 (6%)	1 (2%)	2 (4%)
Stomach, glandular	(49)	(47)	(47)	(48)
Cardiovascular System				
Blood vessel	(47)	(49)	(50)	(49)
Heart	(50)	(50)	(50)	(49)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(47)
Adenoma			1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)			
Capsule, adenoma			1 (2%)	
Adrenal medulla	(49)	(50)	(48)	(48)
Pheochromocytoma benign			1 (2%)	
Pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(50)	(48)	(50)	(50)
Adenoma	1 (2%)		2 (4%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Parathyroid gland	(44)	(48)	(47)	(47)
Pituitary gland	(50)	(47)	(46)	(45)
Pars distalis, adenoma	5 (10%)	5 (11%)	8 (17%)	
Pars intermedia, adenoma		2 (4%)		
Thyroid gland	(50)	(49)	(48)	(47)
C-cell, adenoma	1 (2%)			
Follicular cell, adenoma	1 (2%)			
Follicular cell, carcinoma		1 (2%)		
General Body System				
Peritoneum	(0)	(0)	(0)	(1)
Tissue NOS	(1)	(2)	(0)	(1)
Genital System				
Clitoral gland	(49)	(49)	(50)	(50)
Ovary	(48)	(49)	(50)	(48)
Cystadenoma	2 (4%)	1 (2%)	3 (6%)	
Granulosa cell tumor benign	1 (2%)			
Granulosa cell tumor malignant				1 (2%)
Uterus	(50)	(50)	(50)	(49)
Adenoma	1 (2%)			
Hemangioma	1 (2%)			
Polyp stromal		2 (4%)	3 (6%)	
Bilateral, polyp stromal			1 (2%)	
Vagina	(0)	(1)	(0)	(0)
Squamous cell carcinoma		1 (100%)		
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(49)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)			
Lymph node	(9)	(17)	(12)	(4)
Lymph node, mandibular	(48)	(50)	(50)	(45)
Lymph node, mesenteric	(48)	(45)	(49)	(48)
Spleen	(50)	(47)	(48)	(48)
Capsule, fibrosarcoma, metastatic, skin	1 (2%)			
Thymus	(48)	(45)	(46)	(46)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Rhabdomyosarcoma, metastatic, skeletal muscle			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Mast cell tumor benign				1 (2%)
Lip, mast cell tumor benign				1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (2%)		
Subcutaneous tissue, fibrous histiocytoma, multiple		1 (2%)		
Subcutaneous tissue, rhabdomyosarcoma, metastatic, skeletal muscle			1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Maxilla, rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)			
Skeletal muscle	(2)	(3)	(4)	(1)
Rhabdomyosarcoma	1 (50%)	2 (67%)	2 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(49)
Peripheral nerve	(1)	(1)	(1)	(1)
Spinal cord	(1)	(1)	(3)	(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	5 (10%)	3 (6%)	
Alveolar/bronchiolar carcinoma		1 (2%)	1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)			
Granulosa cell tumor malignant, metastatic, ovary				1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	1 (2%)		1 (2%)
Mediastinum, fibrosarcoma, metastatic, skin	1 (2%)			
Nose	(50)	(50)	(50)	(48)
Pleura	(0)	(0)	(1)	(0)
Trachea	(50)	(50)	(50)	(47)
Special Senses System				
Eye	(47)	(45)	(47)	(48)
Harderian gland	(50)	(49)	(50)	(49)
Adenoma	9 (18%)	1 (2%)	4 (8%)	2 (4%)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(49)	(48)
Urinary bladder	(49)	(50)	(49)	(48)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Lymphoma malignant	7 (14%)	6 (12%)	6 (12%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	34	30	41	49
Total primary neoplasms	44	46	79	83
Total animals with benign neoplasms	24	20	38	46
Total benign neoplasms	28	27	60	52
Total animals with malignant neoplasms	16	15	15	31
Total malignant neoplasms	16	19	19	31
Total animals with metastatic neoplasms	4	1	1	3
Total metastatic neoplasms	13	1	3	3

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

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	9/50 (18%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate[b]	20.7%	2.3%	8.7%	9.6%
Terminal rate[c]	8/33 (24%)	1/35 (3%)	3/37 (8%)	0/0 (0%)
First incidence (days)	656	729 (T)	677	542
Poly-3 test[d]	P=0.379N	P=0.007N	P=0.095N	P=0.251N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	2/50 (4%)	5/50 (10%)	2/50 (4%)
Adjusted rate	23.0%	4.5%	10.9%	9.6%
Terminal rate	9/33 (27%)	1/35 (3%)	4/37 (11%)	0/0 (0%)
First incidence (days)	656	684	677	542
Poly-3 test	P=0.315N	P=0.011N	P=0.105N	P=0.197N
Liver: Hepatocellular Adenoma				
Overall rate	5/50 (10%)	7/49 (14%)	32/50 (64%)	46/49 (94%)
Adjusted rate	11.6%	16.0%	68.0%	97.9%
Terminal rate	5/33 (15%)	7/35 (20%)	26/37 (70%)	0/0 (0%)
First incidence (days)	729 (T)	729 (T)	563	432
Poly-3 test	P<0.001	P=0.385	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	4/50 (8%)	2/49 (4%)	6/50 (12%)	27/49 (55%)
Adjusted rate	9.2%	4.6%	13.0%	75.5%
Terminal rate	3/33 (9%)	1/35 (3%)	4/37 (11%)	0/0 (0%)
First incidence (days)	696	712	598	432
Poly-3 test	P<0.001	P=0.333N	P=0.411	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	8/50 (16%)	8/49 (16%)	33/50 (66%)	47/49 (96%)
Adjusted rate	18.4%	18.3%	69.5%	98.8%
Terminal rate	7/33 (21%)	7/35 (20%)	26/37 (70%)	0/0 (0%)
First incidence (days)	696	712	563	432
Poly-3 test	P<0.001	P=0.602N	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	5/50 (10%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.3%	11.2%	6.5%	0.0%
Terminal rate	1/33 (3%)	3/35 (9%)	2/37 (5%)	0/0 (0%)
First incidence (days)	729 (T)	687	677	—[e]
Poly-3 test	P=0.309N	P=0.108	P=0.327	P=0.638N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	6/50 (12%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.3%	13.4%	8.7%	0.0%
Terminal rate	1/33 (3%)	4/35 (11%)	3/37 (8%)	0/0 (0%)
First incidence (days)	729 (T)	687	677	—
Poly-3 test	P=0.322N	P=0.061	P=0.197	P=0.638N
Ovary: Cystadenoma				
Overall rate	2/48 (4%)	1/49 (2%)	3/50 (6%)	0/48 (0%)
Adjusted rate	4.9%	2.3%	6.6%	0.0%
Terminal rate	2/31 (7%)	1/34 (3%)	3/37 (8%)	0/0 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.540N	P=0.482N	P=0.546	P=0.429N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/50 (10%)	5/47 (11%)	8/46 (17%)	0/45 (0%)
Adjusted rate	11.6%	11.6%	18.4%	0.0%
Terminal rate	5/33 (15%)	5/35 (14%)	7/36 (19%)	0/0 (0%)
First incidence (days)	729 (T)	729 (T)	563	—
Poly-3 test	P=0.408N	P=0.629	P=0.277	P=0.196N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	0.0%	6.7%	2.2%	9.6%
Terminal rate	0/33 (0%)	2/35 (6%)	1/37 (3%)	0/0 (0%)
First incidence (days)	—	690	729 (T)	542
Poly-3 test	P=0.279	P=0.124	P=0.511	P=0.121
Uterus: Stromal Polyp				
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	0.0%	4.5%	8.8%	0.0%
Terminal rate	0/33 (0%)	2/35 (6%)	3/37 (8%)	0/0 (0%)
First incidence (days)	—	729 (T)	726	—
Poly-3 test	P=0.409	P=0.243	P=0.067	—[f]
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.3%	6.5%	2.2%	4.9%
Terminal rate	1/33 (3%)	0/35 (0%)	1/37 (3%)	0/0 (0%)
First incidence (days)	729 (T)	522	729 (T)	459
Poly-3 test	P=0.537N	P=0.327	P=0.748N	P=0.575
All Organs: Malignant Lymphoma				
Overall rate	7/50 (14%)	6/50 (12%)	6/50 (12%)	1/50 (2%)
Adjusted rate	15.8%	13.3%	13.1%	4.9%
Terminal rate	3/33 (9%)	5/35 (14%)	6/37 (16%)	0/0 (0%)
First incidence (days)	593	421	729 (T)	496
Poly-3 test	P=0.232N	P=0.483N	P=0.476N	P=0.234N
All Organs: Benign Neoplasms				
Overall rate	24/50 (48%)	20/50 (40%)	38/50 (76%)	46/50 (92%)
Adjusted rate	54.6%	44.6%	80.4%	97.7%
Terminal rate	22/33 (67%)	17/35 (49%)	30/37 (81%)	0/0 (0%)
First incidence (days)	601	687	563	432
Poly-3 test	P<0.001	P=0.228N	P=0.005	P<0.001
All Organs: Malignant Neoplasms				
Overall rate	16/50 (32%)	15/50 (30%)	15/50 (30%)	31/50 (62%)
Adjusted rate	35.4%	31.6%	31.9%	80.5%
Terminal rate	9/33 (27%)	7/35 (20%)	11/37 (30%)	0/0 (0%)
First incidence (days)	589	421	563	432
Poly-3 test	P<0.001	P=0.436N	P=0.446N	P<0.001

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	34/50 (68%)	30/50 (60%)	41/50 (82%)	49/50 (98%)
Adjusted rate	74.4%	62.8%	85.9%	99.7%
Terminal rate	26/33 (79%)	20/35 (57%)	32/37 (87%)	0/0 (0%)
First incidence (days)	589	421	563	432
Poly-3 test	P<0.001	P=0.156N	P=0.118	P<0.001

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE D3
Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
DE-71 (February 2008)	5/50	4/50	8/50
Ginkgo biloba extract (March 2005)	17/50	9/50	20/50
Indole-3-carbinol (April 2007)	7/50	6/50	12/50
Kava kava extract (August 2004)	8/50	3/50	10/50
N,*N*-dimethyl-*p*-toluidine (October 2004)	17/50	6/50	20/50
Tetrabromobisphenol A (August 2007)	13/50	2/50	15/50
Total (%)	67/300 (22.3%)	30/300 (10.0%)	85/300 (28.3%)
Mean ± standard deviation	22.3% ± 10.5%	10.0% ± 5.1%	28.3% ± 10.2%
Range	10%-34%	4%-18%	16%-40%
Overall Historical Incidence: All Routes			
Total (%)	272/698 (39.0%)	112/698 (16.1%)	320/698 (45.9%)
Mean ± standard deviation	39.1% ± 21.9%	16.1% ± 8.1%	45.9% ± 21.9%
Range	10%-78%	4%-34%	16%-82%

	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies		
DE-71 (February 2008)	0/50	8/50
Ginkgo biloba extract (March 2005)	1/50	20/50
Indole-3-carbinol (April 2007)	0/50	12/50
Kava kava extract (August 2004)	0/50	10/50
N,*N*-dimethyl-*p*-toluidine (October 2004)	0/50	20/50
Tetrabromobisphenol A (August 2007)	0/50	15/50
Total (%)	1/300 (0.3%)	85/300 (28.3%)
Mean ± standard deviation	0.3% ± 0.8%	28.3% ± 10.2%
Range	0%-2%	16%-40%
Overall Historical Incidence: All Routes		
Total (%)	4/698 (0.6%)	320/698 (45.9%)
Mean ± standard deviation	0.6% ± 0.9%	45.9% ± 21.9%
Range	0%-2%	16%-82%

[a] Data as of November 2014

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1		1	
Moribund	10	10	9	46
Natural deaths	6	5	3	4
Survivors				
Terminal kill	33	35	37	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Foreign body			1 (2%)	
Inflammation, granulomatous				1 (2%)
Inflammation, acute			1 (2%)	
Inflammation, chronic		1 (2%)		
Necrosis	1 (2%)			
Muscularis, degeneration	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Gallbladder	(44)	(44)	(47)	(45)
Intestine large, cecum	(46)	(45)	(47)	(47)
Intestine large, colon	(47)	(45)	(47)	(47)
Intestine large, rectum	(47)	(46)	(47)	(47)
Diverticulum			1 (2%)	
Edema		1 (2%)		
Intestine small, duodenum	(46)	(45)	(47)	(47)
Intestine small, ileum	(46)	(45)	(47)	(47)
Hyperplasia, lymphoid	1 (2%)			
Ulcer	1 (2%)			
Intestine small, jejunum	(46)	(45)	(47)	(47)
Inflammation, acute			1 (2%)	
Liver	(50)	(49)	(50)	(49)
Basophilic focus	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Clear cell focus		3 (6%)	2 (4%)	2 (4%)
Eosinophilic focus	3 (6%)	1 (2%)	15 (30%)	8 (16%)
Eosinophilic focus, multiple		1 (2%)	1 (2%)	7 (14%)
Fatty change	18 (36%)	18 (37%)	39 (78%)	20 (41%)
Fibrosis	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	13 (26%)	15 (31%)	8 (16%)	13 (27%)
Hemorrhage	4 (8%)	1 (2%)	4 (8%)	5 (10%)
Inflammation, acute				1 (2%)
Inflammation, chronic	32 (64%)	33 (67%)	34 (68%)	32 (65%)
Mixed cell focus		2 (4%)		1 (2%)
Necrosis, focal	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Tension lipidosis	3 (6%)	2 (4%)	2 (4%)	
Centrilobular, mineralization			1 (2%)	
Centrilobular, necrosis			2 (4%)	
Centrilobular, hepatocyte, hypertrophy		7 (14%)	45 (90%)	47 (96%)
Hepatocyte, cytoplasmic alteration			1 (2%)	
Kupffer cell, pigmentation	3 (6%)	10 (20%)	24 (48%)	27 (55%)
Midzonal, necrosis			1 (2%)	

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Alimentary System (continued)				
Mesentery	(11)	(26)	(12)	(5)
Accessory spleen		1 (4%)		
Cyst		1 (4%)		
Inflammation, chronic		1 (4%)		
Inflammation, chronic active	1 (9%)			
Mineralization		1 (4%)		
Fat, necrosis	10 (91%)	22 (85%)	12 (100%)	3 (60%)
Oral mucosa	(0)	(1)	(0)	(0)
Pancreas	(50)	(48)	(50)	(50)
Atrophy	6 (12%)	8 (17%)	5 (10%)	
Cyst	1 (2%)	3 (6%)	3 (6%)	
Fibrosis		1 (2%)	1 (2%)	
Inflammation, chronic	29 (58%)	31 (65%)	31 (62%)	22 (44%)
Inflammation, chronic active		1 (2%)		
Mineralization, chronic			1 (2%)	
Necrosis	1 (2%)	1 (2%)		1 (2%)
Duct, cyst				1 (2%)
Salivary glands	(50)	(50)	(50)	(48)
Atrophy	1 (2%)		1 (2%)	
Infiltration cellular, mononuclear cell	32 (64%)	37 (74%)	30 (60%)	26 (54%)
Mineralization	1 (2%)	5 (10%)	5 (10%)	1 (2%)
Necrosis	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(49)
Angiectasis, focal				1 (2%)
Cyst	1 (2%)		1 (2%)	
Edema	1 (2%)			
Erosion		1 (2%)	2 (4%)	3 (6%)
Hemorrhage	1 (2%)			
Infiltration cellular, mast cell			1 (2%)	
Inflammation	5 (10%)	3 (6%)	5 (10%)	6 (12%)
Inflammation, acute				1 (2%)
Inflammation, chronic	2 (4%)			
Mineralization		1 (2%)		
Ulcer	5 (10%)	3 (6%)	1 (2%)	1 (2%)
Epithelium, hyperplasia	9 (18%)	5 (10%)	6 (12%)	16 (33%)
Stomach, glandular	(49)	(47)	(47)	(48)
Erosion	1 (2%)			1 (2%)
Inflammation, acute	1 (2%)	1 (2%)	1 (2%)	
Mineralization	5 (10%)	6 (13%)	6 (13%)	1 (2%)
Ulcer		1 (2%)	1 (2%)	
Epithelium, vacuolization cytoplasmic	1 (2%)			
Glands, ectasia, focal	4 (8%)	3 (6%)	4 (9%)	1 (2%)
Cardiovascular System				
Blood vessel	(47)	(49)	(50)	(49)
Inflammation	1 (2%)			
Mineralization	1 (2%)		1 (2%)	
Heart	(50)	(50)	(50)	(49)
Cardiomyopathy	4 (8%)	8 (16%)	5 (10%)	
Hemorrhage			1 (2%)	
Inflammation, chronic		1 (2%)		
Mineralization	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Necrosis, multifocal			1 (2%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Cardiovascular System (continued)				
Heart (continued)	(50)	(50)	(50)	(49)
Thrombosis			1 (2%)	
Valve, thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(47)
Accessory adrenal cortical nodule	5 (10%)	5 (10%)	1 (2%)	3 (6%)
Degeneration, fatty	3 (6%)	2 (4%)	3 (6%)	
Hematopoietic cell proliferation		2 (4%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)			2 (4%)
Hyperplasia	1 (2%)		4 (8%)	
Hyperplasia, focal		1 (2%)		
Hypertrophy, focal	7 (14%)	4 (8%)	9 (18%)	2 (4%)
Hypertrophy, diffuse			4 (8%)	8 (17%)
Capsule, cyst				1 (2%)
Capsule, hyperplasia	49 (98%)	50 (100%)	47 (96%)	47 (100%)
Adrenal medulla	(49)	(50)	(48)	(48)
Hyperplasia	1 (2%)		1 (2%)	
Pigmentation		1 (2%)		
Islets, pancreatic	(50)	(48)	(50)	(50)
Hyperplasia	3 (6%)	3 (6%)	2 (4%)	1 (2%)
Parathyroid gland	(44)	(48)	(47)	(47)
Cyst	2 (5%)	1 (2%)	1 (2%)	
Hyperplasia			1 (2%)	
Pituitary gland	(50)	(47)	(46)	(45)
Pars distalis, angiectasis		1 (2%)	1 (2%)	
Pars distalis, cyst	2 (4%)		1 (2%)	
Pars distalis, hyperplasia	9 (18%)	13 (28%)	13 (28%)	10 (22%)
Rathke's cleft, cyst	1 (2%)			1 (2%)
Thyroid gland	(50)	(49)	(48)	(47)
Infiltration cellular, mononuclear cell		1 (2%)		
Inflammation, chronic	1 (2%)	1 (2%)		
Follicle, cyst	2 (4%)	1 (2%)		
Follicle, degeneration	34 (68%)	28 (57%)	26 (54%)	11 (23%)
Follicle, degeneration, focal		1 (2%)		
Follicle, hypertrophy	24 (48%)	31 (63%)	37 (77%)	42 (89%)
Folliclular cell, hyperplasia				1 (2%)
General Body System				
Peritoneum	(0)	(0)	(0)	(1)
Tissue NOS	(1)	(2)	(0)	(1)
Abscess, chronic				1 (100%)
Fibrosis		1 (50%)		
Foreign body				1 (100%)
Inflammation, chronic		1 (50%)		
Mineralization		1 (50%)		
Fat, fibrosis		1 (50%)		
Fat, inflammation, chronic active		1 (50%)		
Fat, necrosis		1 (50%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Genital System				
Clitoral gland	(49)	(49)	(50)	(50)
Cyst	1 (2%)			
Inflammation, acute	1 (2%)			
Inflammation, chronic active	5 (10%)	13 (27%)	8 (16%)	18 (36%)
Ovary	(48)	(49)	(50)	(48)
Abscess, chronic active	1 (2%)			
Angiectasis	1 (2%)		1 (2%)	
Atrophy				1 (2%)
Cyst	13 (27%)	13 (27%)	6 (12%)	7 (15%)
Thrombosis			1 (2%)	
Uterus	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Cyst	2 (4%)			
Edema	1 (2%)			
Hemorrhage	1 (2%)	2 (4%)		
Hyperplasia, cystic	45 (90%)	47 (94%)	46 (92%)	43 (88%)
Inflammation, histiocytic	1 (2%)			
Inflammation, acute	1 (2%)		1 (2%)	
Necrosis	1 (2%)		1 (2%)	
Thrombosis	1 (2%)			
Serosa, cyst				1 (2%)
Vagina	(0)	(1)	(0)	(0)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(49)
Infiltration cellular, histiocyte	1 (2%)			
Myeloid cell, hyperplasia	1 (2%)	3 (6%)	1 (2%)	
Lymph node	(9)	(17)	(12)	(4)
Ectasia	3 (33%)	6 (35%)	4 (33%)	
Hemorrhage	3 (33%)	5 (29%)	3 (25%)	
Hyperplasia, lymphoid		2 (12%)		1 (25%)
Pigmentation	3 (33%)	5 (29%)	5 (42%)	
Iliac, ectasia			1 (8%)	
Iliac, hemorrhage		1 (6%)	1 (8%)	
Iliac, hyperplasia, lymphoid	1 (11%)		1 (8%)	
Iliac, hyperplasia, plasma cell			1 (8%)	
Lumbar, pigmentation			1 (8%)	
Pancreatic, hyperplasia, lymphoid		1 (6%)		
Renal, hematopoietic cell proliferation		1 (6%)		
Renal, hyperplasia, lymphoid			1 (8%)	
Renal, pigmentation		1 (6%)	1 (8%)	
Lymph node, mandibular	(48)	(50)	(50)	(45)
Atrophy	1 (2%)			
Ectasia	1 (2%)			
Fibrosis	1 (2%)			
Hematopoietic cell proliferation				1 (2%)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	3 (6%)	3 (6%)	2 (4%)	
Hyperplasia, plasma cell	1 (2%)	1 (2%)		
Infiltration cellular, mast cell			1 (2%)	
Inflammation, chronic active	1 (2%)			
Pigmentation	36 (75%)	39 (78%)	41 (82%)	38 (84%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(48)	(45)	(49)	(48)
Atrophy	1 (2%)		1 (2%)	1 (2%)
Ectasia		1 (2%)	3 (6%)	
Hemorrhage		2 (4%)		
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia, plasma cell	1 (2%)			
Necrosis, lymphoid				2 (4%)
Pigmentation				2 (4%)
Spleen	(50)	(47)	(48)	(48)
Accessory spleen		1 (2%)		
Atrophy	1 (2%)			
Hematopoietic cell proliferation	15 (30%)	10 (21%)	11 (23%)	24 (50%)
Necrosis	1 (2%)			
Pigmentation	35 (70%)	26 (55%)	28 (58%)	31 (65%)
Lymphoid follicle, atrophy	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Lymphoid follicle, hyperplasia	12 (24%)	20 (43%)	7 (15%)	21 (44%)
Lymphoid follicle, hyperplasia, plasma cell	1 (2%)			
Lymphoid follicle, hyperplasia, focal	2 (4%)			
Thymus	(48)	(45)	(46)	(46)
Atrophy	25 (52%)	22 (49%)	26 (57%)	13 (28%)
Cyst	8 (17%)	6 (13%)	6 (13%)	2 (4%)
Hyperplasia, lymphoid	16 (33%)	16 (36%)	9 (20%)	10 (22%)
Mineralization	1 (2%)			
Necrosis, lymphoid	2 (4%)	1 (2%)	1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	30 (60%)	28 (56%)	32 (64%)	31 (62%)
Hyperplasia	3 (6%)		5 (10%)	
Inflammation, chronic	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Inflammation, chronic active			1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)			
Edema		2 (4%)	1 (2%)	
Fibrosis	1 (2%)		2 (4%)	
Foreign body	1 (2%)	1 (2%)	1 (2%)	
Hemorrhage	1 (2%)	2 (4%)		
Hyperkeratosis	1 (2%)	1 (2%)		
Infiltration cellular, mast cell		1 (2%)		
Inflammation, granulomatous	1 (2%)	1 (2%)		
Inflammation, acute	2 (4%)	4 (8%)	3 (6%)	
Inflammation, chronic	6 (12%)	8 (16%)	10 (20%)	8 (16%)
Inflammation, chronic active	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Mineralization		2 (4%)	2 (4%)	1 (2%)
Necrosis, fatty, focal				1 (2%)
Ulcer	4 (8%)	4 (8%)	6 (12%)	
Epidermis, hyperplasia			1 (2%)	
Epidermis, tail, hyperkeratosis	1 (2%)		2 (4%)	
Epidermis, tail, hyperplasia	1 (2%)	4 (8%)	3 (6%)	
Hair follicle, atrophy, focal				1 (2%)
Hair follicle, inflammation, chronic active				1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Lip, foreign body		1 (2%)		
Lip, inflammation, chronic	1 (2%)	1 (2%)		
Lip, inflammation, chronic active		1 (2%)		
Subcutaneous tissue, fibrosis		1 (2%)		
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Fibro-osseous lesion	43 (88%)	46 (92%)	49 (98%)	43 (86%)
Tail, callus	1 (2%)		2 (4%)	1 (2%)
Tail, developmental malformation			1 (2%)	
Vertebra, callus			1 (2%)	
Skeletal muscle	(2)	(3)	(4)	(1)
Fibrosis			1 (25%)	
Hemorrhage			2 (50%)	
Inflammation, chronic			1 (25%)	
Mineralization			1 (25%)	
Necrosis			1 (25%)	
Regeneration			1 (25%)	
Nervous System				
Brain	(50)	(50)	(50)	(49)
Compression	1 (2%)	2 (4%)	1 (2%)	
Developmental malformation				1 (2%)
Hemorrhage	3 (6%)	1 (2%)	3 (6%)	1 (2%)
Infiltration cellular, mononuclear cell	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Necrosis			1 (2%)	
Pigmentation			1 (2%)	
Meninges, inflammation, chronic	1 (2%)			
Peripheral nerve	(1)	(1)	(1)	(1)
Degeneration	1 (100%)		1 (100%)	
Sciatic, degeneration		1 (100%)		
Spinal cord	(1)	(1)	(3)	(1)
Degeneration			2 (67%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	2 (4%)	1 (2%)	1 (2%)	
Cyst			1 (2%)	
Edema				1 (2%)
Fibrosis	1 (2%)			
Foreign body			1 (2%)	
Hemorrhage	12 (24%)	3 (6%)	1 (2%)	7 (14%)
Infiltration cellular, histiocyte	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Inflammation, granulomatous				1 (2%)
Inflammation, acute			1 (2%)	
Metaplasia, osseous	2 (4%)			2 (4%)
Mineralization	3 (6%)	2 (4%)	3 (6%)	
Thrombosis	1 (2%)		1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia			1 (2%)	
Alveolus, infiltration cellular, histiocyte				1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(48)
Foreign body	4 (8%)	3 (6%)	3 (6%)	3 (6%)
Inflammation, acute	6 (12%)	6 (12%)	4 (8%)	10 (21%)
Mineralization				1 (2%)
Pleura	(0)	(0)	(1)	(0)
Inflammation, suppurative			1 (100%)	
Trachea	(50)	(50)	(50)	(47)
Special Senses System				
Eye	(47)	(45)	(47)	(48)
Atrophy		1 (2%)		
Cornea, inflammation, acute			1 (2%)	
Harderian gland	(50)	(49)	(50)	(49)
Hyperplasia	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Inflammation, chronic		1 (2%)		
Urinary System				
Kidney	(50)	(50)	(49)	(48)
Infarct			1 (2%)	1 (2%)
Infiltration cellular, mononuclear cell	38 (76%)	44 (88%)	43 (88%)	45 (94%)
Metaplasia, osseous			1 (2%)	1 (2%)
Mineralization	7 (14%)	5 (10%)	5 (10%)	1 (2%)
Nephropathy	14 (28%)	9 (18%)	9 (18%)	3 (6%)
Artery, inflammation, chronic	1 (2%)			
Interstitium, inflammation, chronic	1 (2%)			2 (4%)
Papilla, necrosis		2 (4%)		
Pelvis, inflammation, chronic				1 (2%)
Renal tubule, accumulation, hyaline droplet	2 (4%)	1 (2%)		1 (2%)
Renal tubule, atrophy		1 (2%)	1 (2%)	
Renal tubule, cyst				1 (2%)
Renal tubule, degeneration		1 (2%)	1 (2%)	
Renal tubule, dilatation	31 (62%)	23 (46%)	34 (69%)	43 (90%)
Renal tubule, necrosis			1 (2%)	
Renal tubule, regeneration			1 (2%)	
Renal tubule, vacuolization cytoplasmic			1 (2%)	
Urinary bladder	(49)	(50)	(49)	(48)
Hyperplasia, lymphoid	1 (2%)			

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Bacterial mutagenicity was evaluated in DE-71 and three polybrominated diphenyl ethers, 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). Testing was performed as reported by Zeiger *et al.* (1987) (DE-71) or Zeiger *et al.* (1992) (BDE-47, BDE-99, and BDE-153). Chemicals were sent to the laboratory as coded aliquots and incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535, and TA1537 or the *Escherichia coli* strain WP2 *uvrA*/pKM101, either in buffer or 10% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine (*S. typhimurium* strains) or L-tryptophan (*E. coli* strain) and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of test chemical. The high dose was limited to 10,000 µg/plate by design. No chemical-associated toxicity was observed in any test, though precipitation occurred at the higher doses of most trials.

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

MOUSE MICRONUCLEUS TEST PROTOCOLS

3-Month Study

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month gavage study (Lot 2550OA30A), peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronucleated cells in 2,000 normochromatic erythrocytes (NCEs, mature erythrocytes) per animal. In addition, the percentage of polychromatic erythrocytes (PCEs, reticulocytes, immature erythrocytes) among a population of 1,000 erythrocytes in the peripheral blood was scored for each dose group as a measure of DE-71 associated bone marrow toxicity.

The results from the slide-based evaluation were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the slide-based micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups.

3-Day Study

This study was conducted as described by Witt *et al.* (2008). DE-71 was supplied through the NTP Chemistry Support Contract (Battelle Columbus Laboratories, Columbus, OH) and sent to the testing laboratory (ILS, Inc., Research Triangle Park, NC) as coded aliquots. Adult male B6C3F1/N mice, five per treatment group, were administered DE-71, dissolved in corn oil, by gavage once daily for 3 consecutive days, and peripheral blood and bone marrow samples were obtained 24 hours after the third treatment. Following this same regimen, vehicle control animals received corn oil alone, and the positive control mice received cyclophosphamide at a daily dose of 50 mg/kg. For slide-based analysis of the bone marrow samples, air-dried smears of the contents flushed from the femurs were fixed in absolute methanol, stained with acridine orange, and coded before scoring; 2,000 uniformly stained PCEs were scored for induction of micronucleated cells in each animal. In addition, 500 erythrocytes (mature and immature) were scored to determine the percentage of PCEs among the total erythrocyte population in the bone marrow as a measure of DE-71 associated bone marrow toxicity.

For data collected through flow cytometric methods, blood samples were processed immediately upon collection as described in the MicroFlow® BASIC Kits from Litron Laboratories (Rochester, NY). The kits contain all the supplies and reagents necessary to process blood samples. Briefly, a 60 to 120 µL blood sample was collected from the vena cava after euthanasia, diluted in sodium heparin solution, and fixed in ultracold methanol. Fixed blood samples were immediately placed into a −80° C freezer for storage until flow cytometric analysis was conducted. A FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) was used to carry out the analyses. PCEs were identified by the presence of an active transferrin receptor (CD71+) on the cell surface; mature erythrocytes were identified as CD71-negative. For each animal, 10,000 to 20,000 CD71+ red blood cells were scored for the presence of micronuclei; approximately 10^6 total erythrocytes were counted to determine percent PCEs in blood as a measure of DE-71-associated bone marrow toxicity.

Slide-based evaluations of NCEs and PCEs were conducted as described for NCEs in the 3-month study. Based on prior experience with the large number of cells scored using flow cytometric scoring techniques (Kissling *et al.*, 2007), it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the vehicle control group depend on whether the variances among the groups are equal. The NTP uses Levene's test at α=0.05 to test for equal variances among the treatment groups. In the case of equal variances, linear regression was used to test for a linear trend with dose and Williams' test (Williams, 1971, 1972) was used to test for pairwise differences between each treatment group and the vehicle control group. In the case of unequal variances, Jonckheere's test (Jonckheere, 1954) was used to test for linear trend, and pairwise comparisons of each dosed group with the vehicle control group were tested using Dunn's test (Dunn, 1964). To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P \leq 0.025$.

Factors that must be considered in analyzing micronucleus test data include number of animals per dose group (a minimum of three is required), dose levels and number of doses administered, route of administration, cell type analyzed, sample time (interval between last dosing and harvesting of cells for analysis), frequencies of micronucleated cells in the negative and positive controls, and the results of the statistical analyses. The final conclusion for a micronucleus test is determined by considering the results of statistical analyses, the reproducibility of any observed effects, and the magnitude and biological significance of those effects.

EVALUATION PROTOCOL

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

RESULTS

DE-71 was tested for mutagenic activity in bacteria in three independent studies at three separate laboratories using a total of six different bacterial tester strains (*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537, and *E. coli* WP2 *uvrA*/pKM101) with and without 10% rat or hamster liver metabolic activation enzymes (S9). The study conducted by SITEK Research Laboratories used the same lot of DE-71 (2550OA30A) that was used in the 2-year gavage studies. No evidence of mutagenicity was observed (Zeiger *et al.*, 1987; Tables E1 and E2). In all three studies, dose levels ranged up to 10,000 µg/plate in the absence of observable toxicity, although precipitation occurred in one of the three studies at 1,000 µg/plate and above.

Three related test articles, BDE-47, BDE-99, and BDE-153 were tested for mutagenic activity in three bacterial tester strains (*S. typhimurium* TA98, TA100, and TA102) with and without rat liver S9 mix, and no evidence of mutagenicity was observed with any of the three test articles in any of the tests that were conducted (Tables E3, E4, and E5).

In vivo, no increases in the frequencies of micronucleated NCEs were observed in peripheral blood samples from male or female mice in the 3-month gavage study of DE-71(0.01 to 500 mg/kg; Table E6). Five mice were examined in each dose group except in the 500 mg/kg group only three male mice were available. In a second micronucleus study conducted in male B6C3F1/N mice, no increases in the frequencies of PCEs or NCEs were seen in peripheral blood samples following administration of DE-71 (312.5 to 1,250 mg/kg) by gavage once daily for 3 days; blood samples were evaluated using flow cytometric methods (Witt *et al.*, 2008; Table E7). In these same mice, slide-based data acquisition methods were used to evaluate bone marrow smears for induction of micronucleated PCEs and results were consistent with the results from blood samples (Table E8). In none of the micronucleus tests conducted with DE-71 were significant alterations in the percentage PCEs seen over the dose range tested, suggesting that DE-71 did not induce toxicity in the bone marrow of treated mice. In the 3-day gavage study evaluated using flow cytometric methods, the trend test for percent PCEs gave a significant P value (0.023), but pairwise comparison of the top dose to the vehicle control group was not significant; thus, the small increase detected by flow cytometry (but not by slide scoring in the bone marrow) was not considered to be significant.

Table E1
Mutagenicity of DE-71 in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 10% hamster S9	With 10% rat S9	With 10% rat S9
Study performed at SRI International							
TA100							
	0	121 ± 6	113 ± 7	116 ± 9	116 ± 7	101 ± 8	108 ± 4
	100	98 ± 4	107 ± 9	108 ± 7	122 ± 19	120 ± 10	102 ± 6
	333	87 ± 5	104 ± 9	100 ± 8	111 ± 2	110 ± 9	104 ± 6
	1,000	86 ± 6	102 ± 11	109 ± 13	99 ± 5	111 ± 9	111 ± 9
	3,333	98 ± 7	101 ± 5	109 ± 4	108 ± 8	124 ± 8	98 ± 1
	10,000	101 ± 14	99 ± 10	123 ± 5	89 ± 11	116 ± 5	115 ± 13
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[b]		481 ± 25	397 ± 12	1,419 ± 38	1,834 ± 92	372 ± 34	1,836 ± 102
TA98							
	0	15 ± 1	21 ± 3	33 ± 5	38 ± 3	25 ± 4	28 ± 5
	100	16 ± 1	19 ± 3	36 ± 4	35 ± 3	33 ± 3	29 ± 5
	333	15 ± 2	20 ± 5	33 ± 3	35 ± 3	23 ± 1	24 ± 4
	1,000	13 ± 1	18 ± 2	30 ± 4	30 ± 1	24 ± 4	31 ± 2
	3,333	18 ± 2	18 ± 4	30 ± 3	28 ± 5	24 ± 1	26 ± 2
	10,000	25 ± 5	17 ± 3	21 ± 3	32 ± 2	21 ± 1	28 ± 5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		620 ± 55	378 ± 12	1,278 ± 63	1,551 ± 2	418 ± 14	1,522 ± 83
TA1535							
	0	28 ± 2	30 ± 3	10 ± 3	10 ± 2	6 ± 1	9 ± 2
	100	19 ± 2	24 ± 4	8 ± 2	7 ± 2	9 ± 2	7 ± 0
	333	18 ± 2	25 ± 4	8 ± 1	12 ± 2	8 ± 1	10 ± 1
	1,000	19 ± 1	31 ± 3	8 ± 0	13 ± 1	6 ± 1	9 ± 2
	3,333	25 ± 1	16 ± 1	8 ± 1	11 ± 2	11 ± 3	8 ± 2
	10,000	27 ± 4	14 ± 3	7 ± 1	8 ± 2	9 ± 1	6 ± 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		427 ± 12	472 ± 34	382 ± 19	586 ± 12	132 ± 4	489 ± 17
TA1537							
	0	5 ± 2	8 ± 3	5 ± 1	7 ± 1	7 ± 1	7 ± 1
	100	5 ± 0	4 ± 1	7 ± 3	9 ± 2	7 ± 2	8 ± 1
	333	4 ± 1	6 ± 2	7 ± 1	10 ± 1	7 ± 1	8 ± 1
	1,000	2 ± 0	4 ± 0	8 ± 0	9 ± 1	9 ± 1	9 ± 0
	3,333	9 ± 1	4 ± 1	8 ± 1	8 ± 1	6 ± 1	8 ± 1
	10,000	6 ± 2	8 ± 2	5 ± 1	7 ± 1	6 ± 2	4 ± 0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		222 ± 11	283 ± 43	519 ± 11	268 ± 14	123 ± 4	251 ± 20

Table E1
Mutagenicity of DE-71 in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
Study performed at BioReliance Corporation					
TA102					
	0	307 ± 24	329 ± 26	347 ± 28	221 ± 13
	100	1,219 ± 890	296 ± 11	397 ± 27	224 ± 36
	333	303 ± 3	209 ± 25	396 ± 7	155 ± 17
	1,000	302 ± 10[c]	325 ± 9[c]	310 ± 12[c]	233 ± 7[c]
	3,333	308 ± 13[c]	302 ± 39[c]	339 ± 7[c]	325 ± 14[c]
	10,000	328 ± 16[c]	272 ± 17[c]	355 ± 6[c]	333 ± 16[c]
Trial summary		Negative	Negative	Negative	Equivocal
Positive control		1,003 ± 3	1,324 ± 26	1,434 ± 84	845 ± 22
TA100					
	0	184 ± 7		182 ± 9	
	100	227 ± 12		180 ± 6	
	333	186 ± 19		180 ± 1	
	1,000	208 ± 25[c]		178 ± 3[c]	
	3,333	203 ± 9[c]		180 ± 8[c]	
	10,000	236 ± 18[c]		184 ± 10[c]	
Trial summary		Negative		Negative	
Positive control		689 ± 45		693 ± 22	
TA98					
	0	15 ± 3		20 ± 2	
	100	14 ± 1		22 ± 2	
	333	10 ± 1		20 ± 3	
	1,000	12 ± 0[c]		21 ± 1[c]	
	3,333	11 ± 2[c]		17 ± 1[c]	
	10,000	11 ± 0[c]		19 ± 2[c]	
Trial summary		Negative		Negative	
Positive control		112 ± 9		245 ± 11	

[a] Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol and these data are presented by Zeiger *et al*. (1987). 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), 4-nitro-*o*-phenylenediamine (TA98), and cumene hydroperoxide (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene, except sterigmatocystin was used for TA102.

[c] Precipitate on plate

TABLE E2
Mutagenicity of DE-71 in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	68 ± 8	70 ± 3	90 ± 7	68 ± 3
	1,000	53 ± 2	57 ± 9	82 ± 4	66 ± 2
	2,500	63 ± 3	58 ± 5	72 ± 1	73 ± 6
	5,000	69 ± 2	68 ± 4	85 ± 4	73 ± 3
	7,500	70 ± 3	65 ± 2	70 ± 5	72 ± 6
	10,000	75 ± 8	76 ± 1	82 ± 9	66 ± 1
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		557 ± 8	554 ± 3	868 ± 76	1,101 ± 49
TA98					
	0	27 ± 2	20 ± 2	31 ± 3	24 ± 2
	1,000	18 ± 2	20 ± 5	31 ± 2	27 ± 3
	2,500	17 ± 3	21 ± 2	30 ± 3	36 ± 3
	5,000	19 ± 3	26 ± 5	24 ± 3	32 ± 2
	7,500	22 ± 5	33 ± 2	23 ± 2	27 ± 3
	10,000	22 ± 2	33 ± 8	23 ± 1	38 ± 3
Trial summary		Negative	Negative	Negative	Negative
Positive control		514 ± 13	648 ± 26	1,416 ± 42	1,071 ± 42
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	143 ± 5	167 ± 13	195 ± 12	211 ± 3
	1,000	152 ± 9	163 ± 3	211 ± 13	219 ± 5
	2,500	160 ± 6	181 ± 8	226 ± 10	254 ± 1
	5,000	118 ± 4	193 ± 8	166 ± 8	254 ± 13
	7,500	120 ± 1	210 ± 8	151 ± 6	261 ± 5
	10,000	120 ± 8	249 ± 5	142 ± 15	266 ± 18
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,654 ± 53	1,913 ± 40	996 ± 15	1,046 ± 74

[a] Study was performed at SITEK Research Laboratories using lot 2550OA30A. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 2-nitrofluorene (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E3
Mutagenicity of 2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47) in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9
TA102				
	0	223 ± 9	262 ± 6	285 ± 18
	100	136 ± 68[b]	225 ± 5	276 ± 9
	333	71 ± 71[b]	248 ± 3	273 ± 13
	1,000	216 ± 12[c]	264 ± 3[c]	303 ± 5[c]
	3,333	213 ± 6[c]	268 ± 5[c]	287 ± 1[c]
	10,000	171 ± 22[c]	258 ± 6[c]	276 ± 6[c]
Trial summary		Negative	Negative	Negative
Positive control[d]		1,405 ± 66	1,053 ± 7	1,346 ± 71
TA100				
	0	169 ± 15		149 ± 7
	100	186 ± 10		118 ± 8
	333	172 ± 12		116 ± 7
	1,000	209 ± 11[c]		130 ± 1[c]
	3,333	202 ± 8[c]		121 ± 5[c]
	10,000	146 ± 8[c]		121 ± 10[c]
Trial summary		Negative		Negative
Positive control		589 ± 19		700 ± 48
TA98				
	0	18 ± 0[e]		22 ± 3
	100	14 ± 2		23 ± 2
	333	18 ± 1		19 ± 1
	1,000	13 ± 1[c]		15 ± 1[c]
	3,333	11 ± 1[c]		14 ± 2[c]
	10,000	10 ± 2[c]		18 ± 1[c]
Trial summary		Negative		Negative
Positive control		124 ± 9		273 ± 13

[a] Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Slightly toxic
[c] Precipitate on plate
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and cumene hydroperoxide (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene, except sterigmatocystin was used for TA102.
[e] Contamination

TABLE E4
Mutagenicity of 2,2′,4,4′,5-Pentabromodiphenyl Ether (BDE-99) in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9
TA102				
	0	224 ± 23[b]	269 ± 15	306 ± 15
	100	Toxic	264 ± 9	243 ± 16
	333	194 ± 17[c]	252 ± 13	265 ± 15
	1,000	175 ± 4[c]	231 ± 16[c]	301 ± 4[c]
	3,333	200 ± 9[c]	197 ± 23[c]	283 ± 15[c]
	10,000	132 ± 2[c]	258 ± 8[c]	379 ± 19[c]
Trial summary		Negative	Negative	Negative
Positive control[d]		906 ± 89	992 ± 41	1,218 ± 42
TA100				
	0	178 ± 7		200 ± 6
	100	168 ± 3		183 ± 11
	333	173 ± 6		184 ± 2
	1,000	170 ± 4[c]		188 ± 13[c]
	3,333	170 ± 2[c]		187 ± 12[c]
	10,000	139 ± 27[c]		147 ± 11[c]
Trial summary		Negative		Negative
Positive control		681 ± 45		752 ± 17
TA98				
	0	18 ± 2		28 ± 1
	100	14 ± 4		22 ± 1
	333	14 ± 2		21 ± 4
	1,000	13 ± 1[c]		23 ± 2[c]
	3,333	11 ± 1[c]		24 ± 3[c]
	10,000	7 ± 0[c]		13 ± 1[c]
Trial summary		Negative		Negative
Positive control		120 ± 13		250 ± 22

[a] Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Slightly toxic
[c] Precipitate on plate
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and cumene hydroperoxide (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene, except sterigmatocystin was used for TA102.

TABLE E5
Mutagenicity of 2,2′,4,4′,5,5′-Hexabromodiphenyl Ether (BDE-153) in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	With 10% rat S9
TA102			
	0	307 ± 17	329 ± 11
	100	284 ± 10	348 ± 24
	333	331 ± 3	341 ± 5
	1,000	320 ± 11	351 ± 38[b]
	3,333	289 ± 6[b]	394 ± 7[b]
	5,000	269 ± 24[b]	373 ± 27[b]
Trial summary		Negative	Negative
Positive control[c]		785 ± 21	1,404 ± 104
TA100			
	0	178 ± 7	191 ± 10
	100	203 ± 6	182 ± 7
	333	188 ± 3[b]	186 ± 7[b]
	1,000	235 ± 17[b]	212 ± 3[b]
	3,333	178 ± 5[b]	232 ± 27[b]
	5,000	167 ± 14[b]	186 ± 43[b]
Trial summary		Negative	Negative
Positive control		686 ± 41	644 ± 24
TA98			
	0	16 ± 2	18 ± 1
	100	14 ± 2	19 ± 2
	333	11 ± 3[b]	18 ± 1[b]
	1,000	11 ± 1[b]	16 ± 3[b]
	3,333	9 ± 1[b]	13 ± 1[b]
	5,000	11 ± 2[b]	16 ± 3[b]
Trial summary		Negative	Negative
Positive control		102 ± 4	223 ± 4

[a] Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.

[b] Precipitate on plate

[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and cumene hydroperoxide (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene, except sterigmatocystin was used for TA102.

TABLE E6
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Administered DE-71 by Gavage for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	1.90 ± 0.40		2.360 ± 0.19
DE-71	0.01	5	2.10 ± 0.53	0.3758	2.480 ± 0.26
	5	5	1.80 ± 0.46	0.5654	2.540 ± 0.23
	50	5	1.80 ± 0.34	0.5654	2.300 ± 0.19
	100	5	2.30 ± 0.37	0.2683	2.940 ± 0.34
	500	3	1.83 ± 0.73	0.5376	2.133 ± 0.39
			P=0.537[e]		
Female					
Corn oil[d]	0	5	1.30 ± 0.20		2.840 ± 0.12
DE-71	0.01	5	1.60 ± 0.33	0.2886	3.140 ± 0.38
	5	5	1.50 ± 0.32	0.3526	3.040 ± 0.43
	50	5	1.20 ± 0.46	0.5793	2.360 ± 0.40
	100	5	0.80 ± 0.20	0.8625	2.260 ± 0.12
	500	5	1.40 ± 0.48	0.4236	1.980 ± 0.14
			P=0.510[e]		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte

[b] Mean ± standard error

[c] Pairwise comparison with the vehicle control group; exposed group values are significant at P≤0.005

[d] Vehicle control

[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤ 0.025

TABLE E7
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male Mice Administered DE-71 by Gavage for 3 Days[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)	P Value[c]
Corn oil[d]	0	5	2.59 ± 0.20		1.57 ± 0.04		1.736 ± 0.09	
DE-71	312.5	5	2.16 ± 0.10	0.8731	1.53 ± 0.04	0.6358	1.565 ± 0.12	0.3789
	625	5	2.21 ± 0.12	0.9286	1.59 ± 0.06	0.4287	1.518 ± 0.19	0.2654
	1,250	5	2.33 ± 0.19	0.9447	1.60 ± 0.01	0.4297	1.325 ± 0.13	0.0396
			P=0.767[e]		P=0.223[e]		P=0.023[e].	
Cyclophosphamide[f]	50		32.56 ± 1.58	<0.0001	2.03 ± 0.05	<0.0001	0.175 ± 0.01	<0.0001

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al.* (2008). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the chamber control group; dosed group values are significant at P≤0.025 by Williams' test; positive control values are significant at P≤0.05.
[d] Vehicle control
[e] Dose-related trend; significant at P≤0.025 by Jonckheere's test.
[f] Positive control

TABLE E8
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Administered DE-71 by Gavage for 3 Days[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Corn oil[d]	0	5	2.00 ± 0.42		68.00 ± 2.56
DE-71	312.5	5	1.50 ± 0.32	0.8012	72.50 ± 1.92
	625	5	1.90 ± 0.37	0.5637	71.60 ± 5.03
	1,250	5	2.10 ± 0.19	0.4379	66.80 ± 4.83
			P=0.327[e]		
Cyclophosphamide[f]	50	5	33.70 ± 4.14	<0.0001	31.60 ± 4.62

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al.* (2008). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.008; positive control values are significant at P≤0.05.
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P≤ 0.025
[f] Positive control

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
Hematology						
n						
Day 4	9	9	9	9	8	9
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Automated hematocrit (%)						
Day 4	51.4 ± 1.3	49.7 ± 1.1	49.9 ± 0.8	49.9 ± 0.7	49.8 ± 1.2	50.7 ± 1.0
Day 25	48.1 ± 0.6	48.6 ± 0.5	50.5 ± 0.8	48.5 ± 0.5	49.6 ± 0.9	48.0 ± 0.3
Week 14	44.8 ± 0.4	45.5 ± 0.5	44.4 ± 0.5	43.3 ± 0.4*	43.6 ± 0.4*	42.3 ± 0.4**
Manual hematocrit (%)						
Day 4	50.7 ± 1.2	48.3 ± 1.0	49.0 ± 0.8	48.6 ± 0.7	48.6 ± 1.0	49.6 ± 1.0
Day 25	48.1 ± 0.5	48.6 ± 0.4	50.1 ± 0.7	48.3 ± 0.3	49.5 ± 0.8	48.0 ± 0.3
Week 14	44.3 ± 0.3	45.2 ± 0.4	43.7 ± 0.3	43.2 ± 0.4	43.6 ± 0.3	42.0 ± 0.4**
Hemoglobin (g/dL)						
Day 4	16.7 ± 0.4	16.0 ± 0.3	16.1 ± 0.2	16.4 ± 0.3	16.3 ± 0.3	16.4 ± 0.3
Day 25	15.8 ± 0.1	16.3 ± 0.1	16.5 ± 0.2*	16.1 ± 0.2	16.5 ± 0.3	15.8 ± 0.1
Week 14	15.4 ± 0.1	15.4 ± 0.1	15.1 ± 0.1	14.8 ± 0.1**	14.7 ± 0.1**	14.2 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 4	8.02 ± 0.19	7.78 ± 0.16	7.72 ± 0.12	7.82 ± 0.10	7.80 ± 0.20	8.02 ± 0.16
Day 25	7.92 ± 0.11	8.07 ± 0.10	8.26 ± 0.10	7.99 ± 0.09	8.21 ± 0.15	8.04 ± 0.06
Week 14	9.02 ± 0.06	9.11 ± 0.07	8.90 ± 0.07	8.80 ± 0.09	8.87 ± 0.06	8.87 ± 0.07
Reticulocytes ($10^6/\mu L$)						
Day 4	5.67 ± 0.31	5.48 ± 0.37	5.70 ± 0.37	5.13 ± 0.31	4.33 ± 0.40*	3.59 ± 0.26**
Day 25	2.76 ± 0.12	2.81 ± 0.13	3.03 ± 0.18	2.84 ± 0.10	2.40 ± 0.14	2.00 ± 0.14**
Week 14	1.89 ± 0.06	2.03 ± 0.06	2.07 ± 0.06	1.95 ± 0.07	2.26 ± 0.04**	2.30 ± 0.07**
Nucleated erythrocytes/100 leukocytes						
Day 4	0.70 ± 0.20	0.70 ± 0.30	1.30 ± 0.20	0.80 ± 0.30	0.80 ± 0.20	1.00 ± 0.30
Day 25	0.10 ± 0.10	0.30 ± 0.20	0.10 ± 0.10	0.20 ± 0.10	0.20 ± 0.10	0.10 ± 0.10
Week 14	0.20 ± 0.10	0.30 ± 0.20	0.60 ± 0.30	0.50 ± 0.30	0.40 ± 0.20	0.60 ± 0.30
Mean cell volume (fL)						
Day 4	64.1 ± 0.3	63.9 ± 0.5	64.7 ± 0.3	63.7 ± 0.2	63.9 ± 0.3	63.2 ± 0.2
Day 25	60.6 ± 0.2	60.2 ± 0.2	61.1 ± 0.4	60.8 ± 0.2	60.4 ± 0.3	59.8 ± 0.3
Week 14	49.7 ± 0.1	49.9 ± 0.3	49.8 ± 0.3	49.2 ± 0.2	49.1 ± 0.2*	47.7 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	20.8 ± 0.1	20.6 ± 0.1	20.9 ± 0.1	20.9 ± 0.2	20.9 ± 0.2	20.5 ± 0.1
Day 25	19.9 ± 0.1	20.2 ± 0.2	20.0 ± 0.1	20.1 ± 0.1	20.1 ± 0.2	19.7 ± 0.1
Week 14	17.1 ± 0.0	17.0 ± 0.1	17.0 ± 0.1	16.9 ± 0.1*	16.6 ± 0.0**	16.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.4 ± 0.2	32.3 ± 0.2	32.3 ± 0.1	32.8 ± 0.3	32.8 ± 0.2	32.5 ± 0.1
Day 25	32.8 ± 0.1	33.5 ± 0.2*	32.8 ± 0.1	33.1 ± 0.1	33.3 ± 0.2	32.9 ± 0.1
Week 14	34.4 ± 0.1	34.0 ± 0.1	34.0 ± 0.2	34.2 ± 0.1	33.8 ± 0.1**	33.5 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 4	1,015.0 ± 33.4	1,008.7 ± 29.3	1,011.7 ± 35.9	1,048.3 ± 25.9	1,038.6 ± 38.6	924.2 ± 34.6
Day 25	828.7 ± 23.9	831.8 ± 34.3	847.5 ± 24.2	863.5 ± 14.5	830.4 ± 20.0	784.2 ± 21.0
Week 14	588.3 ± 16.1	595.1 ± 17.9	586.2 ± 18.9	623.5 ± 16.4	672.8 ± 11.9**	633.7 ± 13.9**
Leukocytes ($10^3/\mu L$)						
Day 4	10.38 ± 0.43	9.26 ± 0.22	9.42 ± 0.30	8.98 ± 0.25*	9.12 ± 0.39*	7.88 ± 0.32**
Day 25	9.92 ± 0.33	9.53 ± 0.24	9.68 ± 0.30	8.72 ± 0.23**	9.22 ± 0.40*	8.07 ± 0.36**
Week 14	8.26 ± 0.16	8.31 ± 0.21	8.58 ± 0.29	8.55 ± 0.40	8.65 ± 0.24	7.42 ± 0.28

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	9	9	9	9	8	9
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.03 ± 0.04	1.03 ± 0.03	1.02 ± 0.05	1.04 ± 0.04	1.00 ± 0.06	1.09 ± 0.04
Day 25	0.95 ± 0.05	0.98 ± 0.04	1.10 ± 0.06	0.92 ± 0.03	0.90 ± 0.04	0.79 ± 0.04*
Week 14	1.16 ± 0.03	1.13 ± 0.05	1.25 ± 0.07	1.11 ± 0.05	1.06 ± 0.05	0.86 ± 0.03**
Lymphocytes ($10^3/\mu L$)						
Day 4	8.94 ± 0.41	7.87 ± 0.23	8.02 ± 0.26	7.58 ± 0.22*	7.76 ± 0.32*	6.37 ± 0.27**
Day 25	8.72 ± 0.30	8.28 ± 0.23	8.30 ± 0.27	7.58 ± 0.23**	8.09 ± 0.40*	7.03 ± 0.36**
Week 14	6.74 ± 0.14	6.82 ± 0.20	6.99 ± 0.26	7.09 ± 0.40	7.27 ± 0.25	6.24 ± 0.25
Monocytes ($10^3/\mu L$)						
Day 4	0.24 ± 0.02	0.22 ± 0.01	0.24 ± 0.02	0.21 ± 0.01	0.22 ± 0.02	0.26 ± 0.02
Day 25	0.11 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01
Week 14	0.16 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
Basophils ($10^3/\mu L$)						
Day 4	0.031 ± 0.004	0.027 ± 0.003	0.024 ± 0.002	0.022 ± 0.003	0.024 ± 0.004	0.016 ± 0.002**
Day 25	0.027 ± 0.002	0.021 ± 0.002	0.025 ± 0.002	0.024 ± 0.003	0.024 ± 0.002	0.023 ± 0.002
Week 14	0.028 ± 0.002	0.029 ± 0.002	0.028 ± 0.003	0.039 ± 0.009	0.028 ± 0.002	0.024 ± 0.003
Eosinophils ($10^3/\mu L$)						
Day 4	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01
Day 25	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.03 ± 0.00**	0.03 ± 0.00**	0.02 ± 0.00**
Week 14	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.07 ± 0.01	0.05 ± 0.00**	0.02 ± 0.00**
Large unstained cells ($10^3/\mu L$)						
Day 4	0.101 ± 0.009	0.080 ± 0.004	0.098 ± 0.011	0.091 ± 0.006	0.093 ± 0.008	0.104 ± 0.004
Day 25	0.065 ± 0.004	0.070 ± 0.006	0.067 ± 0.007	0.051 ± 0.005	0.065 ± 0.005	0.069 ± 0.007
Week 14	0.089 ± 0.010	0.089 ± 0.007	0.091 ± 0.008	0.108 ± 0.010	0.099 ± 0.012	0.106 ± 0.014
Clinical Chemistry						
n						
Day 4	9	9	9	9	9	9
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	11.6 ± 0.4	12.2 ± 0.6	11.7 ± 0.2	11.3 ± 0.4	11.2 ± 0.6	12.2 ± 0.3
Day 25	13.8 ± 0.7	13.7 ± 0.6	13.2 ± 0.3	12.3 ± 0.5	13.4 ± 0.2	16.7 ± 0.5*
Week 14	14.7 ± 0.5	15.1 ± 0.3	14.2 ± 0.4	14.3 ± 0.5	14.9 ± 0.4	19.1 ± 0.5**
Creatinine (mg/dL)						
Day 4	0.14 ± 0.02	0.13 ± 0.02	0.17 ± 0.02	0.14 ± 0.02	0.19 ± 0.01	0.20 ± 0.02*
Day 25	0.23 ± 0.02	0.20 ± 0.00	0.22 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	0.25 ± 0.02
Week 14	0.30 ± 0.00	0.31 ± 0.02	0.30 ± 0.02	0.31 ± 0.01	0.33 ± 0.02	0.28 ± 0.01
Glucose (mg/dL)						
Day 4	138 ± 3	136 ± 3	136 ± 4	130 ± 4	131 ± 4	109 ± 2**
Day 25	166 ± 4	148 ± 4**	157 ± 5*	140 ± 3**	137 ± 4**	128 ± 4**
Week 14	122 ± 3	141 ± 14	135 ± 8	119 ± 2	123 ± 5	114 ± 4

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	9	9	9	9	9	9
Day 25	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1
Day 25	5.9 ± 0.0	6.2 ± 0.0**	6.3 ± 0.1**	6.4 ± 0.1**	6.6 ± 0.1**	6.9 ± 0.1**
Week 14	6.8 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	7.5 ± 0.1**	7.7 ± 0.1**	8.1 ± 0.1**
Albumin (g/dL)						
Day 4	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.0*
Day 25	4.4 ± 0.0	4.5 ± 0.0	4.6 ± 0.1*	4.7 ± 0.0**	4.7 ± 0.1**	4.9 ± 0.1**
Week 14	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.0	5.1 ± 0.1**	5.2 ± 0.0**	5.5 ± 0.0**
Cholesterol (mg/dL)						
Day 4	105 ± 4	101 ± 3	106 ± 2	135 ± 3**	148 ± 4**	185 ± 6**
Day 25	77 ± 2	89 ± 2**	89 ± 2**	101 ± 1**	112 ± 2**	217 ± 3**
Week 14	88 ± 1	87 ± 2	83 ± 2	106 ± 3**	117 ± 3**	235 ± 5**
Alanine aminotransferase (IU/L)						
Day 4	67 ± 4	68 ± 2	71 ± 2	69 ± 3	72 ± 4	94 ± 6**
Day 25	47 ± 1	50 ± 2	48 ± 2	52 ± 2	53 ± 2*	109 ± 7**
Week 14	69 ± 4	71 ± 3	49 ± 2	42 ± 1**	46 ± 2**	79 ± 3
Alkaline phosphatase (IU/L)						
Day 4	613 ± 20	605 ± 18	626 ± 13	594 ± 19	603 ± 16	615 ± 16
Day 25	391 ± 9	425 ± 8	427 ± 14	398 ± 13	369 ± 10	437 ± 15[b]
Week 14	226 ± 6	217 ± 4	204 ± 4	185 ± 2**	178 ± 4**	268 ± 5
Creatine kinase (IU/L)						
Day 4	498 ± 36	571 ± 125	448 ± 34	468 ± 44	435 ± 47	416 ± 18
Day 25	372 ± 59	371 ± 42	359 ± 48	341 ± 42	355 ± 27	366 ± 41
Week 14	444 ± 33	443 ± 42	418 ± 46	456 ± 38	523 ± 60	349 ± 30
Sorbitol dehydrogenase (IU/L)						
Day 4	6 ± 1	7 ± 2	7 ± 1	7 ± 1	10 ± 2*	14 ± 1**
Day 25	14 ± 1	14 ± 1	13 ± 1[c]	15 ± 2	16 ± 1	28 ± 4*[b]
Week 14	18 ± 2[c]	15 ± 2[c]	13 ± 2	11 ± 1	15 ± 2	19 ± 3
Bile salts (µmol/L)						
Day 4	20.3 ± 2.0	18.6 ± 1.4	21.5 ± 2.0	27.1 ± 1.2*	31.9 ± 2.1**	33.8 ± 1.6**
Day 25	21.1 ± 2.3	16.4 ± 0.7	22.8 ± 1.8	25.5 ± 1.5*	32.7 ± 1.4**	39.1 ± 2.2**
Week 14	15.5 ± 0.9	20.8 ± 2.2	22.3 ± 1.9*	20.8 ± 0.9**	27.0 ± 1.6**	32.9 ± 1.6**
Total thyroxine (µg/dL)						
Day 4	5.97 ± 0.34[d]	5.72 ± 0.12[d]	5.67 ± 0.29[d]	1.35 ± 0.10**[d]	0.87 ± 0.13**[d]	0.62 ± 0.11**[d]
Day 25	6.55 ± 0.26	6.54 ± 0.48	5.02 ± 0.31**	1.33 ± 0.16**	0.72 ± 0.10**	0.48 ± 0.07**
Week 14	4.25 ± 0.20	4.53 ± 0.18	2.29 ± 0.16**	0.50 ± 0.11**	0.10 ± 0.05**	0.46 ± 0.09**
Total triiodothyronine (ng/dL)						
Day 25	100.9 ± 3.1	113.1 ± 7.6	90.8 ± 6.5	79.4 ± 4.1*	80.0 ± 3.9	108.6 ± 3.9
Week 14	81.1 ± 4.5	75.7 ± 3.7	63.7 ± 5.6	77.9 ± 5.8	73.4 ± 5.3	120.7 ± 5.6
Thyroid stimulating hormone (ng/mL)						
Day 4	5.70 ± 0.41[d]	5.20 ± 0.40[d]	5.04 ± 0.47[d]	5.82 ± 0.55	5.10 ± 0.39[d]	4.42 ± 0.39[d]
Day 25	3.66 ± 0.15	4.69 ± 0.38	5.16 ± 0.64	5.57 ± 0.66	6.55 ± 0.84**	4.63 ± 0.60
Week 14	3.75 ± 0.33	3.61 ± 0.47	3.74 ± 0.46	4.62 ± 0.48	4.69 ± 0.57	6.19 ± 0.84*

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Female						
Hematology						
n						
Day 4	10	10	10	10	8	9
Day 25	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Automated hematocrit (%)						
Day 4	50.4 ± 0.7	50.6 ± 0.6	51.1 ± 1.5	50.0 ± 1.0	50.4 ± 0.9	51.1 ± 0.6
Day 25	49.9 ± 0.6	51.4 ± 0.8	49.2 ± 1.5	50.0 ± 0.8	50.8 ± 1.3	48.0 ± 0.5
Week 14	43.3 ± 0.4	42.8 ± 0.3	42.5 ± 0.5	42.1 ± 0.4	40.3 ± 0.2**	38.2 ± 0.3**
Manual hematocrit (%)						
Day 4	49.0 ± 0.6	49.4 ± 0.7	50.2 ± 1.5	49.2 ± 1.1	48.7 ± 0.9	50.2 ± 0.7
Day 25	49.4 ± 0.5	50.6 ± 0.6	48.7 ± 1.4	49.2 ± 0.7	50.4 ± 1.1	47.8 ± 0.4
Week 14	43.0 ± 0.5	43.1 ± 0.3	42.9 ± 0.3	42.1 ± 0.5	40.7 ± 0.2**	38.6 ± 0.4**
Hemoglobin (g/dL)						
Day 4	16.2 ± 0.2	16.2 ± 0.1	16.4 ± 0.5	16.0 ± 0.3	16.0 ± 0.3	16.3 ± 0.2
Day 25	16.5 ± 0.2	17.0 ± 0.2	16.3 ± 0.5	16.5 ± 0.2	16.7 ± 0.4	15.6 ± 0.2*
Week 14	14.5 ± 0.1	14.4 ± 0.1	14.4 ± 0.1	14.3 ± 0.1	13.7 ± 0.1**	12.8 ± 0.1**
Erythrocytes (10^6/µL)						
Day 4	8.08 ± 0.11	8.04 ± 0.07	8.10 ± 0.26	7.93 ± 0.15	8.06 ± 0.14	8.15 ± 0.11
Day 25	8.38 ± 0.11	8.57 ± 0.12	8.21 ± 0.26	8.35 ± 0.13	8.50 ± 0.18	8.12 ± 0.08
Week 14	8.46 ± 0.07	8.38 ± 0.07	8.31 ± 0.11	8.49 ± 0.08	8.32 ± 0.07	8.27 ± 0.08
Reticulocytes (10^6/µL)						
Day 4	3.30 ± 0.34	4.99 ± 0.48*	4.21 ± 0.34	4.69 ± 0.43	4.71 ± 0.28*	3.47 ± 0.19
Day 25	1.87 ± 0.05	1.71 ± 0.07	1.86 ± 0.10	1.72 ± 0.07	1.80 ± 0.10	1.28 ± 0.04**
Week 14	1.81 ± 0.06	1.89 ± 0.06	1.78 ± 0.04	1.92 ± 0.03	1.69 ± 0.03	2.03 ± 0.05
Nucleated erythrocytes/100 leukocytes						
Day 4	0.80 ± 0.30	0.80 ± 0.40	0.30 ± 0.20	1.10 ± 0.30	0.60 ± 0.40	0.70 ± 0.30
Day 25	0.00 ± 0.00	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.20 ± 0.10	0.20 ± 0.10
Week 14	0.40 ± 0.20	0.30 ± 0.20	0.20 ± 0.20	0.30 ± 0.20	0.30 ± 0.20	0.50 ± 0.20
Mean cell volume (fL)						
Day 4	62.3 ± 0.2	62.9 ± 0.4	63.1 ± 0.4	63.0 ± 0.3	62.5 ± 0.3	62.8 ± 0.4
Day 25	59.6 ± 0.2	59.9 ± 0.3	60.0 ± 0.3	59.9 ± 0.2	59.8 ± 0.4	59.1 ± 0.2
Week 14	51.2 ± 0.2	51.2 ± 0.2	51.1 ± 0.2	49.6 ± 0.2**	48.4 ± 0.2**	46.2 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	20.0 ± 0.1	20.1 ± 0.1	20.2 ± 0.1	20.2 ± 0.1	19.8 ± 0.1	20.0 ± 0.1
Day 25	19.6 ± 0.1	19.8 ± 0.1	19.9 ± 0.1	19.8 ± 0.1	19.6 ± 0.1	19.3 ± 0.1
Week 14	17.2 ± 0.0	17.2 ± 0.1	17.3 ± 0.1	16.8 ± 0.1**	16.5 ± 0.1**	15.5 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.2 ± 0.3	32.0 ± 0.3	32.1 ± 0.3	32.1 ± 0.2	31.8 ± 0.1	31.8 ± 0.2
Day 25	33.0 ± 0.2	33.1 ± 0.2	33.2 ± 0.1	33.0 ± 0.2	32.9 ± 0.2	32.6 ± 0.2
Week 14	33.6 ± 0.1	33.6 ± 0.2	33.9 ± 0.2	33.9 ± 0.2	34.0 ± 0.1*	33.6 ± 0.1
Platelets (10^3/µL)						
Day 4	994.6 ± 62.3	1,096.4 ± 57.9	951.6 ± 29.8	1,048.6 ± 63.8	1,086.9 ± 52.1	881.1 ± 56.1
Day 25	703.9 ± 28.8	775.2 ± 31.8	785.4 ± 30.8	755.6 ± 20.8	788.1 ± 29.1	579.9 ± 19.0
Week 14	562.9 ± 13.4	568.9 ± 14.6	571.1 ± 21.8	594.5 ± 20.0	597.1 ± 23.4	504.4 ± 26.9
Leukocytes (10^3/µL)						
Day 4	10.90 ± 0.40	10.77 ± 0.43	11.16 ± 0.34	10.07 ± 0.31	10.29 ± 0.38	8.84 ± 0.30**
Day 25	9.32 ± 0.51	9.33 ± 0.45	9.53 ± 0.63	9.41 ± 0.44	9.76 ± 0.24	7.02 ± 0.41*
Week 14	7.42 ± 0.29	7.45 ± 0.37	6.63 ± 0.36	6.22 ± 0.21**	6.10 ± 0.39*	6.61 ± 0.28*

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	8	9
Day 25	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Segmented neutrophils (10^3/µL)						
Day 4	1.03 ± 0.06	1.08 ± 0.06	1.10 ± 0.06	0.95 ± 0.09	1.11 ± 0.07	0.92 ± 0.04
Day 25	1.00 ± 0.06	0.91 ± 0.05	0.93 ± 0.06	0.95 ± 0.09	0.93 ± 0.05	0.82 ± 0.06
Week 14	1.16 ± 0.10	1.05 ± 0.06	0.91 ± 0.07*	0.81 ± 0.04**	0.79 ± 0.08**	0.77 ± 0.08**
Lymphocytes (10^3/µL)						
Day 4	9.48 ± 0.37	9.32 ± 0.36	9.67 ± 0.31	8.53 ± 0.27	8.79 ± 0.31	7.54 ± 0.27**
Day 25	8.06 ± 0.52	8.14 ± 0.41	8.31 ± 0.57	8.19 ± 0.38	8.52 ± 0.23	5.97 ± 0.36*
Week 14	5.94 ± 0.23	6.12 ± 0.35	5.44 ± 0.28	5.13 ± 0.18	5.06 ± 0.31	5.52 ± 0.21
Monocytes (10^3/µL)						
Day 4	0.20 ± 0.01	0.20 ± 0.02	0.21 ± 0.02	0.31 ± 0.10	0.24 ± 0.02	0.23 ± 0.02
Day 25	0.11 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.01**	0.13 ± 0.01
Week 14	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.18 ± 0.01
Basophils (10^3/µL)						
Day 4	0.042 ± 0.004	0.036 ± 0.004	0.040 ± 0.004	0.032 ± 0.003	0.030 ± 0.003	0.033 ± 0.005
Day 25	0.027 ± 0.003	0.031 ± 0.003	0.029 ± 0.004	0.027 ± 0.003	0.029 ± 0.003	0.015 ± 0.004
Week 14	0.029 ± 0.003	0.027 ± 0.006	0.023 ± 0.003	0.026 ± 0.003	0.021 ± 0.004	0.033 ± 0.006
Eosinophils (10^3/µL)						
Day 4	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
Day 25	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.04 ± 0.00**	0.04 ± 0.01**	0.03 ± 0.00**
Week 14	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.04 ± 0.01**	0.02 ± 0.00**
Large unstained cells (10^3/µL)						
Day 4	0.093 ± 0.008	0.081 ± 0.008	0.084 ± 0.006	0.205 ± 0.118	0.079 ± 0.007	0.076 ± 0.007
Day 25	0.059 ± 0.003	0.065 ± 0.006	0.070 ± 0.007	0.065 ± 0.005	0.077 ± 0.008	0.060 ± 0.006
Week 14	0.092 ± 0.008	0.072 ± 0.006	0.077 ± 0.008	0.084 ± 0.008	0.075 ± 0.008	0.093 ± 0.008
Clinical Chemistry						
n						
Day 4	3	3	3	4	6	2
Day 25	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	13.1 ± 1.0	13.9 ± 0.9	11.6 ± 1.2[e]	10.4 ± 0.9	11.6 ± 0.7	14.2 ± 0.9
Day 25	14.2 ± 0.5	15.0 ± 0.7	13.9 ± 0.7	12.9 ± 0.6	13.8 ± 0.3	15.8 ± 0.5
Week 14	13.0 ± 0.6	13.5 ± 0.7	13.2 ± 0.3	12.9 ± 0.5	13.9 ± 0.4	21.4 ± 0.9**
Creatinine (mg/dL)						
Day 4	0.24 ± 0.02[c]	0.23 ± 0.02[f]	0.22 ± 0.02[c]	0.23 ± 0.02[d]	0.26 ± 0.03[f]	0.28 ± 0.01[d]
Day 25	0.24 ± 0.02	0.23 ± 0.02	0.20 ± 0.00	0.20 ± 0.01	0.27 ± 0.02	0.24 ± 0.02
Week 14	0.31 ± 0.02	0.30 ± 0.00[c]	0.28 ± 0.01	0.32 ± 0.01	0.28 ± 0.01	0.26 ± 0.02
Glucose (mg/dL)						
Day 4	117 ± 2	126 ± 7	122 ± 10	119 ± 3	118 ± 4	107 ± 6
Day 25	149 ± 7	160 ± 8	148 ± 5	133 ± 4	131 ± 4	120 ± 2**
Week 14	124 ± 3	120 ± 2	117 ± 3	109 ± 2**	109 ± 3**	108 ± 3**

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	3	3	3	4	6	2
Day 25	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.9 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.8 ± 0.3	5.7 ± 0.2	5.6 ± 0.2
Day 25	5.8 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	6.4 ± 0.1**	6.5 ± 0.1**	7.0 ± 0.1**
Week 14	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	7.4 ± 0.1**	7.8 ± 0.1**	7.2 ± 0.1**
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.2	4.2 ± 0.1	4.2 ± 0.1
Day 25	4.5 ± 0.1	4.6 ± 0.0	4.6 ± 0.1	4.8 ± 0.1**	4.8 ± 0.0**	5.0 ± 0.0**
Week 14	4.9 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	5.4 ± 0.1**	5.6 ± 0.0**	5.1 ± 0.1**
Cholesterol (mg/dL)						
Day 4	112 ± 6[c]	108 ± 4[c]	113 ± 3[c]	136 ± 3**[d]	147 ± 7**[b]	176 ± 4**[d]
Day 25	75 ± 2	82 ± 3*	87 ± 3**	117 ± 4**	144 ± 4**[c]	244 ± 5**
Week 14	72 ± 2	74 ± 2	94 ± 3**	145 ± 4**	183 ± 9**	310 ± 9**
Alanine aminotransferase (IU/L)						
Day 4	61 ± 3[c]	61 ± 3[c]	58 ± 4[c]	65 ± 3[d]	69 ± 2*[f]	81 ± 4**[d]
Day 25	44 ± 2	49 ± 2	45 ± 2	42 ± 1	44 ± 2	78 ± 2**
Week 14	52 ± 3	52 ± 4	55 ± 6	35 ± 1*	35 ± 1**	147 ± 33
Alkaline phosphatase (IU/L)						
Day 4	529 ± 14[b]	550 ± 21[g]	560 ± 13[c]	549 ± 23[b]	521 ± 55[e]	570 ± 25[h]
Day 25	328 ± 9	363 ± 10	352 ± 7	333 ± 11	313 ± 9	365 ± 9[i]
Week 14	193 ± 7	184 ± 6	182 ± 6	147 ± 7*	137 ± 4**	315 ± 12
Creatine kinase (IU/L)						
Day 4	473 ± 136	448 ± 69	604 ± 78	727 ± 81	584 ± 72	399 ± 86
Day 25	423 ± 62	509 ± 50	435 ± 48	500 ± 64	421 ± 39	297 ± 39
Week 14	433 ± 48	337 ± 19	381 ± 41	406 ± 42	364 ± 33	322 ± 32
Sorbitol dehydrogenase (IU/L)						
Day 4	4 ± 2[g]	1 ± 0[j]	3 ± 2[e]	4 ± 3[i]	2 ± 0[j]	5 ± 1[f]
Day 25	12 ± 3[c]	9 ± 3[f]	10 ± 2[g]	9 ± 3[b]	9 ± 3[b]	26 ± 3*[g]
Week 14	9 ± 2[c]	15 ± 2[c]	13 ± 3	19 ± 3**	18 ± 1**	31 ± 4**
Bile acids (µmol/L)						
Day 4	16.2 ± 3.7	19.9 ± 2.8	16.4 ± 3.9	27.1 ± 4.9	26.4 ± 2.1	23.4 ± 0.2
Day 25	19.3 ± 1.9	25.4 ± 5.4	18.1 ± 1.9	25.0 ± 1.4*	31.6 ± 1.8**	32.3 ± 1.7**
Week 14	20.2 ± 6.0	16.8 ± 1.5	17.3 ± 0.6*	20.9 ± 1.1**	24.3 ± 0.9**	32.2 ± 2.5**
Total thyroxine (µg/dL)						
Day 4	4.88 ± 0.22[d]	4.90 ± 0.13[d]	4.12 ± 0.20*[d]	0.95 ± 0.12**[d]	0.57 ± 0.07**[d]	0.41 ± 0.08**[d]
Day 25	5.09 ± 0.17	4.89 ± 0.26	4.13 ± 0.25*	1.02 ± 0.11**	0.56 ± 0.14**	0.30 ± 0.07**
Week 14	3.19 ± 0.24	3.36 ± 0.16	1.68 ± 0.12**	0.41 ± 0.06**	0.48 ± 0.09**	0.50 ± 0.07**
Total triiodothyronine (ng/dL)						
Day 25	94.1 ± 5.1	98.1 ± 3.4	91.5 ± 4.5	95.7 ± 4.1	98.7 ± 4.0	120.4 ± 4.6**
Week 14	79.0 ± 5.8	75.2 ± 4.1	62.6 ± 2.0	74.9 ± 4.1	83.6 ± 6.2	137.3 ± 5.7**

TABLE F1
Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	3	3	3	4	6	2
Day 25	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Thyroid stimulating hormone (ng/mL)						
Day 4	4.57 ± 0.46[d]	4.08 ± 0.42[d]	5.80 ± 0.47[d]	4.51 ± 0.44[d]	4.55 ± 0.38[d]	3.61 ± 0.35[d]
Day 25	3.99 ± 0.26	3.96 ± 0.18	4.84 ± 0.32	5.27 ± 0.20**	4.86 ± 0.43*	5.56 ± 0.52*
Week 14	2.69 ± 0.20	2.95 ± 0.29	2.83 ± 0.28	3.40 ± 0.36	4.66 ± 0.72**	4.32 ± 0.34**

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=7
[c] n=9
[d] n=10
[e] n=4
[f] n=8
[g] n=6
[h] n=5
[i] n=3
[j] n=2

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n	10	10	10	10	10	3
Automated hematocrit (%)	49.8 ± 0.8	48.7 ± 0.4	50.0 ± 0.4	48.8 ± 0.5	47.9 ± 0.4	43.6 ± 0.8**
Manual hematocrit (%)	48.5 ± 0.7	48.1 ± 0.4	48.9 ± 0.3	48.1 ± 0.5	47.1 ± 0.5	43.5 ± 0.6**
Hemoglobin (g/dL)	16.3 ± 0.2	16.0 ± 0.1	16.3 ± 0.1	16.1 ± 0.1	15.9 ± 0.1	14.3 ± 0.3*
Erythrocytes (10^6/µL)	10.41 ± 0.15	10.17 ± 0.10	10.38 ± 0.07	10.11 ± 0.10	9.93 ± 0.09*	9.36 ± 0.21**
Reticulocytes (10^6/µL)	2.98 ± 0.02	2.82 ± 0.07	2.85 ± 0.07	2.69 ± 0.09**	2.53 ± 0.04**	2.97 ± 0.22*
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.10 ± 0.10	0.20 ± 0.10	0.00 ± 0.00	0.10 ± 0.10	0.30 ± 0.30
Mean cell volume (fL)	47.8 ± 0.2	47.9 ± 0.2	48.2 ± 0.2	48.3 ± 0.4	48.2 ± 0.3	46.6 ± 0.2
Mean cell hemoglobin (pg)	15.7 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	16.0 ± 0.1	16.1 ± 0.1**	15.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.2	32.9 ± 0.1	32.7 ± 0.1	33.1 ± 0.2	33.3 ± 0.1*	32.8 ± 0.1
Platelets (10^3/µL)	993.1 ± 38.2	1,046.1 ± 44.6	1,131.1 ± 43.0	1,193.5 ± 35.6**	1,331.7 ± 40.0**	1,090.0 ± 28.6*
Leukocytes (10^3/µL)	5.82 ± 0.65	6.45 ± 0.62	6.20 ± 0.57	6.01 ± 0.52	6.51 ± 0.67	7.76 ± 0.91
Segmented neutrophils (10^3/µL)	1.44 ± 0.27	0.98 ± 0.10	0.97 ± 0.12	1.36 ± 0.47	1.20 ± 0.22	2.75 ± 1.02
Lymphocytes (10^3/µL)	4.06 ± 0.68	5.08 ± 0.57	4.84 ± 0.44	4.32 ± 0.53	4.94 ± 0.66	4.57 ± 1.24
Monocytes (10^3/µL)	0.11 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.10 ± 0.01	0.12 ± 0.02	0.21 ± 0.09
Basophils (10^3/µL)	0.026 ± 0.005	0.035 ± 0.007	0.029 ± 0.005	0.030 ± 0.004	0.031 ± 0.009	0.017 ± 0.009
Eosinophils (10^3/µL)	0.16 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.17 ± 0.03	0.18 ± 0.03	0.12 ± 0.03
Large unstained cells	0.035 ± 0.007	0.050 ± 0.009	0.049 ± 0.008	0.034 ± 0.006	0.044 ± 0.008	0.087 ± 0.015*
Female						
n	9	10	10	9	9	5
Automated hematocrit (%)	48.9 ± 0.8	49.0 ± 0.5	49.5 ± 0.4	48.7 ± 0.5	48.1 ± 0.6	43.4 ± 0.7*
Manual hematocrit (%)	48.5 ± 0.7	48.9 ± 0.3	49.4 ± 0.4	49.2 ± 0.4	48.4 ± 0.5	43.4 ± 0.7
Hemoglobin (g/dL)	16.2 ± 0.2	16.2 ± 0.1	16.4 ± 0.1	16.3 ± 0.2	16.2 ± 0.2	14.6 ± 0.2*
Erythrocytes (10^6/µL)	10.24 ± 0.15	10.30 ± 0.09	10.39 ± 0.09	10.19 ± 0.11	10.05 ± 0.11	9.02 ± 0.12**
Reticulocytes (10^6/µL)	3.09 ± 0.11	3.06 ± 0.12	3.28 ± 0.15	2.63 ± 0.07**	2.47 ± 0.13**	2.08 ± 0.24**
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00
Mean cell volume (fL)	47.8 ± 0.2	47.6 ± 0.2	47.6 ± 0.2	47.8 ± 0.2	47.9 ± 0.2	48.1 ± 0.5
Mean cell hemoglobin (pg)	15.8 ± 0.1	15.8 ± 0.0	15.8 ± 0.1	16.0 ± 0.1	16.1 ± 0.1*	16.2 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	33.2 ± 0.1	33.1 ± 0.2	33.1 ± 0.1	33.4 ± 0.1	33.6 ± 0.1	33.7 ± 0.2
Platelets (10^3/µL)	939.9 ± 52.4	937.9 ± 33.8	872.1 ± 25.6	997.8 ± 24.7	1,045.9 ± 51.8	1,129.4 ± 88.4
Leukocytes (10^3/µL)	4.04 ± 0.55	4.58 ± 0.31	4.74 ± 0.37	5.24 ± 0.50	4.79 ± 0.49	6.95 ± 0.97
Segmented neutrophils (10^3/µL)	0.57 ± 0.07	0.53 ± 0.07	0.58 ± 0.04	0.62 ± 0.11	0.57 ± 0.06	1.01 ± 0.17
Lymphocytes (10^3/µL)	3.21 ± 0.45	3.72 ± 0.30	3.83 ± 0.33	4.28 ± 0.39	3.95 ± 0.42	5.55 ± 0.77
Monocytes (10^3/µL)	0.06 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.19 ± 0.03**
Basophils (10^3/µL)	0.018 ± 0.004	0.014 ± 0.002	0.019 ± 0.004	0.022 ± 0.006	0.022 ± 0.008	0.024 ± 0.004
Eosinophils (10^3/µL)	0.16 ± 0.03	0.19 ± 0.03	0.19 ± 0.03	0.19 ± 0.04	0.12 ± 0.01	0.08 ± 0.02
Large unstained cells	0.021 ± 0.005	0.029 ± 0.004	0.030 ± 0.005	0.039 ± 0.007	0.033 ± 0.006	0.102 ± 0.014**

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

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	316 ± 6	335 ± 5	327 ± 6	330 ± 6	318 ± 8	272 ± 5**
Heart						
Absolute	0.76 ± 0.02	0.83 ± 0.01*	0.81 ± 0.02	0.84 ± 0.02**	0.82 ± 0.02	0.76 ± 0.02
Relative	2.401 ± 0.026	2.489 ± 0.025	2.478 ± 0.025	2.552 ± 0.024**	2.573 ± 0.033**	2.801 ± 0.036**
R. Kidney						
Absolute	0.93 ± 0.02	0.99 ± 0.03	1.00 ± 0.03	1.07 ± 0.03**	1.07 ± 0.03**	1.08 ± 0.02**
Relative	2.932 ± 0.023	2.942 ± 0.056	3.050 ± 0.054	3.240 ± 0.036**	3.349 ± 0.027**	3.958 ± 0.035**
Liver						
Absolute	10.09 ± 0.17	11.22 ± 0.33	12.13 ± 0.44**	16.04 ± 0.52**	17.42 ± 0.46**	20.01 ± 0.58**
Relative	31.940 ± 0.252	33.482 ± 0.536	37.037 ± 0.774**	48.628 ± 1.130**	54.787 ± 0.524**	73.381 ± 1.224**
Lung						
Absolute	1.25 ± 0.06	1.46 ± 0.07	1.29 ± 0.05	1.29 ± 0.05	1.27 ± 0.04	1.05 ± 0.03**
Relative	3.956 ± 0.202	4.370 ± 0.197	3.934 ± 0.128	3.908 ± 0.119	4.014 ± 0.155	3.842 ± 0.072
R. Testis						
Absolute	1.314 ± 0.031	1.344 ± 0.019	1.325 ± 0.012	1.372 ± 0.029	1.380 ± 0.021	1.354 ± 0.020
Relative	4.158 ± 0.084	4.023 ± 0.062	4.065 ± 0.079	4.163 ± 0.051	4.352 ± 0.069	4.982 ± 0.084**
Thymus						
Absolute	0.230 ± 0.012	0.243 ± 0.014	0.241 ± 0.012	0.221 ± 0.011	0.245 ± 0.020	0.163 ± 0.014**
Relative	0.727 ± 0.038	0.727 ± 0.041	0.739 ± 0.037	0.672 ± 0.038	0.772 ± 0.059	0.598 ± 0.048
Female						
Necropsy body wt	197 ± 3	191 ± 2	203 ± 4	189 ± 2	181 ± 3**	169 ± 4**
Heart						
Absolute	0.53 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.53 ± 0.01
Relative	2.695 ± 0.056	2.835 ± 0.053	2.654 ± 0.038	2.741 ± 0.026	2.871 ± 0.046*	3.147 ± 0.063**
R. Kidney						
Absolute	0.62 ± 0.01	0.65 ± 0.01	0.68 ± 0.01**	0.68 ± 0.01**	0.68 ± 0.02**	0.79 ± 0.01**
Relative	3.132 ± 0.047	3.378 ± 0.063*	3.333 ± 0.050*	3.617 ± 0.055**	3.737 ± 0.048**	4.716 ± 0.105**
Liver						
Absolute	5.56 ± 0.16	5.92 ± 0.10	6.47 ± 0.13**	8.73 ± 0.16**	9.85 ± 0.27**	12.16 ± 0.35**
Relative	28.191 ± 0.616	31.009 ± 0.599*	31.891 ± 0.490**	46.139 ± 0.590**	54.511 ± 1.135**	72.195 ± 1.448**
Lung						
Absolute	0.91 ± 0.03	0.93 ± 0.04	0.93 ± 0.02	0.88 ± 0.02	0.89 ± 0.06	0.77 ± 0.02**
Relative	4.637 ± 0.172	4.900 ± 0.237	4.581 ± 0.132	4.656 ± 0.062	4.900 ± 0.328	4.598 ± 0.127
Thymus						
Absolute	0.226 ± 0.011	0.212 ± 0.009	0.209 ± 0.007	0.174 ± 0.009**	0.152 ± 0.011**	0.099 ± 0.009**
Relative	1.149 ± 0.055	1.114 ± 0.051	1.032 ± 0.035	0.922 ± 0.051**	0.836 ± 0.055**	0.587 ± 0.050**

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1 Wistar Han Rats at the 3-Month Interim Evaluation in the 2-Year Perinatal and Postnatal Gavage Study[a]

	Vehicle Control	50 mg/kg
n	10	10
Male		
Necropsy body wt	403 ± 10	433 ± 16
Heart		
Absolute	1.02 ± 0.04	1.14 ± 0.05
Relative	2.520 ± 0.073	2.631 ± 0.087
R. Kidney		
Absolute	1.29 ± 0.04	1.57 ± 0.08**
Relative	3.198 ± 0.102	3.618 ± 0.113*
Liver		
Absolute	13.68 ± 0.39	19.53 ± 0.76**
Relative	33.938 ± 0.702	45.180 ± 1.191**
Lung		
Absolute	1.57 ± 0.08	1.72 ± 0.11
Relative	3.907 ± 0.183	4.039 ± 0.357
R. Testis		
Absolute	1.836 ± 0.069	2.168 ± 0.075**
Relative	4.552 ± 0.132	5.011 ± 0.057**
Thymus		
Absolute	0.362 ± 0.022	0.399 ± 0.027
Relative	0.895 ± 0.043	0.928 ± 0.060
Female		
Necropsy body wt	246 ± 4	213 ± 7**
Heart		
Absolute	0.74 ± 0.02	0.68 ± 0.02
Relative	3.021 ± 0.059	3.207 ± 0.062*
R. Kidney		
Absolute	0.89 ± 0.02	0.84 ± 0.02
Relative	3.636 ± 0.067	3.947 ± 0.056**
Liver		
Absolute	7.94 ± 0.18	9.28 ± 0.43*
Relative	32.350 ± 0.579	43.369 ± 0.745**
Lung		
Absolute	1.18 ± 0.03[b]	1.04 ± 0.04**
Relative	4.789 ± 0.105[b]	4.875 ± 0.127
Thymus		
Absolute	0.362 ± 0.020	0.264 ± 0.016**
Relative	1.473 ± 0.071	1.239 ± 0.070*

* Significantly different (P≤0.05) from the vehicle control group by a *t*-test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n	10	10	10	10	10	3
Necropsy body wt	39.3 ± 0.8	38.8 ± 0.7	39.3 ± 1.0	37.3 ± 1.1	35.9 ± 0.7**	28.6 ± 0.9**
Heart						
Absolute	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.11 ± 0.00**
Relative	3.411 ± 0.090	3.562 ± 0.078	3.529 ± 0.093	3.582 ± 0.081	3.648 ± 0.055*	3.966 ± 0.091**
R. Kidney						
Absolute	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.20 ± 0.01**
Relative	6.784 ± 0.133	7.145 ± 0.175	7.067 ± 0.164	7.129 ± 0.148	7.245 ± 0.188	6.995 ± 0.056
Liver						
Absolute	1.38 ± 0.02	1.31 ± 0.05	1.50 ± 0.03	1.79 ± 0.08**	2.18 ± 0.07**	4.11 ± 0.02**
Relative	35.024 ± 0.417	33.701 ± 1.195	38.207 ± 0.870	48.005 ± 1.761**	60.684 ± 1.827**	144.118 ± 4.508**
Lung						
Absolute	0.21 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.16 ± 0.00
Relative	5.306 ± 0.345	4.896 ± 0.402	4.687 ± 0.226	4.947 ± 0.269	4.897 ± 0.200	5.607 ± 0.189
R. Testis						
Absolute	0.115 ± 0.002	0.114 ± 0.002	0.116 ± 0.002	0.116 ± 0.002	0.112 ± 0.003	0.102 ± 0.007*
Relative	2.931 ± 0.065	2.940 ± 0.056	2.969 ± 0.077	3.112 ± 0.071	3.102 ± 0.057	3.553 ± 0.184**
Thymus						
Absolute	0.037 ± 0.003	0.037 ± 0.001	0.034 ± 0.001	0.032 ± 0.002	0.037 ± 0.002	0.035 ± 0.001
Relative	0.922 ± 0.068	0.949 ± 0.041	0.876 ± 0.029	0.875 ± 0.054	1.023 ± 0.044	1.240 ± 0.073**
Female						
n	9	10	10	9	9	5
Necropsy body wt	32.8 ± 0.5	29.9 ± 0.6	29.5 ± 1.1*	30.3 ± 1.0	31.0 ± 1.0	27.3 ± 0.3**
Heart						
Absolute	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.10 ± 0.00**
Relative	3.596 ± 0.084	3.932 ± 0.103	3.849 ± 0.155	3.798 ± 0.083	3.813 ± 0.121	3.803 ± 0.072
R. Kidney						
Absolute	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Relative	4.954 ± 0.151	5.740 ± 0.162**	5.323 ± 0.157**	5.578 ± 0.111**	5.436 ± 0.115**	6.289 ± 0.190**
Liver						
Absolute	1.29 ± 0.20	1.10 ± 0.02	1.10 ± 0.03	1.51 ± 0.04	1.83 ± 0.05**	3.74 ± 0.10**
Relative	39.495 ± 6.272	36.887 ± 0.526	37.404 ± 0.887	50.224 ± 1.481*	59.150 ± 1.078**	137.002 ± 3.891**
Lung						
Absolute	0.19 ± 0.01[b]	0.19 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
Relative	5.659 ± 0.290[b]	6.285 ± 0.303	7.143 ± 0.677	6.407 ± 0.271	5.855 ± 0.353	5.788 ± 0.328
Thymus						
Absolute	0.045 ± 0.003	0.043 ± 0.002	0.044 ± 0.002	0.046 ± 0.002	0.044 ± 0.002	0.040 ± 0.001
Relative	1.380 ± 0.095	1.444 ± 0.085	1.522 ± 0.092	1.534 ± 0.053	1.422 ± 0.082	1.478 ± 0.045

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=8

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	50 mg/kg	100 mg/kg	500 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	316 ± 6	335 ± 7.9	318 ± 8	282 ± 12*
L. Cauda epididymis	0.1289 ± 0.0050	0.1385 ± 0.0119[b]	0.1328 ± 0.0087	0.0724 ± 0.0047**
L. Epididymis	0.4284 ± 0.0102	0.4485 ± 0.0168	0.4184 ± 0.0141	0.3135 ± 0.0128**
L. Testis	1.4061 ± 0.0343	1.5028 ± 0.0337	1.4981 ± 0.0279	1.4818 ± 0.0291
Spermatid measurements				
Spermatid heads (10^6/testis)	181.38 ± 3.90	186.38 ± 7.34	170.50 ± 5.90	164.88 ± 9.49
Spermatid heads (10^6/g testis)	152.48 ± 4.13	151.01 ± 6.13	137.20 ± 3.96*	130.36 ± 6.20**
Epididymal spermatozoal measurements				
Sperm motility (%)	86.6 ± 0.7	86.5 ± 0.9	87.0 ± 0.6	82.7 ± 0.8**
Sperm (10^6/cauda epididymis)	78.3 ± 4.2	63.2 ± 8.9	81.3 ± 4.9	9.9 ± 1.1**
Sperm (10^6/g cauda epididymis)	608.5 ± 25.8	457.2 ± 77.4	591.2 ± 44.2	137.1 ± 14.6**

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's (body weights) or Shirley's (spermatid heads/g testis) test

** Significantly different (P≤0.01) from the vehicle control group by Williams' (cauda epididymis and epididymis weights) or Shirley's (spermatid heads per testis and epididymal spermatazoal measurements) test

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's (testis weights) or Dunn's (spermatid heads/testis) test.

[b] n=9

TABLE H2
Estrous Cycle Characterization for Female F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	50 mg/kg	100 mg/kg	500 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	197 ± 3	189 ± 2	181 ± 3**	169 ± 4**
Proportion of regular cycling females[b]	7/10	8/10	10/10*	0/10
Estrous cycle length (days)	5.8 ± 0.40	5.8 ± 0.29	5.3 ± 0.15	—[c]
Estrous stages (% of cycle)				
Diestrus	61.7	60.0	56.7	100.0
Proestrus	13.3	12.5	18.3	0.0
Estrus	20.0	20.0	18.3	0.0
Metestrus	5.0	7.5	6.7	0.0

* Significantly different (P≤0.05) from the vehicle control group by the Chi-square test

** Significantly different (P≤0.01) from the vehicle control group by Williams' test

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated a significantly higher probability of extended diestrus in the 500 mg/kg group compared to the vehicle control group.

[b] Number of females with a regular cycle/number of females cycling

[c] Estrous cycle was longer than 12 days or unclear in 10 of 10 animals.

Dose (mg/kg)																				
0						D	E	E	D	D	D	D	D	E	D	D	D			
0					D	D	P	E	D	D	D	D	P	E	D	D				
0								P	E	D	D	D	P	E	M	D	D	P	E	
0						E	D	D	D	D	D	D	P	E	D	D	D			
0						E	M	D	D	D	D	D	P	E	M	D	D			
0								D	E	D	D	D	D	E	M	D	D	P	E	
0				D	D	D	D	P	E	D	D	D	D	E	M					
0							D	P	E	D	D	D	P	E	D	D	D	P		
0							D	P	E	D	D	D	P	E	M	D	D	P		
0							D	P	E	D	D	D	D	E	D	D	D	E		
50						D	D	P	E	D	D	D	P	E	D	D	D			
50									E	D	D	D	P	E	D	D	D	E	E	D
50					D	D	P	E	D	D	D	D	P	E	M	D				
50									E	D	D	D	P	E	D	D	D	P	E	D
50						D	D	E	D	D	D	D	D	E	M	D	D			
50					M	D	D	P	E	D	D	D	P	E	M	D				
50				M	D	D	P	E	M	D	D	D	P	E	M					
50			D	D	D	E	M	D	D	D	D	D	P	E						
50					M	D	D	P	E	D	D	D	D	E	E	D				
50						D	P	E	D	D	D	D	P	E	D	D	D			
100								P	E	M	D	D	P	E	D	D	D	P	E	
100							D	P	E	D	D	D	P	E	D	D	D	P		
100						D	P	E	E	M	D	D	P	E	M	D	D			
100						D	D	P	E	D	D	D	D	E	D	D	D			
100					D	D	D	P	E	D	D	D	P	E	D	D				
100			M	D	D	D	P	E	M	D	D	D	P	E						
100								E	M	D	D	D	P	E	M	D	D	D	P	
100					D	D	D	P	E	D	D	D	P	E	D	D				
100							D	P	E	D	D	D	P	E	D	D	D	P		
100					D	D	D	P	E	M	D	D	P	E	D	D				
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							
500		D	D	D	D	D	D	D	D	D	D	D	D							

FIGURE H1
Vaginal Cytology Plots for Female F344/N Rats in the 3-Month Gavage Study of DE-71
D = diestrus, P = proestrus, E = estrus, M = metestrus

TABLE H3
Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female F344/N Rats Administered DE-71 by Gavage for 3 Months

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	<0.001	
Overall Tests	50 mg/kg vs. Vehicle Controls	0.402	
Overall Tests	100 mg/kg vs. Vehicle Controls	0.004	N
Overall Tests	500 mg/kg vs. Vehicle Controls	<0.001	
Extended Estrus	Overall	0.914	
Extended Estrus	50 mg/kg vs. Vehicle Controls	0.595	
Extended Estrus	100 mg/kg vs. Vehicle Controls	1	
Extended Estrus	500 mg/kg vs. Vehicle Controls	0.601	N
Extended Diestrus	Overall	<0.001	
Extended Diestrus	50 mg/kg vs. Vehicle Controls	0.493	N
Extended Diestrus	100 mg/kg vs. Vehicle Controls	0.004	N
Extended Diestrus	500 mg/kg vs. Vehicle Controls	<0.001	
Extended Metestrus	Overall	1	
Extended Metestrus	50 mg/kg vs. Vehicle Controls	1	
Extended Metestrus	100 mg/kg vs. Vehicle Controls	1	
Extended Metestrus	500 mg/kg vs. Vehicle Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	50 mg/kg vs. Vehicle Controls	1	
Extended Proestrus	100 mg/kg vs. Vehicle Controls	1	
Extended Proestrus	500 mg/kg vs. Vehicle Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	50 mg/kg vs. Vehicle Controls	1	
Skipped Estrus	100 mg/kg vs. Vehicle Controls	1	
Skipped Estrus	500 mg/kg vs. Vehicle Controls	1	
Skipped Diestrus	Overall	1	
Skipped Diestrus	50 mg/kg vs. Vehicle Controls	1	
Skipped Diestrus	100 mg/kg vs. Vehicle Controls	1	
Skipped Diestrus	500 mg/kg vs. Vehicle Controls	1	
Summary of Significant Groups			
Overall Tests	100 mg/kg vs. Vehicle Controls	0.004	N
Overall Tests	500 mg/kg vs. Vehicle Controls	<0.001	
Extended Diestrus	100 mg/kg vs. Vehicle Controls	0.004	N
Extended Diestrus	500 mg/kg vs. Vehicle Controls	<0.001	

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

TABLE H4
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	5 mg/kg	50 mg/kg	100 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.3 ± 0.8	39.3 ± 1.0	37.3 ± 1.1	35.9 ± 0.7*
L. Cauda epididymis	0.0274 ± 0.0011	0.0246 ± 0.0015	0.0237 ± 0.0015	0.0214 ± 0.0010**
L. Epididymis	0.0560 ± 0.0019	0.0541 ± 0.0033	0.0554 ± 0.0028	0.0514 ± 0.0017
L. Testis	0.1143 ± 0.0024	0.1149 ± 0.0018	0.1188 ± 0.0028	0.1112 ± 0.0021
Spermatid measurements				
Spermatid heads (10^6/testis)	22.83 ± 0.77	23.39 ± 0.75	22.67 ± 0.58	23.10 ± 0.55
Spermatid heads (10^6/g testis)	221.67 ± 6.18	238.55 ± 9.18	218.16 ± 7.04	238.72 ± 4.68
Epididymal spermatozoal measurements				
Sperm motility (%)	88.5 ± 1.2	89.5 ± 0.2	88.7 ± 0.3	85.3 ± 0.8**
Sperm (10^6/cauda epididymis)	16.7 ± 0.8	15.8 ± 1.6	9.4 ± 2.4	12.1 ± 2.1
Sperm (10^6/g cauda epididymis)	614.1 ± 34.7	676.7 ± 86.4	425.9 ± 120.0	555.3 ± 92.1

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** Significantly different (P≤0.01) from the vehicle control group by Williams' (body weights) or Shirley's (sperm motility) test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (epididymis and testis weights) or Dunn's test (spermatid measurements, sperm/cauda epididymis, and sperm/g cauda epididymis).

TABLE H5
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	5 mg/kg	50 mg/kg	100 mg/kg
Number weighed at necropsy	9	10	9	9
Necropsy body wt (g)	32.8 ± 0.5	29.5 ± 1.1*	30.3 ± 1.0	31.0 ± 1.0
Proportion of regular cycling females[b]	6/8	9/10	7/9	8/9
Estrous cycle length (days)	3.9 ± 0.25[c]	4.3 ± 0.18	4.4 ± 0.19	4.0 ± 0.12
Estrous stages (% of cycle)				
Diestrus	37.0	30.8	34.3	31.5
Proestrus	4.6	4.2	1.9	0.0
Estrus	40.7	45.0	43.5	46.3
Metestrus	17.6	20.0	20.4	22.2

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated dosed females did not have extended estrus or diestrus.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle length was longer than 12 days or unclear in 1 of 9 animals.

Dose (mg/kg)																						
0										E	E	M	D	E	E	D	D	E	E	M	D	
0									E	E	M	D	P	E	E	M	D	E	E	M		
0										E	E	D	D	E	M	D	E	E	M	E	M	
0								M	D	D	E	M	P	E	E	M	P	E	E			
0						M	D	P	E	E	M	D	P	E	E	M	D					
0			D	D	D	D	D	D	D	E	E	M	D	E								
0											E	E	D	E	E	M	D	E	E	M	D	E
0		D	D	D	D	D	D	E	E	D	D	D	D									
0								D	D	D	E	M	D	E	E	M	D	E	E			
5										E	M	D	P	E	E	D	D	P	E	E	M	
5											E	M	D	E	E	M	D	D	E	E	M	D
5										E	M	D	P	E	E	D	D	P	E	E	M	
5								M	D	E	E	M	D	E	E	D	D	D	D			
5										E	E	M	D	E	E	D	D	E	E	M	D	
5									D	E	E	M	D	E	E	M	D	E	E	M		
5								M	D	E	E	M	D	E	E	M	D	E	E			
5										E	M	D	D	E	E	D	D	E	E	E	M	
5											E	M	D	E	E	M	D	E	E	M	D	E
5							D	P	E	E	M	D	D	E	E	M	D	E				
50										E	M	D	D	E	E	D	D	P	E	E	M	
50								E	E	M	D	D	D	E	D	D	D	E	E			
50									D	D	E	M	D	E	E	M	D	E	E	E		
50								M	D	E	E	M	D	E	E	M	D	E	E			
50								M	D	P	E	M	D	E	E	M	D	E	E			
50		M	D	D	D	D	D	M	D	D	E	E	M									
50										E	E	M	D	E	E	D	D	E	E	M	D	
50									D	E	E	M	D	E	E	M	D	E	E	M		
50								M	D	E	E	M	D	E	E	M	D	E	E			
100							D	D	D	E	E	M	D	E	E	M	D	E				
100									D	E	E	M	D	E	E	M	D	E	E	M		
100							M	D	D	D	E	D	D	E	E	M	D	E				
100											E	M	D	E	D	D	D	D	E	E	M	D
100									D	E	E	M	D	E	E	M	D	E	E	M		
100										E	E	M	D	E	E	M	D	E	E	M	D	
100										E	E	M	D	E	E	E	M	D	E	E	M	
100									D	E	E	M	D	E	E	M	D	E	E	M		
100									D	E	E	M	D	E	E	M	D	E	E	M		

FIGURE H2
Vaginal Cytology Plots for Female Mice in the 3-Month Gavage Study of DE-71
D = diestrus, P = proestrus, E = estrus, M = metestrus

TABLE H6
Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Administered DE-71 by Gavage for 3 Months

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	0.009	
Overall Tests	5 mg/kg vs. Vehicle Controls	0.049	N
Overall Tests	50 mg/kg vs. Vehicle Controls	0.339	N
Overall Tests	100 mg/kg vs. Vehicle Controls	0.012	N
Extended Estrus	Overall	0.917	
Extended Estrus	5 mg/kg vs. Vehicle Controls	0.604	
Extended Estrus	50 mg/kg vs. Vehicle Controls	0.995	
Extended Estrus	100 mg/kg vs. Vehicle Controls	0.603	
Extended Diestrus	Overall	0.067	
Extended Diestrus	5 mg/kg vs. Vehicle Controls	0.159	N
Extended Diestrus	50 mg/kg vs. Vehicle Controls	0.213	N
Extended Diestrus	100 mg/kg vs. Vehicle Controls	0.081	N
Extended Metestrus	Overall	1	
Extended Metestrus	5 mg/kg vs. Vehicle Controls	1	
Extended Metestrus	50 mg/kg vs. Vehicle Controls	1	
Extended Metestrus	100 mg/kg vs. Vehicle Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	5 mg/kg vs. Vehicle Controls	1	
Extended Proestrus	50 mg/kg vs. Vehicle Controls	1	
Extended Proestrus	100 mg/kg vs. Vehicle Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	5 mg/kg vs. Vehicle Controls	1	
Skipped Estrus	50 mg/kg vs. Vehicle Controls	0.92	
Skipped Estrus	100 mg/kg vs. Vehicle Controls	1	
Skipped Diestrus	Overall	0.022	
Skipped Diestrus	5 mg/kg vs. Vehicle Controls	0.064	N
Skipped Diestrus	50 mg/kg vs. Vehicle Controls	0.079	N
Skipped Diestrus	100 mg/kg vs. Vehicle Controls	0.079	N
Summary of Significant Groups			
Overall Tests	5 mg/kg vs. Vehicle Controls	0.049	N
Overall Tests	100 mg/kg vs. Vehicle Controls	0.012	N

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

APPENDIX I
TISSUE CONCENTRATION STUDIES

TISSUE CONCENTRATION STUDIES

MATERIALS AND METHODS

3-Month Studies

Groups of 10 male and 10 female special study F344/N rats were randomly assigned to the tissue distribution study at the beginning of the 3-month study. Samples of adipose and liver were collected from vehicle control and each dosed group of special study male and female rats at day 25 and from 10 male and 10 female core study F344/N rats at week 14. Adipose samples were collected from vehicle control and each dosed group of male and female B6C3F1/N mice at week 14 (up to 10 animals/dose group).

All samples were frozen at –70° C and shipped to the analytical chemistry laboratory (Battelle Columbus Operations, Columbus, OH).

2-Year Studies

In Wistar Han [Crl:WI(Han)] rats following perinatal exposure of dams, livers and carcasses from six male and six or seven female F_1 pups from the vehicle control and each dosed group were collected after litter standardization on postnatal day (PND) 4 following decapitation and exsanguination. Groups of six F_0 dams were randomly assigned to the tissue distribution study. On PND 21, adipose and livers from each dam and one pup/sex per litter were collected from all dose groups. Samples of adipose, liver, and plasma were collected at the end of the study from up to 15 F_1 animals/sex per group from all dose groups.

Adipose and liver samples were collected from up to 16 male and 16 female B6C3F1/N mice per dose group at study termination, except that samples from 100 mg/kg male and female mice were collected at approximately 18 months.

All samples were frozen at –70° C and shipped to the analytical chemistry laboratory (Battelle Columbus Operations).

Preparation of Plasma for Analysis

All samples were stored frozen at –70° C until analysis. After thawing at room temperature, a 100 µL aliquot of plasma was transferred into a tube along with the internal standard (100 µL of 11 µg PCB 118/mL toluene). For samples of less than 100 µL, blank plasma was added to bring the final volume to 100 µL. The tubes were mixed and placed in a sonicator for approximately 10 minutes and periodically shaken to remove plasma from the side of the tube. The tubes were subsequently placed on a sample rotator overnight (at 60 rpm), centrifuged for a minimum of 2 minutes at 1,000 rpm, and an aliquot of the supernatant was transferred to an auto injector vial for analysis.

Preparation of Adipose, Liver, and PND 4 Pup Carcass for Analysis

All samples were stored frozen at –70° C until analysis. Prior to preparation, samples were allowed to thaw at room temperature. Adipose, liver, and PND 4 pup carcass were prepared and analyzed similarly to plasma with minor modifications. Pup carcass was homogenized in a 50 mL polypropylene tube for 5 minutes. The internal standard solution (100 µL of 55 µg PCB 118/mL toluene) and 3 mL of toluene were added to approximately 0.1 g of adipose, liver, or pup carcass homogenate and extraction was similar to that described above for plasma. An aliquot of the supernatant was transferred to an auto injector vial for analysis.

Quantitation of BDE-47, BDE-99, and BDE-153

Selected polybrominated diphenyl ether (PBDE) congeners (BDE-47, BDE-99, and BDE-153) were quantified as described below using validated analytical methods.. Authentic standards of BDE-47 (99.6%) , BDE-99 (97.6%) and BDE-153 (99.5%) were obtained from Ceriliant Corporation (Round Rock, TX). All samples were analyzed on an Agilent 6890 gas chromatograph (Agilent, Santa Clara, CA) coupled to an electron capture detector. An RTX®-5 column (30 m × 0.25 mm, 1.0 µm film thickness) (Restek, Bellefonte, PA) was used with a helium carrier gas at a flow rate of 3 mL/minute. The oven temperature was held at 210° C for 2 minutes and then ramped to 330° C at 8° C/minute and held for 3 minutes. Injector and detector temperatures were 300° C and 320° C, respectively.

One µL of each sample extract was analyzed in the splitless mode for plasma and in 1:1 split mode for other matrices.

All matrix calibration standards and quality control (QC) samples were treated and analyzed similar to the study samples. Calibration curves were run on adipose (0.900 to 120 µg/g), liver (0.900 to 120 µg/g), pup carcass (0.900 to 120 µg/g), and plasma (0.0875 to 15 µg/mL) with a minimum of six calibration standards and a calibration blank run before analysis of each set of samples. During the analysis of liver from the 3-month study an additional calibration curve covering the range 0.010 to 1.0 µg/mL was also run. The performance of the calibration curve was evaluated prior to the analysis of each sample set. A successful calibration was indicated by the following: correlation coefficient (r^2) ≥ 0.98; relative standard deviation (RSD) ≤ ± 15% [except at experimental limit of quantitation (LOQs) where RSD ≤ ± 20%]; relative error (RE) ≤ ± 15% (except at experimental LOQ where RE ≤ ± 20%). The experimental LOQs for BDE-47, BDE-99, and BDE-153 were: plasma, 0.0875 µg/mL (except in one run where LOQ for BDE-153 was 0.188 µg/mL); adipose and pup carcass, 0.900 µg/g; and liver, 0.010 (low curve) or 0.900 (high curve) µg/g.

Data from study samples were considered valid if they were bracketed by valid QC sets. In general, for each sample set, method blanks and controls were bracketed by two QC sets, which consisted of a calibration blank and two concentrations of calibration standards (QC low and QC high), with six samples at each concentration. A QC set passed when the measured concentrations for QC standards were within 15% of their nominal values. If the QC standard failed, it was necessary to reanalyze the bracketed samples.

In addition, incurred sample reanalysis was conducted. During the analysis of rat liver samples from the 2-year study, incurred sample reanalysis did not pass all of the acceptance criteria mentioned above. Following an investigation, it was decided to analyze liver samples using up to four replicates when possible. The average value for the replicates was reported when applicable.

The concentration of each analyte was calculated using its individual response, the regression equation, sample weight, and dilution when applicable. Samples with responses greater than the highest calibration standard were diluted with the diluent to get a response within the range. The diluent was prepared similarly to samples but used blank matrix. The concentrations of BDE-43, BDE-99, and BDE-153 in adipose and liver (rats only) from the subchronic studies were expressed as µg/g matrix. The concentrations of BDE-43, BDE-99, and BDE-153 in plasma from the 2-year rat study were expressed as µg/mL plasma. The concentrations of BDE-43, BDE-99, and BDE-153 in adipose and liver from the 2-year studies were expressed as both µg/g matrix and µg/g lipid. The concentrations of BDE-43, BDE-99, and BDE-153 in pup carcass on PND 4 were expressed as µg/g carcass.

Analysis of Adipose and Liver for Lipid Content

All samples were stored frozen at –70° C until analysis. Prior to analysis, samples were allowed to thaw at room temperature. An aliquot of approximately 10 mg of adipose or 50 mg of liver from each study animal was weighed into disposable hand-held homogenizer tubes. Triplicate aliquots were prepared when sufficient sample remained. Following the addition of 4 mL of 1:1 chloroform:methanol (v/v), samples were ground until visibly homogeneous and centrifuged for approximately 5 minutes at 3,000 rpm. The supernatant was transferred into a 5 mL volumetric flask. An additional 0.5 mL of extraction solution was added to each sample tube, and the contents were ground for an additional 30 seconds and centrifuged for 5 minutes at 300 rpm. The supernatant was combined with the first extract and the flask was filled to volume with extraction solution, sealed, and mixed. A 0.25 mL aliquot of each sample extract was evaporated to dryness using a dry block heater at approximately 100° C.

To each residue, 0.2 mL of concentrated sulfuric acid was added and the sample was mixed briefly and placed on the dry block heater at 100° C for 15 minutes. Samples were allowed to cool to room temperature and a vanillin reagent (2.5 mL of 1.2 mg vanillin/mL 68% aqueous phosphoric acid) was added to each hydrolysate. Tubes were vortexed for approximately 3 seconds, covered with an opaque box, and allowed to react for 30 minutes. A 0.2 mL aliquot of the resulting colored solution was pipetted into a 96-well plate, and the absorbance at 490 nm was measured using a DTX 880 Multimode Detector (Beckman Coulter, Inc., Brea, CA) at 25° C. Soybean oil was used as the standard for quantitation of lipids. Standards and blanks (extraction solvent) were carried through the sulfuric acid digestion and the vanillin reaction similar to the study samples. The lipid content of each sample was calculated as a percent of total tissue weight. The average lipid content was calculated for all samples where more than one replicate was analyzed.

TABLE I1
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose and Liver in F344/N Rats in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n	10	10	10	10	10	10
BDE-47 (µg/g)						
Adipose						
Day 25	0.44 ± 0.00	0.61 ± 0.07	63.82 ± 1.57	320.43 ± 6.32	604.67 ± 11.04	3,268.40 ± 107.68
Week 14	0.98 ± 0.04	1.81 ± 0.13	144.06 ± 3.27	596.78 ± 9.04	1,056.65 ± 19.45	4,849.10 ± 106.89
Liver						
Day 25	0.01 ± 0.00	0.04 ± 0.00	2.73 ± 0.33	17.48 ± 2.25	30.96 ± 3.64	186.99 ± 14.40
Week 14	0.07 ± 0.01	0.09 ± 0.02	2.07 ± 0.15	8.85 ± 1.28	16.62 ± 1.88	80.87 ± 9.89
BDE-99 (µg/g)						
Adipose						
Day 25	ND	0.50 ± 0.05	36.29 ± 1.07	256.94 ± 4.95	560.33 ± 12.86	3,012.70 ± 132.59
Week 14	0.82 ± 0.03	1.50 ± 0.10	102.90 ± 2.82	574.49 ± 10.38	1,066.27 ± 18.00	4,867.30 ± 126.70
Liver						
Day 25	0.01 ± 0.00	0.04 ± 0.00	2.12 ± 0.27	15.24 ± 2.34	27.90 ± 4.54	185.85 ± 19.62
Week 14	0.08 ± 0.01	0.08 ± 0.01	1.19 ± 0.21	3.79 ± 0.45	8.65 ± 0.86	58.25 ± 7.14
BDE-153 (µg/g)						
Adipose						
Day 25	ND	ND	7.59 ± 0.42	58.88 ± 1.60	136.24 ± 7.04	653.53 ± 52.46
Week 14	ND	0.45 ± 0.00	27.34 ± 0.66	210.13 ± 6.28	383.75 ± 9.51	1,649.50 ± 38.55
Liver						
Day 25	0.01 ± 0.00	0.02 ± 0.00	1.60 ± 0.14	15.55 ± 1.87	28.12 ± 1.78	139.96 ± 9.76
Week 14	0.03 ± 0.00	0.03 ± 0.01	1.92 ± 0.28	17.23 ± 3.07	34.09 ± 4.55	112.10 ± 16.32

TABLE I1
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose and Liver in F344/N Rats in the 3-Month Gavage Study of DE-71

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Female						
n	10	10	10	10	10	10
BDE-47 (µg/g)						
Adipose						
Day 25	0.44 ± 0.01	0.82 ± 0.05	80.24 ± 2.76	417.80 ± 21.02	721.14 ± 33.18[b]	4,157.40 ± 252.41
Week 14	1.15 ± 0.10	2.23 ± 0.09	180.03 ± 5.38	770.74 ± 15.00	1,363.80 ± 38.58	7,619.30 ± 252.15
Liver						
Day 25	0.01 ± 0.00	0.04 ± 0.01	2.84 ± 0.13	22.75 ± 1.85	37.50 ± 4.13[b]	156.36 ± 14.87
Week 14	0.03 ± 0.01	0.06 ± 0.01	2.30 ± 0.19	11.54 ± 0.99	15.84 ± 1.51	158.65 ± 22.46
BDE-99 (µg/g)						
Adipose						
Day 25	ND	0.62 ± 0.04	48.22 ± 1.43	339.16 ± 17.30	657.59 ± 31.99[b]	4,054.40 ± 253.52
Week 14	1.00 ± 0.09	1.92 ± 0.09	118.30 ± 3.57	681.93 ± 14.11	1,314.00 ± 29.38	7,510.00 ± 255.13
Liver						
Day 25	0.01 ± 0.00	0.04 ± 0.01	2.24 ± 0.18	21.07 ± 2.26	36.28 ± 5.13[b]	164.45 ± 19.94
Week 14	0.03 ± 0.01	0.05 ± 0.01	1.26 ± 0.08	5.23 ± 0.52	7.84 ± 0.83	131.56 ± 22.49
BDE-153 (µg/g)						
Adipose						
Day 25	ND	ND	9.97 ± 0.59	88.19 ± 3.98	183.52 ± 10.58[b]	1,021.26 ± 57.80
Week 14	ND	0.46 ± 0.01	27.21 ± 1.70	269.67 ± 10.98	601.63 ± 27.33	2,685.30 ± 114.75
Liver						
Day 25	0.01 ± 0.00	0.02 ± 0.00	1.30 ± 0.04	15.70 ± 1.93	26.80 ± 1.78[b]	92.68 ± 5.60
Week 14	0.01 ± 0.00	0.02 ± 0.00	1.19 ± 0.11	16.88 ± 1.66	27.68 ± 2.54	148.29 ± 21.44

[a] Data are presented as mean µg analyte/g tissue ± standard error. Values below the experimental limit of quantitation were replaced with ½ the limit of quantitation if there was at least one value in the group that was above the limit of quantitation. ND = all values were missing or below the limit of quantitation; BDE-47 = 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99 = 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-153 = 2,2′,4,4′,5,5′-hexabromodiphenyl ether.

[b] n=9

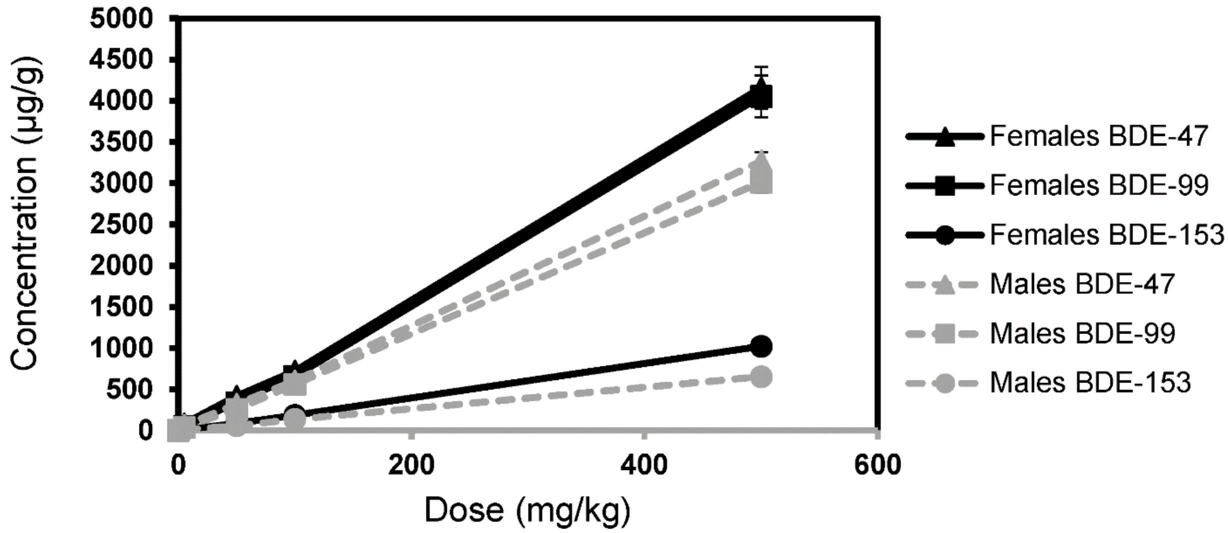


FIGURE I1
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in F344/N Rats on Day 25 in the 3-Month Gavage Study of DE-71

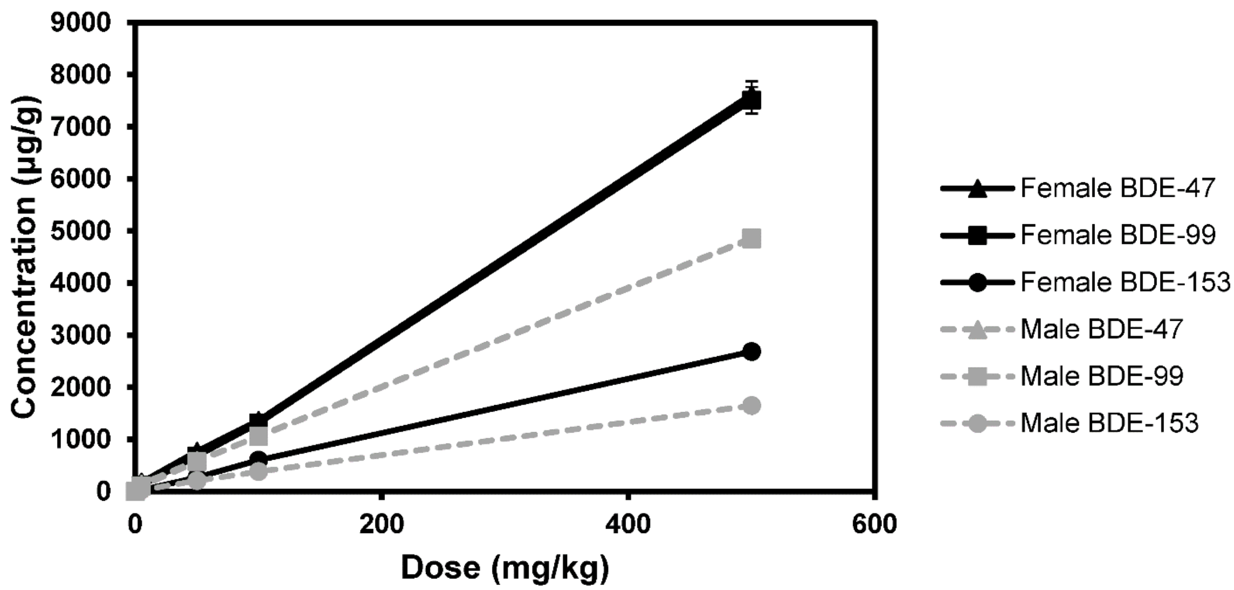


FIGURE I2
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in F344/N Rats at Week 14 in the 3-Month Gavage Study of DE-71

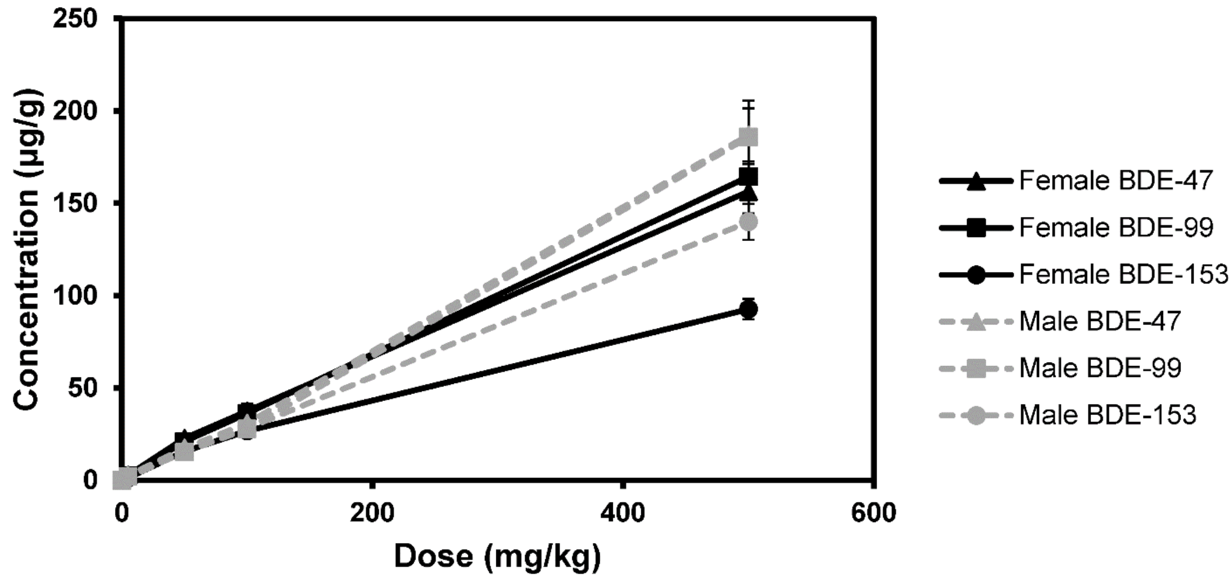


FIGURE I3
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Liver in F344/N Rats on Day 25 in the 3-Month Gavage Study of DE-71

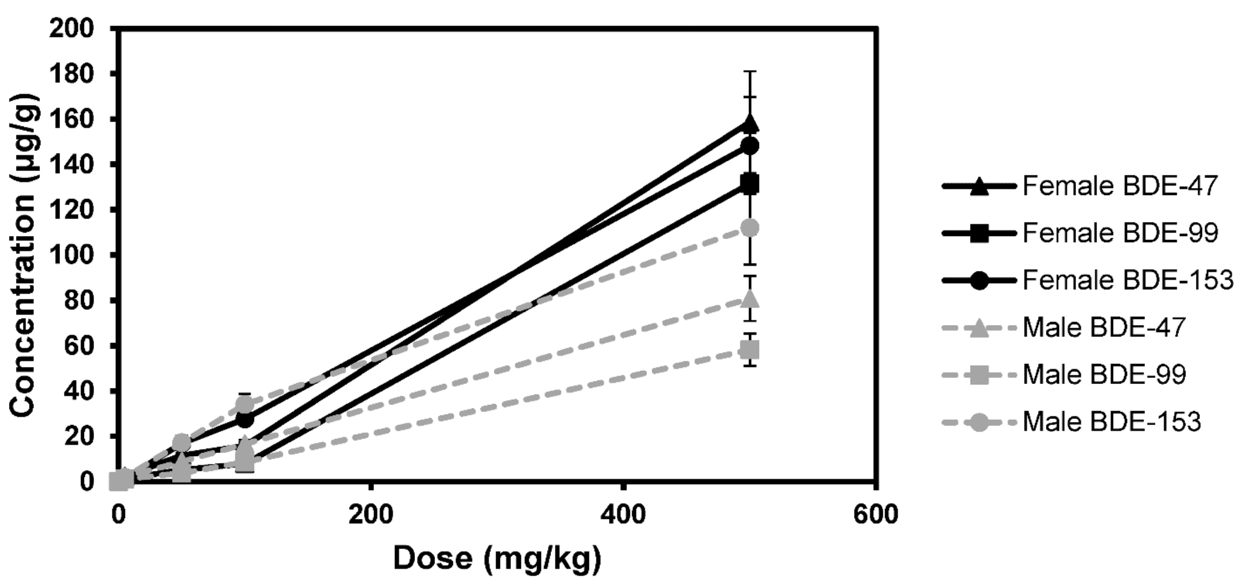


FIGURE I4
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Liver in F344/N Rats at Week 14 in the 3-Month Gavage Study of DE-71

TABLE I2
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose and Liver in Wistar Han Rat Dams on PND 21 in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
n	6	6	6	6
Lipid (%)				
Adipose	91.14 ± 1.64	94.60 ± 2.73	94.83 ± 2.05	94.59 ± 2.40
Liver	6.39 ± 0.10	5.92 ± 0.19	6.17 ± 0.15	5.86 ± 0.12
BDE-47 (µg/g)				
Adipose	ND	40.2 ± 4.9	347.0 ± 41.8	925.7 ± 80.4
Adipose (lipid-adjusted)	ND	43.1 ± 5.9	368.0 ± 44.9	974.1 ± 64.6
Liver	ND	ND	ND	1.3 ± 0.2
Liver (lipid-adjusted)	ND	ND	ND	21.8 ± 3.5
BDE-99 (µg/g)				
Adipose	ND	66.8 ± 6.4	501.8 ± 60.1	1,513.3 ± 186.9
Adipose (lipid-adjusted)	ND	71.4 ± 7.9	535.3 ± 70.6	1,595.7 ± 183.3
Liver	ND	ND	ND	1.1 ± 0.2
Liver (lipid-adjusted)	ND	ND	ND	18.0 ± 3.7
BDE-153 (µg/g)				
Adipose	ND	11.0 ± 1.1	93.1 ± 16.5	304.8 ± 37.8
Adipose (lipid-adjusted)	ND	11.6 ± 1.2	99.9 ± 19.5	322.9 ± 40.5
Liver	ND	ND	ND	0.6 ± 0.1
Liver (lipid-adjusted)	ND	ND	ND	9.5 ± 1.8

[a] Data are presented as mean µg analyte/g matrix ± standard error. Values below the experimental limit of quantitation were replaced with ½ the limit of quantitation if there was at least one value in the group that was above the limit of quantitation. ND = all values were missing or below the limit of quantitation; BDE-47 = 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99 = 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-153 = 2,2′,4,4′,5,5′-hexabromodiphenyl ether.

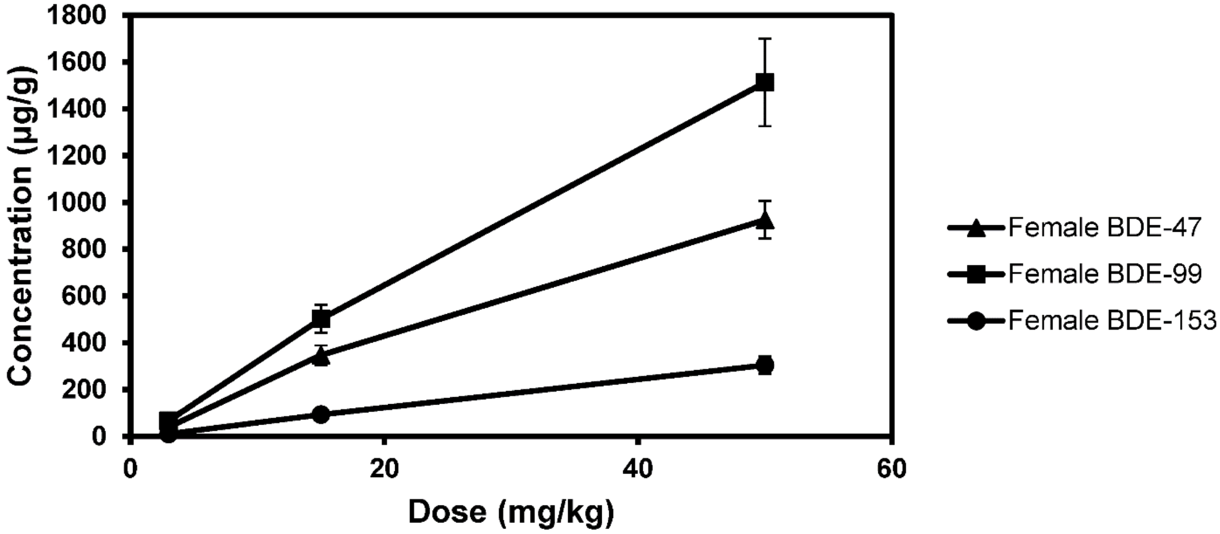


FIGURE I5
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in Wistar Han Rat Dams on PND 21 in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

TABLE I3
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose, Liver, and Carcass in F_1 Wistar Han Rat Pups in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Male				
n	6	6	6	6
Lipid (%)				
PND 4				
Liver	NS	NS	9.82 ± 0.27	9.91 ± 0.82
PND 21				
Adipose	72.59 ± 3.38	67.57 ± 2.63[b]	74.86 ± 4.89[b]	77.61 ± 6.02[b]
Liver	7.90 ± 0.22	8.22 ± 0.23	9.05 ± 0.25	10.49 ± 0.52
BDE-47 (µg/g)				
PND 4				
Liver	ND	1.0 ± 0.3	7.5 ± 1.8	55.8 ± 21.7
Liver (lipid-adjusted)	ND	ND	75.5 ± 16.5	686.3 ± 349.4
Carcass	ND	4.5 ± 0.4	24.2 ± 4.0	58.2 ± 13.3
PND 21				
Adipose	ND	108.2 ± 12.6	403.8 ± 25.5	1,044.3 ± 103.1
Adipose (lipid-adjusted)	ND	142.3 ± 23.1[b]	502.9 ± 51.2[b]	1,266.9 ± 202.5[b]
Liver	ND	ND	2.1 ± 0.3	8.3 ± 1.7
Liver (lipid-adjusted)	ND	ND	23.2 ± 2.7	79.6 ± 14.8
BDE-99 (µg/g)				
PND 4				
Liver	ND	1.1 ± 0.2	7.8 ± 2.2	55.7 ± 19.1
Liver (lipid-adjusted)	ND	ND	77.9 ± 20.1	657.1 ± 288.9
Carcass	ND	3.8 ± 0.4	20.6 ± 3.4	52.2 ± 12.0
PND 21				
Adipose	ND	76.8 ± 9.1	294.0 ± 23.2	846.8 ± 96.7
Adipose (lipid-adjusted)	ND	104.5 ± 18.2[b]	358.8 ± 41.7[b]	1,031.0 ± 184.7[b]
Liver	ND	ND	0.6 ± 0.1	4.6 ± 1.3
Liver (lipid-adjusted)	ND	ND	6.5 ± 1.4	43.5 ± 11.1
BDE-153 (µg/g)				
PND 4				
Liver	ND	ND	3.1 ± 0.8	20.7 ± 6.5
Liver (lipid-adjusted)	ND	ND	31.0 ± 6.9	240.9 ± 96.6
Carcass	ND	ND	3.8 ± 0.6	10.1 ± 1.6
PND 21				
Adipose	ND	13.2 ± 1.7	65.8 ± 6.9	194.5 ± 22.9
Adipose (lipid-adjusted)	ND	17.4 ± 3.5[b]	78.0 ± 8.9[b]	242.6 ± 24.8[b]
Liver	ND	ND	1.3 ± 0.2	7.2 ± 1.2
Liver (lipid-adjusted)	ND	ND	14.7 ± 2.4	68.8 ± 11.0

TABLE I3
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose, Liver, and Carcass in F_1 Wistar Han Rat Pups in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Female				
n	6	6	6	6
Lipid (%)				
PND 4				
Liver	NS	NS	10.71 ± 0.87	10.80 ± 0.34[c]
PND 21				
Adipose	62.55 ± 2.07[d]	70.11 ± 2.73[b]	74.46 ± 2.35	74.65 ± 5.39[b]
Liver	7.36 ± 0.21	7.43 ± 0.23	9.13 ± 0.40	11.28 ± 0.71
BDE-47 (µg/g)				
PND 4				
Liver	ND	1.5 ± 0.4	8.8 ± 2.3	28.5 ± 6.8[c]
Liver (lipid-adjusted)	ND	ND	90.0 ± 26.4	256.5 ± 56.4[c]
Carcass	ND	5.5 ± 0.6	26.0 ± 2.5	60.2 ± 8.5[c]
PND 21				
Adipose	ND	92.1 ± 6.9	377.2 ± 15.0	922.5 ± 106.9
Adipose (lipid-adjusted)	ND	139.6 ± 10.7[b]	508.6 ± 24.3	1,258.3 ± 107.3[b]
Liver	ND	ND	1.9 ± 0.3	7.9 ± 0.8
Liver (lipid-adjusted)	ND	ND	20.5 ± 3.0	70.4 ± 8.0
BDE-99 (µg/g)				
PND 4				
Liver	ND	1.8 ± 0.5	9.1 ± 2.6	33.5 ± 8.2[c]
Liver (lipid-adjusted)	ND	ND	93.7 ± 30.4	300.4 ± 66.9[c]
Carcass	ND	5.0 ± 0.7	22.3 ± 2.3	55.1 ± 8.2[c]
PND 21				
Adipose	ND	67.8 ± 6.7	278.0 ± 14.3	713.5 ± 82.4
Adipose (lipid-adjusted)	ND	102.2 ± 10.4[b]	373.8 ± 17.5	1,013.1 ± 71.8[b]
Liver	ND	ND	0.5 ± 0.1	3.6 ± 0.3
Liver (lipid-adjusted)	ND	ND	6.0 ± 1.0	31.9 ± 2.4

TABLE I3
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose, Liver, and Carcass in F_1 Wistar Han Rat Pups in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Female (continued)				
n	6	6	6	6
BDE-153 (µg/g)				
PND 4				
Liver	ND	0.6 ± 0.1[d]	3.7 ± 1.0	14.4 ± 3.6[c]
Liver (lipid-adjusted)	ND	ND	38.3 ± 12.1	129.9 ± 30.4[c]
Carcass	ND	0.7 ± 0.2	4.1 ± 0.5	10.6 ± 1.2[c]
PND 21				
Adipose	ND	11.6 ± 0.9	65.9 ± 2.9	162.2 ± 9.2
Adipose (lipid-adjusted)	ND	17.2 ± 1.3[b]	88.7 ± 3.7	237.9 ± 21.6[b]
Liver	ND	ND	1.3 ± 0.2	6.6 ± 0.9
Liver (lipid-adjusted)	ND	ND	14.0 ± 1.8	59.1 ± 8.7

[a] Data are presented as mean µg analyte/g matrix ± standard error. Values below the experimental limit of quantitation were replaced with ½ the limit of quantitation if there was at least one value in the group that was above the limit of quantitation. ND = all values were missing or below the limit of quantitation; BDE-47 = 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99 = 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-153 = 2,2′,4,4′,5,5′-hexabromodiphenyl ether. NS = not sampled

[b] n=4

[c] n=7

[d] n=5

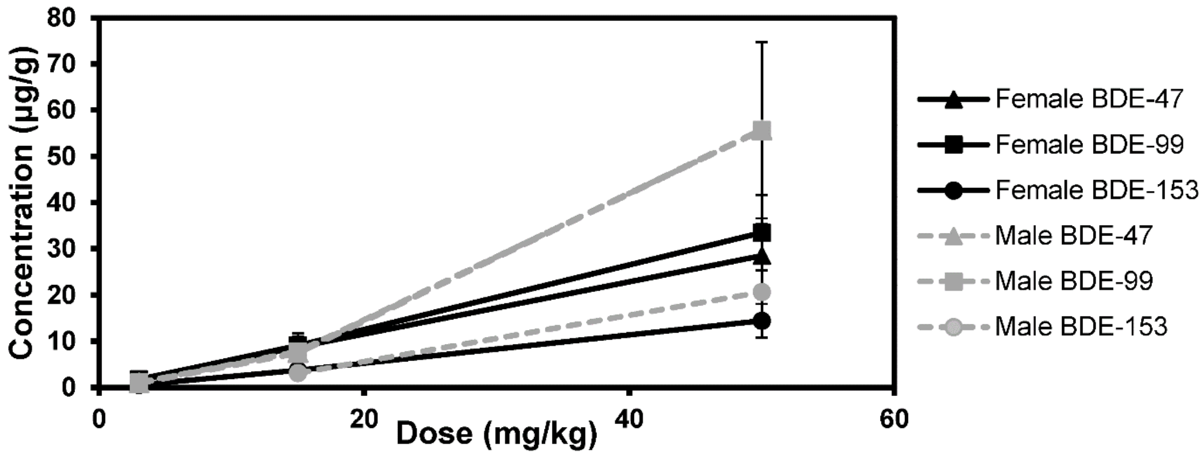


FIGURE I6
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Liver in F_1 Wistar Han Rat Pups on PND 4 in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

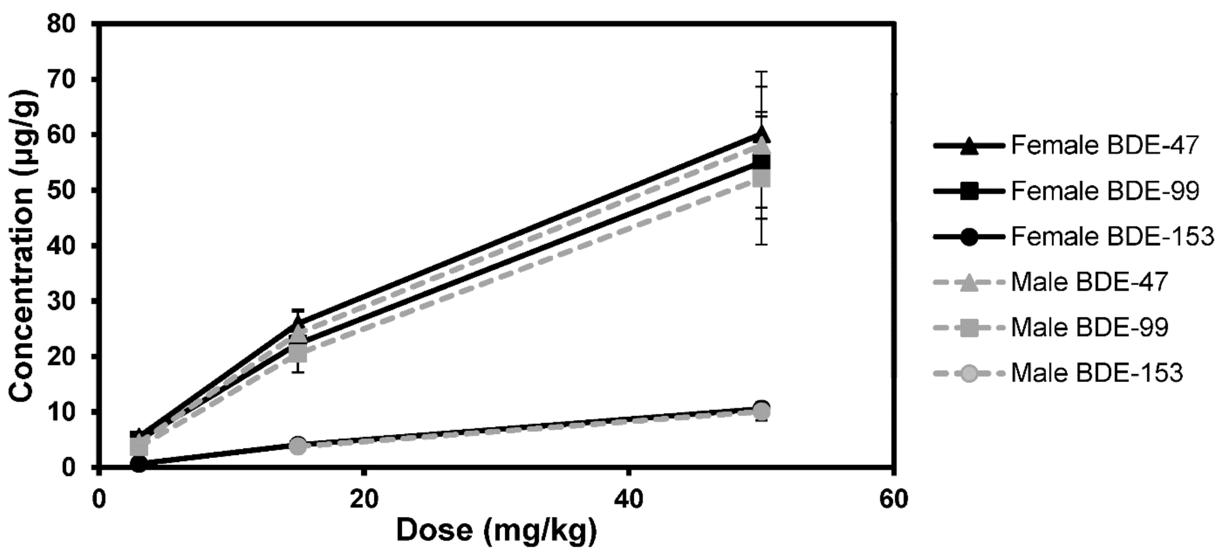


FIGURE I7
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in the Carcass of F_1 Wistar Han Rat Pups on PND 4 in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

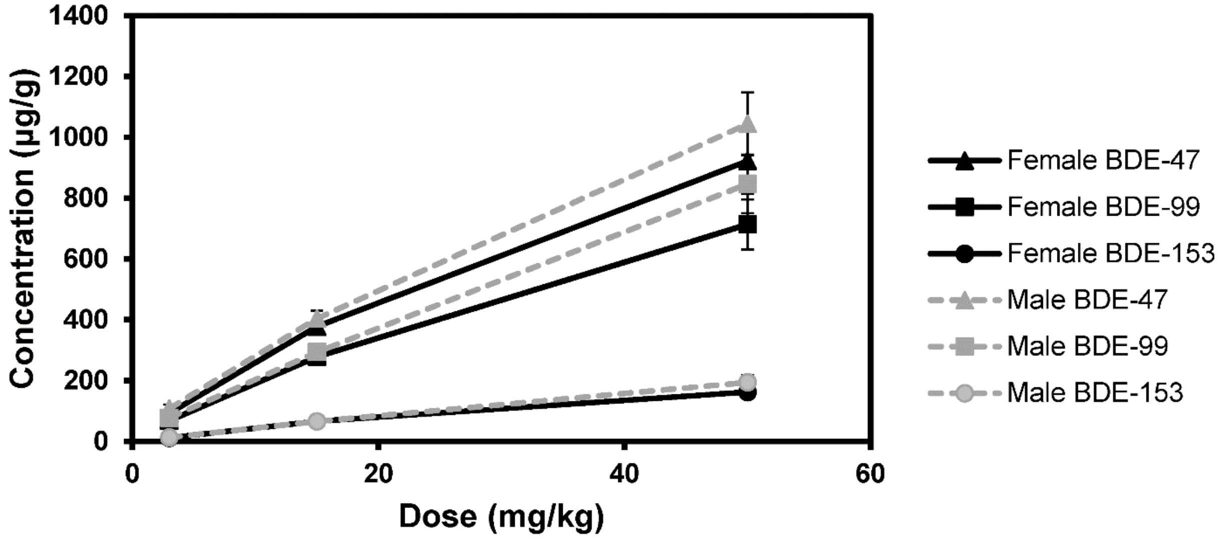


FIGURE I8
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in F_1 Wistar Han Rat Pups on PND 21 in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

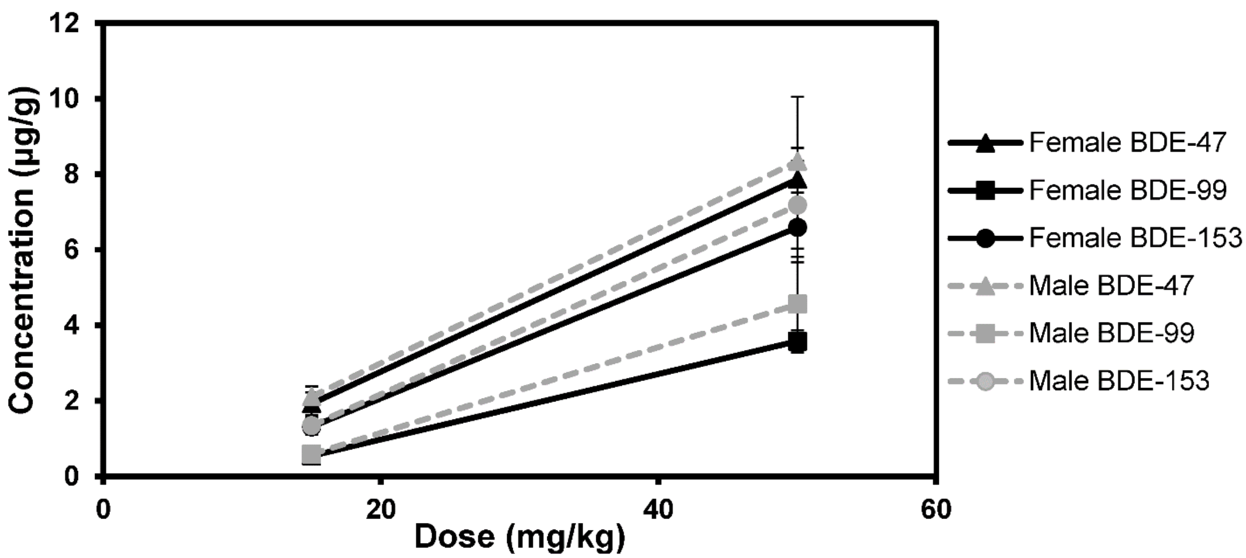


FIGURE I9
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Liver in F_1 Wistar Han Rat Pups on PND 21 in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

TABLE I4
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose, Liver, and Plasma in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Male				
n	14	10	12	15
(lipid-adjusted) (%)				
Adipose	86.71 ± 1.89	87.69 ± 2.45	84.35 ± 3.18	101.20 ± 3.29
Liver	5.08 ± 0.32	6.67 ± 0.61	6.98 ± 0.86	9.91 ± 1.24[b]
BDE-47 (µg/g)				
Adipose	0.6 ± 0.1	187.9 ± 7.5	470.6 ± 24.8	1,671.1 ± 344.8
Adipose (lipid-adjusted)	0.8 ± 0.1	216.0 ± 11.5	564.6 ± 31.2	1,686.0 ± 372.7
Liver	ND	4.1 ± 0.6	8.3 ± 1.1	50.9 ± 13.0
Liver (lipid-adjusted)	ND	64.0 ± 9.3	122.6 ± 15.8	441.4 ± 76.9[b]
Plasma	ND	0.44 ± 0.06	0.95 ± 0.08[c]	4.97 ± 0.43[c]
BDE-99 (µg/g)				
Adipose	0.7 ± 0.1	151.2 ± 5.9	461.5 ± 21.3	1,851.9 ± 416.6
Adipose (lipid-adjusted)	0.8 ± 0.1	173.7 ± 8.7	553.6 ± 26.9	1,869.5 ± 449.2
Liver	ND	2.0 ± 0.5	4.9 ± 0.7	40.6 ± 10.2
Liver (lipid-adjusted)	ND	31.6 ± 7.4	71.6 ± 8.9	361.1 ± 66.7[b]
Plasma	ND	0.22 ± 0.05	0.45 ± 0.05[c]	4.06 ± 0.54[c]
BDE-153 (µg/g)				
Adipose	ND	101.9 ± 5.3	447.8 ± 30.0	1,445.4 ± 227.5
Adipose (lipid-adjusted)	ND	116.7 ± 6.2	538.0 ± 37.7	1,464.3 ± 246.7
Liver	ND	1.8 ± 0.5	9.4 ± 1.6	47.4 ± 10.6
Liver (lipid-adjusted)	ND	29.2 ± 7.9	132.7 ± 19.8	446.2 ± 60.5[b]
Plasma	ND	0.20 ± 0.03	0.71 ± 0.06[c]	6.06 ± 1.40[c]
Female				
n	13	15	13	10
(lipid-adjusted) (%)				
Adipose	100.86 ± 3.61	99.18 ± 2.67	93.82 ± 2.49[d]	97.44 ± 2.69
Liver	5.97 ± 0.28[d]	8.56 ± 1.30[e]	5.85 ± 0.19[f]	6.19 ± 0.36[g]
BDE-47 (µg/g)				
Adipose	0.7 ± 0.1	274.2 ± 21.8	744.0 ± 84.0	2,603.4 ± 542.8
Adipose (lipid-adjusted)	0.7 ± 0.1	279.2 ± 22.7	817.3 ± 98.9[d]	2,619.5 ± 506.2
Liver	ND	5.8 ± 1.6	11.3 ± 2.6	48.9 ± 12.8
Liver (lipid-adjusted)	ND	59.8 ± 8.6[e]	164.6 ± 30.6[f]	819.3 ± 180.4[g]
Plasma	ND	0.73 ± 0.10[f]	2.13 ± 0.42[c]	8.74 ± 1.78[d]
BDE-99 (µg/g)				
Adipose	0.7 ± 0.1	214.5 ± 18.8	742.1 ± 92.0	3,007.7 ± 671.1
Adipose (lipid-adjusted)	0.8 ± 0.1	218.5 ± 19.1	815.3 ± 106.9[d]	3,017.8 ± 628.9
Liver	ND	3.0 ± 1.1	7.5 ± 2.1	45.4 ± 12.4
Liver (lipid-adjusted)	ND	25.3 ± 6.5[e]	102.3 ± 23.2[f]	767.6 ± 182.3[g]
Plasma	ND	0.40 ± 0.06[f]	1.36 ± 0.32[c]	7.67 ± 1.86[d]

TABLE I4
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose, Liver, and Plasma in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

	Vehicle Control	3 mg/kg	15 mg/kg	50 mg/kg
Female (continued)				
n	13	15	13	10
BDE-153 (µg/g)				
Adipose	ND	139.7 ± 15.5	675.7 ± 87.3	2,055.5 ± 226.2
Adipose (lipid-adjusted)	ND	143.2 ± 17.3	734.8 ± 103.1[d]	2,093.5 ± 203.0
Liver	ND	2.8 ± 0.8	10.0 ± 1.8	42.3 ± 8.8
Liver (lipid-adjusted)	ND	28.4 ± 6.7[e]	152.4 ± 22.3[f]	730.1 ± 111.1[g]
Plasma	ND	0.44 ± 0.09[f]	1.99 ± 0.39[c]	8.27 ± 0.83[d]

[a] Data are presented as mean µg analyte/g matrix ± standard error. Values below the experimental limit of quantitation were replaced with ½ the limit of quantitation if there was at least one value in the group that was above the limit of quantitation. ND = all values were missing or below the limit of quantitation; BDE-47 = 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99 = 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-153 = 2,2′,4,4′,5,5′-hexabromodiphenyl ether

[b] n=13

[c] n=10

[d] n=12

[e] n=14

[f] n=11

[g] n=9

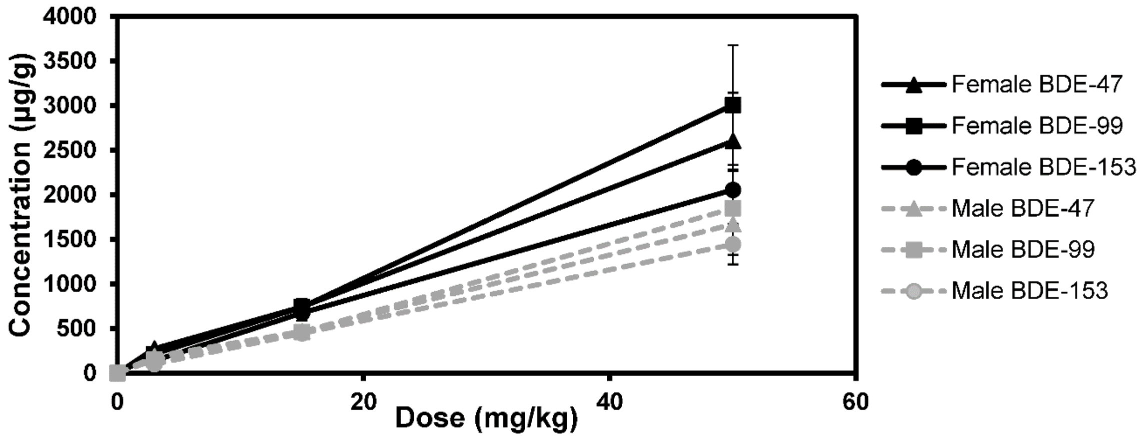


FIGURE I10
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in F1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

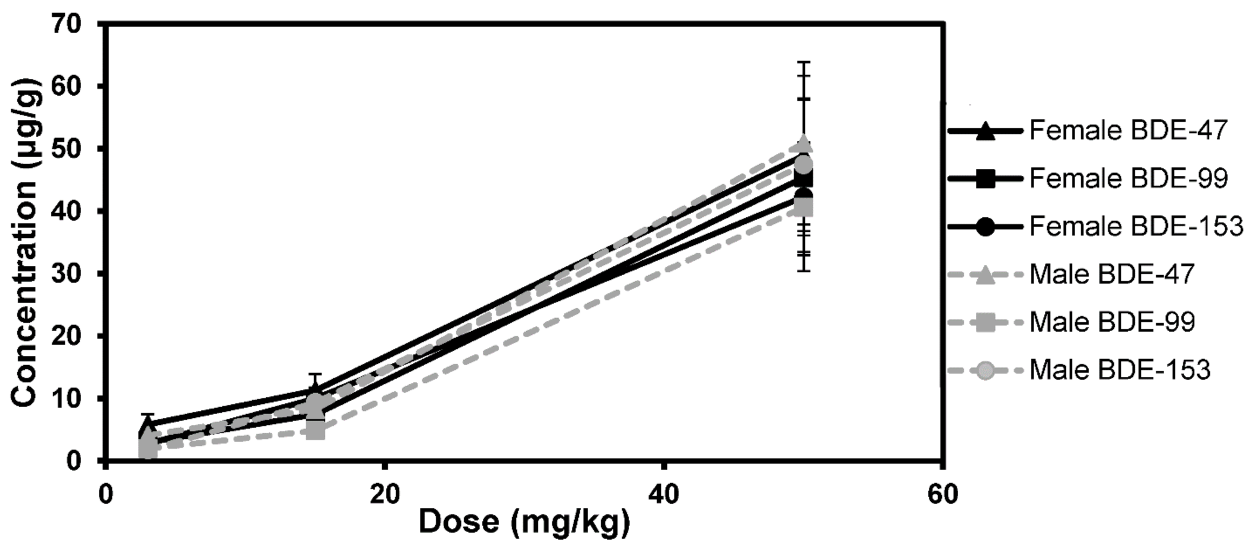


FIGURE I11
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Liver in F_1 Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

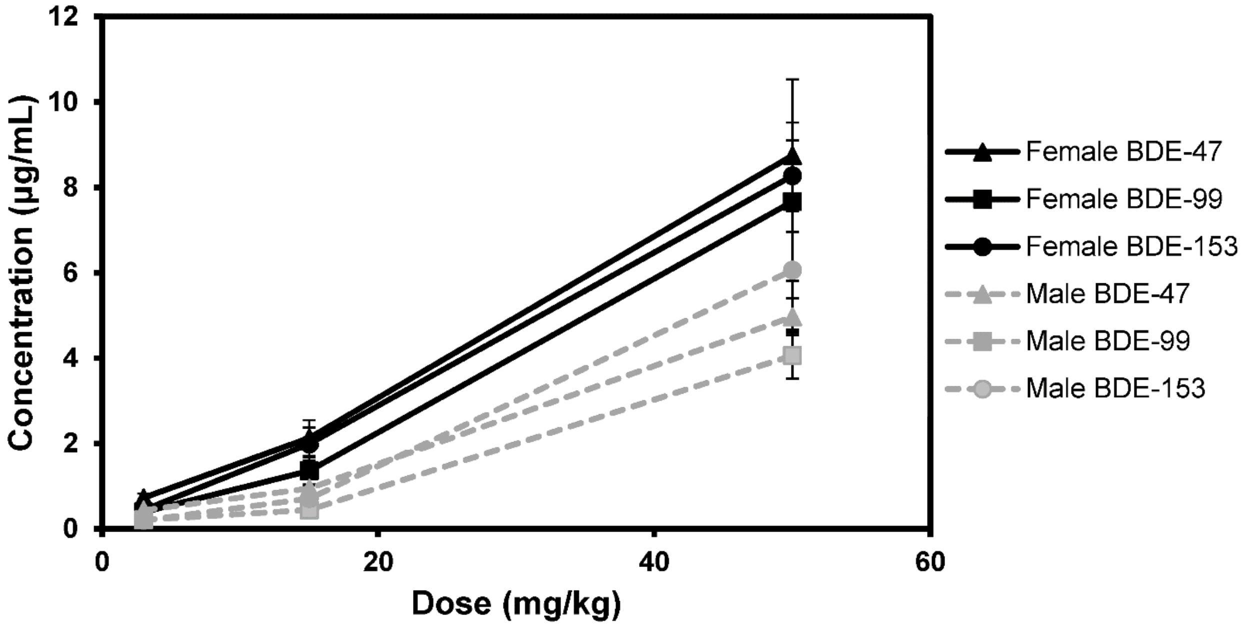


FIGURE I12
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Plasma in F_1 Wistar Han Ratsin the 2-Year Perinatal and Postnatal Gavage Study of DE-71

TABLE I5
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in Mice in the 3-Month Gavage Study of DE-71[a]

	Vehicle Control	0.01 mg/kg	5 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
Male						
n	10	10	10	10	10	3
BDE-47 (µg/g)	ND	ND	20.63 ± 0.87	206.89 ± 5.37	517.71 ± 12.64	6,168.00 ± 1,031.91
BDE-99 (µg/g)	0.68 ± 0.09	1.50 ± 0.63	98.55 ± 3.15	587.83 ± 13.44	1,281.83 ± 58.03	10,588.0 ± 1,414.29
BDE-153 (µg/g)	0.49 ± 0.04	0.62 ± 0.15	23.68 ± 1.15	273.37 ± 14.56	567.51 ± 44.33	9,796.00 ± 1,909.03
Female						
n	9	10	10	9	9	5
BDE-47 (µg/g)	0.47 ± 0.02	0.49 ± 0.03	43.26 ± 2.37	356.08 ± 19.34	846.91 ± 49.51	4,196.80 ± 239.98
BDE-99 (µg/g)	1.09 ± 0.18	1.59 ± 0.18	116.67 ± 8.41	616.96 ± 29.31	1,420.00 ± 98.15	6,729.20 ± 379.72
BDE-153 (µg/g)	0.56 ± 0.09	0.85 ± 0.11	40.65 ± 5.83	343.88 ± 27.61	701.27 ± 91.36	3,936.00 ± 246.00

[a] Data are presented as mean µg analyte/g adipose ± standard error. Values below the experimental limit of quantitation were replaced with ½ the limit of quantitation if there was at least one value in the group that was above the limit of quantitation. ND = all values were missing or below the limit of quantitation; BDE-47 = 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99 = 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-153 = 2,2′,4,4′,5,5′-hexabromodiphenyl ether.

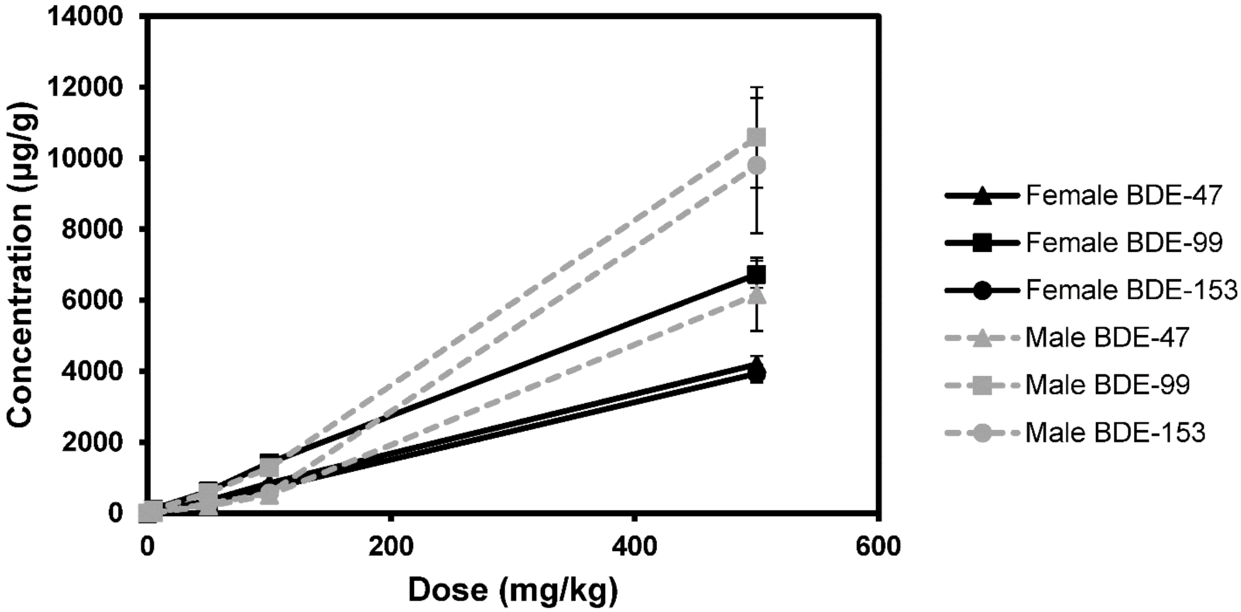


FIGURE I13
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in Mice in the 3-Month Gavage Study of DE-71

TABLE I6
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose and Liver in Mice in the 2-Year Gavage Study of DE-71[a]

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Male				
n	9	4	0[b]	16
Lipid (%)				
Adipose	77.14 ± 5.94	75.50 ± 20.23		83.55 ± 3.19[c]
Liver	6.78 ± 0.67[d]	6.17 ± 0.17[e]		5.55 ± 0.39[f]
BDE-47 (µg/g)				
Adipose	0.9 ± 0.5	22.6 ± 2.4		682.0 ± 64.4
Adipose (lipid-adjusted)	1.3 ± 0.7	42.4 ± 15.6		850.3 ± 84.1[c]
Liver	ND	0.8 ± 0.3		18.3 ± 2.7
Liver (lipid-adjusted)	ND	ND		360.3 ± 61.3[f]
BDE-99 (µg/g)				
Adipose	3.1 ± 2.0	123.0 ± 15.6		1,601.3 ± 171.1
Adipose (lipid-adjusted)	4.5 ± 3.0	223.7 ± 76.2		1,996.5 ± 190.4[c]
Liver	ND	2.4 ± 0.8		32.9 ± 5.0
Liver (lipid-adjusted)	ND	26.7 ± 9.6[e]		678.8 ± 148.5[f]
BDE-153 (µg/g)				
Adipose	1.2 ± 0.6	138.2 ± 27.1		11,031.9 ± 1,579.1
Adipose (lipid-adjusted)	1.7 ± 1.0	231.7 ± 67.1		13,708.1 ± 2,188.0[c]
Liver	ND	4.1 ± 1.1		339.5 ± 54.1
Liver (lipid-adjusted)	ND	55.0 ± 21.0[e]		8,605.9 ± 3,030.5[f]
Female				
n	10	10	9	13
Lipid (%)				
Adipose	94.59 ± 4.00	91.52 ± 6.00	97.63 ± 10.20	81.25 ± 4.69
Liver	7.41 ± 0.41[g]	7.83 ± 0.27[d]	8.90 ± 1.24[g]	7.22 ± 0.61[d]
BDE-47 (µg/g)				
Adipose	0.9 ± 0.2	49.1 ± 3.3	275.1 ± 33.6	1,015.9 ± 104.8
Adipose (lipid-adjusted)	0.9 ± 0.2	57.5 ± 7.4	291.4 ± 39.4	1,293.0 ± 144.9
Liver	ND	1.7 ± 0.3	12.5 ± 3.4	33.4 ± 6.0
Liver (lipid-adjusted)	ND	21.4 ± 5.1[d]	193.1 ± 84.5[g]	388.9 ± 96.4[d]
BDE-99 (µg/g)				
Adipose	1.6 ± 0.3	119.8 ± 5.2	557.5 ± 60.8	2,114.2 ± 159.8
Adipose (lipid-adjusted)	1.7 ± 0.3	137.3 ± 12.8	601.3 ± 74.7	2,707.5 ± 268.3
Liver	ND	3.3 ± 0.5	20.4 ± 6.1	59.7 ± 10.8
Liver (lipid-adjusted)	ND	40.6 ± 8.1[d]	332.0 ± 155.0[g]	674.7 ± 164.3[d]

TABLE I6
Concentrations of Lipids and Selected Polybrominated Diphenyl Ether Congeners in Adipose and Liver in Mice in the 2-Year Gavage Study of DE-71

	Vehicle Control	3 mg/kg	30 mg/kg	100 mg/kg
Female (continued)				
n	10	10	9	13
BDE-153 (µg/g)				
Adipose	ND	113.3 ± 30.6	1,016.5 ± 239.0	5,766.8 ± 882.5
Adipose (lipid-adjusted)	ND	127.1 ± 33.9	1,315.8 ± 519.5	7,793.4 ± 1,528.5
Liver	0.6 ± 0.1	3.4 ± 0.4	53.2 ± 10.2	436.4 ± 189.7
Liver (lipid-adjusted)	8.1 ± 1.8[g]	45.5 ± 7.2[d]	900.0 ± 318.4[g]	3,284.1 ± 856.0[d]

[a] Data are presented as mean µg analyte/g tissue ± standard error. Values below the experimental limit of quantitation were replaced with ½ the limit of quantitation if there was at least one value in the group that was above the limit of quantitation. ND = all values were missing or below the limit of quantitation; BDE-47 = 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99 = 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-153 = 2,2′,4,4′,5,5′-hexabromodiphenyl ether.

[b] Samples were not collected from 30 mg/kg males due to insufficient normal tissue.

[c] n=15

[d] n=6

[e] n=3

[f] n=4

[g] n=7

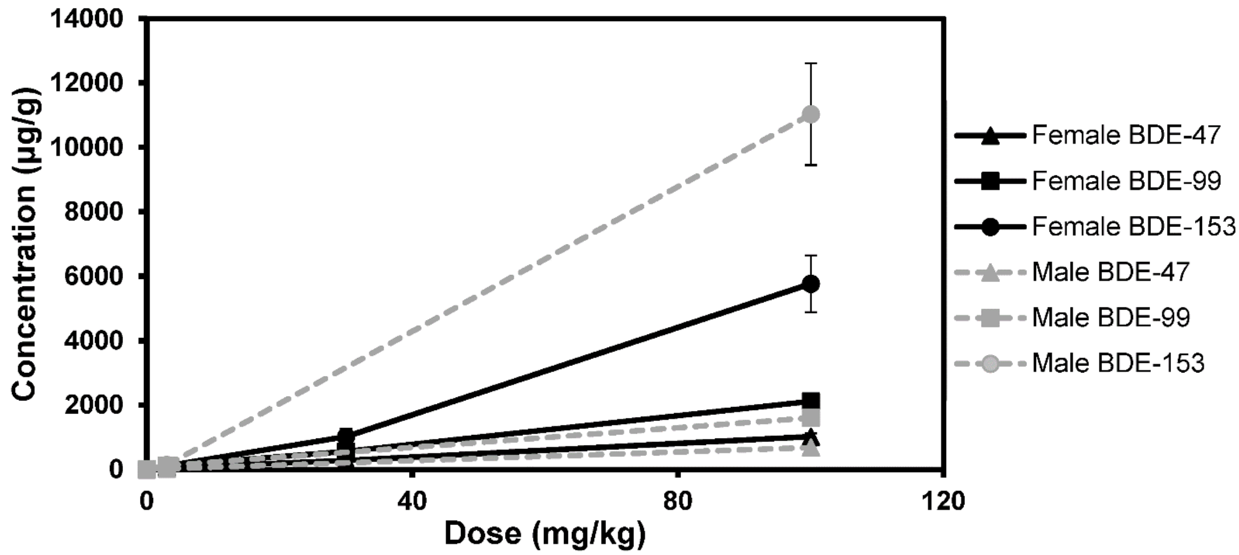


FIGURE I14
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Adipose in Mice in the 2-Year Gavage Study of DE-71

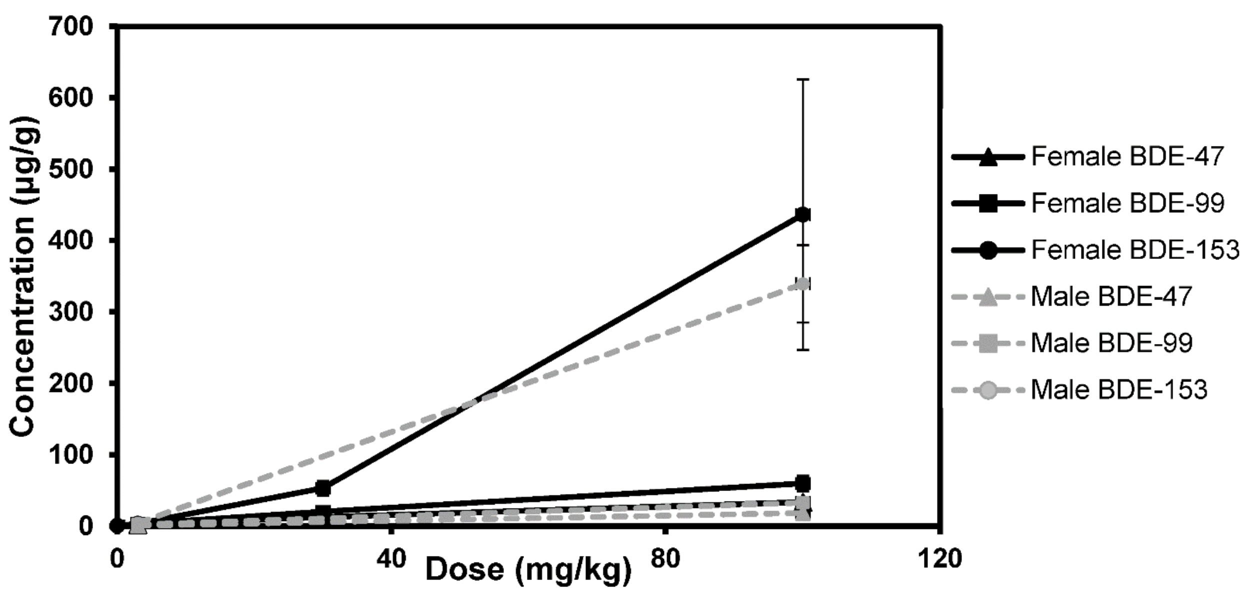


FIGURE I15
Concentrations of Selected Polybrominated Diphenyl Ether Congeners in Liver in Mice in the 2-Year Gavage Study of DE-71

APPENDIX J
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

DE-71

DE-71 was obtained from Great Lakes Chemical Corporation (El Dorado, AR) in two lots (2550OA30A and 1550OK07A). Lot 2550OA30A was used during the 3-month and 2-year studies; lot 1550OK07A was used for dose formulation development studies performed by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) and was not used in any of the animal studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and by the study laboratory at Southern Research Institute (Birmingham, AL). Karl Fischer titration was performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the DE-71 studies are on file at the National Institute of Environmental Health Sciences.

Lot 2550OA30A of the test chemical, a viscous, sticky brown liquid, was identified as DE-71 by the analytical chemistry laboratory using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR spectroscopy. IR spectra were consistent with the literature spectra (Bio-Rad Sadtler, 2003) and the structure of DE-71. Proton and carbon-13 NMR spectra were consistent with computer-calculated spectra and the structures for a polybrominated diphenyl ether mixture. Representative IR and proton NMR spectra are presented in Figures J1 and J2.

For lot 2550OA30A, the moisture content was determined by Karl Fischer titration and the purity profile was determined by the analytical chemistry laboratory using gas chromatography (GC) with flame ionization detection (FID) by system A (Table J1). The purity profile of the bulk chemical was also determined by the study laboratory using a similar GC/FID analysis (system B). In further analyses of the bulk chemical using GC coupled with mass spectrometry (MS) detection, the analytical chemistry laboratory confirmed the identity of the peaks observed in the purity profiles (using system C), and screened for the presence of polychlorinated (using system D) and polybrominated (using system E) dibenzodioxins and furans.

Karl Fischer titration indicated less than 0.1% water. GC/FID using system A (Table J1) yielded a purity profile containing 16 reportable peaks, 11 of which were PBDEs tentatively identified by retention time matching to standards of PBDEs in chloroform obtained from Cambridge Isotope Laboratories, Inc. (CIL, Tewksbury, MA) (Table J2). Six peaks in this profile contained areas exceeding 2% of the total peak area; BDE-99 (41.67%), BDE-47 (35.68%), BDE-100 (10.44%), BDE-154 (3.63%), BDE-153 (3.33%), and BDE-85 (2.03%). GC/FID by a similar system using a column with a thicker film (system B; Table J1) yielded prolonged retention times, but very similar area percents for these six components. The identities of peaks in the GC/FID purity profile were confirmed by GC/MS using authentic PBDE standards for 11 peaks. The specific identity of an individual PBDE was based on the retention time and the mass spectrum of the standard to a peak in DE-71. It should be noted that other positional isomers with the same number of bromines might elute at the same retention time and would give the same mass spectrum. Therefore, the identity of the specific isomer should be considered tentative (Table J2). Using polychlorinated analytical standards purchased from CIL and high resolution GC/MS by system D (Table J1), samples of the bulk chemical were found to contain no polychlorinated dibenzodioxins or furans above the specified limits of quantitation (Table J3). Polybrominated analytical standards obtained from CIL and high resolution GC/MS by system E (Table J1) were used to determine that polybrominated dibenzodioxins and furans were present in the test article; concentrations of 2,3,7,8-TBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, and co-eluting 1,2,3,4,7,8-HxBDF and 1,2,3,6,7,8-HxBDF were quantifiable (Table J4). Taken together, these analyses indicated that the test article consisted of a mixture of approximately 54% pentabromodiphenyl ethers, 36% tetrabromodiphenyl ethers, and 7% hexabromodiphenyl ethers.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC/FID by system A (Table J1). These studies indicated that DE-71 was stable as a bulk chemical for 15 days when stored in sealed amber glass bottles at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in sealed glass containers. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month and 2-year studies with GC/FID by system B and no degradation of the bulk chemical was detected.

Corn Oil

Mazola corn oil was obtained in multiple lots from Red Diamond Foodservice, Inc. (Birmingham, AL), and Sam's Club (Birmingham, AL) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared four times during the 3-month studies and approximately every 4 weeks during the 2-year studies by mixing DE-71 with corn oil to give the required concentrations (Table J5). Dose formulations were stored at approximately 5° C in amber glass containers sealed with Teflon®-lined lids for up to 46 days.

Stability studies of 0.05 mg/mL formulations were performed by the analytical chemistry laboratory using GC with electron capture detection (ECD) by system F (Table J1). Stability was confirmed for at least 46 days for dose formulations stored in amber glass containers sealed with Teflon®-lined lids at temperatures up to 25° C and for 3 hours under simulated animal room conditions. An additional stability study was performed by the study laboratory on the 0.001 mg/mL dose formulation using GC/ECD by a system similar to system F, and stability was confirmed for at least 55 days for dose formulations stored in amber glass containers sealed with Teflon®-lined lids at 5° C and for 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of DE-71 were conducted by the study laboratory using a system similar to system F. Determinations of the concentrations of DE-71 in corn oil were based on quantification of peak areas produced by the marker compound BDE-99. During the 3-month studies, the dose formulations were analyzed three times; all 15 formulations for rats and 14 of 15 for mice were within 10% of the target concentrations (Tables J6 and J7). Animal room samples of these dose formulations were also analyzed; 11 of 15 for rats and 12 of 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months (Tables J8 and J9). Of the dose formulations analyzed and used during the studies, 38 of 39 for rats and all 36 for mice were within 10% of the target concentrations; 23 of 24 animal room samples for rats and 13 of 14 for mice were within 10% of the target concentrations.

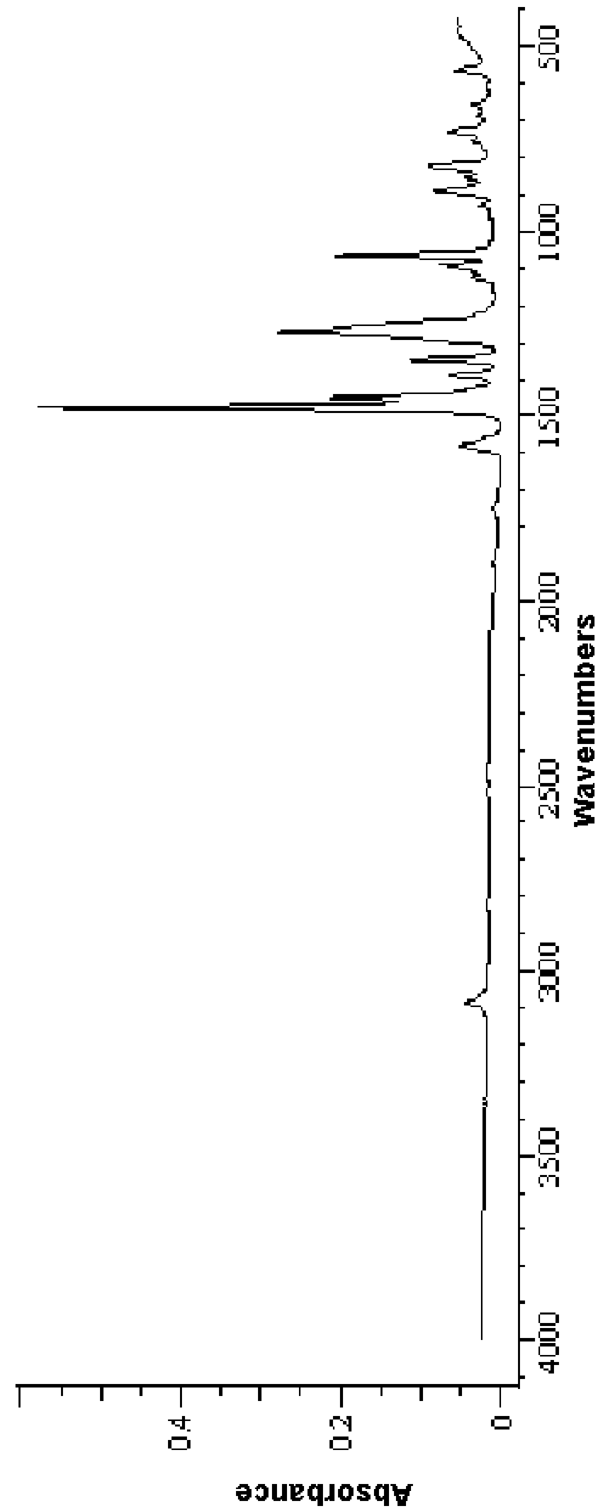


FIGURE J1
Infrared Absorption Spectrum of DE-71

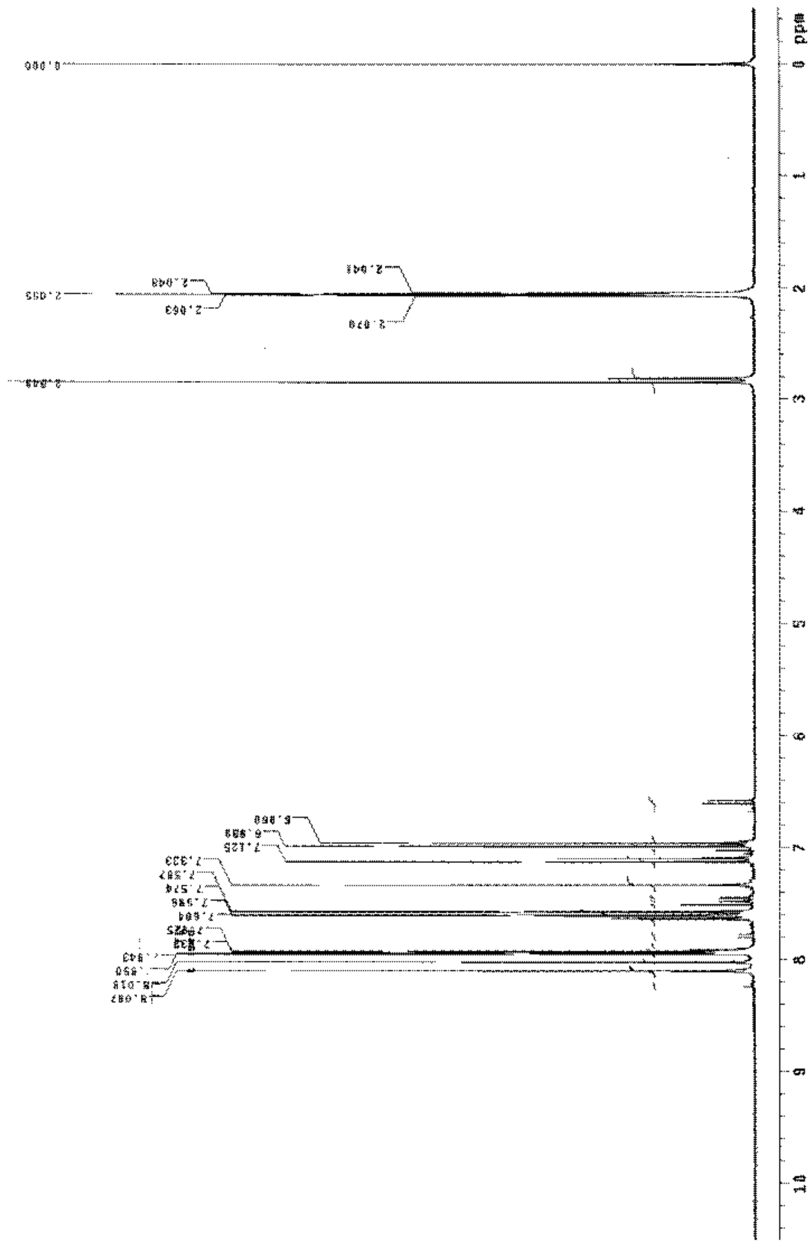


FIGURE J2
Proton Nuclear Magnetic Resonance Spectrum of DE-71

TABLE J1
Gas Chromatography Systems Used in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	Rtx®-5, 30 m × 0.25 mm, 0.25 µm film (Restek, Bellefonte, PA)	Helium at 3 mL/minute	80° C for 1 minute, then 20° C/minute to 200° C, then 10° C/minute to 280° C, held for 10 minutes
System B			
Flame ionization	Rtx®-5, 30 m × 0.25 mm, 1.0 µm film (Restek)	Helium at ~ 3 mL/minute	80° C, then 20° C/minute to 200° C, then 10° C/minute to 300° C, held for 20 minutes
System C			
Mass spectrometry with electron ionization (EI) (50 to 800 amu)	Rtx®-5, 30 m × 0.25 mm, 0.25 µm film (Restek)	Helium at 1 mL/minute	80° C for 1 minute, then 20° C/minute to 200° C, then 10° C/minute to 280° C, held for 20 minutes
System D			
Mass spectrometry with EI and selected ion recording (SIR)	DB-5, 60 m × 0.25 mm, 0.25 µm film (J&W Scientific, Folsom, CA)	Helium at 140 kPa	140° C for 3 minutes, then 20° C/minute to 220° C, held for 16 minutes, then 5° C/minute to 235° C, held for 7 minutes, then 5° C/minute to 320° C, held for 10 minutes
System E			
Mass spectrometry with EI and SIR	DB-5 MS, 30 m × 0.32 mm, 0.25 µm film (J&W Scientific)	Helium at 140 kPa	130° C for 2.5 minutes, then 30° C/minute to 210° C, then 3° C/minute to 315° C, held for 25 minutes
System F			
Electron capture	Rtx®-5, 30 m × 0.25 mm, 1.0 µm film (Restek)	Helium at ~ 3 mL/minute	80° C, then 20° C/minute to 200° C, then 10° C/minute to 300° C, held for 10 minutes

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Palo Alto, CA). The mass spectrometers were manufactured by Agilent Technologies, Inc. (system C) or VG Autospec (Manchester, UK; systems D and E)

TABLE J2
Purity Profile of DE-71 Determined by Gas Chromatography with Flame Ionization Detection

Abbreviation	Name	CAS Number	Retention Time (minutes)	Total Area (%)
BDE-17	2,2′,4′-Tribromodiphenyl ether	147217-75-2	10.66	<0.10
BDE-28	2,4,4′-Tribromodiphenyl ether	41318-75-6	10.91	0.29
—	Unknown A	—	11.90	0.24
—	Unknown B	—	12.48	0.64
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	12.86	35.68
BDE-66	2,3′,4,4′-Tetrabromodiphenyl ether	189084-61-5	13.06	0.48
BDE-100	2,2′,4,4′,6-Pentabromodiphenyl ether	189084-64-8	14.27	10.44
BDE-99	2,2′,4,4′,5-Pentabromodiphenyl ether	60348-60-9	14.73	41.67
BDE-85	2,2′,3,4,4′-Pentabromodiphenyl ether	182346-21-0	15.44	2.03
—	Unknown C	—	15.57	0.21
BDE-154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether	207122-15-4	15.89	3.63
BDE-153	2,2′,4,4′,5,5′-Hexabromodiphenyl ether	68631-49-2	16.61	3.33
—	Unknown D	—	16.98	0.65
—	Unknown E	—	17.21	0.16
BDE-138	2,2′,3,4,4′,5′-Hexabromodiphenyl ether	182677-30-1	17.81	0.45
BDE-183	2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether	207122-16-5	19.62	0.12

TABLE J3
Polychlorinated Dibenzodioxins and Furans in DE-71 Determined by Gas Chromatography with Mass Spectrometry Detection

Abbreviation	Name	CAS Number	LOQ[a] (pg/g)	LOD[b] (pg/g)	Method Blank (pg/g)	DE-71[c] (pg/g)	DE-71[c] (pg/g)
2,3,7,8-TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1746-01-6	35	0.04	ND	ND	ND
1,2,3,7,8-PeCDD	1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	40321-76-4	175	0.08	ND	ND	ND
1,2,3,4,7,8-HxCDD	1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	39227-28-6	175	0.03	ND	ND	ND
1,2,3,6,7,8-HxCDD	1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	57653-85-7	175	0.03	ND	ND	ND
1,2,3,7,8,9-HxCDD	1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	19408-74-3	175	0.03	ND	ND	ND
1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	35822-46-9	175	0.06	ND	ND	ND
OCDD	Octachlorodibenzo-*p*-dioxin	3268-87-9	350	0.02	10.1	ND	ND
2,3,7,8-TCDF	2,3,7,8-Tetrachlorodibenzofuran	51207-31-9	35	0.04	ND	ND	ND
1,2,3,7,8-PeCDF	1,2,3,7,8-Pentachlorodibenzofuran	57117-41-6	175	0.05	ND	ND	ND
2,3,4,7,8-PeCDF	2,3,4,7,8-Pentachlorodibenzofuran	57117-31-4	175	0.04	ND	ND	ND
1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-Hexachlorodibenzofuran	70648-26-9	175	0.03	2.07	ND	ND
1,2,3,6,7,8-HxCDF	1,2,3,6,7,8-Hexachlorodibenzofuran	57117-44-9	175	0.03	ND	ND	ND
1,2,3,7,8,9-HxCDF	1,2,3,7,8,9-Hexachlorodibenzofuran	72918-21-9	175	0.03	ND	ND	ND
2,3,4,6,7,8-HxCDF	2,3,4,6,7,8-Hexachlorodibenzofuran	60851-34-5	175	0.03	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	1,2,3,4,6,7,8 Heptachlorodibenzofuran	67562-39-4	175	0.35	ND	ND	ND
1,2,3,4,7,8,9-HpCDF	1,2,3,4,7,8,9-Heptachlorodibenzofuran	55673-89-7	175	0.42	ND	ND	ND
OCDF	Octachlorodibenzofuran	39001-02-0	350	0.04	6.15	ND	ND

LOQ=limit of quantitation; LOD=limit of detection; ND=not detected

[a] Calculated based on standard levels specified in EPA Method 1613 relative to sample size and sample volume in a clean solvent standard.

[b] Calculated at three times baseline noise in a spiked matrix standard representing optimum conditions. Individual LODs for each sample analyte vary depending on the noise level present in the region of the analyte

[c] Duplicate measurements

TABLE J4
Polybrominated Dibenzodioxins and Furans in DE-71 Determined by Gas Chromatography with Mass Spectrometry Detection

Abbreviation	Name	CAS Number	LOQ[a] (pg/g)	LOD[b] (pg/g)	Method Blank (pg/g)	DE-71[c] (pg/g)	TEF[d]	TEQ (pg/g)
2,3,7,8-TBDD	2,3,7,8-Tetrabromodibenzo-*p*-dioxin	50585-41-6	140	7.02	109.91	130	1	130
1,2,3,7,8-PeBDD	1,2,3,7,8-Pentabromodibenzo-*p*-dioxin	109333-34-8	1,750	118.95	ND	58	1	58
1,2,3,4,7,8-HxBDD and	1,2,3,4,7,8-Hexabromodibenzo-*p*-dioxin	Not found					0.1	
1,2,3,6,7,8-HxBDD	and 1,2,3,6,7,8- Hexabromodibenzo-*p*-dioxin (coeluted)	Not found	3,500	30.20	ND	41	0.1	4.1
1,2,3,7,8,9-HxBDD	1,2,3,7,8,9-Hexabromodibenzo-*p*-dioxin	Not found	3,500	65.48	ND	ND	0.1	
1,2,3,4,6,7,8-HpBDD	1,2,3,4,6,7,8-Heptabromodibenzo-*p*-dioxin	Not found	NS	ND	ND	ND	0.01	
OBDD	Octabromodibenzo-*p*-dioxin	2170-45-8	3,500	26.96	ND	ND		
2,3,7,8-TBDF	2,3,7,8-Tetrabromodibenzofuran	67733-57-7	1,400	144.47	ND	3,680[e]	0.1	368
1,2,3,7,8-PeBDF	1,2,3,7,8-Pentabromodibenzofuran	107555-93-1	7,000	955.08	ND	19,790[e]	0.03	594
2,3,4,7,8-PeBDF	2,3,4,7,8-Pentabromodibenzofuran	131166-92-2	7,000	893.01	23.4	5,381	0.3	1,614
1,2,3,4,7,8-HxBDF and	1,2,3,4,7,8-Hexabromodibenzofuran and	129880-08-6					0.1	
1,2,3,6,7,8-HxBDF	1,2,3,6,7,8-Hexabromodibenzofuran (coeluted)	107555-94-2	5,600	34.72	ND	43,088[e]	0.1	4,309
2,3,4,6,7,8-HxBDF	2,3,4,6,7,8-Hexabromodibenzofuran	161880-50-8	NS	ND	ND	ND	0.1	
1,2,3,7,8,9-HxBDF	1,2,3,7,8,9-Hexabromodibenzofuran	161880-49-5	NS	ND	ND	ND		
1,2,3,4,6,7,8-HpBDF	1,2,3,4,6,7,8-Heptabromodibenzofuran	107555-95-3	14,000	12.10	ND	535	0.1	54
1,2,3,4,7,8,9-HpBDF	1,2,3,4,7,8,9-Heptabromodibenzofuran	161880-51-9	NS	ND	ND	ND	0.1	
OBDF	1,2,3,4,6,7,8,9-Octabromodibenzofuran	103582-29-2	NS		ND	ND		
					TOTAL	71,310	0.1	7,131

LOQ=limit of quantitation; LOD=limit of detection; TEF=toxic equivalency factor; TEQ=toxic equivalents [TEF × DE-71 component (pg/g)]; ND=not detected; NS=no standard available; NA=not available

[a] Calculated based on standard levels specified in EPA Method 1613 relative to sample size and sample volume in a clean solvent standard.

[b] Calculated at three times baseline noise in a spiked matrix standard representing optimum conditions. Individual LODs for each sample analyte vary depending on the noise level present in the region of the analyte

[c] Averages of duplicate measurements are given

[d] van den Berg *et al.* (2013)

[e] Quantifiable, as value exceeds the LOQ

TABLE J5
Preparation and Storage of Dose Formulations in the Gavage and Perinatal and Postnatal Gavage Studies of DE-71

3-Month Studies	2-Year Studies
Preparation	
Prior to making the dose formulations, a bottle of DE-71 was placed into a water bath at approximately 50° C for approximately 1 hour to reduce the viscosity of the test article. For the low concentration dose formulations, 1.00 g of warmed DE-71 was weighed into a beaker and dissolved into corn oil with warmed stirring. The solution was then quantitatively transferred to a 1 L volumetric flask, diluted with corn oil, and thoroughly mixed to prepare a 1 mg/mL stock solution. Using a positive displacement pipette, aliquots of the stock solution were transferred into appropriate volumetric flasks and diluted 1:1,000 with corn oil to achieve final dose formulation concentrations of 0.001 mg/mL (for mice) or 0.002 mg/mL (for rats). For the four highest concentration dose formulations, the appropriate amount of warmed DE-71 was weighed into a beaker, dissolved into corn oil with warmed stirring, quantitatively transferred to an appropriate volumetric flask, diluted to volume with corn oil, and stirred vigorously. The dose formulations were prepared four times.	Same as the four highest concentration dose formulations in the 3-month studies, except the corn oil was also warmed in a water bath to reduce viscosity and aid sampling. The dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number	
2550OA30A	2550OA30A
Maximum Storage Time	
46 days	46 days
Storage Conditions	
Stored in amber glass containers sealed with Teflon®-lined lids at approximately 5° C	Stored in amber glass containers sealed with Teflon®-lined lids at approximately 5° C
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

TABLE J6
Results of Analyses of Dose Formulations Administered to F344/N Rats in the 3-Month Gavage Study of DE-71

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
July 1, 2004	July 2-3, 2004	1.00	0.942	–6
		10.0	9.45	–6
		20.0	18.7	–7
		100	93.7	–6
	August 16-17, 2004[b]	1.00	0.899	–10
		10.0	9.07	–9
		20.0	16.3	–19
		100	92.2	–8
July 8, 2004	July 12-13, 2004	0.002	0.00199	–1
	August 16-17, 2004[b]	0.002	0.00662	+231
August 2, 2004	August 5-6, 2004	0.002	0.00191	–5
		1.00	0.940	–6
		10.0	9.49	–5
		20.0	19.3	–4
		100	98.5	–2
	September 14-15, 2004[b]	0.002	0.00172	–15
		1.00	0.910	–9
		10.0	9.08	–9
		20.0	18.8	–6
		100	93.7	–6
October 4, 2004	October 5-6, 2004	0.002	0.00189	–6
		1.00	0.904	–10
		10.0	9.03	–10
	October 25-26, 2004[b]	0.002	0.00179	–11
		1.00	0.950	–5
		10.0	9.65	–4
October 7, 2004	October 8, 2004	20.0	18.5	–8
		100	97.5	–3
	October 25-26, 2004[b]	20.0	19.4	–3
		100	96.2	–4

[a] Results of duplicate analyses. Dosing volume=5 mL/kg; 0.002 mg/mL=0.01 mg/kg, 1.00 mg/mL=5 mg/kg, 10.0 mg/mL=50 mg/kg, 20.0 mg/mL=100 mg/kg, 100 mg/mL=500 mg/kg

[b] Animal room samples

TABLE J7
Results of Analyses of Dose Formulations Administered to Mice in the 3-Month Gavage Study of DE-71

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
July 1, 2004	July 2-3, 2004	0.001	0.000919	–8
		0.500	0.498	0
		5.00	4.66	–7
		10.0	9.45	–6
	August 16-17, 2004[b]	0.001	0.00102	+2
		0.500	0.475	–5
		5.00	4.07	–19
		10.0	9.46	–5
July 8, 2004	July 12-13, 2004	50.0	51.8	+4
	August 16-17, 2004[b]	50.0	43.0	–14
August 2, 2004	August 5-6, 2004	0.001	0.000970	–3
		0.500	0.485	–3
		5.00	4.75	–5
		10.0	9.49	–5
		50.0	49.1	–2
	September 14-15, 2004[b]	0.001	0.000986	–1
		0.500	0.483	–3
		5.00	4.52	–10
		10.0	9.12	–9
		50.0	43.7	–13
September 27, 2004	September 29-30, 2004	5.00	4.46[c]	–11
	October 25-26, 2004[b]	5.00	4.72	–6
October 4, 2004	October 5-6, 2004	0.001	0.001	0
		0.500	0.468	–6
		10.0	9.03	–10
		50.0	46.0	–8
	October 25-26, 2004[b]	0.001	0.000969	–3
		0.500	0.476	–5
		10.0	9.81	–2
		50.0	48.8	–2

[a] Results of duplicate analyses. Dosing volume=10 mL/kg; 0.001 mg/mL=0.01 mg/kg, 0.500 mg/mL=5 mg/kg, 5.00 mg/mL=50 mg/kg, 10.0 mg/mL=100 mg/kg, 50.0 mg/mL= 500 mg/kg.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration, but used at NTP's direction.

TABLE J8
Results of Analyses of Dose Formulations Administered to Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

Date Prepared[a]	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
July 10, 2008	July 10-11, 2008	3.00	3.215	+7
		10.0	9.84	–2
	August 26-27, 2008[c]	3.00	2.90	–3
		10.0	9.44	–6
July 14, 2008	July 14-15, 2008	0.600	0.584	–3
	August 26-27, 2008[c]	0.600	0.608	+1
August 12, 2008	August 13-14, 2008	0.600	0.627	+5
	September 23-24, 2008[c]	0.600	0.615	+3
August 15, 2008	August 15-16, 2008	3.00	2.93	–2
		10.0	9.23	–8
	September 23-24, 2008[c]	3.00	2.91	–3
		10.0	8.88	–11
October 6, 2008	October 7-8, 2008	0.600	0.607	+1
		3.00	3.17	+6
		10.0	9.35	–7
	November 18-19, 2008[c]	0.600	0.603	+1
		3.00	2.91	–3
		10.0	9.94	–1
December 29, 2008	December 30-31, 2008	3.00	2.93	–2
January 5, 2009	January 5-6, 2009	10.0	9.98	0
January 6, 2009	January 6-7, 2009	0.600	0.622	+4
February 23, 2009	February 24-25, 2009	0.600	0.589	–2
		3.00	2.74	–9
March 2, 2009	March 2-3, 2009	10.0	10.2	+2
May 18, 2009	May 19-20, 2009	0.600	0.609	+2
		3.00	2.97	–1
		10.0	9.72	–3
	June 30-July 1, 2009[c]	0.600	0.617	+3
		3.00	3.08	+3
		10.0	9.53	–5
July 14, 2009	July 16-17, 2009	0.600	0.584	–3
		3.00	2.94	–2
		10.0	9.67	–3
October 6, 2009	October 7-8, 2009	0.600	0.598	0
		3.00	2.95	–2
		10.0	9.47	–5

TABLE J8
Results of Analyses of Dose Formulations Administered to Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
December 1, 2009	December 2-3, 2009	0.600	0.610	+2
		3.00	3.01	0
		10.0	9.69	–3
	January 12-13, 2010[c]	0.600	0.602	0
		3.00	3.01	0
		10.0	9.39	–6
February 24, 2010	February 25-26, 2010	0.600	0.602	0
		3.00	3.03	+1
		10.0	9.61	–4
	April 8-9, 2010[c]	0.600	0.615	+3
		3.00	2.94	–2
		10.0	9.77	–2
April 20, 2010	April 21-22, 2010	0.600	0.589	–2
		3.00	2.97	–1
		10.0	9.61	–4
June 14, 2010	June 15-16, 2010	0.600	0.665[d]	+11
		3.00	2.97	–1
		10.0	9.95	–1
	July 27-28, 2010[c]	0.600	0.595	–1
		3.00	2.91	–3
		10.0	9.28	–7
August 9, 2010	August 12-13, 2010	0.600	0.618	+3
		3.00	2.94	–2
		10.0	9.77	–2
	September 1-2, 2010[c]	0.600	0.610	+2
		3.00	2.97	–1
		10.0	9.97	0

[a] Dose formulations prepared from July 10, 2008, to August 15, 2008, were used for dosing dams and pups; dose formulations prepared on August 12, 2008, and thereafter were used for dosing 2-year study rats.

[b] Results of triplicate analyses. Dosing volume=5 mL/kg; 0.600 mg/mL=3 mg/kg, 3.00 mg/mL=15 mg/kg, 10.0 mg/mL=50 mg/kg.

[c] Animal room samples

[d] Formulation was outside the acceptable range of ± 10% of target concentration but was inadvertently used for dosing animals; the Study Director determined there was no effect on study outcome.

TABLE J9
Results of Analyses of Dose Formulations Administered to Mice in the 2-Year Gavage Study of DE-71

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
February 14, 2008	February 14, 2008	0.30	0.272	–9
		10.0	9.55	–5
	March 10-11, 2008[b]	0.30	0.29778	–1
		10.0	8.8791	–11
February 21, 2008	February 21-22, 2008	3.0	3.03	+1
	March 10-11, 2008[b]	3.0	3.1169	+4
February 26, 2008	February 27-28, 2008	0.30	0.327	+9
		3.0	3.08	+3
		10.0	9.37	–6
March 24, 2008	March 25-26, 2008	0.30	0.295	–2
		3.0	3.06	+2
		10.0	9.22	–8
	May 6-7, 2008[b]	0.30	0.31596	+5
		3.0	3.1738	+6
		10.0	9.8772	–1
May 19, 2008	May 20-21, 2008	3.0	3.189	+6
		10.0	9.75	–3
May 22, 2008	May 22-23, 2008	0.30	0.302	+1
August 12, 2008	August 13-14, 2008	0.30	0.328	+9
August 15, 2008	August 15-16, 2008	3.0	2.93	–2
		10.0	9.23	–8
October 6, 2008	October 7-8, 2008	0.30	0.314	+5
		3.0	3.17	+6
		10.0	9.35	–7
	November 18-19, 2008[b]	0.30	0.31158	+4
		3.0	3.0530	+2
		10.0	10.569	+6
December 29-30, 2008	December 30-31, 2008	0.30	0.302	+1
		3.0	2.93	–2
January 5, 2009	January 5-6, 2009	10.0	9.98	0
February 23, 2009	February 24-25, 2009	0.30	0.299	0
		3.0	2.74	–9
March 2, 2009	March 2-3, 2009	10.0	10.17	+2
May 18, 2009	May 19-20, 2009	0.30	0.288	–4
		3.0	2.97	–1
		10.0	9.72	–3
	June 30-July 1, 2009[b]	0.30	0.305	+2
		3.0	3.045	+2
		10.0	9.531	–5

TABLE J9
Results of Analyses of Dose Formulations Administered to Mice in the 2-Year Gavage Study of DE-71

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
July 14, 2009	July 16-17, 2009	0.30	0.317	+6
		3.0	2.94	–2
		10.0	9.67	–3
October 6, 2009	October 7-8, 2009	0.30	0.294	–2
		3.0	2.95	–2
		10.0	9.47	–5
December 1, 2009	December 2-3, 2009	0.30	0.325	+8
		3.0	3.01	0
		10.0	9.69	–3
	January 12-13, 2010[b]	0.30	0.310	+3
		3.0	3.091	+3

[a] Results of triplicate analyses. Dosing volume=10 mL/kg; 0.30 mg/mL=3 mg/kg, 3.0 mg/mL=30 mg/kg, 10.0 mg/mL=100 mg/kg.
[b] Animal room samples

APPENDIX K
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE K1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

TABLE K2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a] Per kg of finished product

TABLE K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.51	13.7 – 15.9	30
Crude fat (% by weight)	8.2 ± 0.24	7.7 – 8.6	30
Crude fiber (% by weight)	9.3 ± 0.96	7.1 – 11.8	30
Ash (% by weight)	5.1 ± 0.15	4.9 – 5.4	30
Amino Acids (% of total diet)			
Arginine	0.786 ± 0.070	0.67 – 0.97	23
Cystine	0.220 ± 0.024	0.015 – 0.25	23
Glycine	0.700 ± 0.040	0.62 – 0.80	23
Histidine	0.351 ± 0.080	0.27 – 0.68	23
Isoleucine	0.546 ± 0.043	0.43 – 0.66	23
Leucine	1.095 ± 0.066	0.96 – 1.24	23
Lysine	0.700 ± 0.116	0.31 – 0.86	23
Methionine	0.409 ± 0.045	0.26 – 0.49	23
Phenylalanine	0.628 ± 0.039	0.54 – 0.72	23
Threonine	0.506 ± 0.042	0.43 – 0.61	23
Tryptophan	0.150 ± 0.028	0.11 – 0.20	23
Tyrosine	0.405 ± 0.063	0.28 – 0.54	23
Valine	0.664 ± 0.042	0.55 – 0.73	23
Essential Fatty Acids (% of total diet)			
Linoleic	3.96 ± 0.254	3.49 – 4.55	23
Linolenic	0.30 ± 0.031	0.21 – 0.35	23
Vitamins			
Vitamin A (IU/kg)	3,723 ± 87.7	2,110 – 5,720	30
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	80.3 ± 21.6	27.0 – 124.0	23
Thiamine (ppm)[b]	7.1 ± 1.18	5.1 – 11.0	30
Riboflavin (ppm)	7.7 ± 2.87	4.20 – 17.50	23
Niacin (ppm)	79.2 ± 8.97	66.4 – 98.2	23
Pantothenic Acid (ppm)	27 ± 12.35	17.4 – 81.0	23
Pyridoxine (ppm)[b]	9.54 ± 1.94	6.44 – 13.7	23
Folic Acid (ppm)	1.61 ± 0.47	1.15 – 3.27	23
Biotin (ppm)	0.32 ± 0.10	0.20 – 0.704	23
Vitamin B_{12} (ppb)	53.4 ± 38.7	18.3 – 174.0	23
Choline (ppm)[b]	2,773 ± 590	1,160 – 3,790	23
Minerals			
Calcium (%)	0.911 ± 0.043	0.81 – 0.99	30
Phosphorus (%)	0.562 ± 0.057	0.49 – 0.82	30
Potassium (%)	0.667 ± 0.030	0.626 – 0.733	23
Chloride (%)	0.385 ± 0.038	0.300 – 0.474	23
Sodium (%)	0.189 ± 0.016	0.160 – 0.222	23
Magnesium (%)	0.216 ± 0.060	0.185 – 0.490	23
Sulfur (%)	0.170 ± 0.030	0.116 – 0.209	14
Iron (ppm)	186 ± 38.64	135 – 311	23
Manganese (ppm)	51.02 ± 10.19	21.0 – 73.1	23
Zinc (ppm)	53.61 ± 8.34	43.3 – 78.5	23
Copper (ppm)	7.1 ± 2.540	3.21 – 16.3	23
Iodine (ppm)	0.503 ± 0.201	0.158 – 0.972	23
Chromium (ppm)	0.696 ± 0.270	0.330 – 1.380	22
Cobalt (ppm)	0.248 ± 0.163	0.094 – 0.864	21

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

TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.038	0.16 – 0.31	30
Cadmium (ppm)	0.06 ± 0.009	0.04 – 0.10	30
Lead (ppm)	0.11 ± 0.147	0.06 – 0.90	30
Mercury (ppm)	<0.02		30
Selenium (ppm)	0.20 ± 0.043	0.14 – 0.34	30
Aflatoxins (ppb)	<5.00		30
Nitrate nitrogen (ppm)[c]	21.02 ± 8.31	10.0 – 42.3	30
Nitrite nitrogen (ppm)[c]	<0.61		30
BHA (ppm)[d]	<1.0		30
BHT (ppm)[d]	<1.0		30
Aerobic plate count (CFU/g)	10.0 ± 0.0	10	30
Coliform (MPN/g)	3.0 ± 0.0	3.0	30
Escherichia coli (MPN/g)	<10		30
Salmonella (MPN/g)	Negative		30
Total nitrosamines (ppb)[e]	9.64 ± 4.33	2.0 – 17.2	30
N-Nitrosodimethylamine (ppb)[e]	2.65 ± 2.58	0.9 – 11.1	30
N-Nitrosopyrrolidine (ppb)[e]	7.62 ± 3.34	1.0 – 13.9	30
Pesticides (ppm)			
α-BHC	<0.01		30
β-BHC	<0.02		30
γ-BHC	<0.01		30
δ-BHC	<0.01		30
Heptachlor	<0.01		30
Aldrin	<0.01		30
Heptachlor epoxide	<0.01		30
DDE	<0.01		30
DDD	<0.01		30
DDT	<0.01		30
HCB	<0.01		30
Mirex	<0.01		30
Methoxychlor	<0.05		30
Dieldrin	<0.01		30
Endrin	<0.01		30
Telodrin	<0.01		30
Chlordane	<0.05		30
Toxaphene	<0.10		30
Estimated PCBs	<0.20		30
Ronnel	<0.01		30
Ethion	<0.02		30
Trithion	<0.05		30
Diazinon	<0.10		30
Methyl chlorpyrifos	0.119 ± 0.116	0.020 – 0.553	30
Methyl parathion	<0.02		30
Ethyl parathion	<0.02		30
Malathion	0.109 ± 0.092	0.020 – 0.395	30
Endosulfan I	<0.01		30
Endosulfan II	<0.01		30
Endosulfane sulfate	<0.03		30

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] All values were corrected for percent recovery.

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Blood samples were collected from each animal and allowed to clot and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and sent to BioReliance Corporation, Rockville, MD (3-month studies), or the Research Animal Diagnostic Laboratory (RADIL), University of Missouri, Columbia, MO (2-year studies), for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood was collected from five males and five females at all timepoints, except blood was collected from only unmated female rats at arrival for the 2-year perinatal and postnatal gavage study.

Method/Test	**Time of Collection**
RATS	
3-Month Study	
ELISA	
Mycoplasma arthritidis	Study Termination
Mycoplasma pulmonis	Study Termination
Pneumonia virus of mice (PVM)	Study Termination
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	Study Termination
Sendai	Study Termination
Immunofluorescence Assay	
Parvo	Study Termination
2-Year Study	
Multiplex Fluorescent Immunoassay	
Kilham's rat virus (KRV)	Arrival, 1, 6, 12, and 18 months, study termination
M. pulmonis	Arrival, 1, 6, 12, and 18 months, study termination
Parvo NS-1	Arrival, 1, 6, 12, and 18 months, study termination
PVM	Arrival, 1, 6, 12, and 18 months, study termination
RCV/SDA	Arrival, 1, 6, 12, and 18 months, study termination
Rat minute virus (RMV)	Arrival, 1, 6, 12, and 18 months, study termination
Rat parvovirus (RPV)	Arrival, 1, 6, 12, and 18 months, study termination
Rat theilovirus (RTV)	Arrival, 1, 6, 12, and 18 months, study termination
Sendai	Arrival, 1, 6, 12, and 18 months, study termination
Theiler's murine encephalomyelitis virus (TMEV)	Arrival, 1, 6, 12, and 18 months, study termination
Toolan's H-1 virus	Arrival, 1, 6, 12, and 18 months, study termination

Method/Test	Time of Collection
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study Termination
Epizootic diarrhea of infant mice (EDIM)	Study Termination
Theiler's murine encephalomyelitis virus – mouse poliovirus, strain GDVII (TMEV GDVII)	Study Termination
Lymphocytic choriomeningitis virus (LCMV)	Study Termination
Mouse adenoma virus-FL	Study Termination
Mouse hepatitis virus (MHV)	Study Termination
Mouse minute virus viral protein 2 (MMV VP2)	Study Termination
Mouse parvovirus viral protein 2 (MPV VP2)	Study Termination
Mycoplasma pulmonis	Study Termination
PVM	Study Termination
Reovirus	Study Termination
Sendai	Study Termination
Immunofluorescence Assay	
Mouse cytomegalovirus (MCMV)	Study Termination
Ectromelia Virus	Study Termination
2-Year Study	
Multiplex Fluorescent Immunoassay	
Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM	1, 6, 12, and 18 months, study termination
LCMV	1, 6, 12, and 18 months, study termination
M. pulmonis	1, 6, 12, and 18 months, study termination
MHV	1, 6, 12, and 18 months, study termination
Mouse norovirus (MNV)	1, 6, 12, and 18 months, study termination
Parvo NS-1	1, 6, 12, and 18 months, study termination
MPV	1, 6, 12, and 18 months, study termination
MMV	1, 6, 12, and 18 months, study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus	1, 6, 12, and 18 months, study termination
TMEV GDVII	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

APPENDIX M
STUDY ON THE RELATIONSHIP OF THE AhR TO DE-71 LIVER TUMOR FORMATION IN WISTAR HAN RATS

B. Alex Merrick[1], June K. Dunnick[2], Timothy Maynor[5], Amy E. Brix[6], Grace E. Kissling[4], Michael J. Devito[3]

[1]Biomolecular Screening Branch, NTP, NIEHS, Research Triangle Park, NC 27709;
[2]Toxicology Branch, NTP, NIEHS, Research Triangle Park, NC 27709;
[3]NTP Laboratory, NTP, NIEHS, Research Triangle Park, NC 27709;
[4]Biostatistics and Computational Biology Branch, NIEHS, Research Triangle Park, NC 27709;
[5]ILS, Inc., Research Triangle Park, NC 27709;
[6]Experimental Pathology Laboratories, Inc., Research Triangle Park, NC 27709

STUDY ON THE RELATIONSHIP OF THE AhR TO DE-71 LIVER TUMOR FORMATION IN WISTAR HAN RATS

INTRODUCTION

The aim of this study was to determine if a mutation in the aryl hydrocarbon receptor (AhR) genotype was related to DE-71-induced liver tumor formation in female Wistar Han [Crl:WI(Han)] rats. In the current 2-year studies of DE-71, there was clear evidence for liver tumor formation in male and female rats and mice.

The DE-71 test article had a small amount of polybrominated dibenzodioxins and furans (approximately 7×10^{-6} % of the mixture; see Appendix J, Table J4), and it was uncertain if these components could contribute to liver tumor formation via interaction with the AhR. The female rat was selected for study because, based on the original pathology results, high dose (50 mg/kg) female rats had more liver tumors than high dose male rats.

In the current 2-year study, Wistar Han rats were dosed with DE-71, a mixture of pentabromodiphenyl ethers (pentaBDEs). While pentaBDEs have low potential to interact with the AhR (Sanders *et al.*, 2005), there were small amounts of polybrominated dibenzodioxins and furans in the DE-71 mixture. While the polybrominated dibenzodioxins and furans have low toxic equivalency factors compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (van den Berg *et al.*, 2013), they may have some potential to interact with the AhR and affect liver tumorigenesis by this mechanism.

It has been reported that Wistar Han rats show resistance to dioxin-induced hepatocarcinogenesis that may be related to an allelic mutation in the AhR in this strain of rat (Unkila *et al.*, 1993). According to Charles River Laboratories, about 50% of the Wistar Han rats used in the current 2-year study carry or are homozygous for mutation in the AhR allele (presumably at exon 10). An AhR mutation may alter receptor function and result in decreased dioxin-like effects (or polybrominated dibenzodioxins and furan effects) including induction of cancer by activation of the AhR (Pohjanvirta *et al.*, 1993, 1998, 1999).

Two mutations have been found within the DNA sequence in the AhR that may account for the differences in susceptibilities to dioxin-like effects (Pohjanvirta *et al.*, 1998). One of the mutations is in exon 10 and causes a single amino acid change within the variable region of the AhR. The other mutation is in intron 10 and leads to use of cryptic splice sites to form mRNA transcripts that remove short amino acid sequences near the end of the transactivation domain. In this study, DNA sequences in intron 10 and exon 10 were compared with those of Sprague Dawley rats. Using analysis of genotype at exon 10, we tested the possibility that AhR mutations in the Wistar Han rat strain might have functional consequences for AhR activation and liver tumor occurrences. If a wild AhR is necessary for induction of liver tumors by DE-71, it would be expected that DE-71-induced liver tumors would only be seen in animals with the wild AhR genotype.

Therefore, the objective of this study was to compare the AhR genotype (at exon 10) to the DE-71-induced liver tumor incidence in high dose female rats to determine if liver tumor formation correlated with wild AhR genotype and conversely, if the absence of liver tumors correlated with mutant AhR genotype.

MATERIALS AND METHODS

Animals and Tissue Specimens

All archival tissues were from the current 2-year study of DE-71 in Wistar Han rats. The NTP toxicogenomics faculty approved a plan for AhR genotyping of the livers of vehicle (corn oil) controls and 50 mg/kg female rats to determine if there was a correlation between AhR genotype and DE-71-induced liver tumor incidence as well as a non-target blocked tissue (e.g., kidney). Since liver and kidney tissues were only available from formalin-fixed paraffin-embedded (FFPE) blocks for DNA isolation, DNA extracted from a small number of fresh-frozen control liver samples was included to ensure extraction of high quality DNA for the genotyping assay for comparison. Five frozen archival Wistar Han livers were chosen from this study for which there was sufficient original tissue (50 mg) for DNA extraction.

DNA Extraction

In order to perform the genotyping assay, one 20-micron section was taken from paraffin blocks of the liver of female vehicle controls and one 20-micron section was taken from the liver of 50 mg/kg female rats. In addition, to analyze a non-target tissue, similar sections were cut from the kidney, involving both vehicle control and high dose DE-71 kidneys. Paraffin sections from each block were placed in separate screw-top 1.5 mL tubes, centrifuged for 10 to 15 seconds, and stored at 4º C until delivery to ILS, Inc. (Research Triangle Park, NC), for DNA extraction and genotyping.

Propagation and Purification of Authentic Standards

Plasmid controls were generated from genomic DNA (gDNA) and were extracted along with DNA from fresh-frozen tissue collected from five Wistar Han rats for each of the three genotypes (homozygous wild type, heterozygous, and homozygous mutant). Genotyped liver tissue from Wistar Han rats provided the gDNA template for the heterozygous and homozygous mutant controls while one genotyped Sprague Dawley rat fresh-frozen liver provided the gDNA template for the homozygous wild-type control.

AhR Genotyping Assay

The AhR genotyping assay was a polymerase chain reaction (PCR)-based molecular beacon assay adapted from Pohjanvirta *et al.* (1994). The target amplicon and probes are listed in Table M1. Plasmids from Dr. Pohjanvirta for wild-type and mutant AhRs were obtained as standards for the genotyping assay. The gDNA templates were amplified using TaqMan® Genotyping Master Mix (Life Technologies, Carlsbad, CA) according to manufacturer's procedures and cycling conditions (95º C for 10 minutes, followed by 40 cycles of 95º C for 20 seconds, and 60º C for 1 minute) on the ViiA™ 7 Real-Time PCR System 4 (Life Technologies). Of the resultant 108 base pair PCR product, 1 µL was ligated into the pCR™2.1 linear vector using the original TA Cloning® Kit (Life Technologies) according to manufacturer's procedures. The pCR™2.1 plasmid containing the 108 base pair insert was transformed into One Shot® MAX Efficiency® DH5α™-T1® Competent Cells (Life Technologies) according to manufacturer's procedures. The transformed cells were then plated on LB agar plates containing ampicillin (Thermo Fisher Scientific, Inc., Waltham, MA) and incubated overnight at 37º C. After incubation, two colonies for each transformed plasmid were selected from the LB agar plates and incubated separately overnight at 37º C in LB broth containing ampicillin (Thermo Fisher Scientific, Inc.). The six resultant propagated plasmids were purified from the LB broth using the Quantum Prep® Plasmid Midiprep Kit (Bio-Rad Laboratories, Hercules, CA) per manufacturer's procedures. The resultant six purified plasmids (two plasmids per genotype) were assessed for quantity and ratio of absorbances at 260 and 280 nm using the NanoDrop® ND-1000 spectrophotometer (Thermo Fischer Scientific, Inc.). Additionally, the plasmids were Sanger sequenced to confirm the incorporation of the 108 base pair amplicon with the presence or absence of each AhR single nucleotide polymorphism for which homozygous wild type was G/G, heterozygous type was G/A, and homozygous mutant was A/A.

RESULTS

The degraded quality of gDNA extracted from FFPE sections required the use of a nested PCR reaction in order to amplify a 200 base pair region of gDNA containing the AhR mutation of interest. The nested PCR product was then utilized as the template for both quantitative PCR (qPCR) and sequencing. The results of the qPCR and Sanger sequencing were combined to determine the AhR genotype for each sample.

The 118 liver FFPE samples yielded the following genotype totals: 26 (22.0%) homozygous wild-type G/G, 51 (43.2%) heterozygous G/A, 39 (33.1%) homozygous mutant A/A, and 2 (1.7%) undetermined. The 122 kidney FFPE samples yielded the following genotype totals: 21 (17.2%) homozygous wild-type G/G, 51 (41.8%) heterozygous G/A, 38 (31.1%) homozygous mutant A/A, and 12 (9.8%) undetermined.

A number of liver tumors occurred in female Wistar Han rats in the 2-year study ranging from adenomas to cholangiocarcinomas and carcinomas. A statistical analysis was performed for the relationship of genotype and number of animals in each liver tumor type (Table M2). No significant difference was observed for any one genotype and hepatocellular tumors. When various tumors were combined such as adenomas and carcinomas or single and multiple tumors, no significant differences among the AhR genotypes (at exon 10) were observed.

DISCUSSION

DNA extraction was successfully performed from FFPE liver and kidney blocks from the current 2-year DE-71 study using commercial procedures optimized for retrieval of nucleic acids for amplification and sequencing analyses (Janecka *et al.*, 2015). Almost all liver FFPE tissues (60/60 vehicle controls; 58/60 dosed with DE-71) were able to be genotyped; only two samples (from animals dosed with DE-71) were unable to be analyzed for genotype because of poor gDNA sample quality. As indicated in Table M2, there was no statistically significant correlation between liver tumor incidences in the female rats administered DE-71 and the AhR genotype.

The incidences of different tumor types were compared between vehicle control and DE-71-treated rats. Statistical comparisons of incidences were performed for each tumor (e.g., hepatocellular adenoma) according to each genotype in vehicle control versus DE-71-treated tissues. Genotypes were homozygous wild-type (G/G), heterozygous (G/A), or homozygous mutant (A/A). In addition, tumor incidences of combined single or multiple tumor types such as single adenomas and multiple adenomas or after combined different tumor types such as adenomas and carcinomas were also compared. No differences were found in the number of tumor types or combinations of tumor types by AhR genotype in DE-71-dosed female rats.

The distribution of the AhR genotypes in this study shows that 22.0% were wild-type homozygous, 33.1% were mutant homozygous, and 43.2% were heterozygous. This suggests that DE-71-mediated liver tumor formation was independent of a fully functional AhR since over three-fourths of the Wistar rats in this study carried a mutant AhR allele.

Another possibility is that the level of AhR activation was inadequate to contribute to tumor formation during chronic exposure due to a low AhR affinity for the polybrominated diphenyl ethers (PBDEs) found in DE-71 or because of the absence or negligible amounts of dioxin-like contaminants in DE-71. Interestingly, Jiang *et al.* (2009) cloned variants of the AhR of the Wistar Han rat and found the expressed proteins did not vary in their ligand binding capacity from the wild-type AhR protein suggesting that the AhR variants were functionally normal. In addition, the AhR variants were not associated with TCDD-induced developmental toxicity measures in the study reported by these investigators. Overall, the data presented here suggest that under the conditions of the current 2-year study, the AhR genotype was not significantly associated with liver tumor formation after chronic DE-71 administration.

In summary, genotyping of female Wistar Han rats for an AhR mutation from paraffin archival samples did not show an association with the incidences of liver tumors after administration of DE-71 for 2 years.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Raimo Pohjanvirta at the Department of Food Hygiene and Environmental Health, University of Helsinki, Helsinki, Finland, for supplying the cloned vectors containing wild-type and mutated AhR sequences.

REFERENCES

Janecka, A., Adamczyk, A., and Gasińska, A. (2015). Comparison of 8 commercially available kits for DNA extraction from formalin-fixed paraffin-embedded tissues. *Anal. Biochem.* **476**, 8-10.

Jiang, T., Bell, D.R., Clode, S., Fan, M.Q., Fernandes, A., Foster, P.M.D., Loizou, G., MacNicoll, A., Miller, B.G., Rose, M., Tran, L., and White, S. (2009). A truncation in the aryl hydrocarbon receptor of the CRL:WI(Han) rat does not affect the developmental toxicity of TCDD. *Toxicol. Sci.* **107**, 512-521.

Pohjanvirta, R., Unkila, M., Tuomisto, J.T., Vuolteenaho, O., Leppäluoto, J., and Tuomisto, J. (1993). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on plasma and tissue beta-endorphin-like immunoreactivity in the most TCDD-susceptible and the most TCDD-resistant rat strain. *Life Sci.* **53**, 1479-1487.

Pohjanvirta, R., Unkila, M., and Tuomisto, J. (1994). TCDD-induced hypophagia is not explained by nausea. *Pharmacol. Biochem. Behav.* **47**, 273-282.

Pohjanvirta, R., Wong, J.M.Y., Li, W., Harper, P.A., Tuomisto, J., and Okey, A.B. (1998). Point mutation in intron sequence causes altered carboxyl-terminal structure in the aryl hydrocarbon receptor of the most 2,3,7,8-tetrachlorodibenzo-p-dioxin-resistant rat strain. *Mol. Pharmacol.* **54**, 86-93.

Pohjanvirta, R., Viluksela, M., Tuomisto, J.T., Unkila, M., Karasinska, J., Franc, M.-A., Holowenko, M., Giannone, J.V., Harper, P.A., Tuomisto, J., and Okey, A.B. (1999). Physicochemical differences in the AH receptors of the most TCDD-susceptible and the most TCDD-resistant rat strains. *Toxicol. Appl. Pharmacol.* **155**, 82-95.

Sanders, J.M., Burka, L.T., Smith, C.S., Black, W., James, R., and Cunningham, M.L. (2005). Differential expression of CYP1A, 2B, and 3A genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual components. *Toxicol. Sci.* **88**, 127-133.

Unkila, M., Pohjanvirta, R., Honkakoski, P., Törrönen, R., and Tuomisto, J. (1993). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induced ethoxyresorufin-*O*-deethylase (EROD) and aldehyde dehydrogenase ($ALDH_3$) activities in the brain and liver: A comparison between the most TCDD-susceptible and the most TCDD-resistant rat strain. *Biochem. Pharmacol.* **46**, 651-659.

van den Berg, M., Denison, M.S., Birnbaum, L.S., DeVito, M.J., Fiedler, H., Falandysz, J., Rose, M., Schrenk, D., Safe, S., Tohyama, C., Tritscher, A., Tysklind, M., and Peterson, R.E. (2013). Polybrominated dibenzo-p-dioxins, dibenzofurans, and biphenyls: Inclusion in the toxicity equivalency factor concept for dioxin-like compounds. *Toxicol. Sci.* **133**, 197-208.

TABLE M1
Sequence and Primers for Amplification of Wild-Type and Mutant Aryl Hydrocarbon Receptors (AhR) in Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

108bp AhR Sequence
ACACAATAGACTACACGGAGATGCTTGGACCTACAAGGTTTATTCCCTGTAGAAAGCCCTTACCTTGCTTAGGAACGCCTGGG
AGCCTGGAATCTCAGGGCTGTACTG
Rn4: Chr6:54,208,644 - 54,208,751

Reverse Complement (108bp)
CAGTACAGCCCTGAGATTCCAGGCTCCCAGGCGTTCCTAAGCAAGGTAAGGGCTTTCTACAGGGAATAAACCTTGTAGGTCCA
AGCATCTCCGTGTAGTCTATTGTGT

Forward Primer:	CAGTACAGCCCTGAGATTCCAG
Reverse Primer:	ACACAATAGACTACACGGAGATGC (reverse complement)
Wild-Type (G) Probe:	[VIC]-CTAAGCAAG**G**TAAGGGCT
Mutant (A) Probe:	[FAM]-CTAAGCAAG**A**TAAGGGCT

TABLE M2
Summary of Liver Tumor Counts by Genotype in Female Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71

Tumor or Tumor Combination	Heterozygous G/A[a]	Homozygous A/A[a]	Homozygous G/G[a]	P Value[b]
Hepatocellular Adenoma	4 [51]	3 [39]	4 [26]	0.516
Hepatocellular Adenoma, Multiple	5 [51]	1 [39]	2 [26]	0.409
Hepatocellular Carcinoma	1 [51]	2 [39]	0 [26]	0.600
Hepatocellular Carcinoma, Multiple	1 [51]	0 [39]	1 [26]	0.702
Hepatocholangiocarcinoma	2 [51]	1 [39]	1 [26]	1.000
Hepatocholangioma	1 [51]	0 [39]	2 [26]	0.162
Hepatocholangioma, Multiple	0 [51]	1 [39]	0 [26]	0.560
Cholangiocarcinoma	0 [51]	0 [39]	1 [26]	0.224
Cholangiocarcinoma, Multiple	0 [51]	0 [39]	0 [26]	—[c]
Hepatocellular Adenoma + Hepatocellular Adenoma, Multiple	9 [51]	4 [39]	6 [26]	0.342
Hepatocellular Carcinoma + Hepatocellular Carcinoma, Multiple	2 [51]	2 [39]	1 [26]	1.000
Hepatocellular Adenoma + Hepatocellular Adenoma, Multiple + Hepatocellular Carcinoma + Hepatocellular Carcinoma, Multiple	9 [51]	5 [39]	6 [26]	0.512
Hepatocholangioma + Hepatocholangioma, Multiple	1 [51]	1 [39]	2 [26]	0.437
Cholangiocarcinoma + Cholangiocarcinoma, Multiple	0 [51]	0 [39]	1 [26]	0.224

a Number of animals with tumor [total number of animals]
b Fisher's exact test used to compare genotype with number of animals
c Value of statistic cannot be computed.

APPENDIX N
EVALUATION OF *Hras* AND *Ctnnb1* MUTATIONS IN HEPATOCELLULAR TUMORS FROM WISTAR HAN RATS AND B6C3F1/N MICE CHRONICALLY EXPOSED TO DE-71

Hue-Hua L. Hong[1], Thai-Vu Ton[1], June K. Dunnick[2], Amy E. Brix[4], Grace E. Kissling[3], Robert C. Sills[1], Arun R. Pandiri[4]

[1]Cellular & Molecular Pathology Branch, NTP, NIEHS, Research Triangle Park, NC 27709
[2]Toxicology Branch, NTP, NIEHS, Research Triangle Park, NC 27709
[3]Biostatistics and Computational Biology Branch, NIEHS, Research Triangle Park, NC 27709
[4]Experimental Pathology Laboratories, Inc., Research Triangle Park, NC 27709

EVALUATION OF *Hras* AND *Ctnnb1* MUTATIONS IN HEPATOCELLULAR TUMORS FROM WISTAR HAN RATS AND B6C3F1/N MICE CHRONICALLY EXPOSED TO DE-71

INTRODUCTION

Evaluation of genetic mutations in cancer genes from hepatocellular carcinomas that arise either spontaneously or due to chemical exposure can provide some insight into the mechanisms of chemical-induced carcinogenesis. Previous studies have shown that *Ctnnb1* (beta-catenin) mutations and *Hras* mutations are common in liver cancers (Fox *et al.*, 1990; Yamada *et al.*, 1999; Hoenerhoff *et al.*, 2013). Examination of genetic mutations in the hepatocellular tumors in rats and mice resulting from chronic DE-71 exposure might provide some understanding of DE-71-induced hepatocellular tumorigenesis (Jackson *et al.*, 2006).

MATERIALS AND METHODS

Animals and Tissue Sampling

Hepatocellular tumors as well as normal liver samples from rats and mice were obtained from the current DE-71 chronic bioassays. Male and female Wistar Han [Crl:WI(Han)] rats were administered 0, 3, 15, or 50 mg/kg body weight per day and male and female B6C3F1/N mice were administered 0, 3, 30, or 100 mg/kg per day by gavage 5 days per week for 2 years. At necropsy, hepatocellular tumors were fixed in 10% neutral buffered formalin for 18 to 24 hours, and then transferred to 70% ethanol and processed into paraffin blocks, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic analysis. The formalin-fixed paraffin-embedded (FFPE) normal liver tissue and liver tumors representative of spontaneous and DE-71-induced hepatocellular tumors were used for mutation analyses. For rats, due to the paucity of hepatocellular carcinomas, both hepatocellular adenomas (n=33) and carcinomas (n=7) were used for mutation analysis. However, for mice, only hepatocellular carcinomas (n=79) were used for mutation analysis. The hepatocellular tumors chosen for molecular biology analysis were based on their overall size and viability (minimal to no necrosis or hemorrhage observed microscopically) in order to maximize the amount and quality of DNA obtained from FFPE sections. DNA quality was measured using a NanoDrop® spectrophotometer (Thermo Fischer Scientific, Inc., Wilmington, DE) to calculate the ratio of absorbances at 260 and 280 nm, and DNA samples with a purity range of 1.7 to 2.0 were used for analysis. Samples falling outside of this range were reisolated from FFPE sections until a suitable purity measurement was obtained, or were discarded.

DNA Extraction, Polymerase Chain Reaction (PCR), Autosequencing, and Mutation Analysis

Hepatocellular tumors representing all DE-71-dosed groups (35 from Wistar Han rats and 62 from B6C3F1/N mice) and spontaneous hepatocellular tumors (5 from Wistar Han rats and 17 from B6C3F1/N mice) from vehicle controls were evaluated for hot-spot mutations in *Hras* and *Ctnnb1* genes that are relevant in human hepatocellular carcinogenesis. In addition, age-matched non-tumor livers from rats (n=10) and mice (n=8) were also analyzed. FFPE sections at 10-micron thickness were collected into screw top tubes for DNA extraction. DNA was isolated from these FFPE-dissected tissue sections with a DNeasy® Blood and Tissue Kit (QIAGEN, Valencia, CA). Amplification reactions were carried out by semi-nested PCR using primer sets designed for *Hras* and *Ctnnb1* genes for rats (Table N1) and mice (Table N2). Controls lacking DNA were run with all sets of reactions. PCR products were purified using a QIAquick® Gel Extraction Kit (QIAGEN). The purified PCR products were cycled with Terminal Ready Reaction Mix-BigDye® (PerkinElmer Applied Biosystems, Foster City, CA), and the extension products were purified with DyeEx 2.0 Spin Kit (QIAGEN). The lyophilized PCR products were sequenced with an automatic sequencer (PerkinElmer Applied Biosystems ABI Model 3100). The resulting electropherograms were compared to identify mutations in hepatocellular adenomas and carcinomas that either arose spontaneously or were due to DE-71 administration. The mutations were confirmed by sequencing with both forward and reverse primers, and the positive mutations were verified by repeat analysis, starting from amplification of the original DNA extracts.

Statistical Analysis of Mutation Incidences in Hepatocellular Tumors

To compare total mutation incidences in each dosed group to the incidences in the vehicle control groups, one-sided Fisher exact tests were used. Exact one-sided Cochran-Armitage trend tests were used to test for dose-related trends in the incidences of mutations across all dose groups.

RESULTS

Hras mutations in rodent hepatocellular carcinomas are commonly observed within codon 61 (Hoenerhoff *et al.*, 2013). However, in this study, the rat hepatocellular tumors resulting from chronic DE-71 exposure demonstrated mutations exclusively within codon 60 [20% (7/35); Table N3]. Interestingly, all the mutations were the same G to A transition (Gly to Asp). *Ctnnb1* mutations on the other hand were fewer [11% (4/35)], more diverse, identified between codons 33 to 40, and consisted of transitions and transversions. No *Hras* or *Ctnnb1* mutations were noted in the spontaneous hepatocellular adenomas in rats. There were no differences in the incidences of mutations between male and female rats (data not presented) and hence the combined data from both male and female rats are presented in Table N3.

In the mouse hepatocellular carcinomas, the incidences of *Hras* mutations were low [10% (6/62)] and were located within codon 61 mainly C to A or A to T transversions (Table N4). However, there were no significant differences in the incidences of *Hras* mutations or the mutation spectra between hepatocellular carcinomas occurring spontaneously or resulting from chronic treatment with DE-71. Conversely, statistically significant increased incidences of *Ctnnb1* mutations were noted in mouse hepatocellular carcinomas resulting from chronic administration of DE-71. None of the hepatocellular carcinomas arising spontaneously harbored *Ctnnb1* mutations. *Ctnnb1* mutations in spontaneous hepatocellular carcinomas are very rare compared to *Hras* mutations (Table N4). These mutations were present within codons 15 to 46 and contained a mixture of transitions and transversions. In addition, there was a deletion of codons 15 to 46 in one carcinoma. The spontaneous hepatocellular carcinomas did not harbor any mutations in *Ctnnb1*. There were no differences in the incidences of mutations between male and female mice (data not presented) and hence the combined data from both male and female mice are presented in Table N4.

DISCUSSION

The *Hras* mutations in spontaneous and chemically induced rodent tumors are frequently localized within codon 61 (Hoenerhoff *et al.*, 2013). The presence of a novel *Hras* mutation (G to A transition, Gly to Asp) exclusively within codon 60 in rat hepatocellular tumors resulting from chronic gavage administration of DE-71 in a dose dependent manner suggests a possible unique mutational signature for DE-71-induced hepatocellular tumorigenesis. Though mutations in codon 60 are uncommon, this mutation may have a functional significance for HRAS since it serves as a “pivot point” in the conformational change that occurs upon activation of $p21^{ras}$ and it is located in the vicinity of hot-spot regions of codons 59, 61, and 62 that contain GDP/GTP binding domains (Jurnak *et al*., 1990; Radich *et al.*, 1990; Mosteller *et al.*,1994). However, depending on the type of mutation and the resulting substituted amino acid, the functional consequences of codon 60 mutations may be different. For example, a codon 60 Gly to Cys mutation results in decreased GTPase activity of HRAS and hence an activating mutation (Lin *et al.*, 2000) whereas a Gly to Ala mutation abolishes the ability of HRAS to transform NIH 3T3 cells (Sung *et al.*, 1995). In the current study, the codon 60 Gly to Asp mutation will likely result in alteration of HRAS since Asp is a large acidic amino acid compared to the relatively small Gly. Thus, a mutation in codon 60 may likely render mutant HRAS to cause persistent effector signaling even in the absence of extracellular stimuli and cause unperturbed MAPK signaling resulting in sustained hepatocellular proliferation. However, further experiments are needed to prove functional consequences of a codon 60 Gly to Asp mutation.

Statistically significant increased incidences of *Ctnnb1* mutations were noted in mouse hepatocellular carcinomas resulting from chronic administration of DE-71. Though not statistically significant, the incidences of *Hras* mutations were decreased in hepatocellular carcinomas from 100 mg/kg mice. This pattern of increased incidences of *Ctnnb1* mutations and decreased incidences of *Hras* mutations was also noted in hepatocellular carcinomas that resulted from chronic treatment with *Ginkgo biloba* extract (Hoenerhoff *et al.*, 2013). Aydinlik *et al.* (2001) demonstrated a high incidence of *Ctnnb1* mutations in hepatocellular carcinomas that resulted from diethylnitrosamine initiation and phenobarbital promotion. However, in this study, *Ctnnb1* mutations were absent in hepatocellular carcinomas that occurred in mice treated with only the initiating carcinogen (diethylnitrosamine) suggesting that initiated neoplastic hepatocytes harboring *Ctnnb1* mutations had a growth advantage during the phenobarbital promotion (Aydinlik *et al.*, 2001). Using a similar protocol, Strathmann *et al.* (2006), also demonstrated the unique selective pressure on *Ctnnb1*-mutated liver tumors after exposure to PCB153, a nondioxin-like tumor promoter.

PBDE components within DE-71 have been shown to be ligands for the CAR and PXR receptors (Zhou *et al.*, 2001; Sanders *et al.*, 2005; Blanco *et al.*, 2012; Sueyoshi *et al.*, 2014). In addition, especially at high doses, treatment with DE-71 caused an increase in hepatic *Cyp1a1* transcript levels, suggestive of a weak aryl hydrocarbon receptor activation potential for DE-71 (Sanders *et al.*, 2005). DE-71 is nongenotoxic and may not directly cause genetic alterations resulting in mutations and initiating carcinogenesis. Due to the ability of DE-71 to activate multiple nuclear receptors and inhibit apoptosis, it may function as a highly efficient promoter of hepatocarcinogenesis (Pitot *et al.*, 1980; Schwarz *et al.*, 2000; Aydinlik *et al.*, 2001; Schrenk *et al.*, 2004; Schwarz and Appel, 2005). The high incidence of *Ctnnb1* mutations in the mouse hepatocellular carcinomas is likely due to the promotion effects of DE-71 that induce a positive selective pressure on the initiated hepatocytes harboring *Ctnnb1* mutations and result in high tumor incidence. On the other hand, metabolites of DE-71 including dihydroxylated BDEs may cause oxidative stress (Lupton *et al.*, 2009; Blanco *et al.*, 2012) and subsequent DNA damage resulting in mutations in specific genes. Thus, the combination of DNA damage secondary to oxidative stress and the potent tumor promotion effects of DE-71 might have contributed to the DE-71 induced hepatocarcinogenesis.

REFERENCES

Aydinlik, H., Nguyen, T.D., Moennikes, O., Buchmann, A., and Schwarz, M. (2001). Selective pressure during tumor promotion by phenobarbital leads to clonal outgrowth of beta-catenin-mutated mouse liver tumors. *Oncogene* **20**, 7812-7816.

Blanco, J., Mulero, M., Domingo, J.L., and Sánchez, D.J. (2012). Gestational exposure to BDE-99 produces toxicity through upregulation of CYP isoforms and ROS production in the fetal rat liver. *Toxicol. Sci.* **127**, 296-302.

Fox, T.R., Schumann, A.M., Watanabe, P.G., Yano, B.L., Maher, V.M., and McCormick, J.J. (1990). Mutational analysis of the H-ras oncogene in spontaneous C57BL/6 × C3H/He mouse liver tumors and tumors induced with genotoxic and nongenotoxic hepatocarcinogens. *Cancer Res.* **50**, 4014-4019.

Hayashi, S.M., Ton, T.V., Hong, H.H., Irwin, R.D., Haseman, J.K., Devereux, T.R., and Sills, R.C. (2003). Genetic alterations in the Catnb gene but not the H-ras gene in hepatocellular neoplasms and hepatoblastomas of B6C3F(1) mice following exposure to diethanolamine for 2 years. *Chem. Biol. Interact.* **146**, 251-261.

Hoenerhoff, M.J., Pandiri, A.R., Snyder, S.A., Hong, H.H., Ton, T.V., Peddada, S., Shockley, K., Witt, K., Chan, P., Rider, C., Kooistra, L., Nyska, A., and Sills, R.C. (2013). Hepatocellular carcinomas in B6C3F1 mice treated with Ginkgo biloba extract for two years differ from spontaneous liver tumors in cancer gene mutations and genomic pathways. *Toxicol. Pathol.* **41**, 826-841.

Jackson, M.A., Lea, I., Rashid, A., Peddada, S.D., and Dunnick, J.K. (2006). Genetic alterations in cancer knowledge system: Analysis of gene mutations in mouse and human liver and lung tumors. *Toxicol. Sci.* **90**, 400-418.

Jurnak, F., Heffron, S., and Bergmann, E. (1990). Conformational changes involved in the activation of ras p21: Implications for related proteins. *Cell* **60**, 525-528.

Lin, S.R., Hsu, C.H., Tsai, J.H., Wang, J.Y., Hsieh, T.J., and Wu, C.H. (2000). Decreased GTPase activity of K-ras mutants deriving from human functional adrenocortical tumours. *Br. J. Cancer* **82**, 1035-1040.

Lupton, S.J., McGarrigle, B.P., Olson, J.R., Wood, T.D., and Aga, D.S. (2009). Human liver microsome-mediated metabolism of brominated diphenyl ethers 47, 99, and 153 and identification of their major metabolites. *Chem. Res. Toxicol.* **22**, 1802-1809.

Mosteller, R.D., Han, J., and Broek, D. (1994). Identification of residues of the H-ras protein critical for functional interaction with guanine nucleotide exchange factors. *Mol. Cell Biol.* **14**, 1104-1112.

Pitot, H.C., Goldsworthy, T., Campbell, H.A., and Poland, A. (1980). Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. *Cancer Res.* **40**, 3616-3620.

Radich, J.P., Kopecky, K.J., Willman, C.L., Weick, J., Head, D., Appelbaum, F., and Collins, S.J. (1990). N-ras mutations in adult de novo acute myelogenous leukemia: Prevalence and clinical significance. *Blood* **76**, 801-807.

Sanders, J.M., Burka L.T., Smith, C.S., Black, W., James, R., and Cunningham, M.L. (2005). Differential expression of CYP1A, 2B, and 3A genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual components. *Toxicol. Sci.* **88**, 127-133.

Schrenk, D., Schmitz, H.J., Bohnenberger, S., Wagner, B., and Wörner, W. (2004). Tumor promoters as inhibitors of apoptosis in rat hepatocytes. *Toxicol. Lett.* **149**, 43-50.

Schwarz, M., and Appel, K.E. (2005). Carcinogenic risks of dioxin: Mechanistic considerations. *Regul. Toxicol. Pharmacol.* **43**, 19-34.

Schwarz, M., Buchmann, A., Stinchcombe, S., Kalkuhl, A., and Bock, K. (2000). Ah receptor ligands and tumor promotion: Survival of neoplastic cells. *Toxicol. Lett.* **112-113**, 69-77.

Sills, R.C., Boorman, G.A., Neal, J.E., Hong, H.L., and Devereux, T.R. (1999). Mutations in ras genes in experimental tumours of rodents. *IARC Sci. Publ.* **146**, 55-86.

Strathmann, J., Schwarz, M., Tharappel, J.C., Glauert, H.P., Spear, B.T., Robertson, L.W., Appel, K.E., and Buchmann, A. (2006). PCB 135, a non-dioxin-like tumor promoter, selects for β-catenin (*Catnb*)-mutated mouse liver tumors. *Toxicol. Sci.* **93**, 34-40.

Sueyoshi, T., Li, L., Wang, H., Moore, R., Kodavanti, P.R., Lehmler, H.J., Negishi, M., and Birnbaum, L.S. (2014). Flame retardant BDE-47 effectively activates nuclear receptor CAR in human primary hepatocytes. *Toxicol. Sci.* **137**, 292-302.

Sung, Y.J., Carter, M., Zhong, J.M., and Hwang, Y.W. (1995). Mutagenesis of the H-ras p21 at glycine-60 residue disrupts GTP-induced conformational change. *Biochemistry* **34**, 3470-3477.

Yamada, Y., Yoshimi, N., Sugie, S., Suzui, M., Matsunaga, K., Kawabata, K., Hara, A., and Mori, H. (1999). Beta-catenin (Ctnnb1) gene mutations in diethylnitrosamine (DEN)-induced liver tumors in male F344 rats. *Jpn. J. Cancer Res.* **90**, 824-828.

Zhou, T., Ross, D.G., DeVito, M.J., and Crofton, K.M. (2001). Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.* **61**, 76-82.

TABLE N1
Primers Used To Amplify the Hot-Spot Regions of Rat *Hras* and *Ctnnb1* Genes

Exon	Codon	Primer	Strand	Sequence
2	*Hras*-61	RH61F1738	Sense	5′-TGATCCATCAGGGTATGAGAG-3′
		RH61F1752	Sense	5′-ATGAGAGGTGCAAGGGTAG-3′
		RH61R2300	Antisense	5′-TCAATGTAGGGGATGCCATAG-3′
		RH61F1816	Sense	5′-GCTGTGTTCTTTTGCAGG-3′
		RH61R1987	Antisense	5′-GACTTGGTGTTGTTGATGG-3′
2	*Ctnnb1*-5-80	R*β*CatF272	Sense	5′-ACATAATCAACAAGCCACCC-3′
		R*β*CatF431	Sense	5′-ACTCAGGCAGCATTCTCAGTGCAT-3′
		R*β*CatR725	Antisense	5′-GGAAGGTAACACAGAGAGTTGCTT-3′
		R*β*CatR799	Antisense	5′-ATGTGAGACTCCGTTGCC-3′

TABLE N2
Primers Used To Amplify the Hot-Spot Regions of Mouse *Hras* and *Ctnnb1* Genes

Exon	Codon	Primer	Strand	Sequence
2	*Hras*-61	MH61OS	Sense	5′-CCACTAAGCCTGTTGTGTTTTGCAG-3′
		MAPH61S	Sense	5′-GGACTCCTAGCGGAAACAGG-3′
		MH61OA	Antisense	5′-CTGTACTGATGGATGTCCTCGAAGGA-3′
		MAPH61A	Antisense	5′-GGTGTTGTTGATGGCAAATACA-3′
3	*Ctnnb1*-5-55	MbCat1F	Sense	5′-TACAGGTAGCATTTTCAGTTCAC-3′
		MbCat2R	Antisense	5′-TAGCTTCCAAACACAAATGC-3′
		MbCat8R	Antisense	5′-ACATCTTCTTCCTCAGGGTTG-3′
		MbCatF17130	Sense	5′-GATGGAGTTGGACATGGC-3′
		MbCatOR17294	Antisense	5′-ACTTGGGAGGTGTCAACA-3′
		MbCatIR17257	Antisense	5′-TTCTTCCTCAGGGTTGCC-3′

TABLE N3
Summary of *Hras* and *Ctnnb1* Mutations in Non-tumor Liver Tissue and Hepatocellular Adenomas and Carcinomas from Wistar Han Rats in the 2-Year Perinatal and Postnatal Gavage Study of DE-71[a]

Tissue – DE-71 Dose (mg/kg)	**Mutation Frequency** *Hras*[b]	*Ctnnb1*[b]	***Hras* Cdn 60** GGT to GAT	***Ctnnb1*** Cdn 33-40
Non-tumor Liver - 0	0/10 (0)	0/10 (0)	0	0
Hepatocellular Tumors[c] - 0	0/5 (0)	0/5 (0)	0	0
- 3	1/3 (33)	0/3 (0)	1	0
- 15	1/12 (8)	1/12 (8)	1	1
- 50	5/20 (25)	3/20 (15)	5	3[d]
DE-71-treated combined	7/35 (20)	4/35 (11)	7	4

[a] Male and female Wistar Han rats were dosed with 0, 3, 15, or 50 mg DE-71 (mixture of polybrominated diphenyl ethers)/kg body weight by oral gavage for 2 years. Silent mutations are not included. Non-tumor Liver- 0 mg/kg (9 males + 1 female); Hepatocellular Tumors- 0 mg/kg (3 males + 2 females); 3 mg/kg (2 males + 1 female); 15 mg/kg (4 males + 8 females); 50 mg/kg (9 males + 11 females).

[b] Number of tissues with mutations/number of tissues assayed (% with mutation)

[c] Compared to mice, the hepatocellular carcinoma (HCC) incidence was lower in the rats and hence, hepatocellular adenomas (HCA) were also included in the mutation analysis. The rat HCA and HCC included in this study included: controls (5 HCA); 3 mg/kg (3 HCA); 15 mg/kg (11 HCA and 1 HCC); 50 mg/kg [14 HCA and 6 HCC (3 HCC had *Hras* mutations, 1 HCC had *Ctnnb1* mutation)]

[d] Double mutations in one tumor/animal

TABLE N4
Summary of *Hras* and *Ctnnb1* Mutations in Non-tumor Liver Tissue and Hepatocellular Carcinomas from B6C3F1/N Mice in the 2-Year Gavage Study of DE-71[a]

Tissue – DE-71 Dose (mg/kg)	Mutation Frequency		*Hras* Cdn 61 (CAA)			*Ctnnb1* Cdn 15-46
	Hras[b]	*Ctnnb1*[b]	AAA	CGA	CTA	
Non-tumor Liver - 0	0/8 (0)	0/8 (0)	0	0	0	0
Hepatocellular Carcinomas - 0	2/17 (12)	0/17 (0)##	2	0	0	0
- 3	2/14 (14)	3/14 (21)	1	1	0	3
- 30	3/19 (16)	1/19 (5)	2	0	1	1
- 100	1/29 (3)	9/29 (31)**	1	0	0	9
Historical Spontaneous Hepatocellular Carcinomas[c]	276/513 (54)	1/79 (1)	167	80	29	1
DE-71-treated combined	6/62 (10)	13/62 (21)*	4	1	1	13

* Significantly different ($P<0.05$) from the spontaneous hepatocellular carcinomas (from vehicle control) by the Fisher exact test
** $P<0.01$
Significant dose-related trend ($P<0.01$) across the hepatocellular carcinoma groups by the Cochran-Armitage trend test
[a] Male and female B6C3F1/N mice were dosed with 0, 3, 30, or 100 mg DE-71 (mixture of polybrominated diphenyl ethers)/kg body weight by oral gavage for 2 years. Silent mutations are not included. Non-tumor Liver- 0 mg/kg (3 males + 5 females); Hepatocellular Carcinomas- 0 mg/kg (14 males + 3 females); 3 mg/kg (12 males + 2 females); 30 mg/kg (13 males + 6 females); 100 mg/kg (15 males + 14 females).
[b] Number of tissues with mutations/number of tissues assayed (% with mutation)
[c] Historical database for *Hras* and *Ctnnb1* mutations in spontaneous hepatocellular carcinomas (Sills *et al.*, 1999; Hayashi *et al.*, 2003; unpublished data)