

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF A BINARY MIXTURE OF

3,3',4,4',5-PENTACHLOROBIPHENYL (PCB 126)
(CAS NO. 57465-28-8)

AND

2,3',4,4',5-PENTACHLOROBIPHENYL (PCB 118)
(CAS NO. 31508-00-6)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)



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

November 2006

NTP TR 531

NIH Publication No. 07-4467

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 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/>) or in hardcopy upon request from the NTP Central Data Management group at cdm@niehs.nih.gov or (919) 541-3419.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF A BINARY MIXTURE OF

3,3',4,4',5-PENTACHLOROBIPHENYL (PCB 126)
(CAS NO. 57465-28-8)

AND

2,3',4,4',5-PENTACHLOROBIPHENYL (PCB 118)
(CAS NO. 31508-00-6)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)



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

November 2006

NTP TR 531

NIH Publication No. 07-4467

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

N.J. Walker, Ph.D., Study Scientist
 A. Nyska, D.V.M., Study Pathologist
 D.W. Bristol, Ph.D.
 J.R. Bucher, Ph.D.
 J.R. Hailey, D.V.M.
 R.A. Herbert, D.V.M., Ph.D.
 G.E. Kissling, Ph.D.
 R.R. Maronpot, D.V.M.
 D.P. Orzech, M.S.
 C.S. Smith, Ph.D.
 G.S. Travlos, D.V.M.
 M.E. Wyde, Ph.D.

Battelle Columbus Operations

Conducted studies and evaluated pathology findings

M.R. Hejtmancik, Ph.D., Principal Investigator
 D.M. Sells, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology review

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

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Constella Group, Inc.

Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
 L.J. Betz, M.S.
 M.E. Easterling, Ph.D.
 K.P. McGowan, M.B.A.
 J.T. Scott, M.S.
 C.J. Wallwork, Ph.D.

NTP Pathology Working Group

*Evaluated slides and prepared pathology report on rats
 (February 12, 2004)*

M.P. Jokinen, D.V.M., Chairperson
 Pathology Associates, A Charles River Company
 A.E. Brix, D.V.M., Ph.D.
 Experimental Pathology Laboratories
 J.R. Hailey, D.V.M.
 National Toxicology Program
 R.A. Herbert, D.V.M., Ph.D.
 National Toxicology Program
 K. Karli, D.V.M., Observer
 National Toxicology Program
 J. Nold, D.V.M.
 GlaxoSmithKline
 A. Nyska, D.V.M.
 National Toxicology Program
 D.M. Sells, D.V.M., Ph.D.
 Battelle Columbus Operations
 Y. Tani, D.V.M., Ph.D.
 National Toxicology Program

Biotechnical Services, Inc.

Prepared Technical Report

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

CONTENTS

ABSTRACT	5
EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY	11
TECHNICAL REPORTS REVIEW SUBCOMMITTEE	12
SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS	13
OVERVIEW	15
INTRODUCTION	21
MATERIALS AND METHODS	29
RESULTS	37
DISCUSSION AND CONCLUSIONS	65
REFERENCES	77
APPENDIX A Summary of Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	91
APPENDIX B Organ Weights and Organ-Weight-to-Body-Weight Ratios	161
APPENDIX C Chemical Characterization and Dose Formulation Studies	165
APPENDIX D Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration	177
APPENDIX E Sentinel Animal Program	185
APPENDIX F Physiologically Based Pharmacokinetic Model	187
APPENDIX G Transplantation of Liver and Lung Neoplasms	209
APPENDIX H Associated Publications	217

SUMMARY

Background

3,3',4,4',5-Pentachlorobiphenyl (PCB 126) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118) are members of a class of chemicals containing chlorine and related in structure to dioxins. Some dioxins or dioxin-like compounds are highly toxic and cause cancer. Contaminated sites usually contain many different varieties of these dioxin-like compounds. The National Toxicology Program conducted a series of studies to try to gauge the relative toxicity of the more common of these compounds, both alone and in mixtures. This study was originally designed as a test of PCB 118 alone. However, it was discovered that the test material contained a minor (0.6%) contamination with PCB 126, known to be a potent carcinogen. Thus the study was reclassified as a mixture study of PCB 126 and PCB 118.

Methods

We exposed groups of 53, 54, or 66 female rats by depositing mixtures of PCB 126 and PCB 118 dissolved in corn oil through a tube directly into their stomachs five days a week for two years. Daily doses were 10, 30, 100, 300, or 500 micrograms of PCB 118, each with 0.6% PCB 126, per kilogram body weight. Animals receiving the corn oil alone served as the control group. Tissues from more than 40 sites were examined for every animal.

Results

Female rats administered the mixtures of PCB 126 and PCB 118 developed a variety of diseases in several organs, including cancers of the liver, lung, and mouth. A variety of other toxic lesions observed in exposed animals included hypertrophy, hyperplasia, hepatopathy, fibrosis, and necrosis of the liver, hyperplasia of the oral mucosa, metaplasia of the lung, atrophy of the thymus, hypertrophy of the thyroid gland, atrophy of the adrenal cortex, vacuolization and atrophy of the pancreas, kidney nephropathy, hyperplasia of the nose, hyperplasia of the forestomach, hemorrhage of lymph nodes and brain, and atrophy of the spleen.

Conclusions

We conclude that the mixtures of PCB 126 and PCB 118 caused cancer and other toxic effects at several sites in female rats.

ABSTRACT

DIOXIN TOXIC EQUIVALENCY FACTOR EVALUATION OVERVIEW

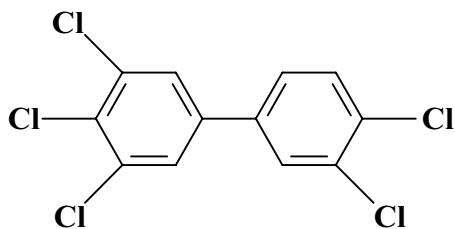
Polyhalogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have the ability to bind to and activate the ligand-activated transcription factor, the aryl hydrocarbon receptor (AhR). Structurally related compounds that bind to the AhR and exhibit biological actions similar to TCDD are commonly referred to as “dioxin-like compounds” (DLCs). Ambient human exposure to DLCs occurs through the ingestion of foods containing residues of DLCs that bio-concentrate through the food chain. Due to their lipophilicity and persistence, once internalized they accumulate in body tissues, mainly adipose, resulting in chronic lifetime human exposure.

Since human exposure to DLCs always occurs as a complex mixture, the toxic equivalency factor (TEF) methodology has been developed as a mathematical tool to assess the health risk posed by complex mixtures of these compounds. The TEF methodology is a relative potency scheme that ranks the dioxin-like activity of a compound relative to TCDD, which is the most potent congener. This allows for the estimation of the potential dioxin-like activity of a mixture of chemicals, based on a common mechanism of action involving an initial binding of DLCs to the AhR.

The toxic equivalency of DLCs was nominated for evaluation because of the widespread human exposure to DLCs and the lack of data on the adequacy of the TEF methodology for predicting relative potency for cancer risk. To address this, the National Toxicology Program

conducted a series of 2-year bioassays in female Harlan Sprague-Dawley rats to evaluate the chronic toxicity and carcinogenicity of DLCs and structurally related polychlorinated biphenyls (PCBs) and mixtures of these compounds.

Mixtures of polychlorinated biphenyls (PCBs) including 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118) were produced commercially before 1977 for the electric industry as dielectric insulating fluids for transformers and capacitors. Manufacture and use of these chemicals were stopped because of increased PCB residues in the environment, but they continue to be released into the environment through the use and disposal of products containing PCBs, as by-products during the manufacture of certain organic chemicals, during combustion of some waste materials, and during atmospheric recycling. This PCB mixture study was conducted as part of the dioxin TEF evaluation that includes conducting multiple 2-year rat bioassays to evaluate the relative chronic toxicity and carcinogenicity of DLCs, structurally related PCBs, and mixtures of these compounds. This study was originally a study of PCB 118 alone. However, midway through the study PCB 126 was identified as one of the minor contaminants (0.622%) of the bulk PCB 118 (98.5% pure). Given the 1,000-fold higher potency of PCB 126 for inducing dioxin-like effects (based on the TEFs for PCB 126 and PCB 118 of 0.1 and 0.0001, respectively), it was expected that the effects of administration of this compound would be due to the combined dioxin-like effects of both PCB 126 and PCB 118. Therefore, this study was reclassified as a mixture study of PCB 126 and PCB 118.

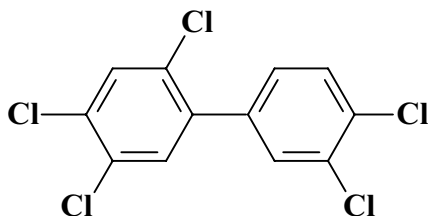


3,3',4,4',5-Pentachlorobiphenyl
PCB 126

CAS No. 57465-28-8

Chemical Formula: $C_{12}H_5Cl_5$ Molecular Weight: 326.42

Synonym: 1,1'-Biphenyl, 3,3',4,4',5-pentachloro-(9Cl)



2,3',4,4',5-Pentachlorobiphenyl
PCB 118

CAS No. 31508-00-6

Chemical Formula: $C_{12}H_5Cl_5$ Molecular Weight: 326.43

Synonyms: 1,1'-Biphenyl, 2,3',4,4',5-pentachloro-(9Cl); 1,1'-biphenyl, 2,3',4,4',5-pentachloro-; 2,3',4,4',5-pentachloro-1,1'-biphenyl; 2,4,5,3',4'-pentachlorobiphenyl; 3,4,2',4',5'-pentachlorobiphenyl; biphenyl, 2,3',4,4',5-pentachloro-; CB 118; PCB 118

2-YEAR STUDY

Groups of female Harlan Sprague-Dawley rats were administered the PCB mixture containing PCB 126 and PCB 118 by gavage in corn oil:acetone (99:1) or vehicle alone, 5 days per week for up to 104 weeks. Dose groups are referred to by the total levels of TCDD toxic equivalents (TEQ) provided by the PCBs in the mixture in each dose group. Groups of 81 female rats were administered 7, 22, 72, or 216 ng TEQ/kg; a group of

86 female rats was administered 360 ng TEQ/kg; and a group of 81 female rats was administered the corn oil:acetone vehicle alone. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. No animals in the 360 ng TEQ/kg group were examined at 53 weeks. A group of 50 female rats was administered 360 ng TEQ/kg for 30 weeks and then the vehicle alone for the remainder of the study. Nominal doses of PCB 118 and levels of PCB 126 in each dose group used were:

7 ng TEQ/kg dose group:	62 ng/kg PCB 126 and 10 µg/kg PCB 118
22 ng TEQ/kg dose group:	187 ng/kg PCB 126 and 30 µg/kg PCB 118
72 ng TEQ/kg dose group:	622 ng/kg PCB 126 and 100 µg/kg PCB 118
216 ng TEQ/kg dose group:	1,866 ng/kg PCB 126 and 300 µg/kg PCB 118
360 ng TEQ/kg dose group:	3,110 ng/kg PCB 126 and 500 µg/kg PCB 118

No animals in the 216 or 360 ng TEQ/kg core study groups survived to the end of the study, and survival in the 360 ng TEQ/kg stop-exposure group was significantly less than in the vehicle control group. Mean body weights of 72 ng TEQ/kg rats were less than those of the vehicle controls after week 33 of the study, and mean body weights of the 216 and 360 ng TEQ/kg core study rats and the 360 ng TEQ/kg stop-exposure group rats were less than those of the vehicle controls throughout most of the study. Clinical findings related to the administration of the binary mixture of PCB 126 and PCB 118 included abnormal breathing, thinness, and ruffled hair.

Thyroid Hormone Concentrations

Alterations in serum thyroid hormone levels were evaluated at the 14-, 31-, and 53-week interim evaluations. Total thyroxine (T_4) and free T_4 were significantly lower in most dose groups than in vehicle controls at the 14- and 31-week interim evaluations. Serum T_3 was significantly lower in the 360 ng TEQ/kg group compared to vehicle controls at 31 weeks only. TSH levels were higher in the 216 and 360 ng TEQ/kg groups than in vehicle controls at 31 weeks only.

Hepatic Cell Proliferation Data

To evaluate hepatocyte replication, analysis of labeling of replicating hepatocytes with 5-bromo-2'-deoxyuridine was conducted at the 14-, 31-, and 53-week interim evaluations. Labeling indices were elevated at doses above 216 ng TEQ/kg at 31 weeks and at doses above 72 ng TEQ/kg at 53 weeks.

Cytochrome P450 Enzyme Activities

CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) and CYP1A2-associated acetanilide-4-hydroxylase (A4H) activities were evaluated at the 14-, 31-, and 53-week interim evaluations to evaluate the expression of known dioxin-responsive genes. In addition, CYP2B-associated pentoxyresorufin-*O*-deethylase (PROD) activity was also analyzed. Hepatic and pulmonary EROD (CYP1A1) activity, hepatic A4H (CYP1A2) activity, and hepatic PROD (CYP2B1) activity were significantly greater in all dosed groups compared to the vehicle controls at weeks 14, 31, and 53.

Determinations of PCB 126 and PCB 118 Concentrations in Tissues

The tissue disposition of PCB 126 and PCB 118 was analyzed in the liver, lung, fat, and blood of up to 10 rats in each group at the 14-, 31-, and 53-week interim evaluations, except for the 360 ng TEQ/kg group at

53 weeks. The tissue disposition of PCB 126 and PCB 118 was also analyzed in 10 rats per group at the end of the 2-year study in the vehicle control, 7, 22, and 72 ng TEQ/kg core study groups and the 360 ng TEQ/kg stop-exposure group. Detectable concentrations of PCB 126 and PCB 118 were observed in the liver, fat, lung, and blood. The highest levels of PCB 126 were seen in the liver whereas the highest levels of PCB 118 were seen in the fat. In general, tissue concentrations increased with increasing doses of the mixture and increasing duration of exposure. Hepatic levels of PCB 126 and PCB 118 in the 72 ng TEQ/kg group at the end of the 2-year study were 284 ng/g and 3,769 ng/g, respectively. On a TCDD equivalents basis this corresponds to 28 ng TEQ/g and 0.4 ng TEQ/g for PCB 126 and PCB 118, respectively. Cessation of administration of the mixture in the stop-exposure group led to declines in the tissue concentrations of both PCB 126 and PCB 118 to levels comparable to those observed in the 7 ng TEQ/kg group at the end of the 2-year study.

Pathology and Statistical Analyses

At 14, 31, and 53 weeks, liver weights were significantly increased in treated groups with more pronounced effects occurring in the higher dose groups. At 14 weeks, hepatocyte hypertrophy and pigmentation were seen at doses less than 72 ng TEQ/kg. Exposure to the PCB mixture led to significant toxicity in the liver. At higher doses, the incidences of toxic hepatopathy were increased as indicated by increased incidences of multinucleated hepatocytes and diffuse fatty change. At 31 weeks, most rats in the 216 and 360 ng TEQ/kg groups had multiple hepatic nonneoplastic lesions. At 53 weeks all animals administered 216 ng TEQ/kg had multiple nonneoplastic lesions. The spectrum of effects and the severity of effects at the interim and 2-year time points increased with dose and duration of exposure. At the end of the 2-year study in all dosed groups, there were significantly increased incidences and severity of toxic hepatopathy characterized by hepatocyte hypertrophy, multinucleated hepatocytes, pigmentation, toxic hepatopathy, diffuse fatty change, nodular hyperplasia, centrilobular fibrosis, cholangiofibrosis, oval cell hyperplasia, bile duct cyst, bile duct hyperplasia, and portal fibrosis. There were also increased incidences of hepatocyte glandular structures, necrosis, centrilobular degeneration, eosinophilic focus, and metaplasia.

The incidences of cholangiocarcinoma (multiple and/or single) were significantly increased in groups administered 22 ng TEQ/kg or greater at 2 years. The incidences

of hepatocellular adenoma were also significantly increased in the 216 and 360 ng TEQ/kg core study groups. In addition, single occurrences of hepatocholangioma, cholangioma, or hepatocellular carcinoma were observed in some dosed groups administered 72 ng TEQ/kg or greater.

In the lung at 53 weeks, the incidences of cystic keratinizing epithelioma and bronchiolar metaplasia were significantly increased in the 216 ng TEQ/kg group. Significantly increased incidences of cystic keratinizing epithelioma (single or multiple) occurred in groups administered 72 ng TEQ/kg or greater at 2 years. Nonneoplastic effects observed in the lung included bronchiolar metaplasia of the alveolar epithelium, squamous metaplasia, serosal fibrosis, and keratin cysts in the stop-exposure group.

Increased incidences of gingival squamous cell carcinoma of the oral mucosa were observed at the end of the 2-year study and were accompanied by increased incidences of gingival squamous hyperplasia of the oral mucosa.

Numerous nonneoplastic effects were seen in other organs at the interim time points including atrophy of the thymus, follicular cell hypertrophy of the thyroid gland, atrophy of the adrenal cortex and acinar cytoplasmic vacuolization of the pancreas. These responses were also affected by administration of PCB 126:PCB 118 at the end of the 2-year study and were accompanied by additional nonneoplastic responses in numerous organs including vacuolization of the adrenal cortex, acinar atrophy of the pancreas, and chronic active inflammation

of the pancreatic acinus. In the kidney, severity of nephropathy was increased. Effects on the cardiovascular system were seen including cardiomyopathy of the heart, chronic active inflammation of the coronary artery, inflammation of the epicardium, and chronic active inflammation of the mesenteric and pancreatic arteries. Other nonneoplastic lesions that were treatment related were hemorrhage of the brain and mandibular, mesenteric, and mediastinal lymph nodes; forestomach hyperplasia; hyperplasia of the nasal respiratory epithelium; metaplasia of the olfactory epithelium; and atrophy of the lymphoid follicle of the spleen.

CONCLUSIONS

Under the conditions of this 2-year gavage study there was *clear evidence of carcinogenic activity** of the mixture of PCB 126 and PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma and hepatocellular neoplasms (predominantly hepatocellular adenomas) of the liver and cystic keratinizing epithelioma of the lung. The marginally increased incidences of gingival squamous cell carcinoma of the oral mucosa were also considered to be related to administration of the mixture of PCB 126 and PCB 118. Occurrences of cholangioma and hepatocholangioma of the liver may have been related to administration of the mixture of PCB 126 and PCB 118.

Administration of the mixture of PCB 126 and PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, thymus, thyroid gland, adrenal cortex, pancreas, kidney, heart, lymph nodes, mesenteric artery, brain, forestomach, spleen, and nose.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Toxicology Study of a Binary Mixture of PCB 126 and PCB 118 in Female Sprague-Dawley Rats

Doses in corn oil/acetone by gavage

0 ng TEQ/kg
7 ng TEQ/kg (62 ng/kg PCB 126:10 µg/kg PCB 118)
22 ng TEQ/kg (187 ng/kg PCB 126:30 µg/kg PCB 118)
72 ng TEQ/kg (622 ng/kg PCB 126:100 µg/kg PCB 118)
216 ng TEQ/kg (1,866 ng/kg PCB 126:300 µg/kg PCB 118)
360 ng TEQ/kg (3,110 ng/kg PCB 126:500 µg/kg PCB 118)
360 ng TEQ/kg (3,110 ng/kg PCB 126:500 µg/kg PCB 118) stop-exposure group

Body weights

72, 216, and 360 ng TEQ/kg core study groups and 360 ng TEQ/kg stop-exposure group less than the vehicle control group

Survival rates

27/53, 17/54, 24/53, 15/53, 0/53, 0/66, 10/50

Nonneoplastic effects

Liver:

hepatocyte hypertrophy (0/53, 16/51, 22/53, 47/53, 52/53, 65/65, 36/50)
multinucleated hepatocyte (0/53, 9/51, 21/53, 48/53, 52/53, 48/65, 38/50)
hepatocyte glandular structure (0/53, 0/51, 0/53, 1/53, 5/53, 25/65, 9/50)
pigmentation (5/53, 18/51, 38/53, 52/53, 53/53, 60/65, 50/50)
toxic hepatopathy (0/53, 8/51, 32/53, 51/53, 52/53, 64/65, 50/50)
diffuse fatty change (3/53, 14/51, 27/53, 45/53, 49/53, 56/65, 35/50)
nodular hyperplasia (0/53, 0/51, 10/53, 45/53, 50/53, 61/65, 34/50)
centrilobular fibrosis (0/53, 0/51, 7/53, 36/53, 52/53, 57/65, 43/50)
necrosis (3/53, 13/51, 6/53, 26/53, 17/53, 27/65, 6/50)
oval cell hyperplasia (4/53, 4/51, 23/53, 49/53, 52/53, 62/65, 43/50)
bile duct cyst (4/53, 3/51, 3/53, 14/53, 25/53, 14/65, 21/50)
bile duct hyperplasia (5/53, 5/51, 7/53, 46/53, 52/53, 60/65, 37/50)
portal fibrosis (0/53, 0/51, 2/53, 37/53, 52/53, 58/65, 42/50)
cholangiofibrosis (1/53, 2/51, 3/53, 21/53, 42/53, 29/65, 27/50)
eosinophilic focus (10/53, 21/51, 23/53, 15/53, 6/53, 10/65, 15/50)
focal fatty change (10/53, 10/51, 6/53, 1/53, 10/53, 6/65, 14/50)
centrilobular degeneration (2/53, 3/51, 8/53, 8/53, 3/53, 8/65, 6/50)
metaplasia (0/53, 0/51, 0/53, 0/53, 4/53, 4/65, 2/50)

Lung:

alveolar epithelium metaplasia bronchiolar (1/53, 14/51, 39/53, 46/53, 35/53, 8/66, 15/50)
squamous metaplasia (0/53, 1/51, 2/53, 14/53, 16/53, 7/66, 8/50)
serosal fibrosis (3/53, 0/51, 0/53, 1/53, 16/53, 8/66, 1/50)
keratin cyst (0/53, 0/51, 0/53, 0/53, 0/66, 9/50)

Oral mucosa:

gingival squamous hyperplasia (11/53, 10/51, 20/53, 24/53, 27/53, 18/66, 18/50)

Thymus:

atrophy (40/53, 44/51, 44/51, 49/51, 50/51, 57/59, 45/46)
severity of atrophy (2.7, 2.5, 3.0, 3.9, 3.8, 3.8, 3.4)

Thyroid gland:

follicular cell hypertrophy (22/53, 35/51, 34/53, 38/52, 44/52, 37/66, 29/49)

Adrenal cortex:

atrophy (2/53, 1/51, 0/53, 15/53, 44/53, 40/65, 12/50)
cytoplasmic vacuolization (14/53, 16/51, 13/53, 24/53, 27/53, 12/65, 16/50)

Pancreas:

acinus cytoplasmic vacuolization (0/53, 1/51, 8/53, 39/53, 49/53, 43/65, 41/50)
chronic active artery inflammation (0/53, 2/51, 2/53, 21/53, 14/53, 4/65, 10/50)
acinus atrophy (1/53, 5/51, 3/53, 5/53, 9/53, 8/65, 8/50)
chronic active inflammation (1/53, 5/51, 4/53, 3/53, 2/53, 6/65, 5/50)

Summary of the 2-Year Carcinogenesis and Toxicology Study of a Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats

Kidney:

nephropathy (41/53, 37/51, 37/53, 48/53, 50/53, 43/65, 34/50)
severity of nephropathy (1.1, 1.2, 1.2, 2.0, 2.0, 1.4, 2.0)

Heart:

cardiomyopathy (20/53, 13/51, 17/53, 34/53, 14/53, 19/65, 15/50)
coronary artery chronic active inflammation (1/53, 1/51, 0/53, 7/53, 10/53, 9/65, 6/50)
epicardium inflammation (1/53, 1/51, 0/53, 6/53, 13/53, 6/65, 1/50)

Mandibular lymph node:

hemorrhage (0/53, 0/51, 1/53, 0/52, 2/52, 7/66, 4/49)

Mediastinal lymph node:

hemorrhage (2/28, 2/16, 4/16, 15/25, 7/20, 25/45, 11/22)

Mesenteric lymph node:

hemorrhage (0/53, 2/51, 2/53, 2/53, 4/52, 14/65, 10/50)

Mesentery:

artery chronic active inflammation (0/53, 1/35, 1/45, 9/49, 9/52, 3/63, 7/43)

Brain:

hemorrhage (0/53, 1/51, 1/53, 4/53, 5/53, 14/66, 8/50)

Forestomach:

squamous hyperplasia (4/53, 1/51, 3/53, 11/53, 4/53, 9/65, 2/50)

Spleen:

lymphoid follicle atrophy (4/53, 5/51, 2/53, 5/53, 3/53, 9/65, 4/50)

Nose:

inflammation (17/53, 15/51, 16/53, 10/53, 22/53, 11/66, 11/50)
respiratory epithelium hyperplasia (8/53, 11/51, 5/53, 8/53, 16/53, 9/66, 10/50)
olfactory epithelium metaplasia (0/53, 2/51, 1/53, 3/53, 8/53, 1/66, 5/50)

Neoplastic effects

Liver:

cholangiocarcinoma (0/53, 0/51, 5/53, 19/53, 28/53, 12/65, 19/50)
hepatocellular adenoma (2/53, 1/51, 0/53, 4/53, 17/53, 5/65, 1/50)
hepatocellular carcinoma (0/53, 0/51, 0/53, 0/53, 1/53, 0/65, 0/50)

Lung:

cystic keratinizing epithelioma (0/53, 0/51, 0/53, 20/53, 49/53, 41/66, 12/50)

Oral mucosa:

gingival squamous cell carcinoma (1/53, 1/51, 2/53, 4/53, 0/53, 1/66, 1/50)

Equivocal findings

Liver:

hepatocholangioma (0/53, 0/51, 0/53, 1/53, 1/53, 1/65, 1/50)
cholangioma (0/53, 0/51, 0/53, 1/53, 0/53, 0/65, 0/50)

Level of evidence of carcinogenic activity

Clear evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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 in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

**NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE**

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on the binary mixture of PCB 126 and PCB 118 on December 9, 2004, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee 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.

Mary Anna Thrall, D.V.M., M.S., Chairperson
Department of Microbiology, Immunology and Pathology
Colorado State University
Fort Collins, CO

James E. Klaunig, Ph.D.
Division of Toxicology
Indiana University School of Medicine
Indianapolis, IN

Diane F. Birt, Ph.D.
Department of Food Science & Human Nutrition
Iowa State University
Ames, IA

Stephen M. Roberts, Ph.D., Principal Reviewer
Center for Environmental & Human Toxicology
University of Florida
Gainesville, FL

Kim Boekelheide, M.D., Ph.D., Principal Reviewer
Division of Biology and Medicine
Department of Pathology and Laboratory Medicine
Brown University
Providence, RI

Richard D. Storer, M.P.H., Ph.D.
Department of Genetic and Cellular Toxicology
Merck Research Laboratories
West Point, PA

Michael R. Elwell, D.V.M., Ph.D., Principal Reviewer
Pathology, Drug Safety Evaluation
Pfizer Global Research and Development
Groton, CT

Mary Vore, Ph.D.
Graduate Center for Toxicology
University of Kentucky
Lexington, KY

Thomas A. Gasiewicz, Ph.D.
Department of Environmental Medicine
Environmental Health Sciences Center
University of Rochester School of Medicine
Rochester, NY

Cheryl Lyn Walker, Ph.D.
Department of Carcinogenesis
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 9, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118) received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. N.J. Walker, NIEHS, presented the background, design, and goals of the NTP study series on the Toxic Equivalency Factor evaluations of mixtures of dioxin-like compounds (dioxins, PCBs, furans).

Dr. Walker, NIEHS, introduced the toxicology and carcinogenesis studies of a binary mixture of PCB 126 and PCB 118 by noting that the study was originally designed to study PCB 118 alone. However, during the course of the study, analyses revealed the presence of 0.622% of the much more potent PCB 126. The study was continued as a mixture study because humans are exposed to both types of PCBs. Dr. Walker described the study design and the spectrum of lesions in the liver,

lung, and oral mucosa, and a variety of nonneoplastic lesions. The proposed conclusions were *clear evidence of carcinogenic activity* of the PCB 126 and PCB 118 mixture in female Harlan Sprague-Dawley rats.

Dr. Roberts, the first principal reviewer, agreed in principle with the proposed conclusions, except he felt that the hepatocellular lesions should just be identified as adenomas, as only one carcinoma was seen in all the groups.

Dr. Boekelheide, the second principal reviewer, also agreed with the proposed conclusions.

Dr. Elwell, the third principal reviewer, inquired if the transplantation studies would be added to the final version of the report. (Appendix G was added.)

Dr. Roberts moved that the conclusions be accepted as written, clear evidence of carcinogenic activity of PCB 126 and PCB 118 in female Harlan Sprague-Dawley rats. Dr. Boekelheide seconded the motion. The motion was passed unanimously with nine votes.

OVERVIEW

DIOXIN TOXIC EQUIVALENCY FACTOR EVALUATION

Polyhalogenated Aromatic Hydrocarbons and Human Exposure

Polyhalogenated aromatic hydrocarbons (PHAHs) comprise a large class of compounds including polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and polybrominated diphenyl ethers (PBDEs).

PCDDs and PCDFs are not manufactured for commercial purposes. They are unwanted by-products of many anthropogenic activities, including combustion processes such as forest and backyard trash fires and manufacturing processes for herbicides and paper. PCB mixtures were commercially produced and used in the electric power industry as dielectric insulating fluids in transformers and capacitors and used in hydraulic fluids, plastics, and paints. PCNs were produced and used as dielectric fluids in capacitors, transformers, and cables. PBDEs are flame retardants, used in the manufacture of items including paints, foams, textiles, furniture, and household plastics (USEPA, 2000a).

Because these compounds are resistant to degradation and persist in the environment, they have the ability to bioaccumulate and become more concentrated. Ambient human exposure to PHAHs occurs through the ingestion of foods containing PHAH residues. Due to their persistence and lipophilicity, once internalized, they accumulate in body tissues, mainly adipose, resulting in chronic lifetime human exposure (Schechter *et al.*, 1994).

Dioxin-like Compounds

Depending on the location and type of the halogenation, some PHAHs, most notably certain PCDDs, PCDFs, and PCBs, have the ability to bind to a cytosolic receptor known as the aryl hydrocarbon receptor (AhR) (Safe, 1990; Whitlock, 1990). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), commonly referred to as “dioxin,” is the most well-characterized member of these structurally related compounds and exhibits the highest potency of

binding to the AhR. Depending upon the number and position of the substitutions, there are potentially 75 PCDDs, 135 PCDFs, and 209 PCBs. Structurally related compounds that bind to the AhR and exhibit biological actions similar to TCDD are commonly referred to as dioxin-like compounds (DLCs). There are seven PCDDs, 10 PCDFs, and 13 PCBs that exhibit such dioxin-like activity (USEPA, 2000b). In addition to the persistent DLCs, there are a wide variety of other compounds that can also bind to the AhR, including polycyclic aromatic hydrocarbons (e.g., benzo(*a*)pyrene found in cigarette smoke), dietary indoles (e.g., indole-3-carbinol found in cruciferous vegetables), dietary flavonoids (e.g., quercetin, kaempferol), and heme degradation products (e.g., bilirubin/biliverdin).

The persistent PHAHs and DLCs have been the subject of an extensive amount of research regarding environmental levels, transport, and fate; human exposure; mechanisms of action; and toxicity that is beyond the scope of this report. The extensive body of knowledge on TCDD and related compounds has been fully reviewed by the International Agency for Research on Cancer (1997), the Agency for Toxic Substances and Disease Registry (1998, 2000), and by the United States Environmental Protection Agency (2000a,b,c); therefore, it will not be re-reviewed in depth in this Technical Report.

Mechanism of Action via the Aryl Hydrocarbon Receptor

Based on the extensive body of research on the induction of the cytochrome P450 1A1 (CYP1A1) gene by TCDD, the primary mechanism of action of DLCs involves initial binding to the AhR (Schmidt and Bradfield, 1996). The AhR is a protein found as a multimeric complex in the cytosol of all vertebrate species and acts as a ligand-activated transcription factor. Initial binding of ligand to the receptor disrupts the receptor complex leading to receptor activation and translocation into the nucleus where it heterodimerizes with the AhR nuclear translocator protein (ARNT) (Gu *et al.*, 2000). The AhR-ARNT heterodimer binds to specific cognate DNA sequence elements known as dioxin/xenobiotic response

elements (DRE/XRE) present in the regulatory region of specific genes such as CYP1A1. Binding of the AhR-ARNT heterodimer to these elements leads to increased transcription of the specific gene. The characteristic response to TCDD is the transcriptional induction of CYP1A1, which is mediated by binding of the heterodimer to DREs present in the 5' flanking region of the gene. The AhR is expressed in all tissues with a definite tissue specificity in terms of level of expression and diversity of response. TCDD has been shown to modulate numerous growth factor, cytokine, hormone, and metabolic pathways in animals and experimental systems. Many, if not all, are parts of pathways involved in cellular proliferation and differentiation and, taken together, they provide a plausible mechanism for toxicity and carcinogenicity. Most of the molecular details for induction of gene expression via the AhR have been characterized for the transcriptional activation of the CYP1A1 gene. The expression of many genes has been shown to be affected by TCDD (Puga *et al.*, 2000; Frueh *et al.*, 2001; Martinez *et al.*, 2002), yet there is evidence for direct transcriptional activation through the AhR for only a very few of these (Sutter and Greenlee, 1992).

Toxicity of Dioxin-like Compounds

High doses and/or continuous exposure to dioxins lead to a broad spectrum of toxic responses including death, immunosuppression, carcinogenicity, and impaired reproduction and development (Whitlock, 1990; ATSDR, 1998; Grassman *et al.*, 1998; USEPA, 2000c). The type of toxicity is dependent on the magnitude of dose, duration and pattern of exposure, timing of exposure, species, and gender. A generalized mode of action for toxicity induced by dioxins is one that involves initial binding of the compounds to the AhR. Subsequent alterations in expression of specific genes and alterations in biological signal transduction pathways lead to an alteration in growth regulation and differentiation that leads to pathology and toxicity.

The broad spectrum of DLC effects on hormone and growth factor systems, cytokines, and signal transduction pathways indicate powerful growth dysregulators. The effect of DLCs on growth regulation may be manifested through alterations in genes involved in cellular growth and homeostasis. Although the relationship between these effects and carcinogenesis can only be inferred, all of these effects are involved in cellular growth and differentiation; disruption of normal cellular processes could be a risk factor for carcinogenicity.

The initial involvement of the AhR in initiating this cascade of events is supported by studies showing the lower potency of structurally related compounds with lower affinity for the AhR, reduction of effects in rodents with lower AhR affinities (Pohjanvirta *et al.*, 1993; Birnbaum, 1994a), and the lack of effects using transgenic mice that lack AhR functionality (Gonzalez *et al.*, 1996; Gonzalez and Fernandez-Salguero, 1998; Gonzalez, 2001; Vorderstrasse *et al.*, 2001). These data indicate that the AhR is necessary, but may not be sufficient, for mediating the toxic action of DLCs.

Polyhalogenated Aromatic Hydrocarbon Mixtures and Toxic Equivalency Factors

PHAHs always exist in the environment as complex mixtures; therefore, normal background human exposure to PHAHs always occurs as a complex mixture. The toxic equivalency factor (TEF) approach has been developed to assess the risk posed by complex mixtures of PCDDs, PCDFs, and PCBs (Ahlborg *et al.*, 1992; Van den Berg *et al.*, 1998; USEPA, 2000c). The TEF methodology is a relative potency scheme to estimate the total exposure and dioxin-like effects of a mixture of chemicals based on a common mechanism of action involving an initial binding of the compound to the AhR. The TEF methodology is currently the most feasible interim approach for assessing and managing the risk posed by these mixtures and has been formally adopted by a number of countries including Canada, Germany, Italy, the Netherlands, Sweden, the United Kingdom, and the United States. The method is also used by the International Programme on Chemical Safety and the World Health Organization. Criteria for inclusion of a compound in the TEF methodology are structural relationship to PCDD/PCDFs, binding to the AhR, elicitation of AhR-mediated biochemical and toxic responses, and persistence and accumulation in the food chain.

The current World Health Organization (WHO) TEFs are based on a subjective evaluation of individual studies that examined the relative potency of a given chemical to the reference compound, TCDD, which is assigned a potency of 1. TEF values are an order of magnitude *estimate* of the overall "toxic potency" of a given compound and therefore do not specifically refer to the potency from any single study with a particular endpoint. By comparison, a relative potency factor is determined for a specific chemical in a single study relative to a specific endpoint. Therefore, a single TEF is based on an evaluation of multiple relative potency factors. The TEF

determination is a subjective assessment because the relative potency factors are derived from the literature and there is considerable variability in the types of studies, endpoints analyzed, and quality of procedures. Types of procedures for calculation of relative potency factors vary from a comparative dose response assessment (e.g., ratio of ED₅₀ or EC₅₀) to a simple administered dose ratio calculation. In evaluating different studies and endpoints, more weight is given to *in vivo* studies than to *in vitro* studies, chronic studies are weighted more than acute studies, and toxic responses are weighted more than simple biochemical responses.

An implicit assumption of the TEF methodology is that the combined effects of the different congeners are dose additive, which is supported by *in vivo* studies with mixtures of PCDDs and PCDFs, mixtures of PCDFs, and mixtures of PCBs and TCDD, and by *in vitro* studies with mixtures of PCBs and PCDFs (Birnbaum *et al.*, 1987; Schrenk *et al.*, 1991, 1994; Birnbaum and DeVito, 1995; USEPA, 2000c). Therefore, the total toxic equivalents (TEQs) for the AhR-mediated toxic potency of a mixture of PCDDs, PCDFs, and PCBs may be estimated by the summation of the mass of each congener in the mixture after adjustment for its potency. Currently only PCDDs, PCDFs, and certain PCBs are included in this TEF scheme.

$$\text{TEQ} = \sum_{ni} (\text{PCDD}_i \times \text{TEF}_i)_n + \sum_{ni} (\text{PCDF}_i \times \text{TEF}_i)_n + \sum_{ni} (\text{PCB}_i \times \text{TEF}_i)_n$$

where *i* = the individual congener and its respective TEF, and *n* = all congeners within each class of DLCs

Uncertainties in the Use of Toxic Equivalency Factors

While TEFs were developed initially as an interim approach to facilitate exposure assessment and hazard identification, there has been an increasing use of this scheme to determine TEQs in human tissues for dose-response assessment of effects in human populations (Flesch-Janys *et al.*, 1998). While the database for development of TEFs for DLCs is extensive, these data are for dioxin-regulated noncancer endpoints that often reflect simply the activation of the AhR. No mammalian studies have formally evaluated relative potency factors for a neoplastic endpoint. The mechanism by which activation of the AhR and subsequent changes in dioxin-responsive events leads to cancer is not known,

and the validity of current TEFs for predicting cancer risk has not been evaluated.

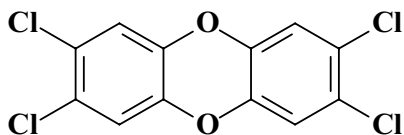
One of the implicit assumptions in the use of TEFs is that the TEQ for different compounds is dose additive. While dose additivity is supported for certain mixtures, for some biological endpoints in some models, this may not be true. As outlined by Van den Berg *et al.* (1998), the TEF methodology is likely valid for biological responses that are clearly AhR dependent, but may not be true for more complex biological responses such as neoplasia.

The Dioxin Toxic Equivalency Factor Evaluation Studies

To test the validity of the TEF approach for the prediction of cancer risk, the National Toxicology Program (NTP) has conducted multiple 2-year bioassays in female Sprague-Dawley rats to evaluate the chronic toxicity and carcinogenicity of DLCs, structurally related PCBs, and mixtures of these compounds. Specific hypotheses to be tested by these studies are:

1. TEFs for PCDDs, PCDFs, and PCBs can predict the relative carcinogenic potency of single congeners in female Sprague-Dawley rats.
2. TEFs for PCDDs, PCDFs, and planar PCBs can predict the relative carcinogenic potency of an environmentally relevant mixture of these chemicals in the female Sprague-Dawley rat.
3. The carcinogenicity of a dioxin-like, non-*ortho* substituted PCB is not altered by the presence of a mono-*ortho*- or di-*ortho*-substituted PCB.
4. Relative potencies for DLCs are dose additive.
5. The relative potencies for activation of biochemical endpoints, such as CYP1A1 induction, in the 2-year studies are equivalent to the relative potency for induction of carcinogenesis when estimated based on administered dose.
6. The relative potencies for activation of biochemical endpoints, such as CYP1A1 induction, in the 2-year studies are equivalent to the relative potency for induction of carcinogenesis when estimated based on target tissue dose.
7. The relative potencies for alteration of a given response are the same, regardless of the dose metric used (e.g., administered dose, serum or whole blood concentrations, or tissue dose).

***Individual Compounds, Mixtures,
and Rationale for Choice***

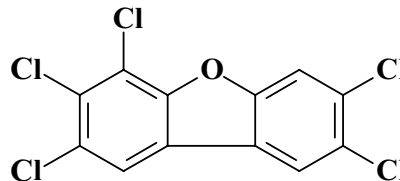


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD

CAS No. 1746-01-6

Chemical Formula: $C_{12}H_4Cl_4O_2$
Molecular weight: 321.98

TCDD is the most potent DLC and the reference compound to which all DLCs are compared in the TEF methodology. As such it has a TEF value of 1. TCDD is classified as a known human carcinogen by the NTP and the International Agency for Research on Cancer.

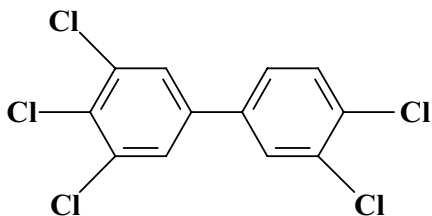


2,3,4,7,8-Pentachlorodibenzofuran
PeCDF

CAS No. 57117-31-4

Chemical Formula: $C_{12}H_3Cl_5O$
Molecular weight: 340.4

PeCDF is a dioxin-like PHAH with high bioaccumulation in the food chain and a TEF value of 0.5. This compound represents the most potent PCDF present in human tissues.

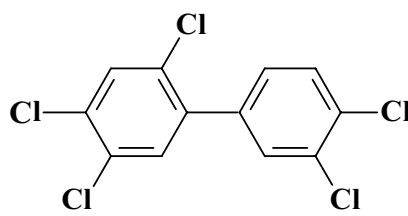


3,3',4,4',5-Pentachlorobiphenyl
PCB 126

CAS No. 57465-28-8

Chemical Formula: $C_{12}H_5Cl_5$
Molecular weight: 326.42

PCB 126 is a non-*ortho*-substituted PCB with high bioaccumulation in the food chain and a TEF value of 0.1. PCB 126 is considered the most potent dioxin-like PCB congener present in the environment and accounts for 40% to 90% of the total toxic potency of PCBs having a “dioxin-like” activity.

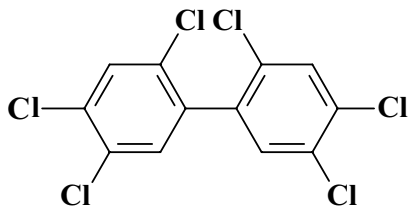


2,3',4,4',5-Pentachlorobiphenyl
PCB 118

CAS No. 31508-00-6

Chemical Formula: $C_{12}H_5Cl_5$
Molecular weight: 326.43

PCB 118 is a mono-*ortho*-substituted PCB that has partial dioxin-like activity. A tentative TEF value of 0.0001 has been assigned although there is controversy over whether mono-*ortho*-substituted PCBs should be included in the TEF methodology.



2,2',4,4',5,5'-Hexachlorobiphenyl
PCB 153

CAS No. 35065-27-1

Chemical Formula: $C_{12}H_4Cl_6$
Molecular weight: 360.88

PCB 153 is a di-*ortho*-substituted nonplanar PCB and is present at the highest concentrations in human samples on a molar basis. Nonplanar PCBs do not have dioxin-like activity and are not included in the TEF methodology; therefore, PCB 153 has no TEF value. Some studies have shown that nondioxin PCBs such as PCB 153 can antagonize the effects of DLCs.

Mixture Studies

Several mixture studies were conducted to assess the dose additivity of DLCs and interactions of PCBs.

Mixture of TCDD, PCB 126, and PeCDF

This mixture was designed to test for dose additivity of the highest potency DLCs in each of the three classes of PHAHs covered by the TEF methodology. The mixture was composed of equal TEQ ratios (1:1:1) of TCDD, PCB 126, and PeCDF. Total TEQ dosages ranged from 10 to 100 ng TEQ/kg per day. These compounds were chosen because they are the most potent members of the PCDDs, PCDFs, and coplanar PCBs. Based on average human tissue levels of these compounds, they represent approximately 48% of the human tissue burden of dioxin TEQs.

Binary mixture study of PCB 126 and PCB 153

Several studies have indicated an antagonism of the effects of DLCs by di-*ortho*-substituted PCBs such as PCB 153. This binary mixture study consisted of two parts:

1. PCB 126 and PCB 153 at the environmentally relevant ratio of 1:1,000. The dosage levels of PCB 126 were chosen to span the range used in the individual dose-response study of PCB 126.
2. Varying ratios of PCB 153 at the mid-dose of PCB 126 (300 ng/kg per day).

Binary mixture study of PCB 118 and PCB 126

This binary mixture was not designed *a priori* as part of the dioxin TEF evaluation. While the individual PCB 118 study was at the in-life phase, it was found that the PCB 118 compound being used contained not only PCB 118 but also 0.622% PCB 126 (PCB 118:PCB 126 of 161:1). Given the large TEF difference between PCB 118 (0.0001) and PCB 126 (0.1), this resulted in a TEQ ratio for PCB 126:PCB 118 of 6:1. As such, the effects of the compound would be expected to be due mainly to dioxin-like effects of PCB 126 rather than effects of PCB 118. In human tissues, the ratio of PCB 126:PCB 118, on a TEQ basis, ranges from 0.9:1 in blood, 3.9:1 in breast milk, and 15:1 in adipose tissue (USEPA, 2000b). The mass ratio of PCB 118:PCB 126 is on average 135:1 in beef fat and 190:1 in milk. Consequently, the PCB 118:PCB 126 ratio in this compound (161:1) represented an environmentally relevant mixture of PCBs on both a mass and TEQ basis. Since PCB 126 was already being studied, and the PCB 118 study was already in life, the PCB 118 study was continued to test for the effect of a mono-*ortho*-substituted PCB on a coplanar PCB at an environmentally relevant ratio. The PCB 118 was resynthesized and checked for the absence of high TEQ contributing compounds, and a new study was started.

STUDY DESIGN, SPECIES, AND DOSE SELECTION RATIONALE

These studies were conducted in female Harlan Sprague-Dawley rats based on the prior observations by Kociba *et al.* (1978) of the carcinogenicity of TCDD in this strain. Female rats were chosen based on the high potency of hepatocarcinogenicity in females in this strain. Male rats were not studied due to the lack of induction of liver and lung neoplasms in the previous studies of Sprague-Dawley rats with TCDD. Animals were dosed by oral gavage because the majority of human exposure is oral.

Dose selection for TCDD of 3 to 100 ng/kg per day was based on the range used in the Kociba *et al.* (1978) study and on the demonstrated induction of liver tumor incidence over this dose range. Dosage levels for other compounds were based on the TCDD dosage range after adjustment for the current TEF values or relative potency values (Table 1). These studies were designed to examine dose additivity rather than response additivity, and dose spacing was weighted in the 10 to 100 ng/kg range to increase dose density in the region where an increase in liver tumors was expected. Doses higher than 100 ng/kg were not used in order to limit the known effects on body weight and liver toxicity seen with TCDD at this dose level. Prior studies of TCDD suggest that this dose (100 ng/kg) is at or near the predicted maximum tolerated dose.

Interim necropsies at 14, 31, and 53 weeks were incorporated into the studies for the examination of mechanistically based biomarkers of AhR- or PCB-mediated effects. These endpoints included alterations in cytochromes P450 1A1, 1A2, and 2B; thyroid hormone levels; and hepatocyte replication. Tissue analyses of the parent compound in the liver, lung, blood, and adipose were included at each interim necropsy and at terminal necropsy for dose response analysis using both administered dose, total body burden, and target tissue dose as the dose metric.

Additional “special study” animals were included at each interim necropsy. Tissues from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies. These animals were not evaluated as part of the core study.

TABLE 1
Compounds and Associated Doses Used in the Dioxin TEF Evaluation Studies

Compound	TEF ^a	Core Study	Stop-Exposure Study
TCDD	1	3, 10, 22, 46, 100 ng/kg	100 ng/kg
PCB 126	0.1	10 ^b , 30, 100, 175, 300, 550, 1,000 ng/kg	1,000 ng/kg
PeCDF	0.5	6, 20, 44, 92, 200 ng/kg	200 ng/kg
TEF Mixture ^c		10 ng TEQ/kg (3.3 ng/kg TCDD, 6.6 ng/kg PeCDF, 33.3 ng/kg PCB 126) 22 ng TEQ/kg (7.3 ng/kg TCDD, 14.5 ng/kg PeCDF, 73.3 ng/kg PCB 126) 46 ng TEQ/kg (15.2 ng/kg TCDD, 30.4 ng/kg PeCDF, 153 ng/kg PCB 126) 100 ng TEQ/kg (33 ng/kg TCDD, 66 ng/kg PeCDF, 333 ng/kg PCB 126)	None
PCB 153	None	10, 100, 300, 1,000, 3,000 µg/kg	3,000 µg/kg
PCB 126/PCB 153 ^d		10/10, 100/100, 300/100, 300/300, 300/3,000, 1,000/1,000	None
PCB 126/PCB 118 ^e		7 ng TEQ/kg (62 ng/kg PCB 126, 10 µg/kg PCB 118) 22 ng TEQ/kg (187 ng/kg PCB 126, 30 µg/kg PCB 118) 72 ng TEQ/kg (622 ng/kg PCB 126, 100 µg/kg PCB 118) 216 ng TEQ/kg (1,866 ng/kg PCB 126, 300 µg/kg PCB 118) 360 ng TEQ/kg (3,110 ng/kg PCB 126, 500 µg/kg PCB 118)	360 ng TEQ/kg
PCB 118	0.0001	10 ^b , 30 ^b , 100, 220, 460, 1,000, 4,600 µg/kg	4,600 µg/kg

^a Van den Berg *et al.* (1998)

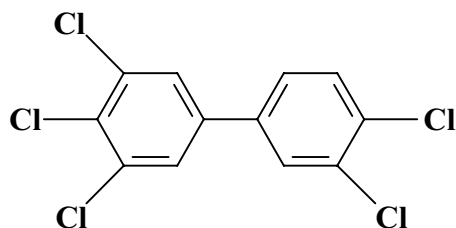
^b 14-, 31-, and 53-week scheduled sacrifices only

^c 10, 22, 46, 100 ng TEQ/kg (TCDD:PeCDF:PCB 126, 1:2:10)

^d PCB 126 dose units are ng/kg, PCB 153 units are µg/kg.

^e PCB 126 dose units are ng/kg, PCB 118 units are µg/kg. Doses are based on PCB 126 levels that are 0.622% of the administered PCB 118 bulk.

INTRODUCTION

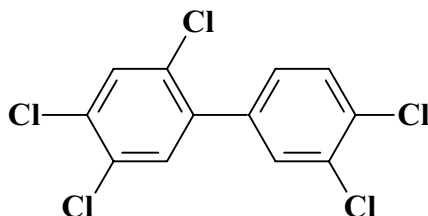


3,3',4,4',5-Pentachlorobiphenyl (PCB 126)

CAS No. 57465-28-8

Chemical Formula: $C_{12}H_5Cl_5$ Molecular Weight: 326.42

Synonym: 1,1'-Biphenyl, 3,3',4,4',5-pentachloro-(9Cl)



2,3',4,4',5-Pentachlorobiphenyl (PCB 118)

CAS No. 31508-00-6

Chemical Formula: $C_{12}H_5Cl_5$ Molecular Weight: 326.43

Synonyms: 1,1'-Biphenyl, 2,3',4,4',5-pentachloro-(9Cl); 1,1'-biphenyl, 2,3',4,4',5-pentachloro-; 2,3',4,4',5-pentachloro-1,1'-biphenyl; 2,4,5,3',4'-pentachlorobiphenyl; 3,4,2',4',5'-pentachlorobiphenyl; biphenyl, 2,3',4,4',5-pentachloro-; CB 118; PCB 118

CHEMICAL AND PHYSICAL PROPERTIES

PCB 126 is a coplanar PCB that was produced commercially before 1977 as a component of technical grade polychlorinated biphenyl (PCB) mixtures, which include Aroclors 1016, 1242, 1248, and 1254 (Mayes *et al.*, 1998). PCB 118 is a mono-*ortho*-substituted PCB that

was commercially produced as a component of Aroclors 1242, 1248, 1254, and 1260 (Frame *et al.*, 1996; ATSDR, 2000). Lower chlorinated Aroclors (1016, 1242, and 1248) are colorless mobile oils. Increasing the chlorine content results in the mixture taking on the consistency of a viscous liquid (Aroclor 1254) or sticky resin

(Aroclors 1260 and 1262) (ATSDR, 2000). PCB 126 has a melting point of 160° to 161° C, a water solubility of 1.03×10^{-3} at 25° C, a vapor pressure of 2.96×10^{-7} at 25° C, and a log octanol:water partition coefficient of 6.89. PCB 118 has a melting point of 108° C, is sparingly soluble, has a vapor pressure of 6.96×10^{-6} at 25° C, and a log octanol:water partition coefficient of 6.42.

PRODUCTION, USE, AND HUMAN EXPOSURE

PCB mixtures, including PCB 126 and PCB 118, were commercially produced between 1929 and 1977 for the electric industry as dielectric insulating fluids for transformers and capacitors. PCBs were also produced for use in hydraulic fluids, solvents, plastics, and paints. The manufacture and use of PCBs in the United States was stopped in 1977 after PCB residues increased in the environment in the 1960s and 1970s. However, PCBs continue to be released into the environment through the use and disposal of products containing PCBs, as by-products during the manufacture of certain organic chemicals, and during combustion of some waste materials (USEPA, 2000a).

Due to their lipophilic nature (high log octanol:water partition coefficient) and resistance to biodegradation, specific PCBs have the ability to bioconcentrate and bioaccumulate. PCBs are widespread in their distribution and are found in virtually all media, including air, soil, water, sediment, and biota (USEPA, 2000b).

The majority of ambient human exposure to PCBs occurs through the ingestion of food containing PCB residues. PCB residues present in fish, milk and dairy products, vegetables, and meat and animal fat are estimated to account for a majority of exposure. Levels of PCB 126 in food range from 0.05 to 0.83 pg/g. Levels of PCB 118 in various food groups ranges from 14 to 1,900 pg/g. Estimated daily exposures from food to PCB 126 and PCB 118 are 130 pg/day and 31 ng/day, respectively (USEPA, 2000b).

Both PCB 126 and PCB 118 are classed as dioxin-like compounds (DLCs) and are included in the World Health Organization (WHO) toxic equivalency factor (TEF) scheme for DLCs (Van den Berg *et al.*, 1998). The WHO TEF values for PCB 126 and PCB 118 are 0.1 and 0.0001, respectively. Exposure to DLCs is usually

calculated in terms of total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs). On a TEQ basis, total exposure to PCDD/PCDF/PCBs from food is estimated to be 62 pg/day of which 22 pg TEQ/day (for a 70 kg person) is from PCBs, including PCB 126 and PCB 118. Of this, PCB 126 (13 pg TEQ/day) accounts for 60% and PCB 118 (3.1 pg TEQ/day) accounts for 14% of the daily intake of TEQ of PCBs. By comparison, intake of TCDD from food is estimated to be 5 pg/day (USEPA, 2000b). Bioaccumulation of PCB 126 and PCB 118 results in persistent levels of these PCBs in human tissues. Average human tissue concentrations of PCB 126 and PCB 118 are 119 pg/g lipid and 32,000 pg/g lipid, respectively. PCB 126 and PCB 118 account for 52% and 14%, respectively, of the PCB TEQ (23 pg/g lipid) present in human tissues (USEPA, 2000b).

TOXICOKINETICS

There is an extensive body of literature examining the toxicokinetics of mixtures and some individual congeners of PCBs (ATSDR, 2000) and DLCs such as PCB 126 (USEPA, 2000c). Since PCB 126 and PCB 118 are DLCs with similar properties to TCDD, the toxicokinetics are expected to be similar to TCDD. In the gastrointestinal tract, PCBs are well absorbed by passive diffusion. Several studies have examined gastrointestinal absorption of TCDD and demonstrate that gastrointestinal absorption of a single dose of 1 µg TCDD/kg body weight in acetone:corn oil (1:25) in Sprague-Dawley rats is 84% (range 66% to 93%) (Piper *et al.*, 1973; Rose *et al.*, 1976). Similar results have been observed after repeated exposure (0.1 to 1 µg/kg per day) and higher doses. Absorption of PCBs has also been estimated to be approximately 90% to 100% from oral routes. Once absorbed, DLCs are transported primarily through the lymphatic systems by chylomicrons and are readily distributed throughout the body. The main sites of distribution of DLCs in rats within the first few days of exposure are the liver, adipose tissue, and to a lesser amount, the skin and thyroid gland (Pohjanvirta *et al.*, 1990). In blood, DLCs are associated mainly with lipoproteins, serum lipids, and to a smaller fraction of albumin and cellular components. The pattern of distribution for DLCs in rats is governed by the lipophilicity of the compound and binding to cytochrome P450 1A2 (Gillner *et al.*, 1987; Diliberto *et al.*, 1997). Cytochrome P450 1A2 is a known binding protein for DLCs and is also inducible by exposure to aryl hydrocarbon receptor (AhR) ligands. Since CYP1A2 is inducible only in the

liver and nasal passages, DLCs tend to sequester in the liver at levels that would not be predicted based on their lipophilicity alone. The hepatic sequestration by TCDD is not seen in CYP1A2 knockout mice, demonstrating the critical involvement of CYP1A2 in this process (Diliberto *et al.*, 1999). There is also evidence for existence of a specific PCB-binding protein in the liver (Buff and Brundl, 1992).

There are limited data available on the distribution and excretion of PCBs in humans (ATSDR, 2000). Absorption of PCBs from the gastrointestinal tract in humans is approximately 90% to 100%. In humans, PCBs are found in the highest concentration in adipose tissue and tend to accumulate to a lesser extent in other lipid-rich tissues, such as liver, skin, and breast milk (ATSDR, 2000).

PCB 126 and PCB 118 Toxic Equivalency Factors

The WHO TEF for PCB 126 is 0.1 (Van den Berg *et al.*, 1998). PCB 118 is a mono-*ortho*-substituted PCB and has a TEF value of 0.0001.

TOXICITY

PCB 126 has a planar structure and is the most potent PCB in terms of its ability to bind and activate the AhR. *In vitro* receptor binding assays show that PCB 126 has an affinity for the AhR of 1.2×10^{-7} M, approximately tenfold lower than that of TCDD (1×10^{-8} M), the most potent AhR ligand. Given this high AhR binding capability, most of the biological responses to PCB 126 are very similar to those of TCDD including altered transcription of TCDD-responsive genes such as CYP1 family cytochromes P450 and induction of UDP-glucuronosyl transferases (ATSDR, 2000). The toxicity profile for PCB 126 is similar to that of TCDD and includes induction of a wasting syndrome, mortality, suppression of body weight gain in subchronic studies, increased liver weight, thymic atrophy, induction of preneoplastic lesions in tumor promotion studies, alteration in porphyrin metabolism, altered retinoid metabolism, and induction of cleft palate (Safe, 1994; Van Birgelen *et al.*, 1994, 1995a; ATSDR, 2000; USEPA, 2000c).

PCB 118 is a mono-*ortho*-substituted nonplanar PCB. PCB congeners with a single chlorine in the *ortho* position have weaker binding affinity for the AhR than

non-*ortho*-substituted PCBs. The affinity of PCB 118 for the AhR has been calculated to be 2.7×10^{-6} M, and it is therefore likely to exhibit dioxin-like activity albeit at higher doses than TCDD or PCB 126. In addition, PCB 118 also exhibits a toxicity profile that has characteristics of di-*ortho*-substituted non-dioxin-like PCB congeners. PCB 118 is a phenobarbital-like inducer of hepatic cytochrome P450 (Denomme *et al.*, 1983). Exposure to PCB 118 also induces hepatic lipid peroxidation and increases glutathione S-transferase activity. Subchronic dietary exposure to PCB 118 in Sprague-Dawley rats reduces hepatic and pulmonary vitamin A, induces histological changes in the thyroid and liver, increases hepatic CYP1A1 expression, and decreases dopamine and its metabolites in the brain (Chu *et al.*, 1995).

Mono-*ortho*-substituted PCBs have been shown to induce neurobehavioral toxicity, neurotoxicity, and endocrine alterations (Fischer *et al.*, 1988; Giesy and Kannan, 1998). Decreased dopamine concentrations in the caudate, putamen, substantia nigra, and hypothalamus regions of the brain are associated with measurable concentrations of the *ortho*-substituted nonplanar congeners PCB 28, PCB 47, and PCB 52 in these brain regions of monkeys exposed to Aroclor 1016 (Seegal *et al.*, 1990). Aroclor 1254 and *ortho*-substituted PCB congeners 4, 52, 88, 95, 103, 104, and 153 disrupt Ca^{2+} transport in central neurons by direct interaction with ryanodine receptors in specific regions of the central nervous system and may contribute mechanistically to the neurotoxicity of these compounds (Wong *et al.*, 1997). PCB 153 decreases neuronal cell viability and induces apoptosis *in vitro* (Sánchez-Alonso *et al.*, 2003). Aroclors 1242 and 1254, which contain relatively low concentrations of dioxin-like PCB congeners, also induce cultured cerebellar granule cell death and reactive oxygen species formation (Mariussen *et al.*, 2002).

CARCINOGENICITY

Experimental Animals

There is an extensive body of literature examining the carcinogenicity of mixtures of PCBs in rodents (Silberhorn *et al.*, 1990). In general, these studies indicate that PCB mixtures have the potential to be carcinogenic, but mainly within the liver (hepatocellular neoplasms). Mixtures of PCBs contain both dioxin-like coplanar PCBs as well as non-dioxin-like PCBs, which may elicit responses via different mechanisms. While

these mixtures of PCBs have been shown to be carcinogenic in rats and mice (Nagasaki *et al.*, 1972; Ito *et al.*, 1973; Kimbrough *et al.*, 1975; Mayes *et al.*, 1998), there have been no individual studies on the carcinogenicity of PCB 118 alone. Until the recent study of PCB 126 as part of the NTP dioxin TEF evaluation (NTP, 2006a), there had been no individual studies on the carcinogenicity of PCB 126. No epidemiology studies of either PCB 126 or PCB 118 alone were found in a review of the literature. With the recent exception of PCB 126 and the study of PCB 153 being conducted as part of the dioxin TEF evaluation (NTP, 2006b), there have been no other published studies examining the carcinogenicity of any individual PCB congener.

In the NTP carcinogenicity study of PCB 126 in female Harlan Sprague-Dawley rats that was conducted as part of the dioxin TEF evaluation, there was clear evidence of carcinogenicity of PCB 126 at doses up to 1,000 ng/kg based on increased incidences of cholangiocarcinoma of the liver, hepatocellular adenoma, squamous neoplasms of the lung (cystic keratinizing epithelioma and squamous cell carcinoma), and gingival squamous cell carcinoma of the oral mucosa (Walker *et al.*, 2005; NTP, 2006a). In addition, there were increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, kidney, heart, thyroid gland, thymus, spleen, clitoral gland, and mesenteric artery that were due to treatment with PCB 126 (NTP, 2006a). A comparative carcinogenicity study of Aroclors 1016, 1242, 1254, and 1260 in male and female Sprague-Dawley rats demonstrated increased incidences of neoplasms, including hepatocellular adenoma, hepatocellular carcinoma, hepatocholangioma, hepatocholangiocarcinoma, and follicular cell adenoma of the thyroid gland (Mayes *et al.*, 1998). The incidences of hepatocellular neoplasms were significantly increased in female rats by PCB exposure with the rank order of Aroclor 1254 > Aroclor 1260 > Aroclor 1242 > Aroclor 1016. In males, thyroid gland tumors were induced by exposure to Aroclors 1242, 1254, and 1260, and liver tumors by Aroclor 1260. Within this context, Aroclor 1254 has the highest dioxin-like activity, measured on a TEQ basis, compared to the other PCB mixtures due to the presence of specific coplanar PCBs, PCDDs, and PCDFs in the mixture. The incidence of liver tumors was more extensive in female rats than in male rats. Female tumor incidence was dependent on hepatic TEQ levels of dioxin-like congeners of PCB (Silkworth *et al.*, 1997). The carcinogenicity of these PCB mixtures in females may entirely, or in part, be attributed to the dioxin-like

components. Based on the similarity in mechanism of dioxin-like PCBs compared to TCDD, it is expected that the carcinogenicity of dioxin-like PCBs in Aroclor mixtures may be similar to the carcinogenicity of TCDD. The carcinogenicity of TCDD has been clearly established in rodents by the dermal, dosed feed, and gavage routes of administration (Kociba *et al.*, 1978; Toth *et al.*, 1979; NTP, 1982a,b; Della Porta *et al.*, 1987; Rao *et al.*, 1988; IARC, 1997; USEPA, 2000c). In a previous NTP study, TCDD administered by gavage significantly increased the incidences of thyroid gland follicular cell adenoma in male and female Osborne-Mendel rats and female B6C3F₁ mice, neoplastic liver nodules in female mice, and hepatocellular carcinoma in male and female mice (NTP, 1982a). TCDD administered by dermal application caused an increased incidence of fibrosarcoma of the integumentary system in female Swiss-Webster mice (but equivocal evidence in male mice) (NTP, 1982b). In the NTP study of TCDD carried out as part of the dioxin TEF evaluation in female Harlan Sprague-Dawley rats there was clear evidence of carcinogenicity based on increased incidences of cholangiocarcinoma of the liver, hepatocellular adenoma, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa (Walker *et al.*, 2005; NTP, 2006c). Increased incidences of squamous cell carcinoma of the uterus were also considered to be related to TCDD exposure, and marginal increased incidences of pancreatic neoplasms and hepatocholangioma and cholangioma of the liver may have been related to TCDD exposure. In addition, there were increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, kidney, heart, thyroid gland, thymus, spleen, clitoral gland, forestomach, and mesenteric artery that were due to treatment.

Humans

Humans have not been exposed to significant amounts of PCB 126 or PCB 118 alone. Exposures to PCB 126 and PCB 118 occur in mixtures combined with other structurally related compounds such as PCDDs, PCDFs, and PCBs. Two accidental poisoning incidents in Japan and Taiwan resulted from exposures to cooking oil that was highly contaminated with PCDFs and PCBs (Masuda, 1985). In addition to extensive reproductive and developmental effects in these populations, early follow-up studies indicated an increased mortality from liver disease and cancer, particularly liver cancer (IARC, 1997). Although later follow-up studies did not show an increased mortality from cancer, mortality from liver disease was still elevated (Yu *et al.*, 1997). However, it

is difficult to determine which contaminants are responsible for these effects.

There have been several studies examining the cancer incidence and mortality in workers exposed to PCBs, although the small cohort sizes in these studies limit the ability to draw any meaningful conclusions (Silberhorn *et al.*, 1990).

TUMOR PROMOTION STUDIES

The majority of studies examining the *in vivo* and *in vitro* genotoxicity of PCBs demonstrate that PCBs are negative (Silberhorn *et al.*, 1990). In the liver, clonal expansion of genetically altered cells leads to the formation of putative preneoplastic altered hepatocellular focal (AHF) lesions identified by alterations in histomorphology or gene expression. These lesions are believed to be precursors in the development of liver tumors (Pitot *et al.*, 1991). There have been numerous studies demonstrating the ability of PCBs to enhance the development of preneoplastic liver lesions (Silberhorn *et al.*, 1990). Studies in Sprague-Dawley rats indicate that DLCs, including PCB 126 and PCB 118, can enhance the development of AHF lesions (Wærn *et al.*, 1991). Haag-Grönlund *et al.* (1997, 1998) demonstrated that PCB 126 alone and in combination with other PCBs enhances the development of AHF lesions. In a comparison study of PCB 126 and TCDD, the relative ability of PCB 126 to enhance altered hepatic foci development was about 1/10th that of TCDD (Hemming *et al.*, 1995). Moreover, the activity of PCB 126 and TCDD, when tested in combination, was additive. The induction of preneoplastic foci by PCB 118 has also been demonstrated. The relative ability of PCB 118, compared to TCDD, to enhance altered hepatic foci development was estimated to be 0.00002 to 0.00005, based on the AHF data and 0.0001 based on the increased 7-ethoxy-resorufin-O-deethylase (EROD) activity (Haag-Grönlund *et al.*, 1997).

Numerous studies have examined the promotion of putative preneoplastic liver lesions by TCDD within the framework of a two-stage initiation-promotion protocol (Dragan and Schrenk, 2000). These studies demonstrate that the effects of TCDD on AHF are dose dependent (Pitot *et al.*, 1980; Maronpot *et al.*, 1993; Teeguarden *et al.*, 1999), duration of exposure dependent, and reversible (Dragan *et al.*, 1992; Walker *et al.*, 1998, 2000). Also, studies show that TCDD induction of

hepatic neoplasms is higher in female rat liver than in male rat liver and that this is due to the enhancing effect of estrogens on the promotion of preneoplastic lesions (Lucier *et al.*, 1991; Wyde *et al.*, 2001a, 2002). Studies in Sprague-Dawley rats also show that PeCDF can enhance the development of AHF lesions (Wærn *et al.*, 1991). Van der Plas *et al.* (1999) indicate that a mixture of PCDDs, PCDFs, and PCBs led to the increased development of putative preneoplastic AHF lesions.

Tests of the tumor initiating and promoting capacity of DLCs have been conducted in two-stage (initiation-TCDD promotion) models of mouse skin tumorigenesis (IARC, 1997; Dragan and Schrenk, 2000; USEPA, 2000c). Dermal painting studies of PeCDF in HRS/J mice indicate that it is a skin tumor promoter (Hebert *et al.*, 1990). Similar studies demonstrate that TCDD is at least two orders of magnitude more potent than the prototypical promoter tetradecanoyl phorbol acetate in those skin tumor promotion models (Poland *et al.*, 1982).

Tumor promotion by PCB 126 or PCB 118 has not been evaluated in transgenic models. However, transgenic models have been used to examine the carcinogenicity of TCDD in mice (Eastin *et al.*, 1998). These include the Tg.AC transgenic mouse that harbors an activated mouse *v-Ha-ras* oncogene (an intermediate in growth factor signaling). Dermal application of TCDD results in a significant increase in the incidence of squamous cell papillomas in male and female Tg.AC mice, which supports the conclusion that TCDD is a tumor promoter. Subsequent studies by NTP showed that the induction of papillomas and squamous cell carcinomas by dermal application of TCDD to hemizygous Tg.AC mice was dose dependent (Van Birgelen *et al.*, 1999; Dunson *et al.*, 2000; Wyde *et al.*, 2004). In addition, the induction of skin papillomas in this model occurs when TCDD is given by oral administration. Based on the similarity of action of PCB 126 and TCDD, it is expected that PCB 126 would act similarly in the Tg.AC model.

In addition to the liver and skin, TCDD and PCDFs are tumor promoters in the lung (Anderson *et al.*, 1991; Beebe *et al.*, 1995). Anderson *et al.* (1986) also demonstrated that PCB mixtures can act as tumor promoters in the lung. However, no studies of PCB 126 or PCB 118 have examined effects on tumor promotion in the lung. In Sprague-Dawley rats, which have a much lower spontaneous incidence of lung tumors, TCDD promotes the development of bronchiolar hyperplasia and alveolar

bronchiolar metaplasia (Tritscher *et al.*, 2000). It was demonstrated that the induction of these lesions was reversible; incidences of these lesions returned to control levels following withdrawal of TCDD for 16 or 30 weeks.

MECHANISM AND BIOCHEMICAL EFFECTS

DLCs, including PCB 126, are generally classified as nongenotoxic and nonmutagenic. The common mechanism of action of DLCs involves an initial binding to the AhR (Poland and Knutson, 1982; Safe, 1990; Whitlock, 1990; Schmidt and Bradfield, 1996). The broad spectrum of effects of TCDD and DLCs on hormone and growth factor systems, cytokines, and other signal transducer pathways indicates that they are powerful growth dysregulators (Birnbaum, 1994a). Since they are not directly genotoxic (Wassom *et al.*, 1977), it is believed that the pathological responses associated with exposure are fundamentally due to binding to and activation of the AhR, subsequent altered expression of AhR-regulated genes, and altered signaling of biological pathways that interact with the AhR signal transduction mechanism.

Alterations in the expression of AhR-regulated genes occurs via a mechanism that involves a high affinity interaction of the ligand with an intracellular protein, the AhR, which functions as a ligand-activated transcription factor (Okey *et al.*, 1994; Schmidt and Bradfield, 1996). Ligand binding initiates a signaling pathway in which the cytosolic AhR dissociates from heat shock proteins and translocates to the nucleus (Whitlock, 1993). At some point subsequent to ligand binding, the AhR associates with another protein, aromatic hydrocarbon nuclear translocator protein (ARNT), to form the nuclear DNA-binding and transcriptionally active AhR complex. Both the AhR and ARNT are members of the basic helix-loop-helix family of transcription factors (Hoffman *et al.*, 1991; Burbach *et al.*, 1992; Ema *et al.*, 1992). The AhR-ARNT heterodimer binds with high affinity to a specific DNA sequence termed the dioxin response element (DRE). DREs have been identified in the enhancer regions of genes encoding several drug-metabolizing enzymes (Lai *et al.*, 1996). The characteristic response to TCDD and DLCs is the transcriptional induction of the cytochrome P450 1A1 gene (CYP1A1), which is mediated by binding of the AhR complex to DREs present in the 5' flanking region of the gene. The

AhR is expressed in all tissues examined (Dolwick *et al.*, 1993) with a definite tissue specificity in terms of level of expression and diversity of response, indicating that DLCs are likely to have some effect in every tissue. However, even with the same receptor and the same ligand, there are both qualitative and quantitative differences in response and these differences in response are likely to be involved in the tissue- and species-specificity of the response. It is still not known how alterations in gene expression ultimately lead to the development of pathologies and adverse health effects associated with dioxin-like compound exposure. However, it is generally accepted that most, if not all, responses require an initial step of binding to the AhR.

The most well-studied response to DLCs is induction of the CYP1A cytochromes P450 (Whitlock, 1999). CYP1A1 is induced in most tissues including liver, lung, kidney, nasal passages, and small intestine with the highest induction in rats occurring in the liver. Induction of CYP1A1 is a sensitive response and serves as a useful marker for exposure to DLCs. DLCs induce CYP1A1 *in vivo* and *in vitro* in human and animal models. CYP1A2 is constitutively expressed in the liver at low levels and inducible by DLCs in liver and possibly the nasal turbinates of rats (Goldstein and Linko, 1984). Induction of EROD activity is a marker of CYP1A1 activity. CYP1A2 is induced by DLCs and expressed primarily in the liver. Induction of acetanilide-4-hydroxylase activity is a marker of CYP1A2 activity. In addition to the well-characterized induction of CYP1A1 and CYP1A2, DLCs also induce another cytochrome P450, CYP1B1, in human cells (Sutter *et al.*, 1994) and rodent tissues (Walker *et al.*, 1995). CYP1B1 is active in the metabolism of numerous polycyclic aromatic hydrocarbons and arylamines and can catalyze the 4-hydroxylation of 17 β -estradiol (Hayes *et al.*, 1996; Murray *et al.*, 2001).

DLCs are believed to disrupt thyroid hormone homeostasis via the induction of the phase II enzymes UDP-glucuronosyl transferases. Thyroxine (T₄) production and secretion are controlled by thyroid stimulating hormone (TSH), which is under negative and positive regulation from the hypothalamus, pituitary gland, and thyroid gland by thyrotrophin releasing hormone, TSH itself, T₄, and triiodothyronine. TCDD induces the synthesis of UDP-glucuronosyl transferase-1 (UGT) mRNA by an AhR-dependent transcriptional mechanism. Consequently, a reduction in serum T₄ levels via an induction of UGT may lead to a decrease in

the negative feedback inhibition on the pituitary gland. This would then lead to a rise in secreted TSH resulting in chronic hyperstimulation of the thyroid gland follicular cells.

DLCs have been shown to modulate numerous growth factor, cytokine, hormone, and metabolic pathways in animals and experimental systems (Birnbaum, 1994b; Sutter and Greenlee, 1992). Many, if not all of these, are parts of pathways involved in cellular proliferation and differentiation. These include the glucocorticoid receptor tyrosine kinases, interleukin-1, plasminogen activator inhibitor-2, urokinase-type plasminogen activator, tumor necrosis factor-alpha, gonadotrophin releasing hormone, testosterone, and prostaglandin endoperoxide H synthase-2. More recently, the application of toxicogenomics analyses have increased our understanding of which genes/proteins are altered by TCDD both *in vitro* (Puga *et al.*, 2000; Martinez *et al.*, 2002) and *in vivo* (Bruno *et al.*, 2002; Kurachi *et al.*, 2002; Zeytun *et al.*, 2002). Most of the molecular details for induction of gene expression via the AhR have been characterized for the transcriptional activation of the CYP1A1 gene (Whitlock, 1999). While the expression of many genes have been shown to be affected by DLCs, there is a detailed characterization of transcriptional activation through the AhR for only a few of these.

In addition to inducing dioxin-like effects, mono-*ortho*-substituted PCBs including PCB 118 induce non-AhR mediated PCB-specific effects. These include increased expression of cytochromes P450 of the 2B family, and associated pentoxyresorufin-*O*-deethylase activity, decreased dopamine levels, and disruption of calcium homeostasis. In addition, hydroxylated PCBs have the ability to induce estrogenic effects mediated via binding to the estrogen receptor (ATSDR, 2000).

STUDY DESIGN, SPECIES, AND DOSE SELECTION RATIONALE

This study is one of a series of studies conducted as part of the dioxin TEF evaluation. The aim of this set of studies was to evaluate the carcinogenicity of DLCs and mixtures of PCBs relative to the most potent dioxin, TCDD, rather than to completely evaluate the carcinogenicity of each respective compound/mixture in a standard NTP two sexes, two species carcinogenicity testing paradigm. Consequently, many of the design rationales are based on the prior observations of the carcinogenicity of TCDD. The Sprague-Dawley female rat was

used for all the dioxin TEF evaluation studies based upon the prior observation of the carcinogenic sensitivity of this strain to TCDD and the extensive literature on the effects of TCDD and related compounds in this model.

The "PCB mixture" study was not designed *a priori* as part of the dioxin TEF evaluation. Initially this study was a study of PCB 118 alone at doses of 0, 100, 220, 460, 1,000, and 4,600 µg/kg. These doses were chosen to give equivalent TEQ doses to those used in the TCDD study conducted as part of the dioxin TEF evaluation (0, 10, 22, 46, 100 ng TCDD/kg; NTP, 2006c), assuming the WHO TEF value for PCB 118 of 0.0001, together with an additional 4,600 µg/kg group to allow for a potentially lower TEF for PCB 118. Within 2 months, premature deaths occurred in the high dose (4,600 µg PCB 118/kg) group suggesting that this was in excess of the maximum tolerated dose.

By 13 weeks of exposure there was a sufficient lack of increase in body weight gain that the study was aborted. Body weights in the 220, 460, and 1,000 µg/kg groups were 97%, 100%, and 85% of controls, respectively. By comparison, at 14 weeks in the study of TCDD alone, the body weights observed in the 22, 46, and 100 ng TCDD/kg groups were 98%, 97%, and 95% of controls, respectively (NTP, 2006c). Based on these body weight changes, the PCB 118 study was restarted using 500 µg PCB 118/kg as the highest dose. Midway through the restarted study, reanalysis of the PCB 118 compound being used indicated that it contained a minor contaminant, PCB 126, at a concentration of 0.622%. For most PCBs, this level of contamination would not be of any consequence. However, given the high doses used, most of the dioxin-like effects observed in the study could be attributed to the PCB 126 contaminant rather than PCB 118. As an example, at the 1,000 µg "PCB 118"/kg dose the PCB 126 dose was 3,110 ng PCB 126/kg (Table 2). On a TEQ basis, the PCB 126 dose would be 311 ng TEQ/kg, compared to the predicted TEQ of the PCB 118 alone of 50 ng/kg (based on the WHO TEF of 0.0001).

It was decided to continue with this study, now termed a PCB mixture of PCB 126 and PCB 118, since PCB 126 and PCB 118 represent the two highest contributors to the PCB-derived TEQ. PCB 126 is the highest contributor to the TEQ (both intake and levels in humans) for all types of PCBs. PCB 118 is the most abundant mono-*ortho*-substituted PCB in human tissues, contributing the highest TEQ for the mono-*ortho*-class of

TABLE 2
Doses Used in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

Dose Group	PCB 118 ($\mu\text{g}/\text{kg}$) ^a	PCB 126 (ng/kg) ^a	TEQ (ng/kg) ^b
7 ng TEQ/kg	10	62	7.2
22 ng TEQ/kg	30	187	21.6
72 ng TEQ/kg	100	622	72.1
216 ng TEQ/kg	300	1,866	216.2
360 ng TEQ/kg	500	3,110	360.4

^a PCB 118 dose is the nominal target dose of PCB 118. PCB 126 dose is based on 0.622% level of PCB 126 in the bulk synthesized PCB 118.

^b Total TCDD toxic equivalents (TEQ) calculated using WHO TEF values of 0.1 (PCB 126) and 0.0001 (PCB 118), 0.0001 (PCB 77), and 0.00001 (PCB 167) and actual levels of PCBs in the bulk material of 98.5% PCB 118, 0.622% PCB 126, 0.2% PCB 77, and 0.5% PCB 167.

PCB congeners. In addition, on a predicted TEQ basis, the TEQ ratio for this “PCB mixture” was 6:1 (PCB 126:PCB 118). In human tissues, the ratio of PCB 126:PCB 118 on a TEQ basis is 0.9:1 in blood, 3.9:1 in breast milk, and 15:1 in adipose tissue (USEPA, 2000b). The mass ratio of PCB 118:PCB 126 is on average 161:1 in beef fat and 190:1 in milk. The mass ratio of PCB 118:PCB 126 in this “PCB mixture” is 161:1. Therefore, the relative ratio of PCB 126 and PCB 118 in this mixture was environmentally relevant on both a mass ratio and a TEQ ratio basis. Since PCB 126 had already been studied, a comparison of data from the

present study to the PCB 126 study could be used to test for the effect of a mono-*ortho*-substituted PCB on a coplanar PCB at an environmentally relevant ratio.

This Technical Report presents the data on this carcinogenicity study of the mixture of PCB 126 and PCB 118. To access the carcinogenicity of PCB 118 alone, PCB 118 was resynthesized and analyzed to ensure that the predicted dioxin-like activity was greater than 98% attributable to PCB 118 alone, and a new study was started. The results of that study will be reported at a later date.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

PCB 118

PCB 118 was obtained from Radian International LLC (Austin, TX) in one lot (31542-46). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (Research Triangle Institute, Research Triangle Park, NC) and the study laboratory (Battelle Columbus Operations, Columbus, OH) (Appendix C). Reports on analyses performed in support of the study of a mixture of PCB 126 and PCB 118 are on file at the National Institute of Environmental Health Sciences.

Lot 31542-46 of the chemical, a white powder, was identified as PCB 118 by the analytical chemistry laboratory using melting point determination and infrared, ultraviolet/visible, proton nuclear magnetic resonance, and low resolution mass spectroscopy and by the study laboratory using infrared spectroscopy. The moisture content of lot 31542-46 was determined by the analytical chemistry laboratory by gas chromatography moisture analysis purge and trap method. The purity of lot 31542-46 was determined by the analytical chemistry laboratory and the study laboratory using gas chromatography. Gas chromatography indicated a water content of approximately 0.06% by weight. Gas chromatography indicated one major peak and two impurity peaks with areas of 0.4% and 0.5% relative to the total integrated peak area. A second gas chromatography system detected one major peak and three impurity peaks with areas of 0.2%, 0.8%, and 0.5% relative to the total integrated peak area. Gas chromatography by a third system indicated a purity of 97.4% relative to a frozen reference sample from the same lot. The overall purity of lot 31542-46 was determined to be greater than 98.5%. The three impurity peaks observed during purity determination were identified by the analytical chemistry laboratory using gas chromatography and low resolution mass spectroscopy. Analysis indicated that the impurities were tetrachlorinated, pentachlorinated, and

hexachlorinated biphenyls. The impurities were identified as PCB 77 (0.2%), PCB 126 (0.8%), and PCB 167 (0.5%).

Because the TEF values of PCB 77 and PCB 167 are 0.0001 and 0.00001, respectively, at these levels of contamination they were not considered to contribute to the total dioxin-like activity of the bulk compound. Because PCB 126 was predicted to be a high contributor to the dioxin-like activity of the bulk material, it was further analyzed to obtain a more accurate assessment of its level in the bulk material. The concentration of PCB 126 in the lot of PCB 118 was determined by the study laboratory by standard addition of PCB 126 using gas chromatography. The concentration of PCB 126 in the lot of PCB 118 was determined to be 0.622%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography. These studies indicated that the chemical was stable for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at 23° C to 25° C protected from light. Stability was monitored during the 2-year study using gas chromatography. No degradation of the bulk chemical was detected.

Formulation Materials

USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) in three lots and was used with corn oil (Spectrum Quality Products) as the vehicle in the 2-year gavage study. The identity of each lot was confirmed by the study laboratory using infrared spectroscopy prior to its use. The purity of each lot was determined by the study laboratory using gas chromatography prior to initial use and at intervals of no more than 6 months thereafter. All acetone lots showed a purity of at least 99.9%. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

A working stock solution (for the 4 µg/mL PCB 118 dose formulation) was prepared, and an aliquot transferred and diluted in the corn oil vehicle. Higher dose formulations were prepared by dissolving the appropriate amount of chemical in acetone and diluting in the corn oil vehicle for the concentration desired. Homogeneity and stability studies of the 4 µg/mL PCB 118 dose formulation were performed by the study laboratory using gas chromatography. Homogeneity was confirmed, and the stability of PCB 118 in the dose formulations was confirmed for at least 42 days at 25° C when stored in amber glass bottles with Teflon®-lined lids and for up to 3 hours when exposed to air and light at room temperature.

Periodic analyses of the dose formulations were conducted at the study laboratory using gas chromatography. The formulations were analyzed every 3 months for PCB 118 concentrations. Of the dose formulations analyzed and used during the 2-year study, all were within 10% of the target concentrations, and all animal room samples analyzed were within 10% of the target concentrations. The formulations were analyzed twice for PCB 126 concentrations using gas chromatography. All preadministration dose formulations and 45% (9/20) of archived dose formulations were within 10% of the expected concentrations.

2-YEAR STUDY

Study Design

Groups of 81 female rats received a binary mixture of PCB 126 and PCB 118 in corn oil:acetone by gavage at doses of 7 ng TEQ/kg, 22 ng TEQ/kg, 72 ng TEQ/kg, or 216 ng TEQ/kg 5 days per week for 104 weeks; a group of 86 female rats received 360 ng TEQ/kg; and a group of 81 female rats received the corn oil:acetone vehicle alone (Table 3). Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. For stop-exposure evaluation, a group of 50 female rats received 360 ng TEQ/kg for 30 weeks and then the vehicle alone for the remainder of the study.

Additional “special study” animals were included at each interim evaluation. Tissues from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies. These animals were not evaluated as part of the core study.

Source and Specification of Animals

Male and female Harlan Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN), for use in the 2-year study. Sufficient male rats were included in this study to ensure normal estrous cycling of female rats. Male rats were not administered the binary mixture. Rats were quarantined

TABLE 3
PCB Concentrations in the Mixture Study of PCB 126 and PCB 118

	PCB 118	PCB 126	PCB 77	PCB 167	PCB 118	PCB 126	PCB 77	PCB 167	Total TEQ
% Bulk mass	98.5%	0.6%	0.2%	0.5%					
% Total TEQ					13.7%	86.3%	0.03%	0.007%	
Dose Group	ug/kg ^c	ng/kg ^a	ng/kg ^a	ng/kg ^a	ng TEQ/kg ^b	ng TEQ/kg ^b	ng TEQ/kg ^b	ng TEQ/kg ^b	ng TEQ/kg ^b
7 ng TEQ/kg	10 ^c	62	20	50	1.0	6.2	0.002	0.0005	7.2
22 ng TEQ/kg	30 ^c	187	60	150	3.0	18.7	0.006	0.0015	21.6
72 ng TEQ/kg	100 ^c	622	200	500	9.9	62.2	0.02	0.005	72.1
216 ng TEQ/kg	300 ^c	1,866	600	1,500	29.6	186.6	0.06	0.015	216.2
360 ng TEQ/kg	500 ^c	3,110	1,000	2,500	49.3	311.0	0.1	0.025	360.4

^a Based on level of each compound present in the bulk synthesized material.

^b Assuming WHO TEF values of 0.1 (PCB 126), 0.0001 (PCB 118), 0.0001 (PCB 77), 0.00001 (PCB 167). TEQ value for PCB 118 calculated assuming 98.5% of bulk material is PCB 118.

^c Nominal dose (µg/kg) of bulk synthesized material.

for 14 days before the beginning of the study. Female rats were approximately 9 weeks old at the beginning of the study. Five male and five female rats were randomly selected for parasite evaluation and gross observation of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix E). Sentinel rats included five males and five females at 1 month, five or six males at 6, 12, and 18 months, and five females from the 72 ng TEQ/kg group at the end of the study.

Animal Maintenance

Male rats were housed three per cage and female rats were housed five per cage except for the last one to two cages in each dose group which housed three females per cage. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 4. Information on feed composition and contaminants is provided in Appendix D.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded on day 29, every 4 weeks, and at the end of the study. Body weights were recorded at the beginning of the study, weekly for the first 13 weeks, then approximately every 4 weeks, and at the end of the study.

At 14, 31, and 53 weeks, blood was taken from the retroorbital sinus of up to ten female rats per group (except stop-exposure) and processed into serum for thyroid hormone determinations. Radioimmunoassays were performed for thyroid stimulating hormone (TSH), total triiodothyronine (T_3), and free thyroxine (T_4) using a Packard Cobra II gamma counter (Packard Instrument Company, Meriden, CT). The assay for total T_4 was performed on a Hitachi 911[®] chemistry analyzer (Boehringer Mannheim, Indianapolis, IN) using a Boehringer Mannheim[®] enzyme immunoassay test system. Thyroid hormone data were summarized using the XYBION system (XYBION Medical Systems Corporation, Cedar Knolls, NJ).

For cell proliferation analysis at 14, 31, and 53 weeks, up to 10 female rats per group (except stop-exposure) received drinking water containing 40 mg BrdU/100 mL Milli-Q water for 5 days. BrdU solutions were administered in amber glass bottles (Allentown Caging Equipment Company, Inc., Allentown, NJ) equipped

with Teflon[®]-lined lids and stainless steel sipper tubes. BrdU solutions were changed every 3 days, and water consumption was measured daily for 5 days. Cell turnover rate in the liver of dosed female rats was compared to the turnover rate in the vehicle control rats by determining the incorporation of BrdU into hepatocytes. A sample of duodenum (positive control) and liver was fixed in 10% neutral buffered formalin for 18 to 24 hours and then transferred to 70% ethanol. Representative sections of the duodenum and liver were trimmed and embedded, and two sections were cut. One of these sections was stained with hematoxylin and eosin and the other with anti-BrdU antibody complexed with avidin and biotin. At the 14-week interim evaluation, potential interlobular variation was determined in the vehicle control and the 360 ng TEQ/kg groups by counting stained cells in the left lobe and right median lobe. Interlobular variation greater than 25% was considered significant. For the remaining rats, stained cells were counted only in the left lobe. At least 2,000 labeled or unlabeled hepatocyte nuclei were counted using a 20 \times objective and ocular grid. The labeling index is expressed as the percentage of total nuclei that were labeled with BrdU.

For determination of cytochrome P450 activities, liver and lung tissue samples were collected from up to 10 female rats per group (except stop-exposure) at 14, 31, and 53 weeks and stored frozen at -70°C . Microsomal suspensions were prepared using the Pearce Method (Pearce *et al.*, 1996). The concentration of protein in each suspension was determined using the microfilter plate method of the Coomassie Plus Protein Assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. Cytochrome P450 1A1 (CYP1A1)-associated 7-ethoxyresorufin-*O*-deethylase (EROD), CYP1A2-associated acetanilide-4-hydroxylase (A4H), and CYP2B-associated pentoxyresorufin-*O*-deethylase (PROD) activities were determined in microsomal proteins and isolated from frozen liver or lung tissue according to established procedures. Data are shown as pmol/min per mg (EROD and PROD) or nmol/min per mg (A4H) microsomal protein.

For analysis of tissue concentrations of PCB 126 and PCB 118, samples of fat, liver, lung, and blood were taken from up to 10 female rats per dose group at 14, 31, and 53 weeks and at 2 years. Tissue sample preparation included overnight saponification with ethanolic potassium hydroxide, extraction of the saponificate with hexanes, and sample extract clean up on columns using

silica gel with hexanes elution by automated solid phase extraction. Blood and lung sample extracts were subjected to a second solid phase clean up on columns using activated carbon with toluene elution. Concentrations of PCB 126 in blood and lung extracts were measured by capillary gas chromatography with high resolution mass spectrometry detection. Concentrations of PCB 126 in fat and liver extracts and of PCB 118 in all tissue extracts were measured by gas chromatography with electron capture detection.

Complete necropsies and microscopic examinations were performed on all rats. At the interim evaluations, the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid gland were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, 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, left ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 4.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated slides from all tumors and all organs with potential chemical-related changes, which included the adrenal cortex, bone marrow, brain, heart, kidney, liver, lung, lymph nodes, mesentery, nose, oral mucosa, ovary, pancreas, pituitary gland, spleen, stomach, thymus, thyroid gland, tooth, and uterus.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the

laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist, the study laboratory pathologist, and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. 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 McConnell *et al.* (1986).

To maintain consistency of diagnoses within and between all the studies on DLCs conducted as part of the dioxin TEF evaluation, the same pathologists were involved in all phases of the pathology evaluation including the initial examination and the pathology peer review. Because of the need for a consistent diagnostic approach across all studies and the unusual nature of some of the lesions, five other studies (PCB 126, PCB 153, TCDD, the TEF Dioxin Mixture, and PeCDF; NTP 2006a,b,c,d,e) were subjected to additional PWG reviews. Within many of these studies, there were hepatocellular proliferative lesions for which the criteria used for common diagnoses did not appear to fit. Furthermore, classification was sometimes confounded by significant liver damage (toxic hepatopathy) that was present in many animals from these studies. Therefore, a PWG was held to ensure that these important proliferative lesions were sufficiently and consistently categorized across all seven studies for which data are to be compared. PWG participants for this review were primarily those involved in previous PWGs. Additionally, a review panel utilizing a different group of pathologists was convened to provide additional guidance relative to the most appropriate classification of the hepatocellular proliferative lesions from these studies of DLCs. Participants included Drs. Jerrold Ward, Ernest McConnell, James Swenberg, Michael Elwell, Peter Bannasch, Douglas Wolf, John Cullen, and Rick Hailey. Final diagnoses for the hepatocellular proliferative lesions reflect the consensus of this complete review process.

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

Study Laboratory

Battelle Columbus Operations (Columbus, OH)

Strain and Species

Harlan Sprague-Dawley rats; Hsd:Sprague-Dawley SD™

Animal Source

Harlan Sprague-Dawley, Inc. (Indianapolis, IN)

Time Held Before Study

14 days

Average Age When Study Began

9 weeks

Date of First Dose (female rats only)

October 14, 1999

Duration of Dosing

5 days/week for 14, 31, or 53 (interim evaluations), 30 (stop-exposure), or 104 weeks

Date of Last Dose

October 7-8, 2001 (core group)

May 10, 2000 (stop-exposure)

Necropsy Dates

October 8-9, 2001

Average Age at Necropsy

113 weeks

Size of Study Groups

81 (vehicle control, 7 ng TEQ/kg, 22 ng TEQ/kg, 72 ng TEQ/kg, 216 ng TEQ/kg); 86 (360 ng TEQ/kg), or 50 (360 ng TEQ/kg stop-exposure)

Method of Distribution

Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage

Male rats: 3

Female rats: 5

Method of Animal Identification

Tail tattoo

Diet

Irradiated NTP-2000 pelleted or wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

Water

Tap water (Columbus municipal supply) via automatic watering system, except via amber glass bottles during BrdU administration, available *ad libitum*

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

Cages

Solid polycarbonate (Lab Products, Inc., Seaford, DE), changed twice weekly

Bedding

Irradiated Sani-Chips[®] (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly

Cage Filters

Dupont 2024 spun-bonded polyester sheets (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks

Racks

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

Animal Room Environment

Temperature: 72° ± 3° F

Relative humidity: 48% ± 17%

Room fluorescent light: 12 hours/day

Room/Chamber air changes: 10/hour

Doses

0 ng TEQ/kg

7 ng TEQ/kg (62 ng/kg PCB 126:10 µg/kg PCB 118)

22 ng TEQ/kg (187 ng/kg PCB 126:30 µg/kg PCB 118)

72 ng TEQ/kg (622 ng/kg PCB 126:100 µg/kg PCB 118)

216 ng TEQ/kg (1,866 ng/kg PCB 126:300 µg/kg PCB 118)

360 ng TEQ/kg (3,110 ng/kg PCB 126:500 µg/kg PCB 118)

Type and Frequency of Observation

Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the study. Clinical findings were recorded on day 29, monthly thereafter, and at the end of the study.

Method of Sacrifice

Carbon dioxide asphyxiation

Necropsy

Necropsy was performed on all female rats. At 14, 31, and 53 weeks, the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid gland were weighed.

Thyroid Hormone Analysis

At 14, 31, and 53 weeks, blood was collected from the retroorbital sinus of up to 10 rats per group (except stop-exposure group at all time points and high dose group at 53 weeks) for thyroid stimulating hormone, total triiodothyronine, and total and free thyroxine determinations.

Cell Proliferation

At 14, 31, and 53 weeks, up to 10 rats per group (except stop-exposure group at all time points and high dose group at 53 weeks) received BrdU in drinking water for 5 days. Samples from the liver and duodenum were measured for BrdU labeling.

Cytochrome P450 Activities

At 14, 31, and 53 weeks, tissue samples from the liver were taken from up to 10 rats per group (except stop-exposure group at all time points and high dose group at 53 weeks) for 7-ethoxyresorufin-*O*-deethylase, 7-pentoxoresorufin-*O*-deethylase, and acetanilide-4-hydroxylase activities. Lung samples from these rats were analyzed for 7-ethoxyresorufin-*O*-deethylase activity.

Tissue Concentration Analysis

At 14, 31, and 53 weeks and 2 years, samples of blood, fat, liver, and lung were taken from up to 10 rats per group (except stop-exposure group at 14, 31, and 53 weeks, high dose group at 53 weeks and 2 years, and 261 ng TEQ/kg group at 2 years) for analysis of PCB 126 and PCB 118 concentrations.

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

Histopathology

Complete histopathology was performed on all core study and stop-exposure rats at 2 years. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, aorta, bone with marrow, brain, clitoral gland, esophagus, eye, harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung with mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, skin, spleen, stomach (forestomach and glandular), thymus, thyroid gland, trachea, urinary bladder, and uterus. The adrenal gland, liver, lung, mammary gland, ovary, pancreas, pituitary gland, spleen, stomach (forestomach and glandular), thymus, thyroid gland, uterus, and vagina of vehicle control and 360 ng TEQ/kg rats were examined at 14 and 31 weeks and of vehicle control and 216 ng TEQ/kg rats at 53 weeks. In the remaining dose groups, the following tissues were examined: the liver, lung, thymus, and thyroid gland at 14, 31, and 53 weeks; the adrenal gland and pancreas at 31 and 53 weeks; and the adrenal gland at 14 weeks (216 ng TEQ/kg).

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 or missing were censored from the survival analyses; 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 A1a, A1b, A5a, and A5b 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 A3a and A3b) 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., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3a and A3b 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 sacrifice.

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 sacrifice; if the animal died prior to terminal sacrifice 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 rats and B6C3F₁ 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 interim evaluation, the Fisher exact test (Gart *et al.*, 1979), a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Thyroid hormone, cell proliferation, and cytochrome P450 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. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

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 database must be generally similar. For female Sprague-Dawley rats, the NTP historical database is currently limited to the seven gavage studies conducted as part of the dioxin TEF evaluation (the current study of PCB 126:PCB 118, PCB 126, PCB 153, TCDD, the TEF mixture, PeCDF, and PCB 126:PCB 153; NTP, 2006a,b,c,d,e,f).

QUALITY ASSURANCE METHODS

The 2-year study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year study were submitted to the NTP Archives, this study was audited retrospectively by an independent quality assurance 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.

RESULTS

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 1). No animals administered 216 or 360 ng TEQ/kg survived to the end of the study. Survival in the 360 ng TEQ/kg stop-exposure group was also significantly less than in the vehicle control group with only 10 animals surviving to the end of the study.

Body Weights and Clinical Findings

Mean body weights of 7 and 22 ng TEQ/kg rats were similar to those of the vehicle controls throughout the

study (Figure 2 and Table 6). Mean body weights of 72 ng TEQ/kg rats were less than those of the vehicle controls after week 33 of the study, mean body weights of 216 ng TEQ/kg rats were less than those of the vehicle controls after week 7 of the study, and mean body weights of 360 ng TEQ/kg core study and stop-exposure group rats were less than those of the vehicle controls after week 5 of the study.

Clinical findings related to the administration of the binary mixture of PCB 126 and PCB 118 included abnormal breathing, thinness, and ruffled fur.

TABLE 5
Survival of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Animals initially in study	81	81	81	81	81	86	50
14-Week interim evaluation ^a	10	10	10	10	10	10	0
31-Week interim evaluation ^a	10	10	10	10	10	10	0
53-Week interim evaluation ^a	8	7	8	8	8	0	0
Accidental deaths ^a	0	0	0	2	0	0	0
Other ^a	0	3	0	0	0	0	0
Moribund	21	20	21	23	43	44	28
Natural deaths	5	14	8	13	10	22	12
Animals surviving to study termination	27	17	24	15	0	0	10
Percent probability of survival at end of study ^b	52	35	46	30	0	0	20
Mean survival (days) ^c	454	454	437	470	388	298	472
Survival analysis ^d	P<0.001	P=0.055	P=0.457	P=0.054	P<0.001	P<0.001	P<0.001

^a Censored from survival analyses

^b Kaplan-Meier determinations

^c Mean of all deaths (uncensored, censored, and terminal sacrifice)

^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. The trend test does not include the 360 ng TEQ/kg stop-exposure group.

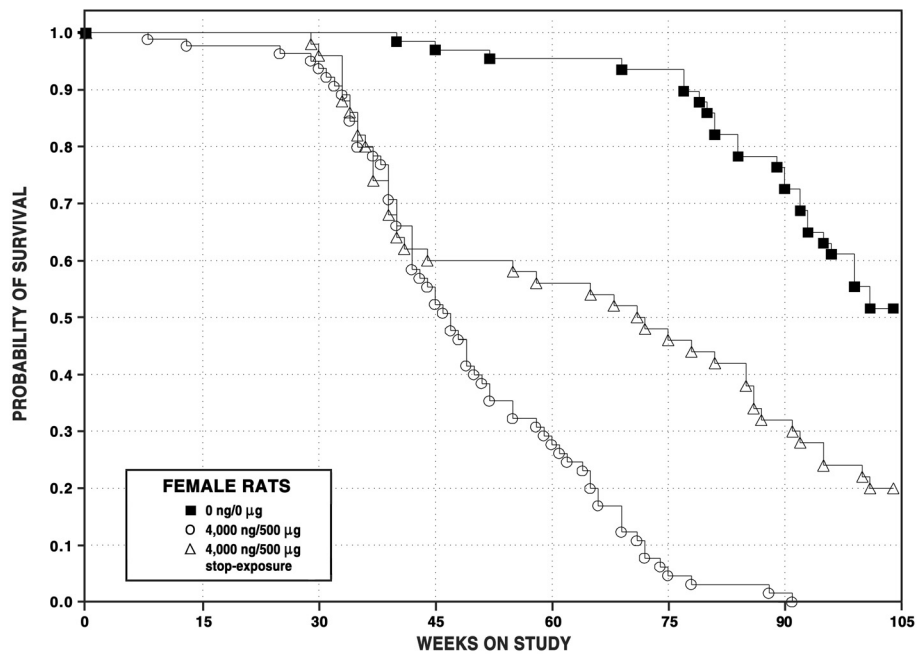
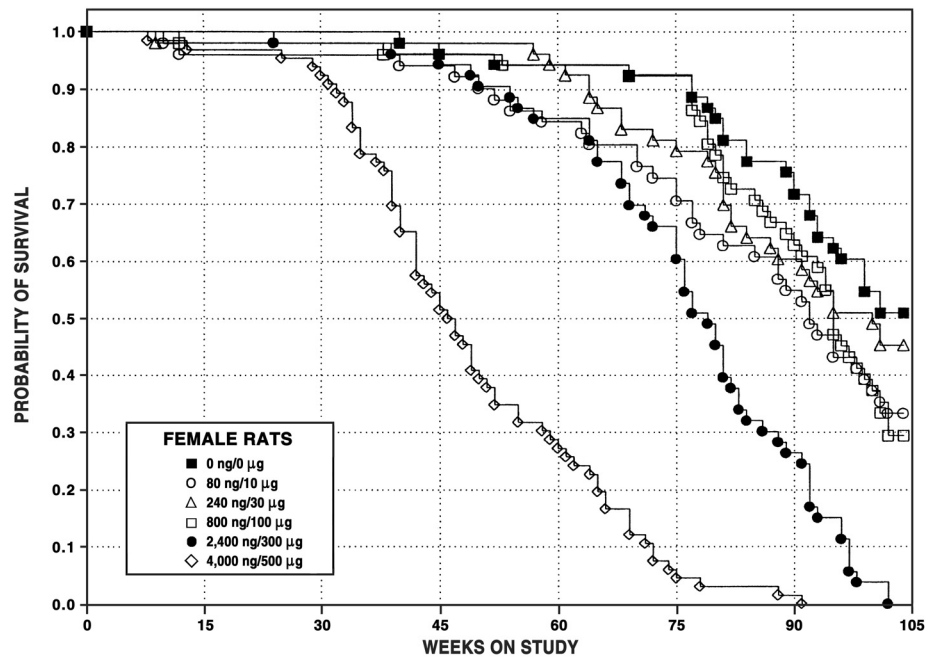


FIGURE 1
Kaplan-Meier Survival Curves for Female Rats Administered
a Binary Mixture of PCB 126 and PCB 118 by Gavage for 2 Years

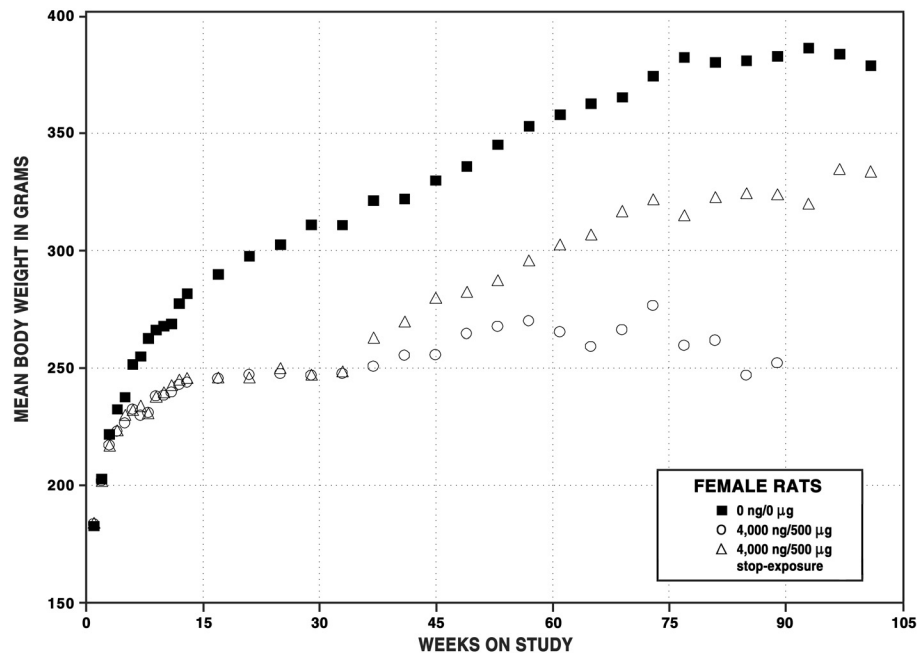
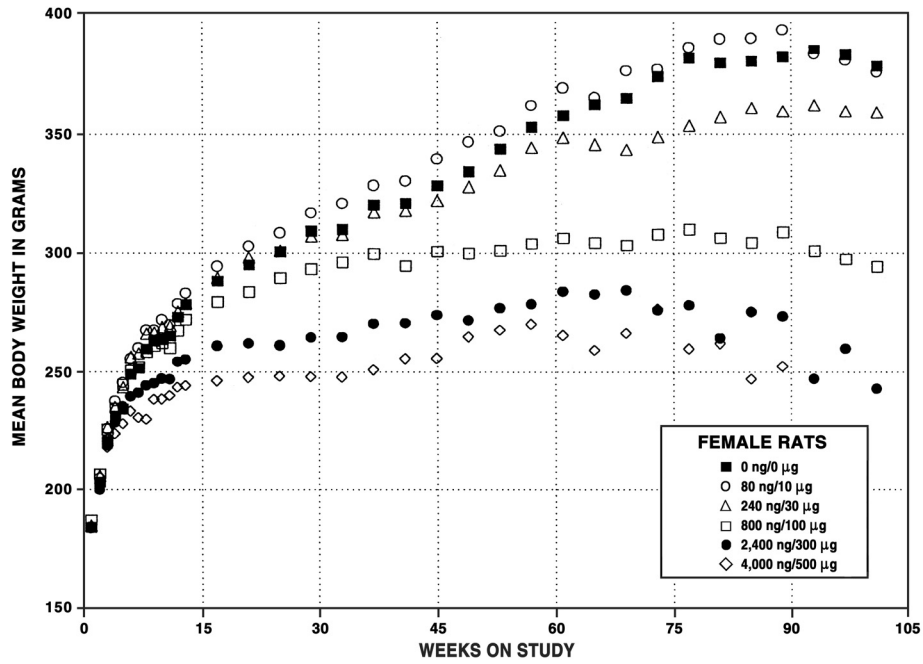


FIGURE 2
Growth Curves for Female Rats Administered a Binary Mixture of PCB 126 and PCB 118 by Gavage for 2 Years

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

Weeks on Study	Vehicle Control		7 ng TEQ/kg			22 ng TEQ/kg			72 ng TEQ/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	183	98	185	101	86	184	101	98	186	102	86
2	203	98	206	102	86	204	100	98	206	102	86
3	222	98	225	101	86	224	101	98	225	101	86
4	233	98	238	102	86	233	100	98	234	101	86
5	238	98	242	102	86	242	102	98	243	102	86
6	252	98	255	101	86	252	100	98	250	99	86
7	255	98	259	102	86	255	100	98	250	98	85
8	263	98	266	101	86	263	100	98	255	97	85
9	266	98	267	101	86	264	99	98	259	97	85
10	268	98	271	101	86	267	100	97	260	97	85
11	269	98	269	100	85	266	99	97	258	96	85
12	278	98	278	100	84	272	98	97	265	96	85
13	282	98	284	101	84	276	98	97	269	95	84
17 ^a	290	82	294	102	74	287	99	81	277	96	74
21	298	82	302	102	74	294	99	81	282	95	74
25	303	82	309	102	74	298	98	81	288	95	74
29	311	82	317	102	74	303	97	81	291	94	74
33 ^a	311	66	321	103	64	307	99	65	294	95	64
37	321	66	329	102	64	316	98	65	298	93	64
41	322	65	330	103	63	317	98	65	293	91	63
45	330	65	339	103	63	321	97	65	300	91	63
49	336	64	347	103	62	327	97	65	298	89	63
53 ^a	345	55	352	102	53	334	97	57	301	87	55
57	353	50	361	102	47	345	98	52	304	86	48
61	358	50	369	103	46	349	98	50	307	86	48
65	363	50	366	101	44	346	95	47	305	84	48
69	365	50	377	103	44	344	94	44	303	83	48
73	375	49	378	101	41	349	93	43	308	82	47
77	382	49	387	101	39	354	93	42	310	81	47
81	380	45	390	103	33	357	94	40	307	81	40
85	381	41	391	103	32	361	95	34	304	80	37
89	383	41	394	103	29	360	94	32	309	81	34
93	387	36	384	99	25	362	94	30	301	78	31
97	384	32	382	99	22	360	94	27	298	78	23
101	379	29	376	99	19	359	95	26	294	78	19
Mean for weeks											
1-13	247		250	101		246	100		243	98	
14-52	314		321	102		308	98		291	93	
53-101	372		377	101		352	95		304	82	

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

Weeks on Study	216 ng TEQ/kg			360 ng TEQ/kg			360 ng TEQ/kg (Stop-Exposure)		
	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	185	101	98	184	101	86	184	101	50
2	202	100	98	202	100	86	202	100	50
3	220	99	98	217	98	86	217	98	50
4	229	98	98	223	96	86	224	96	50
5	236	99	98	227	95	86	230	97	50
6	240	95	98	233	92	86	232	92	50
7	242	95	98	230	90	86	234	92	50
8	245	93	98	231	88	86	231	88	50
9	246	93	98	238	90	85	238	89	50
10	249	93	98	239	89	85	240	90	50
11	248	92	98	240	89	85	243	90	50
12	255	92	98	243	88	85	245	88	50
13	256	91	98	244	87	85	246	87	50
17 ^a	262	90	82	246	85	74	246	85	50
21	263	88	82	247	83	74	246	83	50
25	263	87	81	248	82	74	250	83	50
29	265	85	81	247	80	73	247	80	50
33 ^a	264	85	65	248	80	59	249	80	48
37	270	84	65	251	78	52	263	82	40
41	270	84	64	256	79	43	270	84	32
45	274	83	64	256	78	36	280	85	30
49	273	81	63	265	79	30	283	84	30
53 ^a	276	80	53	268	78	23	287	83	30
57	279	79	46	270	77	21	296	84	29
61	284	79	45	266	74	18	303	85	28
65	283	78	43	259	72	15	307	85	28
69	285	78	39	266	73	11	317	87	26
73	276	74	35	277	74	5	322	86	24
77	278	73	29	260	68	3	315	82	23
81	264	70	24	262	69	2	323	85	21
85	275	72	17	247	65	2	325	85	21
89	274	71	15	252	66	1	324	85	16
93	247	64	9				320	83	14
97	260	68	6				335	87	12
101	243	64	2				334	88	11
Mean for weeks									
1-13	235	95		227	92		228	92	
14-52	267	85		252	80		259	82	
53-101	271	73		263	71		316	85	

^a Interim evaluations occurred during weeks 14, 31, and 53 (except stop-exposure group); until week 53, number of survivors includes 5 (7 and 72 ng TEQ/kg groups) or 17 (vehicle control and 22 and 216 ng TEQ/kg groups) special study animals.

Thyroid Hormone Concentrations

Assays for thyroid stimulating hormone (TSH), total triiodothyronine (T₃), total thyroxine (T₄), and free T₄ were conducted at the 14-, 31-, and 53-week interim evaluations (Table 7). At 14 weeks, serum total T₄ and free T₄ concentrations in the 22, 72, 216, and 360 ng TEQ/kg groups were significantly lower than those in the vehicle controls. No significant differences were observed in total T₃ or TSH concentrations at 14 weeks in any of the dosed groups.

At the 31-week interim evaluation, serum total T₄ and free T₄ concentrations in all dosed groups were

significantly lower than those in the vehicle controls. Serum T₃ concentrations in the 360 ng TEQ/kg group were significantly lower than those in the vehicle controls, and serum TSH concentrations in the 216 and 360 ng TEQ/kg groups were significantly higher than those in the vehicle controls.

At the 53-week interim evaluation, serum total T₄ and free T₄ concentrations in the 22, 72, and 216 ng TEQ/kg groups and total T₄ in the 7 ng TEQ/kg group were significantly lower than those in the vehicle controls. No significant differences were observed in serum T₃ or TSH concentrations at 53 weeks in any dosed group.

TABLE 7
Serum Concentrations of Thyroid Hormones in Female Rats
at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture
of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Week 14						
n	10	10	10	10	10	10
Total T ₄ (µg/dL)	5.640 ± 0.258	4.660 ± 0.389	3.700 ± 0.173**	1.630 ± 0.227**	0.760 ± 0.123**	0.450 ± 0.096**
Free T ₄ (ng/dL)	2.477 ± 0.090	2.233 ± 0.180	2.008 ± 0.140*	1.158 ± 0.093**	0.591 ± 0.072**	0.451 ± 0.027** ^b
Total T ₃ (ng/dL)	126.0 ± 6.9	122.0 ± 6.3	145.9 ± 8.2	147.4 ± 6.6	143.0 ± 6.5	131.9 ± 5.7
TSH (ng/mL)	11.212 ± 0.902	8.994 ± 0.816	11.520 ± 1.027	12.571 ± 1.125	15.462 ± 1.614	13.348 ± 1.520
Week 31						
n	10	10	10	10	10	10
Total T ₄ (µg/dL)	4.270 ± 0.329	2.740 ± 0.270**	1.920 ± 0.215**	0.890 ± 0.145**	0.610 ± 0.136**	0.410 ± 0.085**
Free T ₄ (ng/dL)	2.562 ± 0.177	1.810 ± 0.136*	1.569 ± 0.147**	0.916 ± 0.100**	0.428 ± 0.035**	0.370 ± 0.027**
Total T ₃ (ng/dL)	153.3 ± 5.6	150.8 ± 6.6	157.1 ± 6.1	150.3 ± 11.8	134.8 ± 4.3	101.2 ± 6.6**
TSH (ng/mL)	9.822 ± 0.844	9.518 ± 1.035	11.890 ± 1.247	12.233 ± 0.863	15.433 ± 1.127**	16.729 ± 2.496**
Week 53						
n	8	7	8	7	8	0
Total T ₄ (µg/dL)	3.950 ± 0.302	2.500 ± 0.312**	1.675 ± 0.202**	1.013 ± 0.172** ^c	0.163 ± 0.103**	
Free T ₄ (ng/dL)	2.009 ± 0.140	1.686 ± 0.132	1.144 ± 0.051**	0.746 ± 0.068** ^c	0.339 ± 0.019**	
Total T ₃ (ng/dL)	124.2 ± 7.0	144.9 ± 8.9	134.5 ± 7.1	130.4 ± 5.0	101.0 ± 8.0	
TSH (ng/mL)	20.80 ± 1.79	19.54 ± 2.18	20.57 ± 2.67	22.07 ± 1.93	22.52 ± 1.23	

* 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 T₄=thyroxine; T₃=triiodothyronine; TSH=thyroid stimulating hormone

^c n=7

n=8

Hepatic Cell Proliferation Data

Hepatic cell proliferation data at the 14-, 31-, and 53-week interim evaluations are presented in Table 8. Consumption of the BrdU drinking water solutions prior to each interim evaluation was 20% lower than vehicle controls in the 216 ng TEQ/kg group and 28% lower than vehicle controls in the 360 ng TEQ/kg group (data not shown). No significant differences in labeling indices were observed between the vehicle control and dosed groups at the 14-week interim evaluation. However, at 31 weeks, the labeling indices in the 216 and 360 ng TEQ/kg groups were significantly higher than the vehicle controls, and at 53 weeks, the labeling indices in the 72 and 216 ng TEQ/kg groups were significantly higher than the vehicle controls.

Cytochrome P450 Enzyme Activities

At each interim evaluation, liver and lung samples were collected for determinations of P450 enzyme activity (Table 9). Microsomal suspensions were prepared from

liver samples and were assayed for 7-ethoxyresorufin-*O*-deethylase (EROD) activity (a marker for CYP1A1 activity), 7-pentoxyresorufin-*O*-deethylase (PROD) activity (a marker for CYP2B activity), and acetanilide-4-hydroxylase (A4H) activity (a marker for CYP1A2 activity). Microsomal samples from lung were analyzed for EROD activity only.

Hepatic EROD activity generally increased with dose at 14, 31, and 53 weeks. Significant induction of hepatic EROD was observed in all dosed groups. Hepatic A4H activity was significantly higher in all dosed groups at 14, 31, and 53 weeks, and tended to increase with dose. There was a significant increasing trend in hepatic PROD activity with increasing dose at all three interim evaluations, and the increases were significant in all dosed groups relative to the vehicle controls.

EROD activities in the lung were significantly higher in all dosed groups compared to vehicle controls at 14, 31, and 53 weeks.

TABLE 8
Hepatic Cell Proliferation Data for Female Rats
at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture
of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	7	8	8	8	0
Labeling index (%)						
Week 14	0.818 ± 0.110	0.820 ± 0.220	1.040 ± 0.201	1.016 ± 0.150	1.854 ± 0.364	2.144 ± 0.757
Week 31	0.783 ± 0.130	1.274 ± 0.329	1.171 ± 0.260	1.427 ± 0.297	14.203 ± 1.805**	14.629 ± 0.917**
Week 53	1.127 ± 0.148	1.393 ± 0.346	1.433 ± 0.345	2.939 ± 0.463**	13.723 ± 0.916**	

** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test

^a Data are presented as mean ± standard error.

TABLE 9
Liver and Lung Cytochrome P450 Data for Female Rats
at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture
of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	7	8	8	8	0
Liver Microsomes						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Week 14	0.656 ± 0.046	1.485 ± 0.098**	2.726 ± 0.329**	3.694 ± 0.343**	3.732 ± 0.332**	2.606 ± 0.254**
Week 31	0.662 ± 0.033	1.518 ± 0.112**	2.014 ± 0.113**	2.698 ± 0.172**	2.368 ± 0.069**	1.618 ± 0.105**
Week 53	0.694 ± 0.057	1.597 ± 0.084**	2.078 ± 0.125**	2.663 ± 0.109**	1.593 ± 0.067**	
7-Ethoxresorufin- <i>O</i> -deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	48.07 ± 3.50	1,292.9 ± 119.91**	1,941.2 ± 166.86**	2,453.1 ± 85.60**	2,943.6 ± 186.75**	2,544.3 ± 101.42**
Week 31	41.69 ± 1.78	1,000.3 ± 83.59**	1,266.6 ± 62.87**	1,672.7 ± 85.87**	1,613.9 ± 40.70**	1,418.2 ± 52.33**
Week 53	43.53 ± 1.75	1,064.0 ± 69.51**	1,668.0 ± 71.45**	2,064.4 ± 84.82**	1,295.5 ± 64.32**	
7-Pentoxresorufin- <i>O</i> -deethylase (PROD) (pmol/minute per mg microsomal protein)						
Week 14	2.066 ± 0.083	15.821 ± 0.660**	20.988 ± 1.056**	27.058 ± 1.638**	33.367 ± 2.525**	25.942 ± 1.554**
Week 31	1.935 ± 0.147	12.588 ± 0.739**	16.205 ± 0.615**	19.023 ± 1.668**	18.447 ± 0.679**	16.048 ± 1.020**
Week 53	2.752 ± 0.167	17.003 ± 0.834**	23.113 ± 1.090**	23.607 ± 0.747**	19.718 ± 0.853**	
Lung Microsomes						
7-Ethoxresorufin- <i>O</i> -deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	1.196 ± 0.213	24.266 ± 1.172**	38.097 ± 2.207** ^b	46.033 ± 3.419**	44.569 ± 2.963**	42.250 ± 2.206**
Week 31	1.393 ± 0.153	33.734 ± 2.679**	41.753 ± 2.828**	43.792 ± 3.676**	43.003 ± 2.482**	49.660 ± 3.291**
Week 53	1.160 ± 0.233 ^c	33.107 ± 4.743**	42.117 ± 3.958**	40.905 ± 4.487**	43.693 ± 2.686**	

** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test

^a Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

^b n=9

^c n=7

Determinations of PCB 126 Concentrations in Tissues

Concentrations of PCB 126 were determined in fat, liver, lung, and blood at the 14-, 31-, and 53-week interim evaluations and at the end of the 2-year study (Table 10). The highest concentrations of PCB 126 were observed in the liver and fat.

In the fat of vehicle controls, measurable concentrations of PCB 126 were observed in a single rat at the 14-week interim evaluation. In dosed groups, fat concentrations of PCB 126 increased with increasing dose. In the fat of the 360 ng TEQ/kg stop-exposure group at 2 years, the

PCB 126 concentration was 6 ng/g, which was lower than the level observed in the 7 ng TEQ/kg group (14 ng/g).

Measurable concentrations of PCB 126 were observed in the liver from the vehicle controls at 14, 31, and 104 weeks. PCB 126 concentrations of 3, 4, and 5 ng/g were observed in the liver of single rats in the vehicle control group at 14, 31, and 104 weeks, respectively. In dosed groups, PCB 126 liver concentrations increased with increasing dose. The PCB 126 concentration in the liver of the 360 ng TEQ/kg stop-exposure group (11 ng/g) was lower than the level observed in the 7 ng TEQ/kg group at 2 years (48 ng/g).

TABLE 10
Tissue Concentrations of PCB 126 in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop-Exposure)
n							
Week 14	10	10	10	10	10	10	— ^b
Week 31	10	10	10	10	10	10	—
Week 53	8	7	8	8	8	0	—
Week 104	10	10	10	10	0	0	10
Fat							
Week 14	4.958 ^c	7.439 ± 0.261 ^d	17.29 ± 0.499	48.24 ± 1.716	117.3 ± 3.726	199.7 ± 4.436	
Week 31	BLOQ	8.488 ± 0.361	24.89 ± 0.729	70.41 ± 5.629	257.1 ± 8.793	437.1 ± 15.37	
Week 53	BLOQ	11.29 ± 0.398	28.57 ± 0.890	90.61 ± 3.577	307.4 ± 12.15		
Week 104	BLOQ	14.11 ± 0.840	32.61 ± 2.092	68.86 ± 5.947 ^d			5.545 ± 0.269
Liver							
Week 14	3.33 ^c	19.68 ± 1.46	69.64 ± 2.80	218.37 ± 6.94	520.96 ± 22.46	869.30 ± 35.32	
Week 31	4.47 ^c	34.54 ± 1.79	107.03 ± 5.34	348.41 ± 14.99	801.55 ± 41.17	1,132.62 ± 23.00	
Week 53	BLOQ	34.86 ± 0.62	101.23 ± 5.85	311.79 ± 15.19	598.46 ± 36.15		
Week 104	4.90 ^c	47.94 ± 5.33	118.76 ± 7.79	283.86 ± 19.45			10.69 ± 1.24
Lung							
Week 14	BLOQ	298.2 ± 13.32	614.9 ± 73.06 ^d	1,815.3 ± 302.56 ^d	2,220.3 ± 256.50	4,169.4 ± 528.97	
Week 31	BLOQ	207.6 ± 49.40 ^d	471.9 ± 69.15	1,194.0 ± 145.66	3,928.3 ± 231.56	6,945.1 ± 648.00	
Week 53	BLOQ	275.5 ± 55.12 ^e	553.6 ± 109.13	1,584.3 ± 263.11	3,401.9 ± 246.31		
Week 104	BLOQ	426.2 ± 35.69	1,069.6 ± 254.20	1,211.9 ± 173.75			349.6 ± 128.86
Blood							
Week 14	BLOQ	88.05 ± 10.53 ^c	71.82 ^c	193.91 ± 21.74 ^g	378.10 ± 21.69	571.74 ± 30.30	
Week 31	214.70 ^c	61.74 ± 6.41 ^f	72.43 ± 3.28 ^g	192.84 ± 17.73	572.39 ± 19.88	823.69 ± 51.23	
Week 53	BLOQ	BLOQ	95.52 ± 18.85 ^g	271.53 ± 43.59	586.06 ± 48.57		
Week 104	BLOQ	115.09 ± 18.93	192.69 ± 22.94	578.74 ± 56.75			BLOQ

^a Data are given in ng/g tissue (fat, liver), pg/g (lung), or pg/mL (blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation (BLOQ). LOQ_{fat}=2.1 ng/g, LOQ_{liver}=0.96 ng/g, LOQ_{lung}=192 pg/g, LOQ_{blood}=50 pg/mL

^b Not applicable

^c n=1

^d n=9

^e n=4

^f n=2

^g n=7

No measurable concentrations of PCB 126 were observed in the lungs of vehicle control animals. In dosed groups, concentrations of PCB 126 in lungs increased with increasing doses of PCB 126:PCB 118. In the 360 ng TEQ/kg stop-exposure group, the PCB 126 concentration in lungs at 2 years was 350 pg/g, which was lower than the level observed in the 7 ng TEQ/kg group (426 pg/g).

Measurable concentrations of PCB 126 were observed in the blood from a single vehicle control rat only at the 31-week interim evaluation. In dosed groups, blood concentrations of PCB 126 increased with increasing dose. No measurable concentration of PCB 126 was observed in the 360 ng TEQ/kg stop-exposure group at 2 years.

Determinations of PCB 118 Concentrations in Tissues

Concentrations of PCB 118 were determined in fat, liver, lung, and blood at the 14-, 31-, and 53-week interim evaluations and at the end of the 2-year study (Table 11). PCB 118 was only detected in a single vehicle control animal at 14 weeks. The highest concentrations of PCB 118 were observed in the fat. In the fat of dosed groups, concentrations of PCB 118 increased with increasing dose. In fat of the 360 ng TEQ/kg stop-exposure group at 2 years, the PCB 118 concentration was 9.5 µg/g, which was slightly higher than the level observed in the 7 ng TEQ/kg group (8.7 µg/g).

PCB 118 was undetectable in the liver of vehicle control animals. In dosed groups, concentrations of PCB 118 in the liver increased with increasing doses of PCB 126:PCB 118. In the 360 ng TEQ/kg stop-exposure group, the PCB 118 concentration in liver at

2 years was 391 ng/g, which was lower than the level observed in the 7 ng TEQ/kg group (442 ng/g).

PCB 118 was generally undetectable in the lungs of vehicle control animals; it was detected in a single vehicle control animal at 2 years. PCB 118 concentrations in the lungs of dosed groups increased with increasing dose. The PCB 118 concentration in the lungs of the 360 ng TEQ/kg stop-exposure group (243 ng/g) was higher than the level observed in the 7 ng TEQ/kg group at 2 years (173 ng/g).

PCB 118 was undetectable in the blood of control animals. In dosed groups, concentrations of PCB 118 in the blood increased with increasing doses of PCB 126:PCB 118. In the 360 ng TEQ/kg stop-exposure group, the PCB 118 concentration in blood at 2 years was 77 ng/mL, which was higher than the level observed in the 22 ng TEQ/kg group (68 ng/mL).

TABLE 11
Tissue Concentrations of PCB 118 in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop-Exposure)
n							^b
Week 14	10	10	10	10	10	10	—
Week 31	10	10	10	10	10	10	—
Week 53	8	7	8	8	8	0	—
Week 104	10	10	10	10	0	0	10
Fat							
Week 14	1.892 ^c	2.876 ± 0.134	8.493 ± 0.202	26.46 ± 1.249	69.37 ± 1.878	123.2 ± 3.523	
Week 31	BLOQ	4.316 ± 0.162	12.05 ± 0.463	37.05 ± 2.421	134.4 ± 2.299	239.5 ± 6.786	
Week 53	BLOQ	4.940 ± 0.146	13.63 ± 0.672	45.72 ± 2.688	164.1 ± 6.349		
Week 104	BLOQ	8.732 ± 0.630	19.52 ± 1.688	51.44 ± 4.837 ^d			9.461 ± 0.560
Liver							
Week 14	BLOQ	159.2 ± 5.59 ^e	502.6 ± 29.36	2,047.4 ± 92.86	6,053.2 ± 284.12	12,478.4 ± 696.07	
Week 31	BLOQ	201.8 ± 10.99	673.2 ± 35.54	3,228.9 ± 293.68	10,253.3 ± 580.76	15,449.7 ± 1,169.16	
Week 53	BLOQ	217.2 ± 6.52	807.6 ± 32.12	3,086.0 ± 132.46	12,591.5 ± 1,004.29		
Week 104	BLOQ	442.2 ± 32.42	1,114.7 ± 113.25	3,769.0 ± 202.67		—	390.8 ± 47.02
Lung							
Week 14	BLOQ	212.02 ± 114.17 ^d	250.38 ± 23.73	462.08 ± 63.92	885.32 ± 90.02	1,550.80 ± 110.13	
Week 31	BLOQ	68.90 ± 11.65	148.16 ± 7.64	404.07 ± 24.20	1,254.50 ± 48.53	2,122.80 ± 163.58	
Week 53	BLOQ	79.02 ± 11.85	146.08 ± 10.79	748.60 ± 172.77 ^d	1,429.25 ± 69.14		
Week 104	48.24 ^c	173.46 ± 19.04	686.67 ± 308.61	739.08 ± 59.92 ^d			242.96 ± 59.32
Blood							
Week 14	BLOQ	BLOQ	13.27 ± 0.7889 ^f	46.36 ± 3.6380	140.33 ± 8.0714	240.25 ± 9.3021	
Week 31	BLOQ	13.54 ^c	16.07 ± 0.8499 ^g	54.80 ± 2.4762	196.19 ± 9.9162	282.49 ± 20.0066	
Week 53	BLOQ	BLOQ	22.07 ± 2.3773	114.71 ± 33.7633	255.88 ± 25.9930		
Week 104	BLOQ	35.77 ± 6.3892	67.61 ± 6.1821	282.79 ± 34.0871			77.44 ^c

^a Data are given in µg/g (fat), ng/g tissue (liver, lung), or ng/mL (blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation (BLOQ). LOQ_{fat}=0.42 µg/g, LOQ_{liver}=38 ng/g, LOQ_{lung}=9.6 ng/g, LOQ_{blood}=10 ng/mL

^b Not applicable

^c n=1

^d n=9

^e n=4

^f n=6

^g n=7

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, lung, oral mucosa, thymus, thyroid gland, adrenal cortex, pancreas, kidney, heart, bone marrow, lymph nodes (mandibular, mediastinal, mesenteric), mesentery, brain, forestomach, spleen, nose, mammary gland, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, 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.

Due to decreased survival, animals in the 360 ng TEQ/kg core study group were not evaluated at the 53-week interim evaluation.

Liver: At 14 weeks, the absolute and relative liver weights of the 216 and 360 ng TEQ/kg groups were significantly greater than those of vehicle controls (Table B1). At 31 weeks, the absolute and relative liver weights of all dosed groups were significantly greater than those of vehicle controls. At 53 weeks, the absolute and relative liver weights of the 72 and 216 ng TEQ/kg groups were significantly greater than those of vehicle controls.

At 14 weeks, the incidences of several nonneoplastic lesions were increased in the groups administered 72, 216, or 360 ng TEQ/kg compared to the vehicle controls (Tables 12 and A5a). The incidences of pigmentation were significantly increased in groups administered 22 ng TEQ/kg or greater. Hepatocyte hypertrophy occurred in all dosed groups with significantly increased incidences in the 72, 216, and 360 ng TEQ/kg groups. The incidences of multinucleated hepatocyte, diffuse fatty change, and toxic hepatopathy were significantly increased in the 216 and 360 ng TEQ/kg groups. The severity of these nonneoplastic lesions also tended to increase with increasing dose.

Hepatocyte hypertrophy and pigmentation occurred in all dosed groups at 31 weeks, the incidences were significantly increased in groups administered 22 ng TEQ/kg or greater, and severities increased with increasing dose (Tables 12 and A5a). Also at 31 weeks, significantly increased incidences of hepatocyte hypertrophy, multinucleated hepatocytes, diffuse fatty change,

toxic hepatopathy, bile duct hyperplasia, centrilobular fibrosis, oval cell hyperplasia, and eosinophilic focus occurred in the 216 and 360 ng TEQ/kg groups. The incidences of portal fibrosis and nodular hyperplasia were significantly increased in the 360 ng TEQ/kg group. Cholangiofibrosis was present in two rats each in the 72 and 216 ng TEQ/kg groups, one 360 ng TEQ/kg rat, and one vehicle control rat.

At 53 weeks, the incidences of pigmentation were significantly increased in all dosed groups (Tables 12 and A5a). In the 72 and 216 ng TEQ/kg groups, there were significantly increased incidences of hepatocyte hypertrophy, multinucleated hepatocyte, diffuse fatty change, toxic hepatopathy, and centrilobular fibrosis. There were significantly increased incidences of bile duct and oval cell hyperplasia, portal fibrosis, nodular hyperplasia, and cholangiofibrosis in the 216 ng TEQ/kg group. Cholangiofibrosis was also present in two 72 ng TEQ/kg rats. No rats in the 360 ng TEQ/kg group were evaluated at 53 weeks.

At 2 years, the incidences of cholangiocarcinoma (multiple and/or single) were significantly increased in all groups administered 22 ng TEQ/kg or greater, including the stop-exposure group (Tables 13, A1b, A3a, and A3b); these incidences exceeded the historical vehicle control range (Tables 13 and A4a). The incidences of hepatocellular adenoma were significantly increased in the 216 and 360 ng TEQ/kg groups, and the incidences exceeded the historical vehicle control range. Cholangioma occurred in one rat in the 72 ng TEQ/kg group; hepatocholangioma occurred in one rat each in the 72, 216, and 360 ng TEQ/kg core study groups and in one rat in the 360 ng TEQ/kg stop-exposure group; and hepatocellular carcinoma occurred in one rat in the 216 ng TEQ/kg group (Table A1b).

Cholangiocarcinoma consisted of an irregular, relatively large, noncircumscribed lesion that replaced normal liver parenchyma. 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 displayed degenerative changes. Mitotic figures and localized invasion of adjacent liver parenchyma were also observed (Plate 1).

Hepatocellular adenoma was a nodular mass that usually was larger than a focus, had a distinct border, and

TABLE 12
Incidences of Nonneoplastic Lesions of the Liver in Female Rats
at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture
of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
14-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy ^a	0	3 (1.3) ^b	3 (1.0)	8** (1.0)	10** (1.5)	10** (2.2)
Hepatocyte, Multinucleated	0	0	0	0	9** (1.2)	10** (1.9)
Pigmentation	0	0	9** (1.1)	10** (1.0)	8** (1.0)	9** (1.1)
Fatty Change, Diffuse	0	0	0	0	5* (1.0)	10** (1.4)
Toxic Hepatopathy	0	0	0	1 (1.0)	4* (1.0)	8** (1.0)
31-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	2 (1.0)	4* (1.0)	8** (1.1)	10** (2.8)	10** (3.4)
Hepatocyte, Multinucleated	0	0	0	3 (1.0)	10** (2.2)	10** (2.6)
Pigmentation	2 (1.0)	4 (1.0)	9** (1.1)	10** (1.2)	10** (2.1)	10** (2.6)
Fatty Change, Diffuse	0	0	0	3 (1.7)	8** (1.4)	9** (1.4)
Toxic Hepatopathy	0	0	0	3 (1.0)	10** (2.1)	10** (2.8)
Bile Duct, Hyperplasia	0	0	0	1 (1.0)	10** (1.5)	10** (1.2)
Centrilobular, Fibrosis	0	0	0	3 (1.0)	10** (1.8)	10** (2.3)
Oval Cell, Hyperplasia	0	0	0	0	8** (1.3)	10** (1.3)
Eosinophilic Focus (includes multiple)	0	0	0	0	7**	5*
Portal Fibrosis	0	0	0	0	1 (2.0)	4** (2.3)
Hyperplasia, Nodular	0	0	0	0	1	4*
Cholangiofibrosis	1 (1.0)	0	0	2 (1.5)	2 (1.5)	1 (1.0)
53-Week Interim Evaluation						
Number Examined Microscopically	8	7	8	8	8	0
Hepatocyte, Hypertrophy	0	1 (1.0)	2 (1.5)	8** (1.6)	8** (3.8)	
Hepatocyte, Multinucleated	0	0	3 (1.0)	8** (1.4)	8** (2.6)	
Pigmentation	0	5** (1.0)	8** (1.1)	8** (2.0)	8** (2.5)	
Fatty Change, Diffuse	0	0	0	4* (1.0)	8** (2.1)	
Toxic Hepatopathy	0	0	2 (1.0)	8** (1.3)	8** (3.1)	
Bile Duct, Hyperplasia	0	0	1 (1.0)	1 (1.0)	8** (2.1)	
Centrilobular, Fibrosis	0	0	0	5* (1.2)	8** (2.0)	
Oval Cell, Hyperplasia	0	0	0	0	8** (2.6)	
Portal Fibrosis	0	0	0	0	8** (2.0)	
Hyperplasia, Nodular	0	0	0	1	8**	
Cholangiofibrosis	0	0	0	2 (1.5)	5* (2.4)	

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats
in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Hepatocellular Carcinoma	0	0	0	0	1	0	0
Hepatocholangioma (includes multiple)	0	0	0	1	1	1	1
Cholangioma	0	0	0	1	0	0	0

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test

** $P \leq 0.01$

▲ Significantly different ($P \leq 0.05$) from the 360 ng TEQ/kg core study group by the Poly-3 test

▲▲ $P \leq 0.01$

(T) Terminal sacrifice

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 Sprague-Dawley vehicle control groups (mean \pm standard deviation): 0/371

d Number of animals with neoplasm per number of animals with liver 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; the stop-exposure group is excluded from 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 sacrifice. A lower incidence in a dosed group is indicated by N.

h Pairwise comparison between the 360 ng TEQ/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by N.

i Value of statistic cannot be computed.

j Historical incidence: 4/371 (1.1% \pm 1.5%), range 0%-4%

k Not applicable; no neoplasms in animal group

produced more compression of surrounding normal parenchyma (Plates 2 and 3). Adenoma was composed of a rather uniform population of mildly to moderately pleomorphic hepatocytes that generally were normal size or slightly larger than normal and were arranged in abnormal lobular patterns. The hepatic cords within an adenoma usually intersected the surrounding normal hepatic cords at an oblique angle or sometimes even at a right angle. A few small proliferating bile ducts or oval cells were sometimes seen, but were not as numerous as in nodular hyperplasia. The uniform population of hepatocytes and lack of proliferating bile ducts were important features differentiating adenoma from nodular hyperplasia.

Hepatocholangioma was composed of a mixture of proliferating hepatocellular and bile duct elements. Hepatocholangioma was a rather large, nodular mass with a distinct border that produced compression of the

surrounding normal parenchyma. The hepatocellular element appeared similar to that seen in hepatocellular adenoma and consisted of a rather uniform population of mildly to moderately pleomorphic hepatocytes that were generally normal sized or slightly larger than normal and were arranged in abnormal hepatic cords. Intermixed with the proliferating hepatocytes were numerous small and large biliary structures surrounded by small amounts of dense fibrous tissue stroma that appeared similar to the biliary structures seen within a cholangioma. The smaller biliary structures resembled proliferating small bile ducts, while the large structures were generally irregular and sometimes moderately to markedly dilated. Some of the large structures became confluent, producing highly irregular cystic biliary structures that were incompletely separated by short septae projecting into the lumen. Some of the ductular lumens contained homogenous, lightly eosinophilic material but most were empty. The biliary structures were composed of a single

layer of flattened to cuboidal to low columnar, somewhat pleomorphic, but otherwise relatively normal-appearing bile duct epithelial cells.

Cholangioma was a well-demarcated mass consisting of multiple, densely packed, irregular, bile duct structures, some of which were moderately dilated, within a small amount of fibrous stroma. The bile duct structures were composed of a single layer of densely packed, columnar, somewhat pleomorphic, bile duct epithelial cells.

Hepatocellular carcinoma was a large, poorly demarcated, locally invasive mass composed of atypical hepatocytes that were arranged in trabeculae three or more cells thick and in glandular and solid growth patterns.

In all dosed groups, there were significantly increased incidences of minimal to marked hepatocyte hypertrophy, multinucleated hepatocyte, pigmentation, minimal to marked toxic hepatopathy, and diffuse fatty change (Tables 13 and A5b). The incidences of nodular hyperplasia, centrilobular fibrosis, and oval cell hyperplasia were significantly increased in groups administered 22 ng TEQ/kg or greater. In groups administered 72 ng TEQ/kg or greater, there were significantly increased incidences of bile duct cyst, bile duct hyperplasia, portal fibrosis, and cholangiofibrosis.

There were also significantly increased incidences of hepatocyte glandular structures in groups administered 216 ng TEQ/kg or greater; necrosis in all dosed groups except the 22 ng TEQ/kg and 360 ng TEQ/kg stop-exposure groups; centrilobular degeneration in the core study groups administered 22, 72, and 360 ng TEQ/kg and the 360 ng TEQ/kg stop-exposure group; eosinophilic focus in all dosed groups except the 72 and 216 ng TEQ/kg groups; and metaplasia in the 216 and 360 ng TEQ/kg core study groups (Tables 13 and A5b). The incidences of nonneoplastic liver lesions in the 360 ng TEQ/kg stop-exposure group were generally increased compared to the vehicle controls and significantly decreased compared to the 360 ng TEQ/kg core study group.

Hepatocyte hypertrophy was characterized by hepatocytes that were enlarged with increased amounts of eosinophilic cytoplasm. Minimal hypertrophy affected periportal hepatocytes and as severity increased, hepatocytes in other areas of the hepatic lobule were also affected. The hypertrophy usually was not confined to

periportal hepatocytes, and therefore, the general diagnosis of hepatocyte hypertrophy was used.

Multinucleated hepatocytes were characterized by scattered hepatocytes that were enlarged and contained multiple (more than two and often four to six) nuclei. The presence of binucleated hepatocytes was not sufficient to make this diagnosis.

Pigmentation consisted of light brown to golden pigment present within macrophages and occasionally hepatocytes. The pigmented macrophages were often seen in portal areas but were also seen scattered randomly within the liver. The pigment was shown to stain positive for iron with Perl's stain.

Toxic hepatopathy included all nonneoplastic liver changes under one overall term. The severity of toxic hepatopathy was graded in order to give one overall severity grade for the degree of toxicity in a liver. This was to allow for easier comparison of the degree of toxic change among different dosed groups than would be possible if the severities of all the individual nonneoplastic changes were compared among the different groups. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. The changes included under the diagnosis were hepatocyte hypertrophy, pigmentation, inflammation, multinucleated hepatocyte, diffuse fatty change, bile duct hyperplasia, oval cell hyperplasia, nodular hyperplasia, focal cellular alteration, cholangiofibrosis, bile duct cyst, necrosis, portal fibrosis, and centrilobular degeneration. When only findings of hepatocyte hypertrophy, pigmentation, and slight fatty change were present, no diagnosis was made. Minimal toxic hepatopathy was diagnosed when additional changes indicative of a toxic effect, usually a slight degree of bile duct and/or oval cell hyperplasia, sometimes a few large prominent altered hepatocellular foci, and occasionally a small focus cholangiofibrosis, were present. Mild toxic hepatopathy was characterized by the presence of multiple toxic changes, all of which were of minimal to mild severity. In addition, multiple prominent altered hepatocellular foci (usually mixed cell foci) and an occasional focus of nodular hyperplasia were sometimes present. Moderate toxic hepatopathy was diagnosed when the entire or nearly the entire spectrum of toxic changes was present, with some degree of distortion of the normal liver structure caused by prominent altered hepatocellular foci, nodular hyperplasia, and cholangiofibrosis.

Marked toxic hepatopathy was diagnosed when severe toxic changes were present with pronounced distortion of the liver architecture. Livers with marked toxic hepatopathy often had a multinodular appearance due to the presence of numerous large foci of nodular hyperplasia that replaced much of the liver parenchyma.

Diffuse fatty change was generally a minimal to mild change consisting of discrete clear vacuoles (consistent with lipid) in the cytoplasm of hepatocytes involving foci of hepatocytes scattered diffusely throughout the liver. Bile duct hyperplasia consisted of increased numbers of bile duct nuclei within portal areas. Oval cell hyperplasia consisted of small ovoid cells (with basophilic cytoplasm and round to ovoid nuclei) that were arranged in single or double rows and located predominantly in the portal areas. Bile duct cysts were characterized by either single or multiple dilated bile ducts that were lined by attenuated epithelium. Cholangiofibrosis consisted of proliferating, atypical-appearing bile ducts surrounded by dense fibrous tissue. Cholangiofibrosis appeared relatively small in size and well demarcated and did not show evidence of localized invasion (Plate 4).

Nodular hyperplasia was characterized by few to numerous, small to large, nodular foci generally composed of hepatocytes that were considerably larger than normal hepatocytes (hepatocytic hypertrophy) sometimes mixed with areas of increased numbers of small hepatocytes (hepatocytic hyperplasia). Areas of nodular hyperplasia blended with the surrounding parenchyma, although often they had a distinct border. Large, focal to multifocal areas of nodular hyperplasia were sometimes seen that caused compression of surrounding tissue, and/or bulging of the capsular surface (Plates 5 and 6). The cells within nodular hyperplasia generally were very large, larger than cells seen within adenomas and usually larger than cells seen within foci, with abundant eosinophilic cytoplasm and often with variable degrees of cytoplasmic vacuolization. In a few areas of nodular hyperplasia, however, the cells were of more normal size or sometimes slightly smaller than normal. The cells appeared to be arranged in normal cords, but the cells often were so large as to obscure the sinusoids between the cords, giving the appearance of solid sheets of hepatocytes. Bile duct hyperplasia and portal areas were usually present within nodular hyperplasia. Blood vessels and/or central veins were also sometimes seen within areas of nodular hyperplasia, usually when hepatocytes were not so hypertrophic as to obscure completely the normal architecture. The presence of

hypertrophic, vacuolated hepatocytes together with proliferating bile ducts was considered to be characteristic of nodular hyperplasia and was considered to be useful in the diagnosis of nodular hyperplasia.

Centrilobular fibrosis consisted of fibrous connective tissue accumulation that extended around central veins. Portal fibrosis consisted of fibrous connective tissue accumulation that extended between adjacent portal areas.

Necrosis consisted of scattered necrotic areas of hepatic parenchyma that were often randomly distributed, but occasionally, in more severe cases, were distributed more diffusely.

The hepatic parenchyma was variably effaced with cells forming structures resembling glands (Plate 7) and diagnosed as "hepatocyte, glandular structures." Glandular structures ranged in number from a few scattered structures to very numerous structures that were diffusely scattered throughout the entire liver, including within foci of nodular hyperplasia. The structures were generally quite small with a diameter approximately equal to that of a portal bile duct, but some became quite large with a diameter up to approximately 10 times that of a normal bile duct. The structures were generally lined by large cells resembling hepatocytes but were sometimes smaller and cuboidal, resembling biliary epithelium. Sometimes the central lumen contained granular eosinophilic material or, rarely, some free red blood cells. While the exact nature of these lesions was unclear, it was speculated that they represented abnormal differentiation of hepatic precursor cells resulting in cells that had properties of both hepatocytes and biliary epithelial cells.

Metaplasia (pancreatic) was a slight lesion consisting of one to several well-formed pancreatic acini within the liver, and was presumed to be due to metaplasia of hepatic precursor cells to pancreatic acinar cells (Plate 8).

Eosinophilic, mixed, basophilic, and clear cell foci are characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic focus is composed of cells with eosinophilic cytoplasm. To be classified as an eosinophilic focus, at least 80% of the cells within the focus have to be eosinophilic cells. Otherwise the focus is classified as a mixed cell focus. If two or more foci of a given type are present in a liver, it is diagnosed as multiple.

The treatment-related foci were of eosinophilic and mixed cell type, and often differed somewhat from those in vehicle control animals. Foci in vehicle control animals consisted of hepatocytes that were generally somewhat larger than normal but appeared otherwise normal and were arranged in a relatively normal lobular pattern. The hepatic cords at the periphery of these foci generally merged imperceptibly with surrounding normal liver resulting in an indistinct border and little or no compression of the adjacent liver parenchyma. In contrast, foci in dosed animals often had a more definite border, the cords within the focus often were not smoothly continuous with those in the surrounding parenchyma, and the foci consisted of cells that were often prominently enlarged with abundant eosinophilic or clear vacuolated cytoplasm. If more than 20% of the cells were vacuolated, the focus was classified as mixed cell type, otherwise it was classified as an eosinophilic

focus. In addition, some larger foci caused variable degrees of compression of the surrounding hepatic parenchyma. The cells were arranged in a relatively normal lobular pattern and foci sometimes contained large blood vessels and/or portal areas. The presence of proliferating bile ducts or oval cells was not considered characteristic of a focus. If proliferating bile ducts were present, this was considered indicative of nodular hyperplasia.

Lung: At 53 weeks, the absolute and relative lung weights of the 72 and 216 ng TEQ/kg groups were significantly greater than those of the vehicle controls (Table B1).

At 14 weeks, histiocyte infiltration and inflammation were present in all dosed groups except the stop-exposure group (Tables 14 and A5a). The incidences of

TABLE 14
Incidences of Selected Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats
in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
14-Week Interim Evaluation							
Number Examined							— ^a
Microscopically	10	10	10	10	10	10	
Infiltration, Histiocyte ^b	1 (1.0) ^c	6* (1.0)	7** (1.0)	4 (1.0)	3 (1.0)	4 (1.0)	
Inflammation	1 (1.0)	6* (1.7)	7** (1.6)	4 (1.5)	3 (1.0)	6* (1.3)	
31-Week Interim Evaluation							
Number Examined							—
Microscopically	10	10	10	10	10	10	
Cystic Keratinizing Epithelioma (includes multiple)	0	0	0	0	0	3	
53-Week Interim Evaluation							
Number Examined							—
Microscopically	8	7	8	8	8	0	
Alveolar Epithelium, Metaplasia, Bronchiolar	0	0	3 (1.3)	3 (2.3)	6** (1.7)		
Cystic Keratinizing Epithelioma (includes multiple)	0	0	0	1	5*		

TABLE 14
Incidences of Selected Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats
in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
2-Year Study							
Number Examined							
Microscopically	53	51	53	53	53	66	50
Alveolar Epithelium,							
Metaplasia, Bronchiolar	1 (1.0)	14** (1.3)	39** (1.4)	46** (1.6)	35** (1.1)	8** (1.0)	15** (1.2)
Metaplasia, Squamous	0	1 (1.0)	2 (1.5)	14** (2.1)	16** (2.3)	7** (2.6)	8** (1.9)
Serosa, Fibrosis	3 (1.3)	0	0	1 (3.0)	16** (1.6)	8** (1.8)	1▲ (1.0)
Keratin Cyst	0	0	0	0	0	0	9** (3.3)
Alveolar Epithelium,							
Hyperplasia	18 (1.2)	10 (1.2)	2** (1.0)	1** (1.0)	1** (1.0)	4 (1.3)	5 (1.0)
Cystic Keratinizing							
Epithelioma, Multiple	0	0	0	14**	48**	35**	5*
Cystic Keratinizing							
Epithelioma, (includes multiple) ^d							
Overall rate ^e	0/53 (0%)	0/51 (0%)	0/53 (0%)	20/53 (38%)	49/53 (92%)	41/66 (62%)	12/50 (24%)
Adjusted rate ^f	0.0%	0.0%	0.0%	51.4%	97.7%	97.0%	42.5%
Terminal rate ^g	0/27 (0%)	0/17 (0%)	0/24 (0%)	9/15 (60%)	0/0	0/0	3/10 (30%)
First incidence (days)	— ^j	— ^k	—	594	267	260	240
Poly-3 test ^h	P<0.001	— ^k	—	P<0.001	P<0.001	P<0.001	P<0.001
Poly-3 test ⁱ							P<0.001N

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)

** $P \leq 0.01$

▲ Significantly different ($P \leq 0.01$) from the 360 ng TEQ/kg core study group by the Poly-3 test

a Not applicable

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 Sprague-Dawley vehicle control groups: 0/371

e Number of animals with neoplasm per number of animals with lung 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; the stop-exposure group is excluded from 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 sacrifice.

i Pairwise comparison between the 360 ng TEQ/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by N.

j Not applicable; no neoplasms in animal group

k Value of statistic cannot be computed.

histiocyte infiltration were significantly increased in the 7 and 22 ng TEQ/kg groups. The incidences of inflammation were significantly increased in the 7, 22, and 360 ng TEQ/kg groups.

Cystic keratinizing epithelioma occurred in the 360 ng TEQ/kg group at 31 weeks, but the incidence was not significantly increased (Tables 14 and A1a).

Cystic keratinizing epithelioma occurred in the 72 and 216 ng TEQ/kg groups at 53 weeks with a significantly increased incidence in the 216 ng TEQ/kg group.

At 53 weeks, bronchiolar metaplasia of the alveolar epithelium occurred in the 22, 72, and 216 ng TEQ/kg groups, with a significantly increased incidence in the 216 ng TEQ/kg group.

Significantly increased incidences of cystic keratinizing epithelioma (single or multiple) occurred in groups administered 72 ng TEQ/kg or greater including the 360 ng TEQ/kg stop-exposure group at 2 years (Tables 14, A1b, A3a, and A3b). No instances of cystic keratinizing epithelioma have been observed in historical female Sprague-Dawley vehicle controls (Tables 14 and A4b). The incidence of this lesion was significantly less in the 360 ng TEQ/kg stop-exposure group compared to that in the 360 ng TEQ/kg core study group.

Cystic keratinizing epitheliomas ranged from relatively small to very large lesions that replaced much of the normal lung parenchyma. The epitheliomas were cystic structures consisting of a highly irregular wall of highly keratinized stratified squamous epithelium and a center filled with keratin (Plate 9). The outer portion of the lesion grew by expansion into the adjacent lung but evidence of invasion was not observed.

In all dosed groups at 2 years, there were significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium (Tables 14 and A5b). Squamous metaplasia occurred in all dosed groups and the incidences were significantly increased in groups administered 72 ng TEQ/kg or greater. Significantly increased incidences of serosal fibrosis occurred in the 216 and 360 ng TEQ/kg core study groups. The incidence of keratin cyst was significantly increased in the 360 ng TEQ/kg stop-exposure group when compared to the vehicle controls. Alveolar epithelium hyperplasia occurred in all groups, with significantly decreased incidences in the 22, 72, and 216 ng TEQ/kg groups.

Bronchiolar metaplasia of the alveolar epithelium consisted of replacement of the normal alveolar epithelium by cuboidal to columnar, sometimes ciliated cells, and was often accompanied by abundant mucus production in the affected area (Plate 10). The lesion generally diffusely affected the epithelium located at the bronchiolar-alveolar junction and adjacent alveoli. Aggregates of large alveolar macrophages were sometimes present in areas of bronchiolar metaplasia. This change was differentiated from the alveolar epithelial hyperplasia that was seen in vehicle control animals.

In alveolar epithelial hyperplasia, alveoli were lined by bronchiolar epithelium and, unlike bronchiolar

metaplasia in dosed animals, prominent mucus production was not observed in alveolar epithelial hyperplasia, and very prominent inflammatory cell infiltrate, consisting of large aggregates of alveolar macrophages commonly mixed with focal aggregates of neutrophils, was usually associated with the affected areas.

Squamous metaplasia of the alveolar epithelium was generally a minor change consisting of one or more small, irregular foci of keratinizing stratified squamous epithelium that had replaced the normal alveolar epithelium (Plate 11).

Serosal fibrosis was a lesion occurring in the serosa overlying some of the very large cystic keratinizing epitheliomas. The serosa was mildly to moderately thickened by proliferating fibrous connective tissue. This lesion was considered to be caused by the presence of the large, underlying cystic keratinizing epithelioma.

Oral mucosa: Squamous cell carcinoma occurred in all groups except the 216 ng TEQ/kg group (Tables 15, A1b, and A3a), however none of the incidences were significantly increased. The incidences in the 22 and 72 ng TEQ/kg groups exceeded the historical vehicle control range (Tables 15 and A4c). Gingival squamous hyperplasia occurred in all groups at 2 years with significantly increased incidences in all dosed groups except the 7 ng TEQ/kg group (Tables 15 and A5b).

Squamous cell carcinoma occurred within the oral mucosa of the palate and was located adjacent to the molar tooth in nasal section III. It was characterized by irregular cords and clusters of stratified squamous epithelium cells that invaded deep into the underlying connective tissue and often invaded the bone of the maxilla (Plate 12).

Squamous hyperplasia was a localized lesion that occurred in the stratified squamous epithelium of the gingival oral mucosa adjacent to the molar teeth in nasal section III. It consisted of varying degrees of thickening of the epithelium, often with the formulation of epithelial rete pegs that extended into the underlying connective tissue. Ends of hair shafts and/or some degree of inflammation were often present in the areas of hyperplasia, suggesting the possibility of an association between the hyperplasia and the inflammation and hair shafts.

TABLE 15
Incidences of Selected Neoplasms and Nonneoplastic Lesions of the Oral Mucosa in Female Rats
in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Number Examined							
Microscopically	53	51	53	53	53	66	50
Gingival, Hyperplasia							
Squamous ^a	11 (1.1) ^b	10 (1.2)	20* (1.4)	24** (1.8)	27** (1.7)	18** (1.6)	18** (1.4)
Gingival Squamous Cell Carcinoma ^c							
Overall rate ^d	1/53 (2%)	1/51 (2%)	2/53 (4%)	4/53 (8%)	0/53 (0%)	1/66 (2%)	1/50 (2%)
Adjusted rate ^e	2.4%	3.0%	5.2%	10.6%	0.0%	10.0%	4.7%
Terminal rate ^f	0/27 (0%)	0/17 (0%)	1/24 (4%)	0/15 (0%)	0/0 ^h	0/0	0/10 (0%)
First incidence (days)	687	651	608	594	—	482	636
Poly-3 test ^g	P=0.511	P=0.703	P=0.467	P=0.146	P=0.605N	P=0.447	P=0.594

* 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

^c Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean \pm standard deviation): 4/371 (1.1% \pm 1.0%), range 0%-2%

^d Number of animals with neoplasm per number of animals with oral mucosa 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; the stop-exposure group is excluded from the trend test.

Beneath the dose 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 sacrifice. A lower incidence in a dose group is indicated by N.

^h Not applicable; no neoplasms in animal group

Thymus: At 14 weeks, atrophy occurred in all dosed groups and there were significantly increased incidences in the 216 and 360 ng TEQ/kg groups (Tables 16 and A5a). At 31 weeks, there were significantly increased incidences of atrophy in the 72, 216, and 360 ng TEQ/kg groups, and the severity increased with increasing dose. At 53 weeks, atrophy occurred in all groups examined and, although none of the incidences were significantly increased, the severity was increased in groups administered 72 and 216 ng TEQ/kg. The incidences of atrophy at 2 years were significantly increased in all dosed groups except the 22 ng TEQ/kg group. Atrophy consisted of varying degrees of loss of lymphoid cells from the cortex resulting in reduction of cortical thickness (Plate 13).

Thyroid gland: At 14 weeks, there were significantly increased incidences of follicular cell hypertrophy in all

dosed groups except the 7 ng TEQ/kg group (Tables 16 and A5a). The incidence of follicular cell hypertrophy was significantly increased in the 216 ng TEQ/kg group at 31 weeks. At 53 weeks, follicular cell hypertrophy was present in all groups examined, but the incidences were not significantly increased. The severity of this lesion generally increased with increasing dose at 14, 31, and 53 weeks. The incidences of follicular cell hypertrophy were significantly increased in all dosed groups at 2 years. Follicular cell hypertrophy was a localized to diffuse change, characterized by follicles that were decreased in size and contained decreased amounts of colloid in which aggregates of amphophilic, flocculant-appearing material were often present. The affected follicles were lined by large, prominent, cuboidal follicular epithelial cells that were approximately two to three times normal size, usually with abundant pale cytoplasm containing small, clear vacuoles. Since some degree of

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
14-Week Interim Evaluation							
Thymus ^a	10	10	10	10	10	10	— ^c
Atrophy ^b	0	3 (1.0) ^d	1 (1.0)	3 (1.0)	7** (1.1)	9** (1.6)	
Thyroid Gland	10	10	10	10	10	10	—
Follicular Cell, Hypertrophy	1 (1.0)	1 (1.0)	5* (1.0)	6** (1.3)	8** (1.6)	7** (1.4)	
31-Week Interim Evaluation							
Thymus	10	10	10	9	10	6	—
Atrophy	3 (1.3)	2 (1.5)	5 (1.6)	9** (2.3)	9** (3.4)	6* (3.7)	
Thyroid Gland	10	10	10	10	10	10	—
Follicular Cell, Hypertrophy	3 (1.3)	3 (1.0)	7 (1.3)	7 (1.4)	9** (1.8)	7 (1.7)	
Adrenal Cortex	10	10	10	10	10	10	—
Atrophy	0	0	0	0	2 (1.0)	8** (1.8)	
Pancreas	10	10	10	10	10	10	—
Acinus, Vacuolization Cytoplasmic	0	0	0	1 (1.0)	10** (1.1)	10** (1.1)	
53-Week Interim Evaluation							
Thymus	8	7	8	8	6	0	—
Atrophy	5 (2.0)	7 (2.0)	7 (2.1)	8 (3.0)	6 (3.8)		
Thyroid Gland	8	7	8	8	8	0	—
Follicular Cell, Hypertrophy	3 (1.0)	4 (1.5)	6 (1.0)	7 (1.4)	7 (1.6)		
Adrenal Cortex	8	7	8	8	8	0	—
Atrophy	0	0	0	0	5* (2.2)		
Pancreas	8	7	8	8	8	0	—
Acinus, Vacuolization Cytoplasmic	0	0	0	6** (1.0)	8** (1.8)		

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
2-Year Study							
Thymus	53	51	51	51	51	59	46
Atrophy	40 (2.7)	44* (2.5)	44 (3.0)	49** (3.9)	50** (3.8)	57** (3.8)	45** (3.4)
Thyroid Gland	53	51	53	52	52	66	49
Follicular Cell, Hypertrophy	22 (1.2)	35** (1.4)	34* (1.8)	38** (1.8)	44** (1.9)	37** (1.7)	29** (1.7)
Adrenal Cortex	53	51	53	53	53	65	50
Atrophy	2 (3.0)	1 (4.0)	0	15** (2.4)	44** (2.4)	40** (2.4)	12***▲ (2.3)
Vacuolization Cytoplasmic	14 (1.6)	16 (1.6)	13 (1.5)	24* (1.7)	27** (1.7)	12* (1.5)	16* (1.6)
Pancreas	53	51	53	53	53	65	50
Acinus, Vacuolization Cytoplasmic	0	1 (1.0)	8** (1.0)	39** (1.6)	49** (1.9)	43** (1.6)	41** (1.6)
Artery, Inflammation, Chronic Active	0	2 (3.0)	2 (2.0)	21** (2.3)	14** (2.6)	4** (2.3)	10** (1.8)
Edema	0	0	1 (2.0)	0	4* (1.8)	19** (1.8)	12***▲ (1.7)
Acinus, Atrophy	1 (1.0)	5 (1.8)	3 (1.3)	5 (1.8)	9** (1.2)	8** (1.8)	8** (2.4)
Inflammation, Chronic Active	1 (1.0)	5 (1.8)	4 (1.5)	3 (1.3)	2 (1.5)	6** (1.8)	5* (1.6)
Kidney	53	51	53	53	53	65	50
Nephropathy	41 (1.1)	37 (1.2)	37 (1.2)	48** (2.0)	50** (2.0)	43* (1.4)	34* (2.0)
Transitional Epithelium, Hyperplasia	4 (2.3)	6 (1.8)	6 (1.5)	16** (2.3)	1 (3.0)	0	0
Heart	53	51	53	53	53	65	50
Cardiomyopathy	20 (1.2)	13 (1.1)	17 (1.0)	34** (1.1)	14 (1.0)	19** (1.3)	15▲ (1.1)
Coronary Artery, Inflammation, Chronic Active	1 (1.0)	1 (2.0)	0	7* (2.0)	10** (2.7)	9** (2.4)	6* (2.0)
Epicardium, Inflammation	1 (1.0)	1 (2.0)	0	6* (1.2)	13** (1.5)	6** (1.5)	1▲ (1.0)
Bone Marrow	53	51	53	53	53	66	50
Hyperplasia	37 (2.8)	41 (2.8)	36 (2.8)	48** (2.9)	47** (2.9)	49** (2.6)	40▲ (2.6)
Lymph Node, Mandibular	53	51	53	52	52	66	49
Hemorrhage	0	0	1 (2.0)	0	2 (1.5)	7** (2.0)	4* (1.8)
Lymph Node, Mediastinal	28	16	16	25	20	45	22
Hemorrhage	2 (1.5)	2 (2.0)	4 (1.5)	15** (1.9)	7** (1.9)	25** (1.8)	11***▲ (2.0)
Lymph Node, Mesenteric	53	51	53	53	52	65	50
Hemorrhage	0	2 (1.5)	2 (2.0)	2 (2.0)	4* (1.5)	14** (2.1)	10***▲ (1.9)
Mesentery	53	35	45	49	52	63	43
Artery, Inflammation, Chronic Active	0	1 (3.0)	1 (4.0)	9** (2.7)	9** (2.1)	3* (1.7)	7** (2.0)

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
2-Year Study (continued)							
Brain	53	51	53	53	53	66	50
Hemorrhage	0	1 (1.0)	1 (1.0)	4 (1.3)	5** (2.0)	14** (2.2)	8**▲ (1.8)
Stomach, Forestomach	53	51	53	53	53	65	50
Hyperplasia, Squamous	4 (1.5)	1 (2.0)	3 (2.3)	11* (2.0)	4 (1.8)	9** (1.9)	2▲▲ (1.5)
Spleen	53	51	53	53	53	65	50
Lymphoid Follicle, Atrophy	4 (2.5)	5 (2.0)	2 (2.5)	5 (2.2)	3 (2.3)	9** (2.6)	4▲ (2.5)
Nose	53	51	53	53	53	66	50
Inflammation	17 (1.2)	15 (1.4)	16 (1.3)	10 (1.7)	22* (1.7)	11 (1.4)	11 (1.7)
Respiratory Epithelium,							
Hyperplasia	8 (1.6)	11 (1.8)	5 (1.4)	8 (2.0)	16** (2.4)	9* (1.9)	10 (2.5)
Olfactory Epithelium,							
Metaplasia	0	2 (2.0)	1 (2.0)	3 (2.3)	8** (2.3)	1 (2.0)	5* (2.4)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)

** $P \leq 0.01$

▲ Significantly different ($P \leq 0.05$) from the 360 ng TEQ/kg core study group by the Poly-3 test

▲▲ $P \leq 0.01$

^a Number of animals with tissue examined microscopically

^b Number of animals with lesion

^c Not applicable

^d Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

this change can occur spontaneously, the severity grade of minimal was recorded when 50% to 60% of the follicles were involved, mild when 60% to 75% of the follicles were involved, moderate when 75% to 90% of the follicles were involved, and marked when over 90% of the follicles were involved (Plates 14 and 15).

Adrenal cortex: Atrophy occurred in the 216 and 360 ng TEQ/kg groups at 31 weeks with a significantly increased incidence in the 360 ng TEQ/kg group (Tables 16 and A5a). At 53 weeks, the incidence of atrophy was significantly increased in the 216 ng TEQ/kg group. At 2 years, atrophy occurred in all groups except the 22 ng TEQ/kg group, and significantly increased incidences occurred in groups administered 72 ng TEQ/kg or greater (Tables 16 and A5b). Cytoplasmic vacuolization occurred in all groups at 2 years with significantly increased incidences in groups administered 72 ng TEQ/kg or greater (Tables 16 and A5b).

Cortical atrophy was a locally extensive to diffuse change characterized by loss of cortical epithelial cells within the zona fasciculata and zona reticularis with a subsequent reduction in cortical thickness. The zona glomerulosa was spared. The remaining cells were sometimes vacuolated, especially in the more severe lesions. In severe cases the entire cortex was considerably reduced in thickness resulting in a smaller gland that often was surrounded by a thickened capsule.

Cortical cytoplasmic vacuolization was a focal to multifocal to diffuse change consisting of small, discrete, clear intracytoplasmic vacuoles. Sometimes the cytoplasm contained a large single vacuole that displaced the nucleus. The changes were morphologically consistent with the accumulation of lipid. Cytoplasmic vacuolization occurred most commonly within foci of hypertrophy.

Pancreas: At 31 weeks, the incidences of cytoplasmic vacuolization of the pancreas were significantly increased in the 216 and 360 ng TEQ/kg groups (Tables 16 and A5a). At 53 weeks, the only occurrences of cytoplasmic vacuolization were significantly increased incidences in the 72 and 216 ng TEQ/kg groups.

Cytoplasmic vacuolization, chronic active artery inflammation, edema, acinus atrophy, and chronic active inflammation occurred in most dosed groups at 2 years (Tables 16 and A5b). There were significantly increased incidences of cytoplasmic vacuolization in all dosed groups except the 7 ng TEQ/kg group and chronic active artery inflammation in all dosed groups except the 7 and 22 ng TEQ/kg groups. In the 216 and 360 ng TEQ/kg core study groups and the 360 ng TEQ/kg stop-exposure group, there were significantly increased incidences of edema and acinus atrophy. There were significantly increased incidences of chronic active inflammation in the 360 ng TEQ/kg core and stop-exposure groups.

Cytoplasmic vacuolization consisted of small, clear, discrete intracytoplasmic vacuoles within pancreatic acinar cells. Sometimes these vacuoles coalesced to form larger single vacuoles. The severity of the change was determined by the degree of vacuolization per cell and the amount of tissue involved. Arterial chronic active inflammation was a focal to multifocal change characterized by a thick mantle of macrophages, lymphocytes, and plasma cells around the arteries, with infiltration into the muscular layers of the artery. There was often fibrinoid necrosis of the vessel, and the tunica intima was frequently thickened. Endothelial cells were swollen or decreased in number. This inflammatory reaction often extended into the surrounding parenchyma.

Edema was characterized by separation of the acinar parenchyma by clear spaces that had been filled with edema fluid. Atrophy was a focal to multifocal to diffuse change consisting of a reduction in the amount of acinar tissue with an associated increase in stromal fibrous connective tissue. Chronic active inflammation was generally associated with atrophy and consisted of an infiltrate of mononuclear cells with occasional neutrophils within the stroma (Plate 16).

Kidney: Nephropathy occurred in all groups at 2 years and there were significantly increased incidences in all dosed groups except the 7 and 22 ng TEQ/kg groups

(Tables 16 and A5b). Transitional epithelium hyperplasia occurred in all groups except the 360 ng TEQ/kg core and stop-exposure groups with a significantly increased incidence in the 72 ng TEQ/kg group.

Nephropathy was generally a minimal to mild change, although sometimes moderate to marked nephropathy was seen. It had the typical appearance of this lesion as seen in aging rats, and was similar to that observed in Fischer F344 rats (Barthold, 1998). Nephropathy was characterized by scattered foci of regenerative tubules lined by basophilic epithelium and sometimes surrounded by increased basement membrane, dilated tubules filled with proteinaceous casts and surrounded by fibrous connective tissue, and scattered foci of mixed inflammatory cells. Severity was graded based upon the number and extent of changes described above. Minimal nephropathy was characterized by small numbers of scattered affected tubules, usually involving less than 10% of the renal tubules. On the other extreme, marked nephropathy involved approximately 50% to 60% or more of the tubules.

Transitional epithelium hyperplasia was sometimes focal to multifocal, but generally a diffuse, usually minimal to mild change consisting of varying degrees of thickening of the renal pelvic or papillary epithelium up to approximately 1.5 to 2 times the normal thickness. The significance of this was unclear as it did not appear to correlate with the increased severity of nephropathy since the animals with hyperplasia often had minimal nephropathy.

Heart: At 2 years, cardiomyopathy, chronic active coronary artery inflammation, and epicardium inflammation occurred in most groups (Tables 16 and A5b). Significantly increased incidences of cardiomyopathy occurred in the 72 and 360 ng TEQ/kg core study groups. There were significantly increased incidences of chronic active coronary artery inflammation in all dosed groups except the 7 and 22 ng TEQ/kg groups. Significantly increased incidences of epicardium inflammation occurred in the 72, 216, and 360 ng TEQ/kg core study groups.

Cardiomyopathy had the typical microscopic appearance of this lesion seen in aging rats and appeared similar to the cardiomyopathy seen in aging F344/N rats (MacKenzie and Alison, 1990). It was a multifocal, generally minimal to mild lesion consisting of hyper-eosinophilic myofibers that lacked cross striations,

infiltrates of mononuclear cells, separation of myofibers by myxomatous material (bluish material on H and E stain), and eventually replacement of myofibers by fibrous connective tissue. The severity was graded based upon the number and extent of foci of myocardial degeneration. Minimal cardiomyopathy consisted of a few scattered foci while mild cardiomyopathy consisted of a greater number of lesions more diffusely scattered within the myocardium.

Chronic active inflammation of the coronary artery generally was characterized by deposition of hyaline material beneath the intima, and small amounts of mixed inflammatory cell infiltrate were seen within the wall and the adventitia. Epicardium inflammation was usually a minimal to mild change characterized by focal to multifocal to diffuse infiltration of the epicardium overlying the outer surface of the heart with small to moderate numbers of mixed mononuclear inflammatory cells.

Bone marrow: The incidences of hyperplasia at 2 years were significantly increased in the 72, 216, and 360 ng TEQ/kg core study groups (Tables 16 and A5b). Bone marrow hyperplasia was characterized by a diffuse increase in myeloid hematopoietic cells and was graded as follows: marked when the entire marrow cavity was filled with dense marrow, moderate when marrow elements filled about 90% of the cavity (the remaining 10% was fat), mild when marrow elements filled approximately 60% to 90% of the marrow cavity, and minimal which was rarely recorded because of the normal variation in the amount of bone marrow. Normal bone marrow was used when the distal end of the femur section contained 20% to 60% marrow.

Lymph nodes: At 2 years, hemorrhage in the mandibular lymph node occurred in the 22 and 216 ng TEQ/kg groups and the 360 ng TEQ/kg core study and stop-exposure groups with significantly increased incidences in the 360 ng TEQ/kg groups (Tables 16 and A5b). Hemorrhage in the mediastinal lymph node occurred in all groups with significantly increased incidences in groups that were administered 72 ng TEQ/kg or greater. Hemorrhage in the mesenteric lymph node occurred in all dosed groups with significantly increased incidences in the core study groups administered 216 or 360 ng TEQ/kg and the 360 ng TEQ/kg stop-exposure group. For these lymph nodes, hemorrhage was characterized by varying numbers of free red blood cells within the medullary sinuses.

Mesentery: Chronic active inflammation of the mesenteric artery occurred in all dosed groups at 2 years, with significantly increased incidences in all dosed groups except the 7 and 22 ng TEQ/kg groups (Tables 16 and A5b), and appeared similar to that seen in the pancreas.

Brain: At 2 years, hemorrhage occurred in all dosed groups with significantly increased incidences in groups administered 216 ng TEQ/kg or greater (Tables 16 and A5b). Hemorrhage was generally a minimal to mild change consisting of few to several scattered small foci of free red blood cells within the brain parenchyma. Hemorrhagic foci tended to be more common in the white matter and in the more casual portions of the brain (cerebellum and caudal thalamus) but was also sometimes seen within the gray matter including the cerebral cortex.

Forestomach: Squamous hyperplasia occurred in all groups at 2 years with significantly increased incidences in the 72 and 360 ng TEQ/kg core study groups (Tables 16 and A5b). Squamous hyperplasia of the forestomach epithelium was generally a minimal to mild, focal, or occasionally multifocal change characterized by varying degrees of thickening of the stratified squamous epithelium up to approximately five times normal thickness in more severe cases. Sometimes the hyperplasia occurred around a focal ulcer, although most cases occurred without the presence of an apparent ulcer.

Spleen: Atrophy of the lymphoid follicle occurred in all groups at 2 years, but the only significantly increased incidence occurred in the 360 ng TEQ/kg core study group (Tables 16 and A5b).

Nose: At 2 years, inflammation, respiratory epithelial hyperplasia, and olfactory epithelial metaplasia occurred in all dosed groups (Tables 16 and A5b). There were significantly increased incidences of these lesions in the 216 ng TEQ/kg group. In addition, there were significantly increased incidences of respiratory epithelial hyperplasia in the 360 ng TEQ/kg core study group and significantly increased incidences of olfactory epithelial metaplasia in the 360 ng TEQ/kg stop-exposure group.

Inflammation was usually seen in section III and was generally characterized by accumulation of varying numbers of neutrophils mixed with mucus and debris within the nasal cavity. Olfactory epithelial metaplasia, consisting of replacement of normal olfactory epithelium

by respiratory-type epithelium, and respiratory epithelial hyperplasia, consisting of varying degrees of thickening of the respiratory epithelium due to an increase in the number of epithelial cells, were generally seen in association with inflammation and appeared to be secondary to the inflammation.

Mammary gland: In all dosed groups at 2 years, the incidences of fibroadenoma (single or multiple) (vehicle control, 42/53; 7 ng TEQ/kg, 38/51; 22 ng TEQ/kg, 36/53; 72 ng TEQ/kg, 17/53; 216 ng TEQ/kg, 4/53; 360 ng TEQ/kg, 6/66; 360 ng TEQ/kg, 12/50), adenoma

(2/53, 1/51, 0/53, 0/53, 0/53, 0/66, 0/50), and carcinoma (single or multiple) (5/53, 1/51, 4/53, 0/53, 0/53, 0/66, 1/50) generally decreased with increasing dose (Tables A1b, A3a, and A3b). The incidences of fibroadenoma (single or multiple) in all groups administered 72 ng TEQ/kg or greater and the incidence of carcinoma in the 72 ng TEQ/kg group were significantly decreased.

Pituitary gland: The incidences of adenoma of the pars distalis were significantly decreased in all dosed groups at 2 years (30/53, 11/51, 16/52, 10/53, 0/53, 0/65, 0/50; Tables A1b, A3a, and A3b).

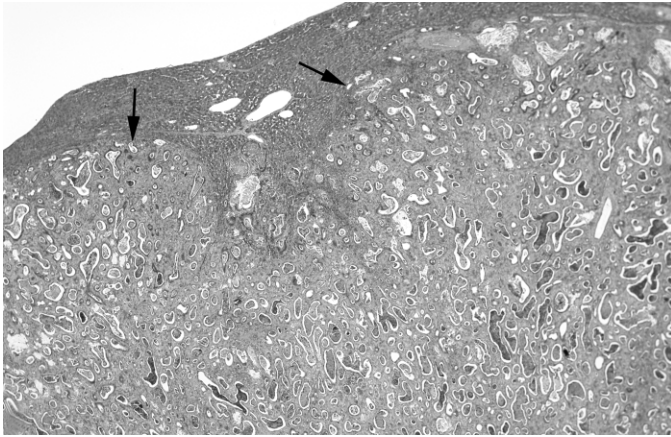


PLATE 1

Cholangiocarcinoma in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. Note the invasion of the neoplastic tissue into the surrounding hepatic tissue (arrows). H&E; 4×

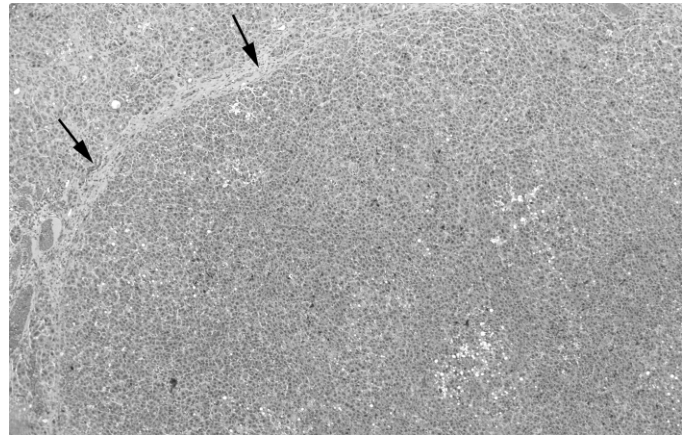


PLATE 2

Hepatocellular adenoma in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The hepatocellular neoplasm has a distinct border, producing compression of surrounding normal parenchyma (arrows). H&E; 10×

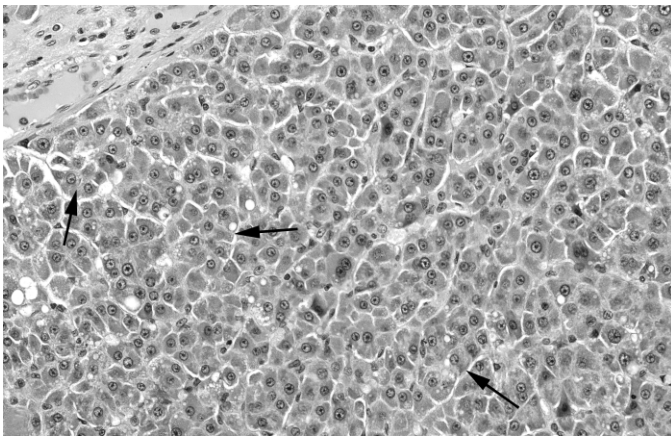


PLATE 3

Hepatocellular adenoma in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The hepatocellular adenoma is composed of a rather uniform population of mildly pleomorphic hepatocytes that generally are normal in size or slightly larger than normal and arranged in thickened trabeculae (arrows). H&E; 50×

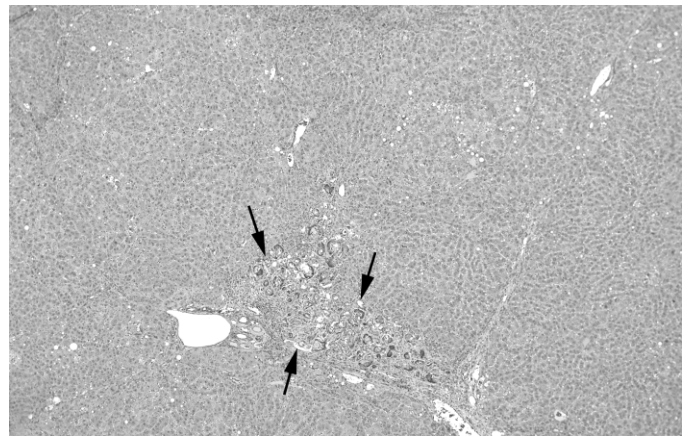


PLATE 4

Cholangiofibrosis in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The cholangiofibrosis is relatively smaller in size (arrows) compared to the cholangiocarcinoma presented in Plate 1. H&E; 4×

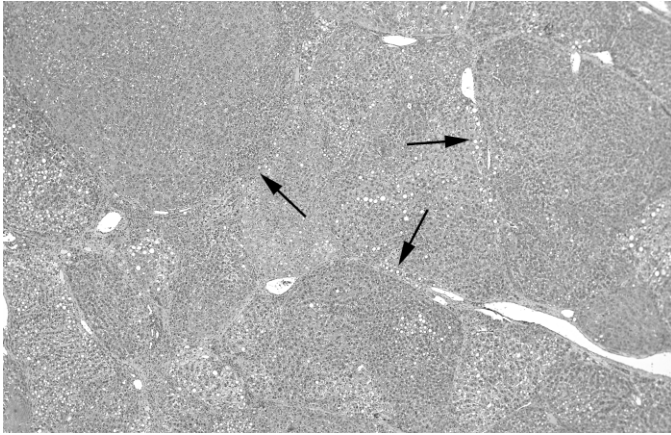


PLATE 5

Nodular hyperplasia in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. Note the multiple nodules of different sizes (arrows). H&E; 2.5×

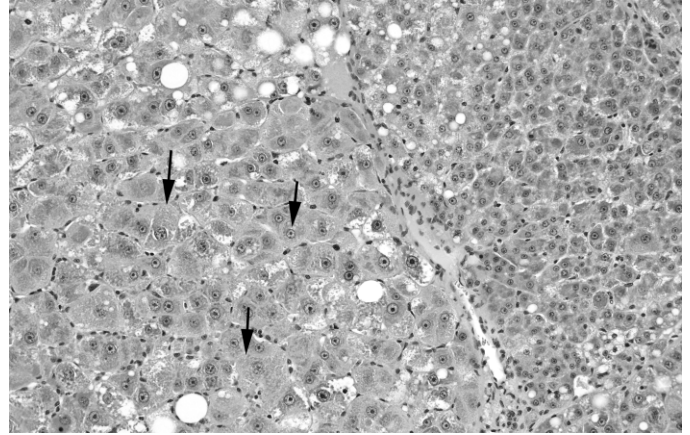


PLATE 6

Nodular hyperplasia in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The nodule is composed of hepatocytes that are considerably larger than normal hepatocytes (hepatocyte hypertrophy, arrows). H&E; 20×

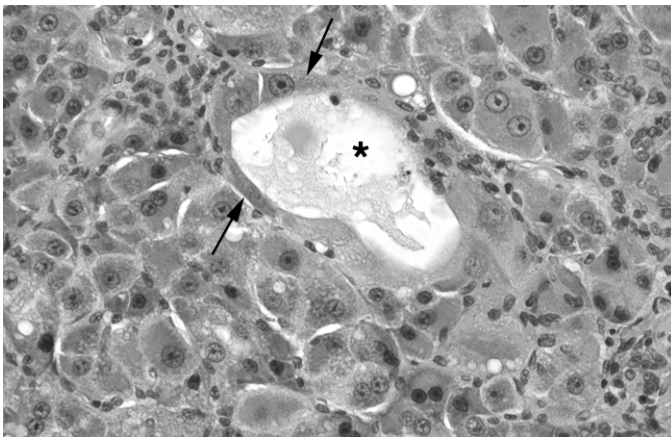


PLATE 7

Glandular structure in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The lesion is characterized by a circular ring of hepatocytes (arrows) surrounding a central lumen (asterisk) that produces a structure resembling a gland. The central lumen contains granular eosinophilic material. H&E; 50×

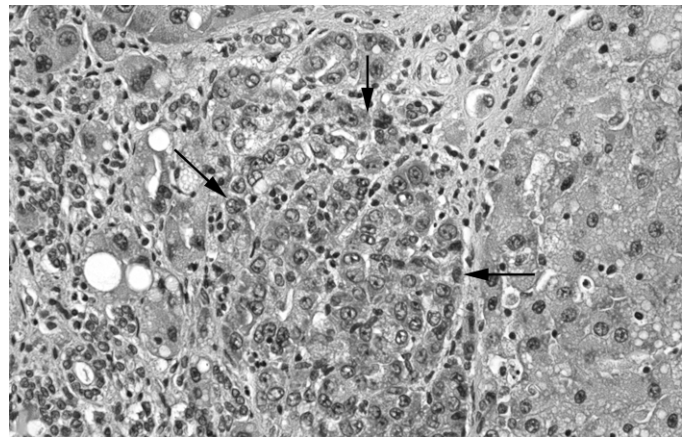


PLATE 8

Pancreatic metaplasia in the liver of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The lesion is characterized by a well-circumscribed area of cells (arrows) whose morphology is consistent with pancreatic acinar cells. H&E; 50×

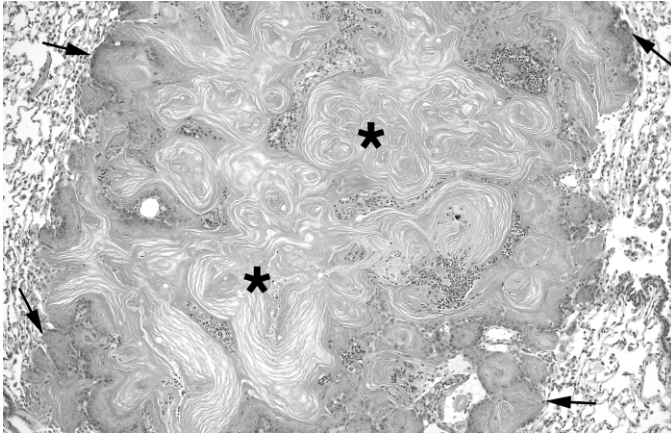


PLATE 9

Cystic keratinizing epithelioma in the lung of a female rat administered 360 ng TEQ/kg by gavage for 2 years. Note the cystic structure consisting of an irregular wall (arrows) of highly keratinized stratified squamous epithelium and a center filled with keratin (asterisks). H&E; 10×

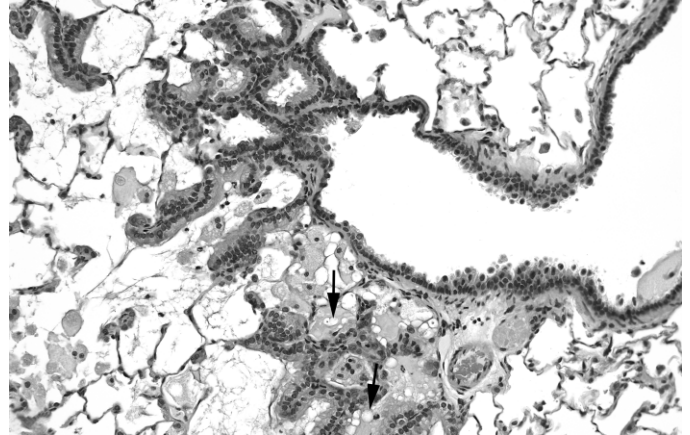


PLATE 10

Alveolar epithelium, bronchiolar metaplasia in the lung of a female rat administered 72 ng TEQ/kg by gavage for 2 years. The normal alveolar epithelium is replaced by cuboidal to columnar epithelium, accompanied by abundant mucus production in the affected area (arrows). H&E; 20×

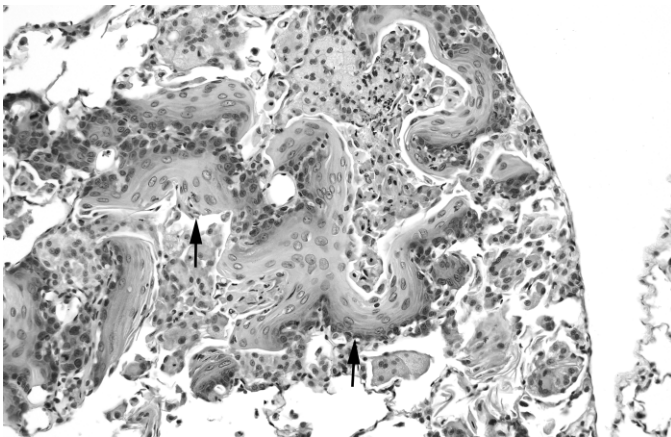


PLATE 11

Squamous metaplasia in the lung of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The normal alveolar epithelium has been replaced by keratinizing stratified squamous epithelium (arrows). H&E; 10×

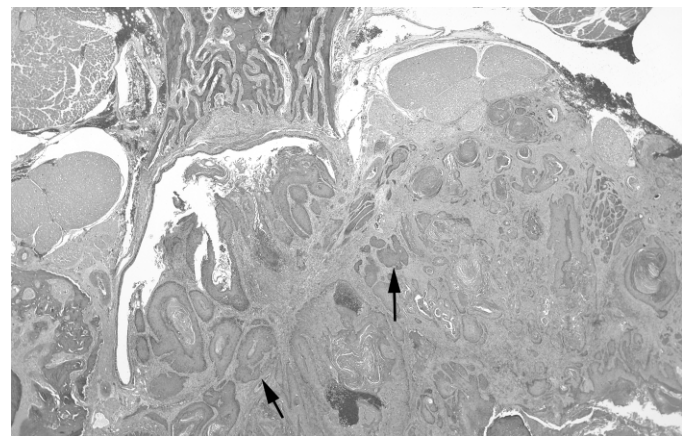


PLATE 12

Gingival squamous cell carcinoma in the oral mucosa of a female rat administered 360 ng TEQ/kg by gavage for 2 years. The carcinoma is characterized by irregular cords and clusters of stratified squamous epithelial cells that invade deep into the underlying connective tissue (arrows). H&E; 2.5×

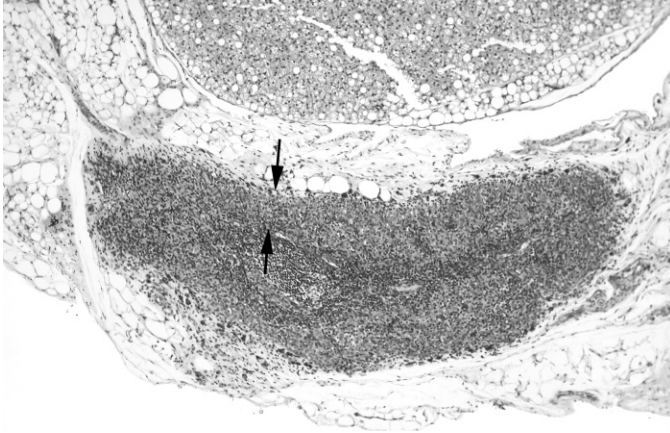


PLATE 13

Severe atrophy (grade 4) in the thymus of a female rat administered 360 ng TEQ/kg by gavage for 2 years. There is a severe loss of lymphoid cells from the cortex resulting in reduction of cortical thickness (the area between the arrows). H&E; 13.2×

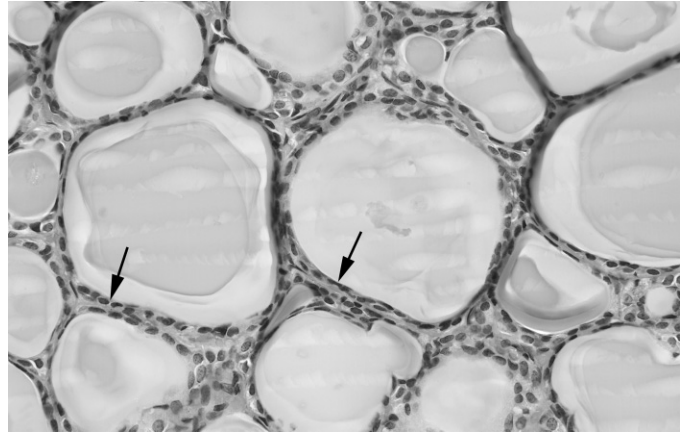


PLATE 14

Normal thyroid gland follicles in a vehicle control female rat sacrificed at 31 weeks in the 2-year study of a binary mixture of PCB 126 and PCB 118. Note that the follicles are distended with homogeneous colloid and the lining epithelium is flattened (arrows). H&E; 40×

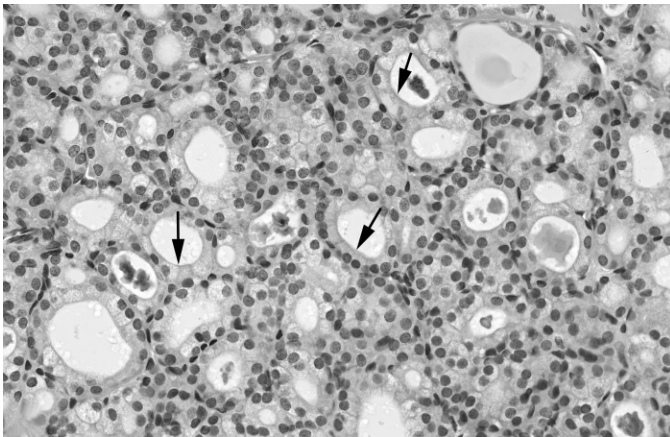


PLATE 15

Follicular hypertrophy in the thyroid gland of a female rat administered 360 ng TEQ/kg by gavage for 31 weeks. The follicles are smaller in size, lined by cuboidal epithelium (arrows), and contain decreased amounts of colloid in which aggregates of amphophilic, flocculant-appearing material are present (compare with Plate 13). H&E; 40×

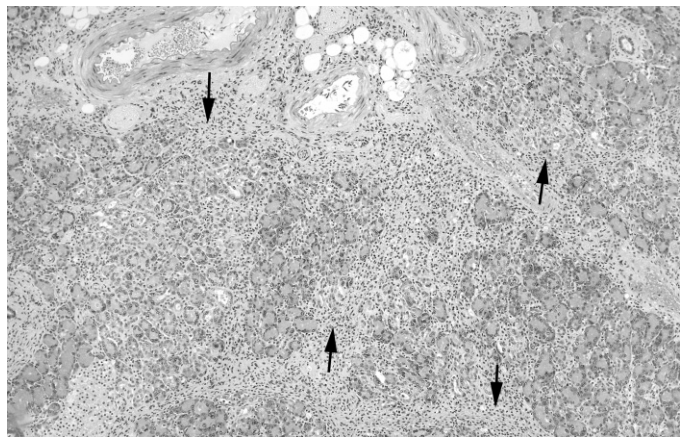


PLATE 16

Exocrine gland chronic active inflammation and acinar atrophy in the pancreas of a female rat administered 360 ng TEQ/kg by gavage for 2 years. There is a reduction in the amount of acinar tissue, with an associated increase in stromal fibrous connective tissue and infiltration of mixed inflammatory cells (arrows). H&E; 10×

DISCUSSION AND CONCLUSIONS

This 2-year study of the chronic toxicity and carcinogenicity of the mixture of PCB 126 and PCB 118 in female Harlan Sprague-Dawley rats is one in a series of studies carried out as part of a multistudy NTP initiative examining the relative chronic toxicity and carcinogenicity of dioxin-like compounds (DLCs) and structurally related polychlorinated biphenyls (PCBs) (see Overview section).

This mixture study was not intentionally designed as part of the dioxin toxic equivalency factor (TEF) evaluation. Initially it was a specifically designed study of PCB 118 alone. PCB 118 is a mono-*ortho*-substituted PCB that has only partial dioxin-like activity and has a World Health Organization (WHO) TEF of 0.0001. However during this PCB 118 study it was discovered that one of the minor mass contaminants of the bulk PCB 118 was PCB 126. PCB 126 is the most potent coplanar PCB for the induction of TCDD-like activities and has a WHO TEF of 0.1. Given the 1,000-fold higher relative dioxin-like activity of PCB 126 compared to PCB 118 and based on the proportion (0.622%) of PCB 118 that was actually PCB 126, it was predicted that a large proportion of the expected effects of the compound would be attributable to PCB 126 rather than PCB 118. Evaluation of the potential interactions between dioxin-like PCBs and non-dioxin-like PCBs was one of the original goals of the dioxin TEF evaluation and was being studied using a mixture of PCB 126 and PCB 153. Rather than terminating this "PCB 118" study, the study was continued because it afforded an opportunity to now evaluate the carcinogenicity of a mixture of a mono-*ortho*-substituted PCB and a non-*ortho*-substituted PCB. PCB 118 was resynthesized and checked for the absence of high TEQ-contributing compounds and a new study will be reported separately.

The range of doses used in the study and the proportion of PCB 126 and PCB 118 used were not those that would have been chosen had this study been specifically designed to test for an interaction between PCB 126 and PCB 118. Specifically, the relative contributions of PCB 126 and PCB 118 to the predicted dioxin-like activity are not equal as was the case in the TEF mixture

study conducted as part of the dioxin TEF evaluation (NTP, 2006d). In that study, TCDD, PeCDF, and PCB 126, were administered in concentrations predicted to deliver equivalent amounts of the total TCDD toxic equivalents (TEQ) from each compound. In addition, the doses used in this mixture were higher than those that would have been selected *a priori* because the TEF for PCB 118 is relatively low, and this compound was predicted to contribute the entire dioxin-like activity. Doses for other studies in the dioxin TEF evaluation were based on the known effects of TCDD in the Sprague-Dawley rat. By comparison, in the study of PCB 126 alone conducted as part of the dioxin TEF evaluation, the highest dose of PCB 126 used was 1,000 ng/kg (NTP, 2006a). This represents a dose of 100 ng TEQ/kg, a dose known to increase the incidence of liver neoplasms in female rats (Kociba *et al.*, 1978). In the NTP study, there was clear evidence of carcinogenicity of PCB 126 based on increased incidences of cholangiocarcinoma of the liver and squamous neoplasms of the lung. Increased incidences of hepatocellular adenoma and hepatocholangioma were also considered to be related to treatment with PCB 126. The total predicted TEQ of the two highest doses of the PCB 126:PCB 118 mixture (216 ng TEQ/kg and 360 ng TEQ/kg) are two to fourfold higher than the highest TEQ dose used in the NTP study of PCB 126 alone (100 ng TEQ/kg). Therefore it was anticipated that, if there was no antagonism of the effect of PCB 126 by PCB 118, the site specificity and spectrum of effects seen would be very similar to those seen in the study of PCB 126 alone, and moreover that responses seen in the two highest dose groups would be more severe than those seen in the NTP study of PCB 126 alone. For ease of comparison to other studies, dose groups are referred to by the predicted administered total TEQ dose, even though this is not a measurable "dose" *per se*, and it is yet to be determined if the weak dioxin-like activity of PCB 118 applies to the induction of neoplastic endpoints.

Once the composition of PCB 118 was known, the hypothesis actually being tested was the effect of the presence of PCB 118 (a mono-*ortho*-substituted PCB)

on the carcinogenicity of PCB 126 (a coplanar dioxin-like PCB). Testing of this hypothesis requires a quantitative comparison of the effects seen in the present study with those seen in the NTP study of PCB 126 alone (NTP, 2006a). In this Technical Report, only the results of the toxicology and carcinogenicity study of the mixture of PCB 126 and PCB 118 are described and, where appropriate, a qualitative comparison to neoplastic responses seen in the gavage studies of PCB 126 and TCDD alone (NTP, 2006a,c) conducted as part of the dioxin TEF evaluation. The quantitative comparison of the potency of carcinogenic effects observed in this study to responses observed with PCB 126 alone and other compounds studied as part of the dioxin TEF evaluation will be presented elsewhere.

In the current study, survival in the groups administered 216 and 360 ng TEQ/kg was significantly less than that in the vehicle controls with no animals surviving to the end of the study. Daily administration at doses greater than 72 ng TEQ/kg led to a marked reduction in body weight gain over the course of the 2-year study. Reduction in body weight gain is a characteristic toxic response to dioxin-like compounds and was also seen in the study of PCB 126 alone. The reduction in body weight gain was treatment related and required daily continuous administration. In the 360 ng TEQ/kg stop-exposure group, administration of the mixture ceased after 30 weeks and was followed by gavage with corn oil:acetone (99:1) alone 5 days per week. Thereafter, the rate of body weight gain in these animals returned to normal and was similar to vehicle control animals. However survival in this group was significantly less than vehicle controls, primarily due to the effect on survival of dosing with the mixture during the first 30 weeks of the study.

The doses of PCB 126 and total TEQ in these groups (216 and 360 ng TEQ/kg) were more than twofold higher than the highest dose used in the study of PCB 126 alone (NTP, 2006a). While these groups showed excessive toxicity, the findings from these groups were not central to the conclusions drawn from the study, as significant but less severe effects were also seen at lower doses. Rather they allowed for an interpretive evaluation of the full range of the continuum of effects seen in these studies. With few exceptions, the target sites for both neoplastic and nonneoplastic effects were the same as those seen at the lower doses where there were no effects on survival.

The principal findings of the current study were increased incidences of neoplasms in several organs, specifically in the liver (predominantly cholangiocarcinoma and hepatocellular adenoma, but also cholangioma, hepatocholangioma, and hepatocellular carcinoma), lung (cystic keratinizing epithelioma) and oral mucosa (gingival squamous cell carcinoma). The principal nonneoplastic findings in this study were significant increases in the incidences and severities of hepatotoxicity in the liver. In addition, numerous organs exhibited increased incidences of nonneoplastic lesions; notably the lung, thymus, thyroid gland, adrenal cortex, pancreas, oral mucosa, kidney, heart, mesenteric artery, brain, forestomach, spleen, and nose.

Chronic exposure led to significant accumulation of PCB 126 and PCB 118 in liver, fat, and lung and detectable levels in blood. The significant accumulation in fat is consistent with the lipophilic nature of these compounds. Previous studies of DLCs indicate that the liver and fat are the main depots for DLCs in rodents and together contribute approximately 70% to 80% of the total body burden in rodents (DeVito *et al.*, 1995). As expected, the levels of PCB 126 in liver were two to fourfold higher than those in fat on a wet weight basis. This hepatic sequestration is a characteristic of persistent DLCs such as TCDD and PCB 126 and is believed to be the result of binding of the compound to CYP1A2 that is inducible by DLCs in the liver (Diliberto *et al.*, 1997). By comparison PCB 118 levels in the liver were in general less than 10% of the levels seen in the fat, indicating minimal CYP1A2-mediated sequestration of PCB 118 in the liver. The distribution of PCB 118 is determined by the lipophilic nature of the compound and fat content of the tissue of concern.

The tissue levels observed for PCB 126 in the present study were similar to those seen at comparable doses in the NTP study of PCB 126 alone (NTP, 2006a). While the two studies do not have directly comparable dose groups, analysis of the PCB 126 in the current study using a PBPK model developed from the data from the study of PCB 126 alone indicates that the liver and fat levels of PCB 126 seen in the mixture study can be adequately predicted based on the doses used in the current study. This suggests that the tissue distribution of PCB 126 was not affected by the presence of PCB 118. On a TEQ basis (using the WHO TEF factor of 0.1 for PCB 126) the liver burdens of PCB 126 in the present study in the 7 and 72 ng TEQ/kg groups were 4.8 and

28 ng TEQ/g, respectively. By comparison, terminal liver levels of TCDD in the TCDD study conducted as part of the dioxin TEF evaluation were 2.2 and 9.3 ng/g at the 10 and 100 ng/kg doses, respectively (NTP, 2006c).

Cessation of daily treatment with the mixture of PCB 126 and PCB 118 in the stop-exposure group led to a decline in the levels of PCB 126 and PCB 118 in all tissues examined. In general, levels of both PCB 126 and PCB 118 in the stop-exposure group at 2 years were comparable to levels seen in the groups administered 22 ng TEQ/kg or less. Therefore, interpretation of the pathology results in the stop-exposure group animals has to be made in light of the fact that exposure *per se* does not end on cessation of daily administration of the compounds. While PCB 126 levels in the stop-exposure group declined significantly over the remainder of the study, animals were still continually exposed throughout the course of the study to the compounds still resident in the animals.

There was measurable PCB 126 in the tissues of some vehicle control animals. These concentrations can be attributed to the ingestion of very low levels of PCBs that are present in rodent chow (Feeley and Jordan, 1998; Jordan and Feeley, 1999). Accumulation of PCDDs and PCDFs has also been observed in vehicle control animals in other studies (Vanden Heuvel *et al.*, 1994). PCB 126 levels of 0.4 ppt (pg/g feed) have been reported in rat feed (Jordan and Feeley, 1999). This level of PCB 126 would result in a daily intake of approximately 200 pg/kg per day (for a 200 g rat ingesting 10 g feed per day). PCB levels were measured in the NTP 2000 feed (Table D5). PCB 126 levels were below the limits of quantitation (< 9 pg/g) and mean PCB 118 levels were 130 pg/g.

In all the TEF studies experimental treatments are made on top of a background of prior exposure to DLCs normally present in feed and, therefore, the vehicle control group exposure is not zero. However, as one can see from the estimated intake, the background intake is several orders of magnitude lower than the doses where neoplastic responses were observed. Consequently, the additional contribution of this background exposure to the observed neoplastic responses to the administered mixture of PCB 126 and PCB 118 is negligible.

Increased expression of CYP1A1 and CYP1A2 are characteristic responses to DLCs in the liver and are directly

linked to binding and activation of the aryl hydrocarbon receptor (AhR) by DLCs (Whitlock, 1993). In many cases, the relative potency for induction of CYP1A1 *in vivo* is an appropriate surrogate for the dioxin-like activity of a given compound and provides the basis for many TEFs (Van den Berg *et al.*, 1998). In this study, increased CYP1A1 and CYP1A2 activities as a result of exposure were observed at all time points and at all doses used. PCB 126 is a coplanar PCB that has the highest affinity of this class of PCBs for the AhR. Consequently, the finding that the liver was a target following exposure to this mixture was expected and was observed in the study of PCB 126 alone (Toyoshima *et al.*, 2004). 7-Pentoxoresorufin-*O*-deethylase (PROD) activity was significantly induced in all dosed groups. It is known that mono-*ortho*- and di-*ortho*-substituted PCBs such as PCB 118 and PCB 153 can exhibit phenobarbital-like effects such as increased expression of CYP2B-mediated PROD activity. However, the level of PROD activity was similar, at comparable PCB 126 doses, to that seen in the study of PCB 126 alone and therefore it is likely that the increased PROD activity may be due to combined TEQ-mediated effects on cytochrome P450 expression rather than a non-dioxin-like effect of PCB 118 alone.

Numerous studies have examined the toxicity of DLCs and PCBs and have demonstrated that the liver is a principal target organ for the action of these compounds. In the present study of the mixture of PCB 126 and PCB 118, the principal hepatic neoplasms observed were cholangiocarcinoma and hepatocellular adenoma. Single incidences of cholangioma, hepatocholangioma, and hepatocellular carcinoma were also seen in the groups administered 72 ng TEQ/kg or greater. In general, the spectrum of hepatic neoplasms seen in this study is consistent with those seen in the dioxin TEF evaluation studies of PCB 126, TCDD, and the TEF mixture (Hailey *et al.*, 2005; Walker *et al.*, 2005; NTP, 2006a,c,d). In these studies the primary neoplastic response was the increased incidence of cholangiocarcinoma. This indicates that the effects of this PCB 126 and PCB 118 mixture were due to the dioxin-like effects of these PCBs. However in comparison to the other studies, the effects seen in the two highest dose groups of the present mixture study were the most severe and exhibited the highest incidences of any of the dioxin TEF evaluation studies. This was not surprising given that the total TEQ in these groups were two to fourfold higher than those used in any of the other studies.

The incidence and pattern of hepatic toxicity exhibited a clear dose and duration dependence and preceded neoplastic effects in the liver. In this study, there were significant increases in hepatic toxicity with increased severity occurring at higher doses and longer durations of treatment. Hepatic toxicity was characterized by numerous lesions including hepatocyte hypertrophy, pigmentation, multinucleated hepatocytes, diffuse and focal fatty change, necrosis, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, cholangiofibrosis, hepatocyte degeneration, oval cell hyperplasia, foci of cellular alteration, and portal fibrosis. A comprehensive term of toxic hepatopathy was also used, reflecting the overall severity of all the nonneoplastic effects combined. The purpose of this term was to allow for easier comparison of the toxic changes among different dose groups than would be possible if the severities of all the individual nonneoplastic changes had to be compared among the different groups. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. Some treated animals occasionally had just a few of these changes present, but this was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy.

The increased incidences and severities of hepatotoxicity and increased incidences of hepatocellular adenoma are consistent with previously observed effects of TCDD and hexachlorodioxins in the liver (Kociba *et al.*, 1978; NCI, 1980; NTP, 1982a). However, in the present study the most significant neoplastic response seen in the liver was in the incidences of cholangiocarcinoma. The increased incidences of cholangiocarcinoma and hepatocellular adenoma are consistent with the effects of PCB 126 and TCDD seen in the NTP studies conducted as part of the dioxin TEF evaluation (NTP, 2006a,c). Cholangiocarcinomas have been seen only rarely in previous studies of DLCs and PCBs despite data showing that bile ducts are targets for DLCs. In an initiation-promotion study, cholangiocarcinoma was seen in one of 14 DEN-initiated female rats exposed to 100 ng TCDD/kg body weight per day for 60 weeks (Walker *et al.*, 2000). No cholangiocarcinomas were observed in the 2-year bioassay of Aroclor 1254 (Mayes *et al.*, 1998). In addition, there was no increased incidence of cholangiocarcinoma in the TCDD feed study by Kociba *et al.* (1978).

In the original report of the data from the study of Kociba *et al.* (1978), there was a 47% incidence of "hepatocellular hyperplastic nodules" in the 100 ng TCDD/kg body weight group compared to a 9% incidence in control animals. Subsequent to the Kociba

et al. (1978) study, there was an evolution of nomenclature for hepatocellular proliferative lesions and a reevaluation of the slides from the study. In that evaluation, neoplastic lesions were classified as adenoma or carcinoma. Using the newer nomenclature, the incidence of hepatocellular adenoma at the highest dose of 100 ng TCDD/kg body weight was 31% (Goodman and Sauer, 1992). By comparison, the survival-adjusted incidences of hepatocellular adenoma in the current PCB 126:PCB 118 mixture study were 11% and 56% in the 72 ng and 216 ng TEQ/kg groups, respectively. A summary of the pathology reevaluation is provided in the NTP Technical Report for TCDD (NTP, 2006c).

The spectrum of hepatocellular proliferative lesions observed in the present study is not common in NTP studies, and there is a lack of biological information relative to the progression and behavior of these lesions. These lesions generally occurred on a background of toxic hepatopathy, the components of which are listed above and described in the Results section. It is generally accepted that in the rat, hepatocellular adenoma and hepatocellular carcinoma represent a morphological and biological continuum (Narama *et al.*, 2003; Hailey *et al.*, 2005). Foci of cellular alteration are often part of that continuum, but not always. In the high dose groups, proper categorization of the lesions was further complicated by the presence of severe toxic hepatopathy. While the biological behavior of hepatocellular lesions within this study and other studies conducted as part of the dioxin TEF evaluation is uncertain, the morphology suggests that in this study, eosinophilic foci, nodular hyperplasia, and potentially adenoma were a continuum. Despite the high doses used and the observation of high incidences of hepatocellular adenoma in this study, only a single occurrence of hepatocellular carcinoma was observed. This suggests that there was only a weak ability of the observed adenomas to progress into carcinomas.

The foci of cellular alteration seen in dosed animals generally differed from the typical foci seen in vehicle controls. Foci seen in vehicle controls were usually smaller, lacked discrete borders and blended with the surrounding parenchyma, produced little or no compression, and consisted of cells that were normal-sized or slightly smaller or larger than normal. In contrast, foci in the livers of dosed animals generally had discrete borders, produced some compression of the adjacent parenchyma, and consisted of large, hypertrophic, often vacuolated cells. The significantly increased incidences of hypertrophy seen in foci from dosed animals resulted in a greater degree of compression of adjacent hepatic

parenchyma than is often seen with typical foci of hepatocellular alteration. At 2 years, focal lesions were observed that resembled foci of hepatocellular alteration, but were larger and often nodular, with greater compression of surrounding hepatic parenchyma and more disorganization of hepatic cords. As with foci, these lesions generally contained a somewhat normal hepatic structure including portal triads with biliary tracts. Additionally, these focal lesions contained variable numbers of randomly scattered biliary epithelium that often formed profiles of small glands/ductules. The large size of the lesions and presence of scattered biliary epithelium suggested a proliferative response of both hepatocellular and biliary cells, and therefore, these lesions were considered to have progressed beyond a simple focus of cellular alteration. However, because of the somewhat normal hepatic structure and the dual cellular composition of hepatocytes and biliary cells, the lesions were considered to be hyperplastic rather than neoplastic and were diagnosed as nodular hyperplasia.

In the higher dose animals with severe toxic hepatopathy, there was evidence of hepatocyte degeneration and loss, and a regenerative response by the damaged liver. The term "hyperplasia, nodular" was selected as the inclusive term, and was characterized by areas of focal hypertrophy and hyperplasia of hepatocytes that also contained proliferating biliary epithelium. Nodular hyperplasia varied in size, but generally appeared morphologically similar whether in a high dose animal with severe toxic hepatopathy or in a lower dose animal where the toxic hepatopathy was minimal to non-existent. In the dioxin TEF evaluation studies, nodular hyperplasia was seen most commonly in the higher dose groups in which prominent toxic changes were present. However, a lesser degree of nodular hyperplasia was sometimes seen in lower dose animals in which the only evidence of liver pathology may have been hepatocyte hypertrophy.

Morphologically, a hyperplastic nodule associated with regeneration cannot be distinguished from a hyperplastic nodule of another pathogenesis. The morphological alterations suggest that regeneration is a significant contributor to the proliferative response in animals with toxic hepatopathy. However, this does not explain these responses in animals that lack significant hepatic toxicity. This indicates that some other type of stimulus, rather than regeneration secondary to degeneration and

necrosis of the hepatic parenchyma, may have contributed to the proliferative lesions observed in this study.

The earliest treatment-related hepatocellular change, noted at the 14-, 31-, and 53-week interim evaluations, was a diffuse hepatocyte hypertrophy. With continued dosing, poorly demarcated foci of prominent hypertrophic, often vacuolated hepatocytes, resembling those seen in foci and nodular hyperplasia, were seen superimposed on the background of diffuse hypertrophy. It appeared that with continued dosing, the poorly demarcated foci of hypertrophic cells grew, giving rise to lesions diagnosed as foci. With continued dosing, in some instances aided by toxic changes, these foci may have progressed to nodular hyperplasia.

In contrast to nodular hyperplasia, hepatocellular adenoma was a nodular mass that usually was larger than a focus, had a distinct border, and produced more compression of surrounding normal hepatic parenchyma. Adenomas were composed of mildly to moderately pleomorphic hepatocytes with a subjectively increased nucleus to cytoplasmic ratio. Cells lacked the normal architectural arrangements of hepatic lobules, and while a few bile ducts may have been present within an adenoma, they were usually found at the periphery of the lesion and were considered entrapped. Proliferating biliary epithelium or oval cells were generally absent. The lack of proliferating bile duct epithelium or oval cells was an important feature differentiating adenoma from nodular hyperplasia.

The highly increased incidence of cholangiocarcinoma following exposure was unexpected based on prior studies of dioxins and PCBs but was consistent with observations made in other studies conducted as part of the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f). Spontaneous cholangioma and cholangiocarcinoma are apparently rare in Harlan Sprague-Dawley rats and were not observed in 371 vehicle control animals from these seven studies. These neoplasms are characterized by glandular structures lined by a single layer of well-differentiated epithelium (benign lesions) or single or multiple layers of epithelial cells that have malignant characteristics (e.g., high nuclear to cytoplasmic ratio, pleomorphism and anisokaryosis, and an increased mitotic rate).

In the present study, cholangiocarcinoma was diagnosed, and while it differed morphologically from spontaneous cholangiocarcinoma, it was similar to chemical-induced cholangiocarcinoma in a study by Maronpot *et al.* (1991). In this study, cholangiocarcinomas were variably sized, often multiple lesions composed of irregular and atypical bile ducts in a matrix of fibrous connective tissue. The bile ducts themselves were often incomplete or crescent-shaped and lined by very basophilic, cuboidal to columnar cells with large, euchromatic nuclei. Stratification of these epithelial cells was present in some areas. Atypical biliary epithelium was often identified within the adjacent hepatic parenchyma, suggesting invasion. The fibrous connective tissue component was frequently profound, much more than that seen in the scirrhous reaction that may be observed with spontaneous cholangiocarcinoma. The lesions seen in this study were sometimes large, effacing an entire liver lobe.

Cholangiofibrosis was the term used to describe small lesions that somewhat resembled cholangiocarcinoma, but were less aggressive in appearance. Cholangiofibrosis often originated in the portal area and tended to have a more mature fibrous connective tissue component, and less atypia associated with the epithelial cells. Most often, cholangiofibrosis and cholangiocarcinomas seen in this study did not compress the surrounding hepatic parenchyma or expand beyond the existing hepatic profile. However, cholangiocarcinomas often expanded within the liver lobe.

While cholangiofibrosis and cholangiocarcinoma appear to be a morphological continuum, there is limited biological information relative to the pathogenesis or progression of these lesions. As a result, the most appropriate classification scheme for these lesions is somewhat uncertain and controversial. While the characteristic of malignancy, distant metastasis, was not observed in any animals in the present study, other characteristics of malignancy were present such as atypical appearance of the epithelial cells and apparent localized invasion. It was clear that some of these cholangiolar lesions were small and very benign appearing and warranted a non-neoplastic diagnosis, and there were lesions at the other end of the spectrum that appeared aggressive. While there were specific diagnostic criteria for cholangiofibrosis versus cholangiocarcinoma, some of the lesions did not readily fit the criteria and posed a diagnostic challenge.

Other chemicals, including furan, have increased the incidence of lesions similar to those observed in the present study. In the Maronpot *et al.* (1991) furan study, the lesions appeared more aggressive, yet even in that study, where there was nearly a 100% incidence in treated animals, there were few metastases. In this study, it appears that the cholangiocarcinomas were slow-growing neoplasms of relatively low-grade malignancy. Transplantation studies in the furan study were positive for growth and metastases.

In the current study there were single occurrences of cholangioma and hepatocholangioma in several dose groups. Both neoplasms appear to be rare and did not occur in any vehicle control animals from any study conducted as part of the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f). Hepatocholangiomas were mixed neoplasms with areas of hepatocytes that appeared identical to hepatocellular adenoma and areas of ductular structures lined by biliary epithelium that appeared identical to cholangioma. The pluripotent nature of these neoplasms was demonstrated by occasional ductular structures lined by cells resembling both hepatocytes and biliary epithelium. In contrast to the cholangiofibrosis and cholangiocarcinomas, a scirrhous response was not present within these neoplasms. While the histogenesis of hepatocholangioma is not clear, there was evidence of proliferation of hepatocytes, biliary epithelium, and oval cells within these studies. Therefore, the occurrence of hepatocholangioma and cholangioma may have been related to the administration of the mixture of PCB 126 and PCB 118.

The mechanism underlying the increased incidences of cholangiocarcinoma is likely to be multifactorial. There was clearly an effect on bile duct proliferation in the current study. This may be an indirect response to the toxicity observed as a result of the action of the mixture of PCB 126 and PCB 118 on the hepatocytes or due to a direct effect on the biliary cells themselves. Tritscher *et al.* (1995) showed a high degree of staining for TGF α in bile duct cells after TCDD administration in female rats. The observed bile duct proliferation may represent a process of excessive and long term repair following specific damage to hepatocytes and leading to the death of hepatocytes and perhaps also of the bile duct epithelium. The proliferative response may be a reparative response of proliferating hepatocytes, bile duct cells, and scarring tissue (cholangiofibrosis). The inflammation

also observed can produce oxidative stress that may also result in promotion of DNA damage. Consequently, the oxidative stress may be only a secondary phenomenon due to the ongoing response to the toxic hepatopathy. In addition, there may be a direct stimulatory effect on the oval cells themselves. This is supported by the increased incidence of oval cell hyperplasia in the present study. Because oval cells may differentiate into hepatocytes and/or biliary epithelium this may explain why both hepatocellular proliferative and biliary lesions were associated with exposure.

There has been a considerable amount of research examining the potential mode of action of DLCs such as TCDD and PCB 126 in the liver. There is a general scientific consensus that almost all responses require initial binding to the AhR. Recent data indicate that the acute toxic responses (including hepatotoxicity) to TCDD require AhR binding and nuclear localization (Bunger *et al.*, 2003). In addition, transgenic mouse studies indicate that constitutive activation of the AhR alone can lead to an induction of stomach tumors (Andersson *et al.*, 2002) and can promote the pathogenesis of liver neoplasms in animals treated with initiating agents.

The dioxin-like effects of PCB 126 and PCB 118 are likely AhR mediated. Due to the lack of direct genotoxicity, the action is likely by promoting the development of spontaneously initiated cells. There are essentially three potential modes of action via the AhR: increased numbers of initiated cells capable of undergoing promotion, increased net growth rate of initiated cells due to selective growth advantage, and decreased rates of cell death via suppression of apoptosis. Numerous initiation-promotion models of hepatocarcinogenesis have shown that PCDDs, PCDFs, and PCBs such as PCB 126 can promote the development of altered hepatic foci. Given that TCDD and related compounds are not direct acting genotoxic agents and are potent growth dysregulators, it is believed that their predominant mode of action is promoting the development of preneoplastic and neoplastic lesions. Within a conceptual multistage model of carcinogenesis, promotion of neoplasia mediated by these compounds via the AhR may be due to an increase in net growth rate of initiated cells due to selective growth advantage or decreased rate of cell death via suppression of apoptosis. In studies with TCDD there are significant increases in hepatocyte replication as judged by BrdU labeling studies (Maronpot *et al.*, 1993; Walker *et al.*, 1998; Wyde *et al.*, 2001a). Increases in BrdU labeling indices have also been observed in the

present study of PCB 126 and PCB 118 and in other studies in the dioxin TEF evaluation. Studies by Stinchcombe *et al.* (1995), Worner and Schrenk (1996), and Bohnenberger *et al.* (2001) have also shown a suppression of apoptosis by TCDD and PCBs. In addition, altered growth regulation may be due to alterations in intercellular communication, which have also been observed in the livers of rats exposed to DLCs (Baker *et al.*, 1995; Warngard *et al.*, 1996; Bager *et al.*, 1997). While DLCs are not direct acting genotoxic agents, there are data indicating that persistent AhR-active compounds may be indirectly genotoxic. This may contribute to an increase in the number of cells within the liver capable of undergoing promotion (Moolgavkar *et al.*, 1996; Portier *et al.*, 1996). It is hypothesized that the indirect genotoxicity may be via an AhR-dependent induction of CYP1-family cytochromes P450 that leads to an induction of oxidative stress due to either inefficient electron transfer during P450 metabolism (Park *et al.*, 1996) or the production of redox-active estradiol metabolites as a result of CYP1-mediated estrogen metabolism (Lucier *et al.*, 1991; Kohn *et al.*, 1993). Studies have shown an induction of oxidative stress and DNA damage by high dose acute exposure to TCDD (Stohs *et al.*, 1990). The induction of lipid peroxidation and single stranded DNA breaks was also observed in tissues from studies conducted as part of the dioxin TEF evaluation (Hassoun *et al.*, 2000, 2001). Other studies on the female-specific tumor promotion response in rats have shown an induction of oxidative DNA damage and hepatocyte replication by TCDD that is female specific and estrogen dependent (Lucier *et al.*, 1991; Tritscher *et al.*, 1996; Wyde *et al.*, 2001a,b).

In the present study, the highest increased incidence of any neoplasm was cystic keratinizing epithelioma (CKE) of the lung. Histopathologically these lesions varied in size and number and appeared as cystic structures consisting of a highly irregular wall of highly keratinized stratified squamous epithelium with a center filled with keratin. These lesions were absent in vehicle control animals and have not been observed in any vehicle controls in the dioxin TEF evaluation studies (NTP, 2006a,b,c,d,e,f). Significantly increased incidences of lung CKE were also observed in PCB 126-treated and TCDD-treated animals (NTP, 2006a,c). In the 2-year feed study of TCDD, conducted by Kociba *et al.* (1978), an increased incidence of keratinizing squamous cell carcinoma of the lung was observed following exposure to 100 ng TCDD/kg body weight per day. In the present study, squamous cell carcinomas were not observed.

While direct comparison has not been made between CKE and the keratinizing squamous cell carcinoma observed in the Kociba *et al.* (1978) study, given the keratinizing nature of the lesion it is possible that these may be similar lesions. It should be noted that CKE was not a diagnostic term consistently used at the time of the Kociba *et al.* (1978) evaluation. Diagnostic criteria for classification of CKE as a lesion distinct from squamous cell carcinoma were later developed at a workshop held in the mid 1990s (Boorman *et al.*, 1996). In contrast to the present study, a recent study of the carcinogenicity of the high TEQ PCB mixture Aroclor 1254 demonstrated no increased incidences of any type of lung tumor (Mayes *et al.*, 1998). While Aroclor 1254 contains a significant TEQ contribution by PCB 126, this mixture also contains mono-*ortho*- and di-*ortho*-substituted PCBs.

CKEs were observed in three of 10 animals at the 53-week interim evaluation in the 360 ng TEQ/kg dose group. At 2 years, there was a significantly increased incidence of CKE in the 360 ng TEQ/kg stop-exposure group, although the incidence was significantly lower than that observed in the core study group at 2 years. In the stop-exposure group, there was a significantly increased incidence of keratin cysts in addition to the significantly increased incidences of CKE. Keratin cysts were not observed in the 360 ng TEQ/kg core study group. A transplantation study was conducted using some fragments of CKE from the current study, and in most cases there was either marked regression or some remaining cystic structures containing keratin (Appendix G). Therefore, it is possible that the observed keratin cysts in the 360 ng TEQ/kg stop-exposure group may have arisen from regression of CKE that developed earlier in the study as a result of declining lung burdens after cessation of treatment.

In addition to the increased incidences of CKE in the current study, there were significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium in all dosed groups at 2 years. This lesion was also increased in all dosed groups in the study of PCB 126 alone (NTP, 2006a). These findings are consistent with prior observations of increased incidences of alveolar bronchiolar metaplasia following exposure to TCDD within the framework of a two-stage initiation-promotion model in Sprague-Dawley rat lung (Tritscher *et al.*, 2000).

Alveolar ducts and alveoli are normally composed of type I alveolar epithelial cells and type II alveolar epithelial cells, which are cuboidal. Type I cells are very susceptible to damage, and the typical response in the lung, subsequent to the damage to the type I cells, is a proliferation of the type II cells. This is often diagnosed as alveolar epithelial hyperplasia. There were significantly decreased incidences of alveolar epithelial hyperplasia in all dosed groups in the present study. PCB 126 induced a multifocal lesion that was found throughout the lung at the junction of the terminal bronchioles and alveolar ducts. The epithelium was cuboidal to columnar and ciliated in contrast to type II alveolar epithelial cells. Also, scattered throughout the ciliated cells were dome-shaped nonciliated cells, consistent with Clara cells. Clara cells are normally found in the lining of the bronchioles, but not alveoli or alveolar ducts. Histo-chemical analyses of mucin and GSTPi in lung tissue from the dioxin TEF evaluation studies indicates that this does appear to be similar to bronchiolar epithelium and is distinct from alveolar epithelial hyperplasia (Brix *et al.*, 2004). It is not clear if this lesion represents a destruction of type I alveolar epithelial cells with replacement by bronchiolar-type epithelium (bronchiolar metaplasia) or an extension of bronchiolar epithelium from the terminal bronchiole (bronchiolar hyperplasia).

There are at least two potential mechanisms involved in the increased incidences of these neoplasms and nonneoplastic lesions in the lung. CYP1A1 is known to be inducible in the lung by TCDD in several species (Beebe *et al.*, 1990; Walker *et al.*, 1995). This was confirmed in the present study by the observed increase in lung CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) activity. The inducibility of CYP1A1 by TCDD is observable in Clara cells and bronchiolar cells and to a lesser degree in type II cells (Tritscher *et al.*, 2000). This indicates that the bronchiolar epithelium is clearly responsive to AhR ligands and suggests the potential for a direct effect on the lung. *In vitro* studies of normal human lung epithelial cells (mixed type II, Clara cell type) also demonstrate the alteration of numerous cell signaling pathways by TCDD including the Ah battery, altered retinoid signaling, and altered cytokine signaling pathways (Martinez *et al.*, 2002).

Another possible mechanism for the action of DLCs on the lung may be an indirect effect due to the disruption

of retinoid homeostasis in the liver. It is known that in rodents, mobilization of retinoid stores by TCDD and DLCs leads to a disruption in retinoid homeostasis and vitamin A deficiency (Van Birgelen *et al.*, 1994, 1995b; Fiorella *et al.*, 1995; Fattore *et al.*, 2000; Schmidt *et al.*, 2003). A characteristic of retinoid deficiency is abnormal epithelial differentiation to a keratinized squamous phenotype (Lancillotti *et al.*, 1992; Lotan, 1994). The action of DLCs may therefore be a disruption of retinoid action leading to altered growth and differentiation of the lung epithelium resulting in squamous metaplasia and ultimately neoplasia.

Gingival squamous cell carcinoma of the oral mucosa was observed in some dosed groups. Similarly, in the PCB 126 gavage study conducted as part of the dioxin TEF evaluation (NTP, 2006a), there were significantly increased incidences of gingival squamous cell carcinoma of the oral mucosa. In the TCDD feed study by Kociba *et al.* (1978), there were increased incidences of stratified squamous cell carcinoma of the hard palate/nasal turbinates in both male and female rats. The location of the squamous cell carcinomas in the present study was adjacent to the molars and invaded into the hard palate/nasal turbinate areas. This suggests that the lesions seen in the NTP (2006a) and Kociba *et al.* (1978) studies are similar.

In recent years there has been an increasing awareness of the sensitivity of the oral cavity to the effects of DLCs. In two PCB/PCDF human poisoning episodes, one of the toxic responses observed was early tooth eruption (Grassman *et al.*, 1998). More recent studies have shown that TCDD can accelerate incisor tooth eruption and delay molar eruption. Proliferation of the periodontal squamous epithelium has been seen in juvenile mink exposed to PCB 126 (Render *et al.*, 2001) but not in juvenile Otsuka Long-Evans Tokushima Fatty (OLETF) rats exposed to 100 ppb PCB 126 or 10 ppb TCDD for 101 days (Aulerich *et al.*, 2001). Studies suggest that the effect of TCDD on tooth development is due to a disruption in EGFR-mediated signaling (Partanen *et al.*, 1998) as has been shown for other developmental effects of TCDD such as cleft palate (Abbott *et al.*, 2003). In addition, as noted above for the effects of the mixture on the lung, the squamous lesions in the oral cavity may also be related to the alteration in retinoid homeostasis that is known to be induced by PCB 126.

In the present study, there were increased incidences of adrenal cortical atrophy and cytoplasmic vacuolization.

These are consistent with effects seen in the study of PCB 126 alone (NTP, 2006a). While the incidences of adrenal cortical neoplasm occurred with a positive trend, there was no effect of treatment in this study with a mixture of PCB 126 and PCB 118. In the Kociba *et al.* (1978) feed study of TCDD, there was a significantly increased incidence of adrenal cortical adenoma in male but not female rats at the 100 ng/kg dose. In the dioxin TEF evaluation TCDD study (NTP, 2006c), there were sporadic cases of adenoma of the adrenal cortex, in both vehicle control and TCDD-treated animals, but no significantly increased TCDD-related incidences. The cortical atrophy seen in the present study was a prominent effect and may reflect the continued stress in these animals, leading to depletion of corticosteroid hormones or some other unknown mechanisms (Sapolsky *et al.*, 1987).

In the present PCB 126:PCB 118 study, the incidences of pancreatic inflammation, acinar atrophy, acinar cytoplasmic vacuolization, and pancreatic arterial inflammation were all increased in dosed animals compared to vehicle controls. Similar treatment-related increased incidences of these lesions were observed in the NTP study of PCB 126 alone (NTP, 2006a). Edema was significantly increased in the two highest dose groups in the present study but was not observed with PCB 126 alone. Acinar atrophy of the pancreas may be related to the down regulation of cholecystikinin (CCK). As shown by Lee *et al.* (2000) in samples from the present PCB 126:PCB 118 study, intestinal CCK is reduced by PCB 126 exposure. Down-regulation of CCK is likely due to a general endocrine effect as a result of the reduction in body weight gain following exposure to PCB 126. CCK is an important regulator of pancreatic growth and function (Baldwin, 1995; Varga *et al.*, 1998). Previous studies have shown that increased apoptosis and pancreatic acinar atrophy is observed in OLETF rats that lack the CCK-A receptor gene (Jimi *et al.*, 1997). In addition, antagonism of CCK action can lead to reduced pancreatic growth (Ohlsson *et al.*, 1995).

In the present study, the incidences of nephropathy in dosed groups were significantly increased compared to vehicle controls. While it is known that the kidney is directly responsive to the AhR agonist TCDD, the kidney historically has not been a target for DLC-induced neoplasia.

Administration of PCB 126 and TCDD to female Harlan Sprague-Dawley rats significantly increased the

incidences of cardiomyopathy in a dose-related manner (Jokinen *et al.*, 2003; NTP, 2006a,c). Similarly, in the present mixture study, there were increased incidences of cardiomyopathy. However, the average severity of cardiomyopathy was unaffected. Cardiomyopathy is a common, spontaneously occurring degenerative change of myocardial fibers that is seen in rats as they age. Its cause in the rat is unknown, but age of onset and severity are affected by diet, environment, and stress. The microscopic appearance of cardiomyopathy was the same in both the vehicle control and treated animals and was typical of that described for spontaneous lesions. This finding may suggest that exposure to the chemicals increased the occurrence of the spontaneous change. The heart is a target for TCDD and related DLCs in both rodents and humans (Peterson *et al.*, 1993; Flesch-Janys *et al.*, 1995; Walker and Catron, 2000; Heid *et al.*, 2001). In the 2-year study in which Sprague-Dawley rats were administered up to 0.1 µg/kg per day of TCDD in the feed, Kociba *et al.* (1978) also reported an increase in the incidences of myocardial degenerative change above background levels in females only.

In this mixture of PCB 126 and PCB 118, the incidences of thymic atrophy were significantly increased. Thymic atrophy is one of the hallmark immunotoxic responses to DLCs (Poland and Knutson, 1982) and is due to an AhR-mediated alteration in lymphocyte growth and differentiation (Staples *et al.*, 1998; Gasiewicz *et al.*, 2000). Thymic atrophy and other hematopoietic changes may in part be related to the reduction in body weight gain observed in these animals as seen in short term feed restriction studies (Levin *et al.*, 1993).

In the current study, there were increased incidences of thyroid gland follicular cell hypertrophy in the higher dose groups at the interim evaluation time points and in all dose groups at the end of the study. However, there was no significant effect on follicular cell neoplasms. By comparison, in a 2-year gavage study of TCDD in Osborne-Mendel rats, there were significantly increased incidences of follicular cell adenomas in male rats and nonsignificantly increased incidences in females (NTP, 1982a). Alteration in thyroid hormone homeostasis by PCB 126 and TCDD is well established (Van Birgelen *et al.*, 1994,1995a; Schmidt *et al.*, 2003). Analyses of thyroid hormones in the present study confirmed the alterations in thyroid hormone homeostasis. The disruption of thyroid hormone homeostasis by DLCs is believed to be due to the increase in thyroxine (T₄) glucuronidation as a result of increased expression of

UDP-GT. This leads to a decreased negative feedback inhibition of the thyroid gland leading to overexpression of thyroid stimulating hormone (TSH) (Curran and DeGroot, 1991). Kohn *et al.* (1996) developed a mathematical model of the effects of TCDD on UDP-GT expression and thyroid hormone homeostasis that is consistent with this mechanism. It has been hypothesized that overstimulation of the thyroid gland by TSH may be involved in the mechanism of follicular cell carcinogenesis (Hill *et al.*, 1989). In the present study, it was observed that, despite alterations in T₄ at all time points, there were inconsistent effects on TSH.

In this current study, there were decreased trends in the incidences of mammary gland fibroadenoma and carcinoma. Fibroadenoma is a spontaneous lesion in female Sprague-Dawley rats and occurred at high incidence in the vehicle control group (79%). Mammary gland carcinoma was also seen in vehicle control animals at a lower incidence (9%). In addition, there were significantly lower incidences of spontaneous pituitary gland (pars distalis) adenoma in all dosed groups. It is believed that the lower incidences of mammary gland and pituitary gland neoplasms in dosed rats are related to a general endocrine effect as a result of reductions in body weight gain associated with exposure. A significant association between reduced body weight gain and lower incidence of mammary gland and pituitary gland neoplasms has been observed in many NTP studies (Seilkop, 1995). Significantly lower incidences of mammary gland and pituitary gland neoplasms were also observed in animals exposed to 100 ng TCDD/kg body weight in the 2-year feed study of Kociba *et al.* (1978). Similarly, there were significantly lower incidences of spontaneous mammary gland and pituitary gland neoplasms in the NTP (2006a,c) studies of PCB 126 and TCDD. Reductions in IGF-1 may underlie the inhibitory effect of reduced body weight gain on tumor development. It is known that caloric restriction leads to lower levels of IGF-1 and a reduction in background tumor rates (Hursting *et al.*, 2003). One of the major intestinal hormones expressed in the proximal gastrointestinal tract is CCK. CCK regulates gallbladder contraction, pancreatic secretion, stomach emptying, and intestinal motility and can also inhibit food intake. In an analysis of intestinal tissue obtained from the NTP study of PCB 126, Lee *et al.* (2000) showed lower levels of intestinal CCK and an induction of IGFBP3 by PCB 126. Alterations in CCK-processing enzymes by TCDD were also observed in cultured intestinal cells, suggesting direct effects of PCB 126 on intestinal cells. The authors hypothesized

that alterations in CCK may be due to alterations in processing enzymes and lower IGF-1 levels as a result of alterations in IGFBP3.

Overall, the spectrum of lesions observed in this mixture study of PCB 126 and PCB 118 was generally consistent with observations made in other studies of DLCs carried out as part of the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f). Given that PCB 118 is known to have weak dioxin-like activity, it suggests that the effects of the mixture were likely due to the combined dioxin-like activity of the mixture and not due to PCB 126 alone. Comparison of data from the restarted NTP study of PCB 118 alone with these dioxin TEF evaluation studies will allow for a more definitive assessment of the dioxin-like carcinogenicity of PCB 118. In addition, quantitative dose response modeling of the data from the present study will be needed to assess whether predicted potency of the current study is consistent with the total dioxin-like activity of this mixture.

CONCLUSIONS

Under the conditions of this 2-year gavage study there was *clear evidence of carcinogenic activity** of the mixture of PCB 126 and PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma and hepatocellular neoplasms (predominantly hepatocellular adenomas) of the liver and cystic keratinizing epithelioma of the lung. The marginally increased incidences of gingival squamous cell carcinoma of the oral mucosa were also considered to be related to administration of the mixture of PCB 126 and PCB 118. Occurrences of cholangioma and hepatocholangioma of the liver may have been related to administration of the mixture of PCB 126 and PCB 118.

Administration of the mixture of PCB 126 and PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, thymus, thyroid gland, adrenal cortex, pancreas, kidney, heart, lymph nodes, mesenteric artery, brain, forestomach, spleen, and nose.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

REFERENCES

- Abbott, B.D., Buckalew, A.R., DeVito, M.J., Ross, D., Bryant, P.L., and Schmid, J.E. (2003). EGF and TGF- α expression influence the developmental toxicity of TCDD: Dose response and AhR phenotype in EGF, TGF- α , and EGF + TGF- α knockout mice. *Toxicol. Sci.* **71**, 84-95.
- Agency for Toxic Substances and Disease Registry (ATSDR) (1998). Toxicological Profile for Chlorinated dibenzo-*p*-dioxins. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.
- Agency for Toxic Substances and Disease Registry (ATSDR) (2000). Toxicological Profile for Polychlorinated Biphenyls (PCBs). U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.
- Ahlborg, U.G., Brouwer, A., Fingerhut, M.A., Jacobson, J.L., Jacobson, S.W., Kennedy, S.W., Kettrup, A.A., Koeman, J.H., Poiger, H., and Rappe, C. (1992). Impact of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. *Eur. J. Pharmacol.* **228**, 179-199.
- Anderson, L.M., Ward, J.M., Fox, S.D., Issaq, H.J., and Riggs, C.W. (1986). Effects of a single dose of polychlorinated biphenyls to infant mice on N-nitrosodimethylamine-initiated lung and liver tumors. *Int. J. Cancer* **38**, 109-116.
- Anderson, L.M., Beebe, L.E., Fox, S.D., Issaq, H.J., and Kovatch, R.M. (1991). Promotion of mouse lung tumors by bioaccumulated polychlorinated aromatic hydrocarbons. *Exp. Lung Res.* **17**, 455-471.
- Andersson, P., McGuire, J., Rubio, C., Gradin, K., Whitelaw, M.L., Pettersson, S., Hanberg, A., and Poellinger, L. (2002). A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 9990-9995.
- Aulerich, R.J., Yamini, B., and Bursian, S.J. (2001). Dietary exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) does not induce proliferation of squamous epithelium or osteolysis in the jaws of weanling rats. *Vet. Hum. Toxicol.* **43**, 170-171.
- Bager, Y., Kato, Y., Kenne, K., and Wärngård, L. (1997). The ability to alter the gap junction protein expression outside GST-P positive foci in liver of rats was associated to the tumour promotion potency of different polychlorinated biphenyls. *Chem. Biol. Interact.* **103**, 199-212.
- 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.
- Baker, T.K., Kwiatkowski, A.P., Madhukar, B.V., and Klaunig, J.E. (1995). Inhibition of gap junctional intercellular communication by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rat hepatocytes. *Carcinogenesis* **16**, 2321-2326.
- Baldwin, G.S. (1995). The role of gastrin and cholecystokinin in normal and neoplastic gastrointestinal growth. *J. Gastroenterol. Hepatol.* **10**, 215-232.
- Barthold, S.W. (1998). Chronic progressive nephropathy, rat. In *Urinary System*, 2nd ed. (T.C. Jones, G.C. Hard, and U. Mohr, Eds.), pp. 228-233. Springer, Berlin.

- Beebe, L., Park, S.S., and Anderson, L.M. (1990). Differential enzyme induction of mouse liver and lung following a single low or high dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Biochem. Toxicol.* **5**, 211-219.
- Beebe, L.E., Anver, M.R., Riggs, C.W., Fornwald, L.W., and Anderson, L.M. (1995). Promotion of N-nitrosodimethylamine-initiated mouse lung tumors following single or multiple low dose exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Carcinogenesis* **16**, 1345-1349.
- Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.
- Birnbaum, L.S. (1994a). Evidence for the role of the Ah receptor in response to dioxin. *Prog. Clin. Biol. Res.* **387**, 139-154.
- Birnbaum, L.S. (1994b). The mechanism of dioxin toxicity: Relationship to risk assessment. *Environ. Health Perspect.* **102** (Suppl. 9), 157-167.
- Birnbaum, L.S., and DeVito, M.J. (1995). Use of toxic equivalency factors for risk assessment for dioxins and related compounds. *Toxicology* **105**, 391-401.
- Birnbaum, L.S., Harris, M.W., Crawford, D.D., and Morrissey, R.E. (1987). Teratogenic effects of polychlorinated dibenzofurans in combination in C57BL/6N mice. *Toxicol. Appl. Pharmacol.* **91**, 246-255.
- Bohnenberger, S., Wagner, B., Schmitz, H.J., and Schrenk, D. (2001). Inhibition of apoptosis in rat hepatocytes treated with 'non-dioxin-like' polychlorinated biphenyls. *Carcinogenesis* **22**, 1601-1605.
- 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.
- Boorman, G.A., Brockmann, M., Carlton, W.W., Davis, J.M., Dungworth, D.L., Hahn, F.F., Mohr, U., Reichhelm, H.B., Turusov, V.S., and Wagner, B.M. (1996). Classification of cystic keratinizing squamous lesions of the rat lung: Report of a workshop. *Toxicol. Pathol.* **24**, 564-572.
- Brix, A.E., Jokinen, M.P., Walker, N.J., Sells, D.M., and Nyska, A. (2004). Characterization of bronchiolar metaplasia of the alveolar epithelium in female Sprague-Dawley rats exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB126). *Toxicol. Pathol.* **32**, 333-337.
- Brundl, A., and Buff, K. (1993). Partial purification and characterization of a rat liver polychlorinated biphenyl (PCB) binding protein. *Biochem. Pharmacol.* **45**, 885-891.
- Bruno, M.E., Borchers, C.H., Dial, J.M., Walker, N.J., Hartis, J.E., Wetmore, B.A., Barrett, J.C., Tomer, K.B., and Merrick, B.A. (2002). Effects of TCDD upon IkappaB and IKK subunits localized in microsomes by proteomics. *Arch. Biochem. Biophys.* **406**, 153-164.
- Buff, K., and Brundl, A. (1992). Specific binding of polychlorinated biphenyls to rat liver cytosol protein. *Biochem. Pharmacol.* **43**, 965-970.
- Bunger, M.K., Moran, S.M., Glover, E., Thomae, T.L., Lahvis, G.P., Lin, B.C., and Bradfield, C.A. (2003). Resistance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor. *J. Biol. Chem.* **278**, 17,767-17,774.
- Burbach, K.M., Poland, A., and Bradfield, C.A. (1992). Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8185-8189.
- Chu, I., Villeneuve, D.C., Yagminas, A., Lecavalier, P., Hakansson, H., Ahlborg, U.G., Valli, V.E., Kennedy, S.W., Bergman, A., Seegal, R.F., and Feeley, M. (1995). Toxicity of PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 118 (2,3',4,4',5-pentachlorobiphenyl) in the rat following subchronic dietary exposure. *Fundam. Appl. Toxicol.* **26**, 282-292.

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

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

Curran, P.G., and DeGroot, L.J. (1991). The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocr. Rev.* **12**, 135-150.

Della Porta, G., Dragani, T.A., and Sozzi, G. (1987). Carcinogenic effects of infantile and long-term 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment in the mouse. *Tumori* **73**, 99-107.

Denomme, M.A., Bandiera, S., Lambert, I., Copp, L., Safe, L., and Safe, S. (1983). Polychlorinated biphenyls as phenobarbitone-type inducers of microsomal enzymes. Structure-activity relationships for a series of 2,4-dichloro-substituted congeners. *Biochem. Pharmacol.* **32**, 2955-2963.

DeVito, M.J., Birnbaum, L.S., Farland, W.H., and Gasiewicz, T.A. (1995). Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ. Health Perspect.* **103**, 820-831.

Diliberto, J.J., Burgin, D., and Birnbaum, L.S. (1997). Role of CYP1A2 in hepatic sequestration of dioxin: Studies using CYP1A2 knock-out mice. *Biochem. Biophys. Res. Commun.* **236**, 431-433.

Diliberto, J.J., Burgin, D.E., and Birnbaum, L.S. (1999). Effects of CYP1A2 on disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, and 2,2',4,4',5,5'-hexachlorobiphenyl in CYP1A2 knockout and parental (C57BL/6N and 129/Sv) strains of mice. *Toxicol. Appl. Pharmacol.* **159**, 52-64.

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.

Dolwick, K.M., Schmidt, J.V., Carver, L.A., Swanson, H.I., and Bradfield, C.A. (1993). Cloning and expression of a human Ah receptor cDNA. *Mol. Pharmacol.* **44**, 911-917.

Dragan, Y.P., and Schrenk, D. (2000). Animal studies addressing the carcinogenicity of TCDD (or related compounds) with an emphasis on tumour promotion. *Food Addit. Contam.* **17**, 289-302.

Dragan, Y.P., Xu, X.H., Goldsworthy, T.L., Campbell, H.A., Maronpot, R.R., and Pitot, H.C. (1992). Characterization of the promotion of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the female rat. *Carcinogenesis* **13**, 1389-1395.

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.

Dunson, D.B., Haseman, J.K., van Birgelen, A.P.J.M., Stasiewicz, S., and Tennant, R.W. (2000). Statistical analysis of skin tumor data from Tg.AC mouse bioassays. *Toxicol. Sci.* **55**, 293-302.

Eastin, W.C., Haseman, J.K., Mahler, J.F., and Bucher, J.R. (1998). The National Toxicology Program evaluation of genetically altered mice as predictive models for identifying carcinogens. *Toxicol. Pathol.* **26**, 461-473.

Ema, M., Sogawa, K., Watanabe, N., Chujoh, Y., Matsushita, N., Gotoh, O., Funae, Y., and Fujii-Kuriyama, Y. (1992). cDNA cloning and structure of mouse putative Ah receptor. *Biochem. Biophys. Res. Commun.* **184**, 246-253.

Fattore, E., Trossvik, C., and Håkansson, H. (2000). Relative potency values derived from hepatic vitamin A reduction in male and female Sprague-Dawley rats following subchronic dietary exposure to individual polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners and a mixture thereof. *Toxicol. Appl. Pharmacol.* **165**, 184-194.

Feeley, M.M., and Jordan, S.A. (1998). Dietary and tissue residue analysis and contaminant intake estimations in rats consuming diets composed of Great Lakes salmon: A multigenerational study. *Regul. Toxicol. Pharmacol.* **27**, S8-S17.

- Fiorella, P.D., Olson, J.R., and Napoli, J.L. (1995). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin induces diverse retinoic acid metabolites in multiple tissues of the Sprague-Dawley rat. *Toxicol. Appl. Pharmacol.* **134**, 222-228.
- Fischer, L.J., Seegal, R.F., Ganey, P.E., Pessah, I.N., and Kodavanti, P.R. (1988). Symposium overview: Toxicity of non-coplanar PCBs. *Toxicol. Sci.* **41**, 49-61.
- Flesch-Janys, D., Berger, J., Gurn, P., Manz, A., Nagel, S., Waltsgott, H., and Dwyer, J.H. (1995). Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. *Am. J. Epidemiol.* **142**, 1165-1175.
- Flesch-Janys, D., Steindorf, K., Gurn, P., and Becher, H. (1998). Estimation of the cumulated exposure to polychlorinated dibenzo-*p*-dioxins/furans and standardized mortality ratio analysis of cancer mortality by dose in an occupationally exposed cohort. *Environ. Health Perspect.* **106** (Suppl. 2), 655-662.
- Frame, G.M., Wagner, R.E., Carnahan, J.C., Brown, J.F., May, R.J., Smullen, L.A., and Bedard, D.L. (1996). Comprehensive, quantitative, congener-specific analyses of eight arochlors and complete PCB congener assignments on DB-1 capillary GC columns. *Chemosphere* **33**, 603-623.
- Frueh, F.W., Hayashibara, K.C., Brown, P.O., and Whitlock, J.P., Jr. (2001). Use of cDNA microarrays to analyze dioxin-induced changes in human liver gene expression. *Toxicol. Lett.* **122**, 189-203.
- 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.
- Gasiewicz, T.A., Thurmond, T.S., Staples, J.E., Murante, F.G., and Silverstone, A.E. (2000). Use of bone marrow chimeras to identify cell targets in the immune system for the actions of chemicals. *Ann. N.Y. Acad. Sci.* **919**, 300-303.
- Giesy, J.P., and Kannan, K. (1998). Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): Implications for risk assessment. *Crit. Rev. Toxicol.* **28**, 511-569.
- Gillner, M., Brittebo, E.B., Brandt, I., Soderkvist, P., Appelgren, L.E., and Gustafsson, J.A. (1987). Uptake and specific binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the olfactory mucosa of mice and rats. *Cancer Res.* **47**, 4150-4159.
- Goldstein, J.A., and Linko, P. (1984). Differential induction of two 2,3,7,8-tetrachlorodibenzo-*p*-dioxin forms of cytochrome P-450 in extrahepatic versus hepatic tissues. *Mol. Pharmacol.* **25**, 185-191.
- Gonzalez, F.J. (2001). The use of gene knockout mice to unravel the mechanisms of toxicity and chemical carcinogenesis. *Toxicol. Lett.* **120**, 199-208.
- Gonzalez, F.J., and Fernandez-Salguero, P. (1998). The aryl hydrocarbon receptor: Studies using the AHR-null mice. *Drug Metab. Dispos.* **26**, 1194-1198.
- Gonzalez, F.J., Fernandez-Salguero, P., and Ward, J.M. (1996). The role of the aryl hydrocarbon receptor in animal development, physiological homeostasis and toxicity of TCDD. *J. Toxicol. Sci.* **21**, 273-277.
- Goodman, D.G., and Sauer, R.M. (1992). Hepatotoxicity and carcinogenicity in female Sprague-Dawley rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): A pathology working group reevaluation. *Regul. Toxicol. Pharmacol.* **15**, 245-252.
- Grassman, J.A., Masten, S.A., Walker, N.J., and Lucier, G.W. (1998). Animal models of human response to dioxins. *Environ. Health Perspect.* **106** (Suppl. 2), 761-775.
- Gu, Y.Z., Hogenesch, J.B., and Bradfield, C.A. (2000). The PAS superfamily: Sensors of environmental and developmental signals. *Annu. Rev. Pharmacol. Toxicol.* **40**, 519-561.

- Haag-Grönlund, M., Wärngård, L., Flodström, S., Scheu, G., Kronevi, T., Ahlborg, U.G., and Fransson-Steen, R. (1997). Promotion of altered hepatic foci by 2,3',4,4',5-pentachlorobiphenyl in Sprague-Dawley female rats. *Fundam. Appl. Toxicol.* **35**, 120-130.
- Haag-Grönlund, M., Johansson, N., Fransson-Steen, R., Håkansson, H., Scheu, G., and Wärngård, L. (1998). Interactive effects of three structurally different polychlorinated biphenyls in a rat liver tumor promotion bioassay. *Toxicol. Appl. Pharmacol.* **152**, 153-165.
- Hailey, J.R., Walker, N.J., Sells, D.M., Brix, A.E., Jokinen, M.P., and Nyska, A. (2005). Classification of proliferative hepatocellular lesions in Harlan Sprague-Dawley rats chronically exposed to dioxin-like compounds. *Toxicol. Pathol.* **33**, 165-174.
- Hassoun, E.A., Li, F., Abushaban, A., and Stohs, S.J. (2000). The relative abilities of TCDD and its congeners to induce oxidative stress in the hepatic and brain tissues of rats after subchronic exposure. *Toxicology* **145**, 103-113.
- Hassoun, E.A., Li, F., Abushaban, A., and Stohs, S.J. (2001). Production of superoxide anion, lipid peroxidation and DNA damage in the hepatic and brain tissues of rats after subchronic exposure to mixtures of TCDD and its congeners. *J. Appl. Toxicol.* **21**, 211-219.
- Hayes, C.L., Spink, D.C., Spink, B.C., Cao, J.Q., Walker, N.J., and Sutter, T.R. (1996). 17 β -Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9776-9781.
- Hebert, C.D., Harris, M.W., Elwell, M.R., and Birnbaum, L.S. (1990). Relative toxicity and tumor-promoting ability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PCDF), and 1,2,3,4,7,8-hexachlorodibenzofuran (HCDF) in hairless mice. *Toxicol. Appl. Pharmacol.* **102**, 362-377.
- Heid, S.E., Walker, M.K., and Swanson, H.I. (2001). Correlation of cardiotoxicity mediated by halogenated aromatic hydrocarbons to aryl hydrocarbon receptor activation. *Toxicol. Sci.* **61**, 187-196.
- Hemming, H., Bager, Y., Flodström, S., Nordgren, I., Kronevi, T., Ahlborg, U.G., and Wärngård, L. (1995). Liver tumor promoting activity of 3,4,5,3',4'-pentachlorobiphenyl and its interaction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Eur. J. Pharmacol.* **292**, 241-249.
- Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L., and Wilkinson, C.F. (1989). Thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.* **12**, 629-697.
- Hoffman, E.C., Reyes, H., Chu, F.F., Sander, F., Conley, L.H., Brooks, B.A., and Hankinson, O. (1991). Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* **252**, 954-958.
- Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.
- Hursting, S.D., Lavigne, J.A., Berrigan, D., Perkins, S.N., and Barrett, J.C. (2003). Calorie restriction, aging, and cancer prevention: Mechanisms of action and applicability to humans. *Annu. Rev. Med.* **54**, 131-152.
- International Agency for Research on Cancer (IARC) (1997). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Polychlorinated Dibenzopara-dioxins and Polychlorinated Dibenzofurans*, Vol. 69. IARC, Lyon, France.
- Ito, N., Nagasaki, H., Arai, M., Makiura, S., Sugihara, S., and Hirao, K. (1973). Histopathologic studies on liver tumorigenesis induced in mice by technical polychlorinated biphenyls and its promoting effect on liver tumors induced by benzene hexachloride. *J. Natl. Cancer Inst.* **51**, 1637-1646.
- Jimi, A., Kojiro, M., Miyasaka, K., Kono, A., and Funakoshi, A. (1997). Apoptosis in the pancreas of genetically diabetic rats with a disrupted cholecystokinin (CCK-A) receptor gene. *Pancreas* **14**, 109-112.

- Jokinen, M.P., Walker, N.J., Brix, A.E., Sells, D.M., Haseman, J.K., and Nyska, A. (2003). Increase in cardiovascular pathology in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl. *Cardiovasc. Toxicol.* **3**, 299-310.
- Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.
- Jordan, S.A., and Feeley, M.M. (1999). PCB congener patterns in rats consuming diets containing Great Lakes salmon: Analysis of fish, diets, and adipose tissue. *Environ. Res.* **80**, S207-S212.
- Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.
- Kimbrough, R.D., Squire, R.A., Linder, R.E., Strandberg, J.D., Montalli, R.J., and Burse, V.W. (1975). Induction of liver tumor in Sherman strain female rats by polychlorinated biphenyl aroclor 1260. *J. Natl. Cancer Inst.* **55**, 1453-1459.
- Kociba, R.J., Keyes, D.G., Beyer, J.E., Carreon, R.M., Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Park, C.N., Barnard, S.D., Hummel, R.A., and Humiston, C.G. (1978). Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol. Appl. Pharmacol.* **46**, 279-303.
- Kohn, M.C., Lucier, G.W., Clark, G.C., Sewall, C., Tritscher, A.M., and Portier, C.J. (1993). A mechanistic model of effects of dioxin on gene expression in the rat liver. *Toxicol. Appl. Pharmacol.* **120**, 138-154.
- Kohn, M.C., Sewall, C.H., Lucier, G.W., and Portier, C.J. (1996). A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol. Appl. Pharmacol.* **136**, 29-48.
- Kohn, M.C., Walker, N.J., Kim, A.H., and Portier, C.J. (2001). Physiological modeling of a proposed mechanism of enzyme induction by TCDD. *Toxicology* **162**, 193-208.
- Kurachi, M., Hashimoto, S., Obata, A., Nagai, S., Nagahata, T., Inadera, H., Sone, H., Tohyama, C., Kaneko, S., Kobayashi, K., and Matsushima, K. (2002). Identification of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-responsive genes in mouse liver by serial analysis of gene expression. *Biochem. Biophys. Res. Commun.* **292**, 368-377.
- Lai, Z.W., Pineau, T., and Esser, C. (1996). Identification of dioxin-responsive elements (DREs) in the 5' regions of putative dioxin-inducible genes. *Chem. Biol. Interact.* **100**, 97-112.
- Lancillotti, F., Darwiche, N., Celli, G., and De Luca, L.M. (1992). Retinoid status and the control of keratin expression and adhesion during the histogenesis of squamous metaplasia of tracheal epithelium. *Cancer Res.* **52**, 6144-6152.
- Lee, H.M., He, Q., Englander, E.W., and Greeley, G.H., Jr. (2000). Endocrine disruptive effects of polychlorinated aromatic hydrocarbons on intestinal cholecystokinin in rats. *Endocrinology* **141**, 2938-2944.
- Lee, S.K., Ou, Y.C., and Yang, R.S.H. (2002). Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in nonpregnant mice, lactating mice, and suckling pups. *Toxicol. Sci.* **65**, 26-34.
- Levin, S., Semler, D., and Ruben, Z. (1993). Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. *Toxicol. Pathol.* **21**, 1-14.
- Lotan, R. (1994). Suppression of squamous cell carcinoma growth and differentiation by retinoids. *Cancer Res.* **54**, 1987s-1990s.
- Lucier, G.W., Tritscher, A., Goldworthy, T., Foley, J., Clark, G., Goldstein, J., and Maronpot, R. (1991). Ovarian hormones enhance 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated increases in cell proliferation and preneoplastic foci in a two-stage model for rat hepatocarcinogenesis. *Cancer Res.* **51**, 1391-1397.

- McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.
- MacKenzie, W.F., and Alison, R. (1990). Heart. 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. 461-471. Academic Press, San Diego, CA.
- Mariussen, E., Myhre, O., Reistad, T., and Fonnum, F. (2002). The polychlorinated biphenyl mixture aroclor 1254 induces death of rat cerebellar granule cells: The involvement of the *N*-methyl-D-aspartate receptor and reactive oxygen species. *Toxicol. Appl. Pharmacol.* **179**, 137-144.
- Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.
- Maronpot, R.R., Giles, H.D., Dykes, D.J., and Irwin, R.D. (1991). Furan-induced hepatic cholangiocarcinomas in Fischer 344 rats. *Toxicol. Pathol.* **19**, 561-570.
- Maronpot, R.R., Foley, J.F., Takahashi, K., Goldsworthy, T., Clark, G., Tritscher, A., Portier, C., and Lucier, G. (1993). Dose response for TCDD promotion of hepatocarcinogenesis in rats initiated with DEN: Histologic, biochemical, and cell proliferation endpoints. *Environ. Health Perspect.* **101**, 634-642.
- Martinez, J.M., Afshari, C.A., Bushel, P.R., Masuda, A., Takahashi, T., and Walker, N.J. (2002). Differential toxicogenomic responses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in malignant and nonmalignant human airway epithelial cells. *Toxicol. Sci.* **69**, 409-423.
- Masuda, Y. (1985). Health status of Japanese and Taiwanese after exposure to contaminated rice oil. *Environ. Health Perspect.* **60**, 321-325.
- Mayes, B.A., McConnell, E.E., Neal, B.H., Brunner, M.J., Hamilton, S.B., Sullivan, T.M., Peters, A.C., Ryan, M.J., Toft, J.D., Singer, A.W., Brown, J.F., Jr., Menton, R.G., and Moore, J.A. (1998). Comparative carcinogenicity in Sprague-Dawley rats of the polychlorinated biphenyl mixtures Aroclors 1016, 1242, 1254, and 1260. *Toxicol. Sci.* **41**, 62-76.
- Moolgavkar, S.H., Luebeck, E.G., Buchmann, A., and Bock, K.W. (1996). Quantitative analysis of enzyme-altered liver foci in rats initiated with diethylnitrosamine and promoted with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* **138**, 31-42.
- Murray, G.I., Melvin, W.T., Greenlee, W.F., and Burke, M.D. (2001). Regulation, function, and tissue-specific expression of cytochrome P450 CYP1B1. *Annu. Rev. Pharmacol. Toxicol.* **41**, 297-316.
- Nagasaki, H., Tomii, S., Mega, T., Marugami, M, and Ito, N. (1972). Hepatocarcinogenicity of polychlorinated biphenyls in mice. *Gann* **63**, 805.
- Narama, I., Imaida, K., Iwata, H., Nakae, D., Nishikawa, A., and Harada, T. (2003). A review of nomenclature and diagnostic criteria for proliferative lesions in the liver of rats by a working group of the Japanese Society of Toxicologic Pathology. *J. Toxicol. Pathol.* **16**, 1-17.
- National Cancer Institute (NCI) (1980). Bioassay of a Mixture of 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (Gavage) for Possible Carcinogenicity (CAS Nos. 57653-85-7 and 19408-74-3). Technical Report Series No. 198. NIH Publication No. 80-1754. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD, and Research Triangle Park, NC.
- National Toxicology Program (NTP) (1982a). Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (CAS No. 1746-01-6) in Osborne-Mendel Rats and B6C3F1 Mice (Gavage Study). Technical Report Series No. 209, NIH Publication No. 82-1765. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, and Bethesda, MD.
- National Toxicology Program (NTP) (1982b). Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (CAS No. 1746-01-6) in Swiss-Webster Mice (Dermal Study). Technical Report Series No. 201, NIH Publication No. 82-1757. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, and Bethesda, MD.

- National Toxicology Program (NTP) (2006a). Toxicology and Carcinogenesis Studies of 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 520, NIH Publication No. 06-4454. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- National Toxicology Program (NTP) (2006b). Toxicology and Carcinogenesis Studies of 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153) (CAS No. 35065-27-1) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 529, NIH Publication No. 06-4465. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- National Toxicology Program (NTP) (2006c). 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.
- National Toxicology Program (NTP) (2006d). Toxicology and Carcinogenesis Studies of a Mixture of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6), 2,3,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4), and 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 526, NIH Publication No. 06-4462. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- National Toxicology Program (NTP) (2006e). Toxicology and Carcinogenesis Studies of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 525, NIH Publication No. 06-4461. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- National Toxicology Program (NTP) (2006f). Toxicology and Carcinogenesis Studies of a Binary Mixture of 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) and 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153) (CAS No. 35065-27-1) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 530, NIH Publication No. 06-4466. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- Ohlsson, B., Axelson, J., Sternby, B., Rehfeld, J.F., and Ihse, I. (1995). Time-course of the pancreatic changes following long-term stimulation or inhibition of the CCK-A receptor. *Int. J. Pancreatol.* **18**, 59-66.
- Okey, A.B., Riddick, D.S., and Harper, P.A. (1994). The Ah receptor: Mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. *Toxicol. Lett.* **70**, 1-22.
- Park, J.Y.K., Shigenaga, M.K., and Ames, B.N. (1996). Induction of cytochrome P4501A1 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or indolo(3,2-*b*)carbazole is associated with oxidative DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2322-2327.
- Partanen, A.M., Alaluusua, S., Miettinen, P.J., Thesleff, I., Tuomisto, J., Pohjanvirta, R., and Lukanmaa, P.L. (1998). Epidermal growth factor receptor as a mediator of developmental toxicity of dioxin in mouse embryonic teeth. *Lab. Invest.* **78**, 1473-1481.
- Pearce, R.E., McIntyre, C.J., Madan, A., Sanzgiri, U., Draper, A.J., Bullock, P.L., Cook, D.C., Burton, L.A., Latham, J., Nevins, C., and Parkinson, A. (1996). Effects of freezing, thawing, and storing human liver microsomes on cytochrome P450 activity. *Arch. Biochem. Biophys.* **331**, 145-169.
- Peterson, R.E., Theobald, H.M., and Kimmel, G.L. (1993). Developmental and reproductive toxicity of dioxins and related compounds: Cross-species comparisons. *Crit. Rev. Toxicol.* **23**, 283-335.
- Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology*, Section 6.3.2. Chapman and Hall, London.

- Piper, W.N., Rose, J.Q., and Gehring, P.J. (1973). Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Environ. Health Perspect.* **5**, 241-244.
- 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.
- Pitot, H.C., Dragan, Y., Sargent, L., and Xu, Y.H. (1991). Biochemical markers associated with the stages of promotion and progression during hepatocarcinogenesis in the rat. *Environ. Health Perspect.* **93**, 181-189.
- Pohjanvirta, R., Vartiainen, T., Uusi-Rauva, A., Monkkonen, J., and Tuomisto, J. (1990). Tissue distribution, metabolism, and excretion of ¹⁴C-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain. *Pharmacol. Toxicol.* **66**, 93-100.
- 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.
- Poland, A., and Knutson, J.C. (1982). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**, 517-554.
- Poland, A., Palen, D., and Glover, E. (1982). Tumour promotion by TCDD in skin of HRS/J hairless mice. *Nature* **300**, 271-273.
- 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.
- Portier, C.J., Sherman, C.D., Kohn, M., Edler, L., Kopp-Schneider, A., Maronpot, R.M., and Lucier, G. (1996). Modeling the number and size of hepatic focal lesions following exposure to 2,3,7,8-TCDD. *Toxicol. Appl. Pharmacol.* **138**, 20-30.
- Puga, A., Maier, A., and Medvedovic, M. (2000). The transcriptional signature of dioxin in human hepatoma HepG2 cells. *Biochem. Pharmacol.* **60**, 1129-1142.
- Rao, M.S., Subbarao, V., Prasad, J.D., and Scarpelli, D.G. (1988). Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Syrian golden hamster. *Carcinogenesis* **9**, 1677-1679.
- Render, J.A., Bursian, S.J., Rosenstein, D.S., and Aulerich, R.J. (2001). Squamous epithelial proliferation in the jaws of mink fed diets containing 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Vet. Hum. Toxicol.* **43**, 22-26.
- Rose, J.Q., Ramsey, J.C., Wentzler, T.H., Hummel, R.A., and Gehring, P.J. (1976). The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to the rat. *Toxicol. Appl. Pharmacol.* **36**, 209-226.
- Safe, S.H. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.* **21**, 51-88.
- Safe, S.H. (1994). Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* **24**, 87-149.
- Sánchez-Alonzo, J.A., López-Aparicio, P., Recio, M.N., and Pérez-Albarsanz, M.A. (2003). Apoptosis-mediated neurotoxic potential of a planar (PCB 77) and a non-planar (PCB 153) polychlorinated biphenyl congeners in neuronal cell cultures. *Toxicol. Lett.* **144**, 337-349.
- Sapolsky, R., Armanini, M., Packan, D., and Tombaugh, G. (1987). Stress and glucocorticoids in aging. *Endocrinol. Metab. Clin. North Am.* **16**, 965-980.

- Schechter, A., Fürst, P., Fürst, C., Papke, O., Ball, M., Ryan, J.J., Cau, H.D., Dai, L.C., Quayh, H.T., Cuong, H.Q., Phoung, N.T.N., Phiet, P.H., Beim, A., Constable, J., Startin, J., Samedy, M., and Seng, Y.K. (1994). Chlorinated dioxins and dibenzofurans in human tissue from general populations: A selective review. *Environ. Health Perspect.* **102** (Suppl. 1), 159-171.
- Schmidt, C.K., Hoegberg, P., Fletcher, N., Nilsson, C.B., Trossvik, C., Håkansson, H., and Nau, H. (2003). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) alters the endogenous metabolism of *all-trans-retinoic acid* in the rat. *Arch. Toxicol.* **77**, 371-383.
- Schmidt, J.V., and Bradfield, C.A. (1996). Ah receptor signaling pathways. *Annu. Rev. Cell Dev. Biol.* **12**, 55-89.
- Schrenk, D., Lipp, H.P., Wiesmuller, T., Hagenmaier, H., and Bock, K.W. (1991). Assessment of biological activities of mixtures of polychlorinated dibenzo-*p*-dioxins: Comparison between defined mixtures and their constituents. *Arch. Toxicol.* **65**, 114-118.
- Schrenk, D., Buchmann, A., Dietz, K., Lipp, H.P., Brunner, H., Sirma, H., Munzel, P., Hagenmaier, H., Gebhardt, R., and Bock, K.W. (1994). Promotion of preneoplastic foci in rat liver with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8-hepatochlorodibenzo-*p*-dioxin and a defined mixture of 49 polychlorinated dibenzo-*p*-dioxins. *Carcinogenesis* **15**, 509-515.
- Seegal, R.F., Bush, B., and Shain, W. (1990). Lightly chlorinated ortho-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. *Toxicol. Appl. Pharmacol.* **106**, 136-144.
- Seilkop, S. K. (1995). The effect of body weight on tumor incidence and carcinogenicity testing in B6C3F1 mice and F344 rats. *Fundam. Appl. Toxicol.* **24**, 247-259.
- Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.
- Silberhorn, E.M., Glauert, H.P., and Robertson, L.W. (1990). Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *Crit. Rev. Toxicol.* **20**, 440-496.
- Silkworth, J., Mayes, B., Fish, K., and Brown, J.F., Jr. (1997). Tumor responses, PCB tissue concentrations and PCB hepatic binding in S-D rats fed Aroclors 1016, 1242, 1254 or 1260. *Organohalogen Compounds* **34**, 164-166.
- Staples, J.E., Murante, F.G., Fiore, N.C., Gasiewicz, T.A., and Silverstone, A.E. (1998). Thymic alterations induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are strictly dependent on aryl hydrocarbon receptor activation in hemopoietic cells. *J. Immunol.* **160**, 3844-3854.
- Stinchcombe, S., Buchmann, A., Bock, K.W., and Schwarz, M. (1995). Inhibition of apoptosis during 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated tumour promotion in rat liver. *Carcinogenesis* **16**, 1271-1275.
- Stohs, S.J., Shara, M.A., Alsharif, N.Z., Wahba, Z.Z., and al-Bayati, Z.A. (1990). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced oxidative stress in female rats. *Toxicol. Appl. Pharmacol.* **106**, 126-135.
- Sutter, T.R., and Greenlee, W.F. (1992). Classification of members of the Ah gene battery. *Chemosphere* **25**, 223-226.
- Sutter, T.R., Tang, Y.M., Hayes, C.L., Wo, Y.Y.P., Jabs, E.W., Li, X., Yin, H., Cody, C.W., and Greenlee, W.F. (1994). Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J. Biol. Chem.* **269**, 13,092-13,099.
- Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.
- Teegarden, J.G., Dragan, Y.P., Singh, J., Vaughan, J., Xu, Y.H., Goldsworthy, T., and Pitot, H.C. (1999). Quantitative analysis of dose- and time-dependent promotion of four phenotypes of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague-Dawley rats. *Toxicol. Sci.* **51**, 211-223.

- Toth, K., Somfai-Relle, S., Sugar, J., and Bence, J. (1979). Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. *Nature* **278**, 548-549.
- Toyoshiba, H., Walker, N.J., Bailer, A.J., and Portier, C.J. (2004). Evaluation of toxic equivalency factors for induction of cytochromes P450 CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. *Toxicol. Appl. Pharmacol.* **194**, 156-168.
- Tritscher, A.M., Clark, G.C., Sewall, C., Sills, R.C., Maronpot, R., and Lucier, G.W. (1995). Persistence of TCDD-induced hepatic cell proliferation and growth of enzyme altered foci after chronic exposure followed by cessation of treatment in DEN initiated female rats. *Carcinogenesis* **16**, 2807-2811.
- Tritscher, A.M., Seacat, A.M., Yager, J.D., Groopman, J.D., Miller, B.D., Bell, D., Sutter, T.R., and Lucier, G.W. (1996). Increased oxidative DNA damage in livers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treated intact but not ovariectomized rats. *Cancer Lett.* **98**, 219-225.
- Tritscher, A.M., Mahler, J., Portier, C.J., Lucier, G.W., and Walker, N.J. (2000). Induction of lung lesions in female rats following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Pathol.* **28**, 761-769.
- U.S. Environmental Protection Agency (USEPA) (2000a). Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds (September 2000 Draft). Part I: Estimating exposure to dioxin-like compounds. Volume 2: Sources of dioxin-like compounds in the United States. EPA/600/P-00/001 Bb. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
- U.S. Environmental Protection Agency (USEPA) (2000b). Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds (September 2000 Draft). Part I: Estimating exposure to dioxin-like compounds. Volume 3: Properties, environmental levels and background exposures. EPA/600/P-00/011 Bc. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
- U.S. Environmental Protection Agency (USEPA) (2000c). Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds (September 2000 Draft). Part II: Health assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. EPA/600/P-001/001 Be. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
- Van Birgelen, A.P.J.M., van der Kolk, J., Fase, K.M., Bol, I., Poiger, H., Brouwer, A., and van den Berg, M. (1994). Toxic potency of 3,3',4,4',5-pentachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. *Toxicol. Appl. Pharmacol.* **127**, 209-221.
- Van Birgelen, A.P., Smit, E.A., Kampen, I.M., Groeneveld, C.N., Fase, K.M., van der Kolk, J., Poiger, H., van den Berg, M., Koeman, J.H., and Brouwer, A. (1995a). Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: Use in risk assessment. *Eur. J. Pharmacol.* **293**, 77-85.
- Van Birgelen, A.P.J.M., Van der Kolk, J., Fase, K.M., Bol, I., Poiger, H., Brouwer, A., and Van den Berg, M. (1995b). Subchronic dose-response study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague-Dawley rats. *Toxicol. Appl. Pharmacol.* **132**, 1-13.
- Van Birgelen, A.P.J.M., Ross, D.G., Devito, M.J., and Birnbaum, L.S. (1996). Interactive effects between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,2',4,4',5,5'-hexachlorobiphenyl in female B6C3F₁ mice: Tissue distribution and tissue-specific enzyme induction. *Fundam. Appl. Toxicol.* **34**, 118-131.
- Van Birgelen, A.P.J.M., Johnson, J.D., Fuciarelli, A.F., Toft, J.D., Mahler, J., and Bucher, J.R. (1999). Dose and time-response of TCDD in Tg.AC mice after dermal and oral exposure. In *Dioxin '99: 19th International Symposium on Halogenated Environmental Organic Pollutants and POPs*. (ISBN 88-87772-02-9), Vol. 42 Organohalogen Compounds, pp. 235-239, Venice, Italy.

- Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Wærn, F., and Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **106**, 775-792.
- Vanden Heuvel, J.P., Clark, G.C., Tritscher, A.M., and Lucier, G.W. (1994). Accumulation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in liver of control laboratory rats. *Fundam. Appl. Toxicol.* **23**, 465-469.
- Van der Plas, S.A., Haag-Grönlund, M., Scheu, G., Wärngård, L., van den Berg, M., Wester, P., Koeman, J.H., and Brouwer, A. (1999). Induction of altered hepatic foci by a mixture of dioxin-like compounds with and without 2,2',4,4',5,5'-hexachlorobiphenyl in female Sprague-Dawley rats. *Toxicol. Appl. Pharmacol.* **156**, 30-39.
- Varga, G., Kisfalvi, K., Pelosini, I., D'Amato, M., and Scarpignato, C. (1998). Different actions of CCK on pancreatic and gastric growth in the rat: Effect of CCK(A) receptor blockade. *Br. J. Pharmacol.* **124**, 435-440.
- Vorderstrasse, B.A., Steppan, L.B., Silverstone, A.E., and Kerkvliet, N.I. (2001). Aryl hydrocarbon receptor-deficient mice generate normal immune responses to model antigens and are resistant to TCDD-induced immune suppression. *Toxicol. Appl. Pharmacol.* **171**, 157-164.
- Wærn, F., Flodström, S., Busk, L., Kronevi, T., Nordgren, I., and Ahlborg, U.G. (1991). Relative liver tumour promoting activity and toxicity of some polychlorinated dibenzo-*p*-dioxin- and dibenzofuran-congeners in female Sprague-Dawley rats. *Pharmacol. Toxicol.* **69**, 450-458.
- Walker, M.K., and Catron, T.F. (2000). Characterization of cardiotoxicity induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related chemicals during early chick embryo development. *Toxicol. Appl. Pharmacol.* **167**, 210-221.
- Walker, N.J., Gastel, J.A., Costa, L.T., Clark, G.C., Lucier, G.W., and Sutter, T.R. (1995). Rat CYP1B1: An adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. *Carcinogenesis* **16**, 1319-1327.
- Walker, N.J., Miller, B.D., Kohn, M.C., Lucier, G.W., and Tritscher, A.M. (1998). Differences in kinetics of induction and reversibility of TCDD-induced changes in cell proliferation and CYP1A1 expression in female Sprague-Dawley rat liver. *Carcinogenesis* **19**, 1427-1435.
- Walker, N.J., Tritscher, A.M., Sills, R.C., Lucier, G.W., and Portier, C.J. (2000). Hepatocarcinogenesis in female Sprague-Dawley rats following discontinuous treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Sci.* **54**, 330-337.
- Walker, N.J., Crockett, P.W., Nyska, A., Brix, A.E., Jokinen, M.P., Sells, D.M., Hailey, J.R., Easterling, M., Haseman, J.K., Yin, M., Wyde, M.E., Bucher, J.R., and Portier, C.J. (2005). Dose-additive carcinogenicity of a defined mixture of "dioxin-like compounds." *Environ. Health Perspect.* **113**, 43-48.
- Warngard, L., Bager, Y., Kato, Y., Kenne, K., and Ahlborg, U.G. (1996). Mechanistical studies of the inhibition of intercellular communication by organochlorine compounds. *Arch. Toxicol. Suppl.* **18**, 149-159.
- Wassom, J.S., Huff, J.E., and Loprieno, N. (1977). A review of the genetic toxicology of chlorinated dibenzo-*p*-dioxins. *Mutat. Res.* **47**, 141-160.
- Whitlock, J.P., Jr. (1990). Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* **30**, 251-277.
- Whitlock, J.P., Jr. (1993). Mechanistic aspects of dioxin action. *Chem. Res. Toxicol.* **6**, 754-763.
- Whitlock, J.P., Jr. (1999). Induction of cytochrome P4501A1. *Annu. Rev. Pharmacol. Toxicol.* **39**, 103-125.
- 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.
- Wong, P.W., Brackney, W.R., and Pessah, I.N. (1997). *Ortho*-substituted polychlorinated biphenyls alter microsomal calcium transport by direct interaction with ryanodine receptors of mammalian brain. *J. Biol. Chem.* **272**, 15,145-15,153.
- Worner, W., and Schrenk, D. (1996). Influence of liver tumor promoters on apoptosis in rat hepatocytes induced by 2-acetylaminofluorene, ultraviolet light, or transforming growth factor- β 1. *Cancer Res.* **56**, 1272-1278.
- Wyde, M.E., Eldridge, S.R., Lucier, G.W., and Walker, N.J. (2001a). Regulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced tumor promotion by 17 β -estradiol in female Sprague-Dawley rats. *Toxicol. Appl. Pharmacol.* **173**, 7-17.
- Wyde, M.E., Wong, V.A., Kim, A.H., Lucier, G.W., and Walker, N.J. (2001b). Induction of hepatic 8-oxodeoxyguanosine adducts by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Sprague-Dawley rats is female-specific and estrogen-dependent. *Chem. Res. Toxicol.* **14**, 849-855.
- Wyde, M.E., Cambre, T., Lebetkin, M., Eldridge, S.R., and Walker, N.J. (2002). Promotion of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 17 β -estradiol in male Sprague-Dawley rats. *Toxicol. Sci.* **68**, 295-303.
- Wyde, M.E., Braen, A.P.J.M., Hejtmancik, M., Johnson, J.D., Toft, J.D., Blake, J.C., Cooper, S.D., Mahler, J., Vallant, M., Bucher, J.R., and Walker, N.J. (2004). Oral and dermal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces cutaneous papillomas and squamous cell carcinomas in female hemizygous Tg.AC transgenic mice. *Toxicol. Sci.* **82**, 34-45.
- Yu, M.L., Guo, Y.L., Hsu, C.C., and Rogan, W.J. (1997). Increased mortality from chronic liver disease and cirrhosis 13 years after the Taiwan "yucheng" ("oil disease") incident. *Am. J. Ind. Med.* **31**, 172-175.
- Zeytun, A., McKallip, R.J., Fisher, M., Camacho, I., Nagarkatti, M., and Nagarkatti, P.S. (2002). Analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced gene expression profile in vivo using pathway-specific cDNA arrays. *Toxicology* **178**, 241-260.

APPENDIX A
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR GAVAGE STUDY
OF A BINARY MIXTURE OF PCB 126 AND PCB 118

TABLE A1a	Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	92
TABLE A1b	Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	95
TABLE A2	Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	100
TABLE A3a	Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	138
TABLE A3b	Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of a Binary Mixture of PCB 126 and PCB 118	142
TABLE A4a	Historical Incidence of Liver Neoplasms in Vehicle Control Female Sprague-Dawley Rats	144
TABLE A4b	Historical Incidence of Cystic Keratinizing Epithelioma in the Lung of Vehicle Control Female Sprague-Dawley Rats	144
TABLE A4c	Historical Incidence of Squamous Cell Carcinoma in the Oral Mucosa of Vehicle Control Female Sprague-Dawley Rats	145
TABLE A5a	Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	146
TABLE A5b	Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	152

TABLE A1a
Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Disposition Summary						
Animals initially in study	28	27	28	28	28	20
<i>14-Week interim evaluation</i>	10	10	10	10	10	10
<i>31-Week interim evaluation</i>	10	10	10	10	10	10
<i>53-Week interim evaluation</i>	8	7	8	8	8	0
Animals examined microscopically	28	27	28	28	28	20
<i>14-Week Interim Evaluation</i>						
Urinary System						
Kidney				(1)		
Nephroblastoma				1 (100%)		
<i>Systems Examined at 14 Weeks with No Neoplasms Observed</i>						
Alimentary System						
Cardiovascular System						
Endocrine System						
General Body System						
Genital System						
Hematopoietic System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Respiratory System						
Special Senses System						
<i>31-Week Interim Evaluation</i>						
Genital System						
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Hemangioma						1 (10%)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Cystic keratinizing epithelioma						2 (20%)
Cystic keratinizing epithelioma, multiple						1 (10%)

TABLE A1a

Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Systems Examined at 31 Weeks with No Neoplasms Observed						
Alimentary System						
Cardiovascular System						
Endocrine System						
General Body System						
Hematopoietic System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						
Urinary System						
53-Week Interim Evaluation						
Endocrine System						
Adrenal medulla	(8)		(1)		(8)	
Pheochromocytoma benign			1 (100%)			
Genital System						
Uterus	(8)	(7)	(8)	(8)	(8)	
Polyp stromal, multiple					1 (13%)	
Integumentary System						
Mammary gland	(8)	(2)	(1)	(1)	(8)	
Fibroadenoma		1 (50%)	1 (100%)	1 (100%)	1 (13%)	
Respiratory System						
Lung	(8)	(7)	(8)	(8)	(8)	
Cystic keratinizing epithelioma					4 (50%)	
Cystic keratinizing epithelioma, multiple				1 (13%)	1 (13%)	
Systems Examined at 53 Weeks with No Neoplasms Observed						
Alimentary System						
Cardiovascular System						
General Body System						
Hematopoietic System						
Musculoskeletal System						
Nervous System						
Special Senses System						
Urinary System						

TABLE A1a
Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Neoplasm Summary						
Total animals with primary neoplasms ^b						
14-Week interim evaluation				1		
31-Week interim evaluation						3
53-Week interim evaluation		1	2	2	5	
Total primary neoplasms						
14-Week interim evaluation				1		
31-Week interim evaluation						4
53-Week interim evaluation		1	2	2	7	
Total animals with benign neoplasms						
31-Week interim evaluation						3
53-Week interim evaluation		1	2	2	5	
Total benign neoplasms						
31-Week interim evaluation						4
53-Week interim evaluation		1	2	2	7	
Total animals with malignant neoplasms						
14-Week interim evaluation				1		
Total malignant neoplasms						
14-Week interim evaluation				1		

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Disposition Summary							
Animals initially in study	53	54	53	53	53	66	50
Early deaths							
Accidental deaths				2			
Moribund	21	20	21	23	43	44	28
Natural deaths	5	14	8	13	10	22	12
Survivors							
Terminal sacrifice	27	17	24	15			10
Other		3					
Animals examined microscopically	53	51	53	53	53	66	50
Alimentary System							
Intestine large, colon	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Intestine large, cecum	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Intestine small, duodenum	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Intestine small, jejunum	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Leiomyoma		1 (2%)	1 (2%)				
Intestine small, ileum	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Liver	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Carcinoma, metastatic, pancreas			1 (2%)				
Cholangiocarcinoma			4 (8%)	7 (13%)	7 (13%)	5 (8%)	7 (14%)
Cholangiocarcinoma, multiple			1 (2%)	12 (23%)	21 (40%)	7 (11%)	12 (24%)
Cholangioma				1 (2%)			
Hepatocellular carcinoma					1 (2%)		
Hepatocellular adenoma	2 (4%)	1 (2%)		2 (4%)	7 (13%)	3 (5%)	1 (2%)
Hepatocellular adenoma, multiple				2 (4%)	10 (19%)	2 (3%)	
Hepatocholangioma				1 (2%)		1 (2%)	1 (2%)
Hepatocholangioma, multiple					1 (2%)		
Oral mucosa	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Gingival, squamous cell carcinoma	1 (2%)	1 (2%)	2 (4%)	4 (8%)		1 (2%)	1 (2%)
Pancreas	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Carcinoma			1 (2%)				
Schwannoma malignant, metastatic, uterus		1 (2%)					
Acinus, adenoma			1 (2%)				
Salivary glands	(53)	(51)	(53)	(52)	(52)	(66)	(49)
Schwannoma malignant, metastatic, skin		1 (2%)					
Stomach, forestomach	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Squamous cell carcinoma	1 (2%)	1 (2%)					
Squamous cell papilloma							1 (2%)
Stomach, glandular	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Carcinoma				1 (2%)			
Neuroendocrine tumor, malignant				1 (2%)			

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Cardiovascular System							
Blood vessel	(53)	(51)	(52)	(53)	(53)	(66)	(50)
Heart	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Schwannoma benign				1 (2%)			
Endocrine System							
Adrenal cortex	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Adenoma						1 (2%)	
Carcinoma	1 (2%)						
Subcapsular, adenoma			1 (2%)				
Adrenal medulla	(53)	(51)	(53)	(53)	(53)	(64)	(49)
Pheochromocytoma malignant			1 (2%)				
Pheochromocytoma benign	3 (6%)	3 (6%)	2 (4%)		1 (2%)		
Islets, pancreatic	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Adenoma	1 (2%)						
Carcinoma	1 (2%)						
Pituitary gland	(53)	(51)	(52)	(53)	(53)	(65)	(50)
Pars distalis, adenoma	30 (57%)	11 (22%)	16 (31%)	10 (19%)			
Pars distalis, carcinoma			1 (2%)				
Pars intermedia, adenoma	1 (2%)						
Thyroid gland	(53)	(51)	(53)	(52)	(52)	(66)	(49)
Bilateral, C-cell, adenoma	1 (2%)		1 (2%)				
Bilateral, C-cell, carcinoma	1 (2%)						
C-cell, adenoma	8 (15%)	5 (10%)	7 (13%)	8 (15%)	4 (8%)	1 (2%)	4 (8%)
C-cell, carcinoma	3 (6%)	2 (4%)	2 (4%)				
Follicular cell, adenoma			2 (4%)		2 (4%)	1 (2%)	
Follicular cell, carcinoma	1 (2%)				1 (2%)		
General Body System							
None							
Genital System							
Clitoral gland	(53)	(51)	(51)	(52)	(52)	(66)	(50)
Carcinoma			1 (2%)				
Ovary	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Granulosa cell tumor malignant	1 (2%)						
Luteoma	1 (2%)						
Periovarian tissue, carcinoma, metastatic, pancreas			1 (2%)				

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Genital System (continued)							
Uterus	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Carcinoma	1 (2%)	1 (2%)		1 (2%)			
Carcinoma, metastatic, pancreas			1 (2%)				
Fibrosarcoma				1 (2%)			
Hemangiosarcoma		1 (2%)					
Leiomyoma			1 (2%)				
Polyp stromal	8 (15%)	4 (8%)	6 (11%)	8 (15%)	6 (11%)	3 (5%)	7 (14%)
Polyp stromal, bilateral							1 (2%)
Schwannoma malignant		1 (2%)					
Schwannoma malignant, metastatic, vagina			1 (2%)				
Squamous cell carcinoma				1 (2%)			
Bilateral, polyp stromal	3 (6%)	2 (4%)	1 (2%)		1 (2%)		
Cervix, schwannoma malignant	1 (2%)						
Cervix, schwannoma malignant, metastatic, vagina			1 (2%)				
Vagina			(2)				
Schwannoma malignant			1 (50%)				
Schwannoma malignant, metastatic, skin			1 (50%)				
Hematopoietic System							
Bone marrow	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Lymph node	(28)	(16)	(16)	(25)	(20)	(45)	(22)
Pancreatic, carcinoma, metastatic, stomach, glandular				1 (4%)			
Lymph node, mandibular	(53)	(51)	(53)	(52)	(52)	(66)	(49)
Schwannoma malignant, metastatic, skin		1 (2%)					
Lymph node, mesenteric	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Spleen	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Thymus	(53)	(51)	(51)	(51)	(51)	(59)	(46)
Thymoma benign	1 (2%)						
Integumentary System							
Mammary gland	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Adenolipoma				1 (2%)			
Adenoma	2 (4%)	1 (2%)					
Carcinoma	4 (8%)	1 (2%)	3 (6%)				1 (2%)
Carcinoma, multiple	1 (2%)		1 (2%)				
Fibroadenoma	27 (51%)	23 (45%)	21 (40%)	14 (26%)	4 (8%)	6 (9%)	11 (22%)
Fibroadenoma, multiple	15 (28%)	15 (29%)	15 (28%)	3 (6%)			1 (2%)
Skin	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Basal cell adenoma	1 (2%)						
Fibroma	1 (2%)	1 (2%)	1 (2%)	3 (6%)			
Fibrosarcoma			1 (2%)				
Schwannoma malignant		1 (2%)	1 (2%)				
Squamous cell papilloma	1 (2%)						

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Musculoskeletal System							
Skeletal muscle		(1)	(1)				
Carcinoma, metastatic, pancreas			1 (100%)				
Schwannoma malignant, metastatic, uterus		1 (100%)					
Nervous System							
Brain	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Carcinoma, metastatic, pituitary gland			1 (2%)				
Carcinoma, metastatic, oral mucosa			1 (2%)				
Medulloblastoma malignant	1 (2%)						
Cranial nerve, squamous cell carcinoma, metastatic, oral mucosa							1 (2%)
Cranial nerve, schwannoma malignant		1 (2%)					
Respiratory System							
Lung	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)	1 (2%)				
Carcinoma, metastatic, pancreas			1 (2%)				
Carcinoma, metastatic, thyroid gland	1 (2%)						
Carcinoma, metastatic, uncertain primary site		1 (2%)					
Cystic keratinizing epithelioma				6 (11%)	1 (2%)	6 (9%)	7 (14%)
Cystic keratinizing epithelioma, multiple				14 (26%)	48 (91%)	35 (53%)	5 (10%)
Nose	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Schwannoma malignant, metastatic, brain		1 (2%)					
Special Senses System							
Harderian gland	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Carcinoma, metastatic, oral mucosa			1 (2%)				

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Urinary System							
Kidney	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Carcinoma, metastatic, pancreas			1 (2%)				
Papilloma		1 (2%)					
Renal tubule, adenoma	1 (2%)						
Renal tubule, carcinoma		1 (2%)					
Urinary bladder	(53)	(51)	(53)	(53)	(52)	(66)	(50)
Schwannoma malignant, metastatic, uterus		1 (2%)					
Systemic Lesions							
Multiple organs ^b	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Adenolipoma				1 (2%)			
Leukemia granulocytic						1 (2%)	
Leukemia mononuclear	1 (2%)						
Lymphoma malignant		1 (2%)					1 (2%)
Neoplasm Summary							
Total animals with primary neoplasms ^c	53	47	51	42	51	45	36
Total primary neoplasms	126	80	96	102	115	73	61
Total animals with benign neoplasms	53	43	47	40	51	45	28
Total benign neoplasms	107	68	76	74	85	59	39
Total animals with malignant neoplasms	16	12	18	21	28	14	20
Total malignant neoplasms	19	12	20	27	30	14	22
Total animals with metastatic neoplasms	1	5	6	1			1
Total metastatic neoplasms	1	8	13	1			1
Total animals with malignant neoplasms of uncertain primary site		1					
Total animals with uncertain neoplasms- benign or malignant				1			
Total uncertain neoplasms				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
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118: Vehicle Control

Number of Days on Study	2	3	3	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	7		
	7	1	6	8	3	3	4	5	6	6	8	8	2	2	2	3	3	4	5	6	7	8	8	8	0		
	5	3	2	2	4	4	8	5	4	7	2	6	3	8	8	9	9	9	9	1	5	2	7	7	8	7	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	8	9	6	3	6	9	2	6	3	1	2	0	5	2	9	4	6	5	6	4	2	0	9	4	1		
	8	1	2	1	1	3	7	7	5	4	3	3	4	1	5	3	8	5	5	5	4	2	6	8	2		
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hepatocellular adenoma																											
Mesentery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gingival, squamous cell carcinoma																										X	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Squamous cell carcinoma																											
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Tooth					+	+				+	+			+	+			+	+	+	+		+	+		+	
Cardiovascular System																											
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma																										X	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pheochromocytoma benign																											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																											
Carcinoma																										X	
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pars distalis, adenoma															X	X	X	X		X					X	X	
Pars intermedia, adenoma																										X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bilateral, C-cell, adenoma																										X	
Bilateral, C-cell, carcinoma																										X	
C-cell, adenoma																										X	
C-cell, carcinoma																											
Follicular cell, carcinoma																											
General Body System																											
None																											

+: Tissue examined microscopically
A: Autolysis precludes examination

M: Missing tissue
I: Insufficient tissue

X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118: 360 ng TEQ/kg

Number of Days on Study	4 4 4 4 4 4 4 4 4 5 5 5 5 5 6 6	4 5 5 5 6 7 8 8 9 0 0 1 2 4 1 3	6 2 3 6 1 8 2 2 1 4 4 3 4 5 1 6														
Carcass ID Number	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	8 2 2 5 6 6 5 7 6 1 2 2 0 0 4 4	6 7 3 4 6 4 1 7 7 5 9 8 2 7 9 8														
			Total Tissues/ Tumors														
Alimentary System																	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66	
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Cholangiocarcinoma					X					X	X			X		5	
Cholangiocarcinoma, multiple									X			X	X		X	7	
Hepatocellular adenoma																3	
Hepatocellular adenoma, multiple									X					X		2	
Hepatocholangioma															X	1	
Mesentery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Gingival, squamous cell carcinoma									X								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Tooth	+	+	+		+		+	+	+				+	+	+	+	25
Cardiovascular System																	
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Adenoma					X												1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	62
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
C-cell, adenoma																X	1
Follicular cell, adenoma			X														1
General Body System																	
None																	
Genital System																	
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	66
Polyp stromal														X			3

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118: 360 ng TEQ/kg

Number of Days on Study	4 4 4 4 4 4 4 4 4 5 5 5 5 5 6 6	
	4 5 5 5 6 7 8 8 9 0 0 1 2 4 1 3	
	6 2 3 6 1 8 2 2 1 4 4 3 4 5 1 6	
Carcass ID Number	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Total
	8 2 2 5 6 6 5 7 6 1 2 2 0 0 4 4	Tissues/
	6 7 3 4 6 4 1 7 7 5 9 8 2 7 9 8	Tumors
Hematopoietic System		
Bone marrow	+ + + + + + + + + + + + + + + +	66
Lymph node	M + + + + + M M + + M + + + + +	45
Lymph node, mandibular	+ + + + + + + + + + + + + + + +	66
Lymph node, mesenteric	+ + + + + + + + + + + + + + + +	65
Spleen	+ + + + + + + + + + + + + + + +	65
Thymus	+ M M + + + + + + + + + + + +	59
Integumentary System		
Mammary gland	+ + + + + + + + + + + + + + + +	66
Fibroadenoma		6
Skin	+ + + + + + + + + + + + + + + +	66
Musculoskeletal System		
Bone	+ + + + + + + + + + + + + + + +	66
Nervous System		
Brain	+ + + + + + + + + + + + + + + +	66
Peripheral nerve		1
Spinal cord		1
Respiratory System		
Lung	+ + + + + + + + + + + + + + + +	66
Cystic keratinizing epithelioma		6
Cystic keratinizing epithelioma, multiple	X X X X X X X X X X X X X X X	35
Nose	+ + + + + + + + + + + + + + + +	66
Trachea	+ + + + + + + + + + + + + + + +	66
Special Senses System		
Eye	+ + + + + + + + + + + + + + + +	66
Harderian gland	+ + + + + + + + + + + + + + + +	66
Urinary System		
Kidney	+ + + + + + + + + + + + + + + +	65
Urinary bladder	+ + + + + + + + + + + + + + + +	66
Systemic Lesions		
Multiple organs	+ + + + + + + + + + + + + + + +	66
Leukemia granulocytic		1

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Adrenal Medulla: Benign Pheochromocytoma						
Overall rate ^a	3/53 (6%)	3/51 (6%)	2/53 (4%)	0/53 (0%)	1/53 (2%)	0/64 (0%)
Adjusted rate ^b	7.2%	9.1%	5.3%	0.0%	4.1%	0.0%
Terminal rate ^c	3/27 (11%)	2/17 (12%)	2/24 (8%)	0/15 (0%)	0/0	0/0
First incidence (days)	726 (T)	659	726 (T)	— ^e	520	—
Poly-3 test ^d	P=0.187N	P=0.546	P=0.547N	P=0.143N	P=0.508N	P=0.484N
Adrenal Medulla: Benign or Malignant Pheochromocytoma						
Overall rate	3/53 (6%)	3/51 (6%)	3/53 (6%)	0/53 (0%)	1/53 (2%)	0/64 (0%)
Adjusted rate	7.2%	9.1%	7.8%	0.0%	4.1%	0.0%
Terminal rate	3/27 (11%)	2/17 (12%)	2/24 (8%)	0/15 (0%)	0/0	0/0
First incidence (days)	726 (T)	659	408	—	520	—
Poly-3 test	P=0.165N	P=0.546	P=0.625	P=0.143N	P=0.508N	P=0.484N
Liver: Cholangiocarcinoma						
Overall rate	0/53 (0%)	0/51 (0%)	5/53 (9%)	19/53 (36%)	28/53 (53%)	12/65 (18%)
Adjusted rate	0.0%	0.0%	13.2%	47.9%	80.0%	69.3%
Terminal rate	0/27 (0%)	0/17 (0%)	5/24 (21%)	6/15 (40%)	0/0	0/0
First incidence (days)	—	—	726 (T)	567	450	294
Poly-3 test	P<0.001	— ^f	P=0.022	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Adenoma						
Overall rate	2/53 (4%)	1/51 (2%)	0/53 (0%)	4/53 (8%)	17/53 (32%) ^g	5/65 (8%)
Adjusted rate	4.8%	3.1%	0.0%	10.6%	55.8%	37.9%
Terminal rate	2/27 (7%)	0/17 (0%)	0/24 (0%)	2/15 (13%)	0/0	0/0
First incidence (days)	726 (T)	659	—	541	474	245
Poly-3 test	P<0.001	P=0.584N	P=0.260N	P=0.288	P<0.001	P=0.021
Lung: Cystic Keratinizing Epithelioma						
Overall rate	0/53 (0%)	0/51 (0%)	0/53 (0%)	20/53 (38%)	49/53 (92%)	41/66 (62%)
Adjusted rate	0.0%	0.0%	0.0%	51.4%	97.7%	97.0%
Terminal rate	0/27 (0%)	0/17 (0%)	0/24 (0%)	9/15 (60%)	0/0	0/0
First incidence (days)	—	—	—	594	267	260
Poly-3 test	P<0.001	—	—	P<0.001	P<0.001	P<0.001
Mammary Gland: Fibroadenoma						
Overall rate	42/53 (79%)	38/51 (75%)	36/53 (68%)	17/53 (32%)	4/53 (8%)	6/66 (9%)
Adjusted rate	81.0%	85.1%	75.3%	42.0%	15.3%	41.8%
Terminal rate	19/27 (70%)	13/17 (77%)	16/24 (67%)	7/15 (47%)	0/0	0/0
First incidence (days)	275	347	426	261	479	222
Poly-3 test	P<0.001N	P=0.389	P=0.324N	P<0.001N	P<0.001N	P=0.016N
Mammary Gland: Fibroadenoma or Adenoma						
Overall rate	42/53 (79%)	39/51 (76%)	36/53 (68%)	17/53 (32%)	4/53 (8%)	6/66 (9%)
Adjusted rate	81.0%	86.2%	75.3%	42.0%	15.3%	41.8%
Terminal rate	19/27 (70%)	13/17 (77%)	16/24 (67%)	7/15 (47%)	0/0	0/0
First incidence (days)	275	347	426	261	479	222
Poly-3 test	P<0.001N	P=0.334	P=0.324N	P<0.001N	P<0.001N	P=0.016N

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Mammary Gland: Carcinoma						
Overall rate	5/53 (9%)	1/51 (2%)	4/53 (8%)	0/53 (0%)	0/53 (0%)	0/66 (0%)
Adjusted rate	11.7%	3.0%	10.2%	0.0%	0.0%	0.0%
Terminal rate	4/27 (15%)	0/17 (0%)	2/24 (8%)	0/15 (0%)	0/0	0/0
First incidence (days)	362	275	426	—	—	—
Poly-3 test	P=0.030N	P=0.167N	P=0.556N	P=0.045N	P=0.120N	P=0.382N
Mammary Gland: Adenoma or Carcinoma						
Overall rate	6/53 (11%)	2/51 (4%)	4/53 (8%)	0/53 (0%)	0/53 (0%)	0/66 (0%)
Adjusted rate	13.9%	5.9%	10.2%	0.0%	0.0%	0.0%
Terminal rate	4/27 (15%)	0/17 (0%)	2/24 (8%)	0/15 (0%)	0/0	0/0
First incidence (days)	362	275	426	—	—	—
Poly-3 test	P=0.016N	P=0.222N	P=0.433N	P=0.025N	P=0.085N	P=0.346N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma						
Overall rate	42/53 (79%)	40/51 (78%)	37/53 (70%)	17/53 (32%)	4/53 (8%)	6/66 (9%)
Adjusted rate	81.0%	86.6%	76.5%	42.0%	15.3%	41.8%
Terminal rate	19/27 (70%)	13/17 (77%)	16/24 (67%)	7/15 (47%)	0/0	0/0
First incidence (days)	275	275	426	261	479	222
Poly-3 test	P<0.001N	P=0.312	P=0.377N	P<0.001N	P<0.001N	P=0.016N
Oral Cavity (Gingiva): Squamous Cell Carcinoma						
Overall rate	1/53 (2%)	1/51 (2%)	2/53 (4%)	4/53 (8%)	0/53 (0%)	1/66 (2%)
Adjusted rate	2.4%	3.0%	5.2%	10.6%	0.0%	10.0%
Terminal rate	0/27 (0%)	0/17 (0%)	1/24 (4%)	0/15 (0%)	0/0	0/0
First incidence (days)	687	651	608	594	—	482
Poly-3 test	P=0.511	P=0.703	P=0.467	P=0.146	P=0.605N	P=0.447
Pituitary Gland (Pars Distalis): Adenoma						
Overall rate	30/53 (57%)	11/51 (22%)	16/52 (31%)	10/53 (19%)	0/53 (0%)	0/65 (0%)
Adjusted rate	67.3%	32.2%	39.9%	25.9%	0.0%	0.0%
Terminal rate	21/27 (78%)	6/17 (35%)	10/24 (42%)	4/15 (27%)	0/0	0/0
First incidence (days)	582	541	548	537	—	—
Poly-3 test	P<0.001N	P<0.001N	P=0.007N	P<0.001N	P<0.001N	P=0.008N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma						
Overall rate	30/53 (57%)	11/51 (22%)	17/52 (33%)	10/53 (19%)	0/53 (0%)	0/65 (0%)
Adjusted rate	67.3%	32.2%	41.9%	25.9%	0.0%	0.0%
Terminal rate	21/27 (78%)	6/17 (35%)	10/24 (42%)	4/15 (27%)	0/0	0/0
First incidence (days)	582	541	548	537	—	—
Poly-3 test	P<0.001N	P<0.001N	P=0.011N	P<0.001N	P<0.001N	P=0.008N
Skin: Fibroma						
Overall rate	1/53 (2%)	1/51 (2%)	1/53 (2%)	3/53 (6%)	0/53 (0%)	0/66 (0%)
Adjusted rate	2.4%	3.0%	2.6%	8.1%	0.0%	0.0%
Terminal rate	1/27 (4%)	0/17 (0%)	0/24 (0%)	2/15 (13%)	0/0	0/0
First incidence (days)	726 (T)	639	703	659	—	—
Poly-3 test	P=0.531N	P=0.705	P=0.738	P=0.261	P=0.604N	P=0.696N

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Skin: Fibroma or Fibrosarcoma						
Overall rate	1/53 (2%)	1/51 (2%)	2/53 (4%)	3/53 (6%)	0/53 (0%)	0/66 (0%)
Adjusted rate	2.4%	3.0%	5.3%	8.1%	0.0%	0.0%
Terminal rate	1/27 (4%)	0/17 (0%)	1/24 (4%)	2/15 (13%)	0/0	0/0
First incidence (days)	726 (T)	639	703	659	—	—
Poly-3 test	P=0.467N	P=0.705	P=0.465	P=0.261	P=0.604N	P=0.696N
Thyroid Gland (C-Cell): Adenoma						
Overall rate	9/53 (17%)	5/51 (10%)	8/53 (15%)	8/52 (15%)	4/52 (8%)	1/66 (2%)
Adjusted rate	20.6%	14.7%	20.7%	21.5%	16.2%	10.4%
Terminal rate	4/27 (15%)	1/17 (6%)	5/24 (21%)	5/15 (33%)	0/0	0/0
First incidence (days)	548	611	632	619	639	636
Poly-3 test	P=0.420N	P=0.353N	P=0.605	P=0.571	P=0.452N	P=0.429N
Thyroid Gland (C-Cell): Carcinoma						
Overall rate	4/53 (8%)	2/51 (4%)	2/53 (4%)	0/52 (0%)	0/52 (0%)	0/66 (0%)
Adjusted rate	9.5%	6.1%	5.3%	0.0%	0.0%	0.0%
Terminal rate	3/27 (11%)	2/17 (12%)	2/24 (8%)	0/15 (0%)	0/0	0/0
First incidence (days)	688	726 (T)	726 (T)	—	—	—
Poly-3 test	P=0.044N	P=0.460N	P=0.387N	P=0.080N	P=0.169N	P=0.425N
Thyroid Gland (C-Cell): Adenoma or Carcinoma						
Overall rate	13/53 (25%)	7/51 (14%)	9/53 (17%)	8/52 (15%)	4/52 (8%)	1/66 (2%)
Adjusted rate	29.7%	20.6%	23.3%	21.5%	16.2%	10.4%
Terminal rate	7/27 (26%)	3/17 (18%)	6/24 (25%)	5/15 (33%)	0/0	0/0
First incidence (days)	548	611	632	619	639	636
Poly-3 test	P=0.141N	P=0.256N	P=0.341N	P=0.277N	P=0.181N	P=0.298N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma						
Overall rate	1/53 (2%)	0/51 (0%)	2/53 (4%)	0/52 (0%)	3/52 (6%)	1/66 (2%)
Adjusted rate	2.4%	0.0%	5.3%	0.0%	12.0%	9.9%
Terminal rate	1/27 (4%)	0/17 (0%)	1/24 (4%)	0/15 (0%)	0/0	0/0
First incidence (days)	726 (T)	—	632	—	565	452
Poly-3 test	P=0.053	P=0.550N	P=0.467	P=0.528N	P=0.156	P=0.448
Uterus: Stromal Polyp						
Overall rate	11/53 (21%)	6/51 (12%)	7/53 (13%)	8/53 (15%)	7/53 (13%)	3/66 (5%)
Adjusted rate	24.7%	17.9%	17.5%	21.1%	26.4%	25.5%
Terminal rate	4/27 (15%)	4/17 (24%)	3/24 (13%)	5/15 (33%)	0/0	0/0
First incidence (days)	534	534	399	555	530	300
Poly-3 test	P=0.347	P=0.331N	P=0.294N	P=0.450N	P=0.548	P=0.588
All Organs: Benign Neoplasms						
Overall rate	53/53 (100%)	43/51 (84%)	47/53 (89%)	40/53 (75%)	51/53 (96%)	45/66 (68%)
Adjusted rate	100.0%	94.2%	93.7%	87.2%	99.7%	98.0%
Terminal rate	27/27 (100%)	15/17 (88%)	22/24 (92%)	13/15 (87%)	0/0	0/0
First incidence (days)	275	347	399	261	267	222
Poly-3 test	P=0.136	P=0.099N	P=0.074N	P=0.003N	P=1.000N	P=0.542N

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
All Organs: Malignant Neoplasms						
Overall rate	16/53 (30%)	13/51 (25%)	18/53 (34%)	22/53 (42%)	28/53 (53%)	14/66 (21%)
Adjusted rate	36.3%	34.0%	43.1%	53.8%	80.0%	74.0%
Terminal rate	10/27 (37%)	3/17 (18%)	11/24 (46%)	6/15 (40%)	0/0	0/0
First incidence (days)	362	275	408	567	450	294
Poly-3 test	P<0.001	P=0.505N	P=0.330	P=0.071	P<0.001	P=0.014
All Organs: Benign or Malignant Neoplasms						
Overall rate	53/53 (100%)	47/51 (92%)	51/53 (96%)	42/53 (79%)	51/53 (96%)	45/66 (68%)
Adjusted rate	100.0%	97.6%	98.1%	90.5%	99.7%	98.0%
Terminal rate	27/27 (100%)	16/17 (94%)	23/24 (96%)	13/15 (87%)	0/0	0/0
First incidence (days)	275	275	399	261	267	222
Poly-3 test	P=0.472	P=0.424N	P=0.496N	P=0.013N	P=1.000N	P=0.542N

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, 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 the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in dose group is indicated by N.

^e Not applicable; no neoplasms in animal group

^f Value of statistic cannot be computed.

^g One hepatocellular carcinoma occurred in a rat that also had a hepatocellular adenoma

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Liver: Hepatocellular Adenoma			
Overall rate ^a	2/53 (4%)	5/65 (8%)	1/50 (2%)
Adjusted rate ^b	4.8%	37.9%	4.6%
Terminal rate ^c	2/27 (7%)	0/0	0/10 (0%)
First incidence (days)	726 (T)	245	451
Poly-3 test ^d		P=0.088	P=0.805N
Poly-3 test ^e			P=0.038N
Liver: Cholangiocarcinoma			
Overall rate	0/53 (0%)	12/65 (18%)	19/50 (38%)
Adjusted rate	0.0%	69.3%	75.4%
Terminal rate	0/27 (0%)	0/0	8/10 (80%)
First incidence (days)	— ^f	294	303
Poly-3 test		P<0.001	P<0.001
Poly-3 test			P=0.516
Lung: Cystic Keratinizing Epithelioma			
Overall rate	0/53 (0%)	41/66 (62%)	12/50 (24%)
Adjusted rate	0.0%	97.0%	42.5%
Terminal rate	0/27 (0%)	0/0	3/10 (30%)
First incidence (days)	—	260	240
Poly-3 test		P<0.001	P<0.001
Poly-3 test			P<0.001
Mammary Gland: Fibroadenoma			
Overall rate	42/53 (79%)	6/66 (9%)	12/50 (24%)
Adjusted rate	81.0%	41.8%	50.6%
Terminal rate	19/27 (70%)	0/0	5/10 (50%)
First incidence (days)	275	222	504
Poly-3 test		P=0.066N	P=0.015N
Poly-3 test			P=0.491
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	30/53 (57%)	0/65 (0%)	0/50 (0%)
Adjusted rate	67.3%	0.0%	0.0%
Terminal rate	21/27 (78%)	0/0	0/10 (0%)
First incidence (days)	582	—	—
Poly-3 test		P=0.067N	P<0.001N
Poly-3 test			— ^g
Thyroid Gland (C-Cell): Adenoma			
Overall rate	9/53 (17%)	1/66 (2%)	4/49 (8%)
Adjusted rate	20.6%	10.4%	19.3%
Terminal rate	4/27 (15%)	0/0	3/9 (33%)
First incidence (days)	548	636	474
Poly-3 test		P=0.776N	P=0.653N
Poly-3 test			P=0.640

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Thyroid Gland (C-Cell): Adenoma or Carcinoma			
Overall rate	13/53 (25%)	1/66 (2%)	4/49 (8%)
Adjusted rate	29.7%	10.4%	19.3%
Terminal rate	7/27 (26%)	0/0	3/9 (33%)
First incidence (days)	548	636	474
Poly-3 test		P=0.624N	P=0.367N
Poly-3 test			P=0.640
Uterus: Stromal Polyp			
Overall rate	11/53 (21%)	3/66 (5%)	8/50 (16%)
Adjusted rate	24.7%	25.5%	33.9%
Terminal rate	4/27 (15%)	0/0	2/10 (20%)
First incidence (days)	534	300	541
Poly-3 test		P=0.809	P=0.368
Poly-3 test			P=0.571
All Organs: Benign Neoplasms			
Overall rate	53/53 (100%)	45/66 (68%)	28/50 (56%)
Adjusted rate	100.0%	98.0%	84.3%
Terminal rate	27/27 (100%)	0/0	9/10 (90%)
First incidence (days)	275	222	240
Poly-3 test		P=0.700N	P=0.002N
Poly-3 test			P=0.006
All Organs: Malignant Neoplasms			
Overall rate	16/53 (30%)	14/66 (21%)	20/50 (40%)
Adjusted rate	36.3%	74.1%	76.4%
Terminal rate	10/27 (37%)	0/0	8/10 (80%)
First incidence (days)	362	294	209
Poly-3 test		P=0.026	P<0.001
Poly-3 test			P=0.635
All Organs: Benign or Malignant Neoplasms			
Overall rate	53/53 (100%)	45/66 (68%)	36/50 (72%)
Adjusted rate	100.0%	98.0%	97.8%
Terminal rate	27/27 (100%)	0/0	10/10 (100%)
First incidence (days)	275	222	209
Poly-3 test		P=0.700N	P=0.793N
Poly-3 test			P=0.961

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, 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 dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group.

^e The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by N.

^f Pairwise comparison between the 360 ng TEQ/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by N.

^f Not applicable; no neoplasms in animal group

^g Value of statistic cannot be computed.

TABLE A4a
Historical Incidence of Liver Neoplasms in Vehicle Control Female Sprague-Dawley Rats^a

Study	Incidence in Controls				
	Hepatocholangioma	Cholangioma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Cholangiocarcinoma
PCB 126	0/53	0/53	1/53	0/53	0/53
TCDD	0/53	0/53	0/53	0/53	0/53
PeCDF	0/53	0/53	1/53	0/53	0/53
TEF Dioxin Mixture	0/53	0/53	0/53	0/53	0/53
PCB 153	0/53	0/53	0/53	0/53	0/53
Binary Mixture PCB 126/PCB 153	0/53	0/53	0/53	0/53	0/53
Binary Mixture PCB 126/PCB 118	0/53	0/53	2/53	0/53	0/53
Overall Historical Incidence					
Total (%)	0/371	0/371	4/371 (1.1%)	0/371	0/371
Mean ± standard deviation			1.1% ± 1.5%		
Range			0%-4%		

^a Data as of February 27, 2005

TABLE A4b
Historical Incidence of Cystic Keratinizing Epithelioma in the Lung of Vehicle Control Female Sprague-Dawley Rats^a

Study	Incidence in Controls
	PCB 126
TCDD	0/53
PeCDF	0/53
TEF Dioxin Mixture	0/53
PCB 153	0/53
Binary Mixture PCB 126/PCB 153	0/53
Binary Mixture PCB 126/PCB 118	0/53
Overall Historical Incidence	
Total	0/371

^a Data as of February 27, 2005

TABLE A4c
Historical Incidence of Squamous Cell Carcinoma in the Oral Mucosa of Vehicle Control Female Sprague-Dawley Rats^a

Study	Incidence in Controls
PCB 126	0/53
TCDD	1/53
PeCDF	1/53
TEF Dioxin Mixture	1/53
PCB 153	0/53
Binary Mixture PCB 126/PCB 153	0/53
PCB Mixture PCB 126/PCB 118	1/53
Overall Historical Incidence	
Total (%)	4/371 (1.1%)
Mean ± standard deviation	1.1% ± 1.0%
Range	0%-2%

^a Data as of February 27, 2005

TABLE A5a

Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Disposition Summary						
Animals initially in study	28	27	28	28	28	20
<i>14-Week interim evaluation</i>	10	10	10	10	10	10
<i>31-Week interim evaluation</i>	10	10	10	10	10	10
<i>53-Week interim evaluation</i>	8	7	8	8	8	0
Animals examined microscopically	28	27	28	28	28	20
14-Week Interim Evaluation						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Clear cell focus, multiple		1 (10%)				
Eosinophilic focus			1 (10%)	1 (10%)	1 (10%)	
Fatty change, diffuse					5 (50%)	10 (100%)
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus		1 (10%)	1 (10%)	1 (10%)	2 (20%)	
Necrosis		1 (10%)	1 (10%)	1 (10%)	3 (30%)	1 (10%)
Pigmentation			9 (90%)	10 (100%)	8 (80%)	9 (90%)
Toxic hepatopathy				1 (10%)	4 (40%)	8 (80%)
Centrilobular, degeneration						1 (10%)
Centrilobular, fibrosis						1 (10%)
Hepatocyte, hypertrophy		3 (30%)	3 (30%)	8 (80%)	10 (100%)	10 (100%)
Hepatocyte, multinucleated					9 (90%)	10 (100%)
Stomach, glandular	(10)					(10)
Atypia cellular, focal						1 (10%)
Cyst, squamous						1 (10%)
Endocrine System						
Adrenal cortex	(10)	(10)	(10)	(10)	(10)	(10)
Hypertrophy					2 (20%)	
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy	1 (10%)	1 (10%)	5 (50%)	6 (60%)	8 (80%)	7 (70%)
Genital System						
Uterus	(10)					(10)
Endometrium, hyperplasia, cystic	1 (10%)					
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	10 (100%)					10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy		3 (30%)	1 (10%)	3 (30%)	7 (70%)	9 (90%)

^a Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A5a

Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Musculoskeletal System						
Skeletal Muscle			(1)			
Hemorrhage			1 (100%)			
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage	2 (20%)				3 (30%)	2 (20%)
Infiltration cellular, histiocyte	1 (10%)	6 (60%)	7 (70%)	4 (40%)	3 (30%)	4 (40%)
Inflammation	1 (10%)	6 (60%)	7 (70%)	4 (40%)	3 (30%)	6 (60%)
Alveolar epithelium, hyperplasia			1 (10%)		1 (10%)	2 (20%)
Alveolar epithelium, metaplasia, bronchiolar						1 (10%)
Systems Examined at 14 Weeks with No Nonneoplastic Lesions Observed						
Cardiovascular System						
General Body System						
Integumentary System						
Nervous System						
Special Senses System						
Urinary System						
31-Week Interim Evaluation						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus	1 (10%)					
Cholangiofibrosis	1 (10%)			2 (20%)	2 (20%)	1 (10%)
Eosinophilic focus					3 (30%)	2 (20%)
Eosinophilic focus, multiple					4 (40%)	3 (30%)
Fatty change, diffuse				3 (30%)	8 (80%)	9 (90%)
Hepatodiaphragmatic nodule	1 (10%)			1 (10%)		
Hyperplasia, nodular					1 (10%)	4 (40%)
Infarct					1 (10%)	
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus	3 (30%)	5 (50%)	1 (10%)			
Mixed cell focus, multiple	2 (20%)	3 (30%)	1 (10%)	6 (60%)		
Necrosis	1 (10%)	1 (10%)	1 (10%)		2 (20%)	
Pigmentation	2 (20%)	4 (40%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Toxic hepatopathy				3 (30%)	10 (100%)	10 (100%)
Bile duct, fibrosis	1 (10%)			1 (10%)		
Bile duct, hyperplasia				1 (10%)	10 (100%)	10 (100%)
Centrilobular, fibrosis				3 (30%)	10 (100%)	10 (100%)
Hepatocyte, hypertrophy		2 (20%)	4 (40%)	8 (80%)	10 (100%)	10 (100%)
Hepatocyte, multinucleated				3 (30%)	10 (100%)	10 (100%)
Oval cell, hyperplasia					8 (80%)	10 (100%)
Portal, fibrosis					1 (10%)	4 (40%)

TABLE A5a

Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Alimentary System (continued)						
Pancreas	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active						2 (20%)
Acinus, atrophy					1 (10%)	2 (20%)
Acinus, vacuolization cytoplasmic				1 (10%)	10 (100%)	10 (100%)
Stomach, forestomach	(10)		(1)			(10)
Serosa, inflammation, chronic active			1 (100%)			
Endocrine System						
Adrenal Cortex	(10)	(10)	(10)	(10)	(10)	(10)
Angiectasis		6 (60%)	2 (20%)		1 (10%)	1 (10%)
Atrophy					2 (20%)	8 (80%)
Degeneration, cystic		1 (10%)				
Hypertrophy	5 (50%)	4 (40%)	3 (30%)	3 (30%)	3 (30%)	1 (10%)
Pituitary gland	(10)	(1)				(10)
Angiectasis		1 (100%)				
Cyst	1 (10%)					1 (10%)
Cytoplasmic alteration						1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active						1 (10%)
Follicular cell, hypertrophy	3 (30%)	3 (30%)	7 (70%)	7 (70%)	9 (90%)	7 (70%)
Genital System						
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy	6 (60%)	7 (70%)	7 (70%)	4 (40%)	1 (10%)	
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Decidual reaction			1 (10%)			
Inflammation, suppurative	1 (10%)					
Metaplasia, squamous	5 (50%)	6 (60%)	6 (60%)	4 (40%)	1 (10%)	
Endometrium, hyperplasia, cystic	5 (50%)	5 (50%)	6 (60%)	3 (30%)	1 (10%)	1 (10%)
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	10 (100%)					10 (100%)
Thymus	(10)	(10)	(10)	(9)	(10)	(6)
Atrophy	3 (30%)	2 (20%)	5 (50%)	9 (100%)	9 (90%)	6 (100%)
Cyst				1 (11%)		
Integumentary System						
Mammary gland	(10)					(10)
Hyperplasia	2 (20%)					

TABLE A5a

Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage	2 (20%)			1 (10%)	1 (10%)	
Infiltration cellular, histiocyte	3 (30%)	2 (20%)	4 (40%)	2 (20%)	5 (50%)	2 (20%)
Inflammation		1 (10%)				
Inflammation, granulomatous				1 (10%)	2 (20%)	2 (20%)
Metaplasia, squamous						1 (10%)
Alveolar epithelium, hyperplasia						1 (10%)
Alveolar epithelium, metaplasia, bronchiolar					3 (30%)	3 (30%)
Interstitium, fibrosis, focal					1 (10%)	
Systems Examined at 31 Weeks with No Nonneoplastic Lesions Observed						
Cardiovascular System						
General Body System						
Musculoskeletal System						
Nervous System						
Special Senses System						
Urinary System						
53-Week Interim Evaluation						
Alimentary System						
Liver	(8)	(7)	(8)	(8)	(8)	
Angiectasis					1 (13%)	
Basophilic focus	2 (25%)	3 (43%)	1 (13%)	1 (13%)	1 (13%)	
Cholangiofibrosis				2 (25%)	5 (63%)	
Clear cell focus, multiple			1 (13%)			
Eosinophilic focus	1 (13%)		1 (13%)			
Fatty change, diffuse				4 (50%)	8 (100%)	
Fatty change, focal	1 (13%)					
Hepatodiaphragmatic nodule				1 (13%)		
Hyperplasia, nodular				1 (13%)	8 (100%)	
Inflammation	7 (88%)	7 (100%)	8 (100%)	8 (100%)	8 (100%)	
Mixed cell focus	2 (25%)	2 (29%)	2 (25%)			
Mixed cell focus, multiple	5 (63%)	3 (43%)	5 (63%)	7 (88%)		
Necrosis	1 (13%)	1 (14%)	3 (38%)	2 (25%)	2 (25%)	
Pigmentation		5 (71%)	8 (100%)	8 (100%)	8 (100%)	
Toxic hepatopathy			2 (25%)	8 (100%)	8 (100%)	
Bile duct, cyst					1 (13%)	
Bile duct, fibrosis					1 (13%)	
Bile duct, hyperplasia			1 (13%)	1 (13%)	8 (100%)	
Centrilobular, fibrosis				5 (63%)	8 (100%)	
Hepatocyte, hypertrophy		1 (14%)	2 (25%)	8 (100%)	8 (100%)	
Hepatocyte, multinucleated			3 (38%)	8 (100%)	8 (100%)	
Oval cell, hyperplasia					8 (100%)	
Portal, fibrosis					8 (100%)	

TABLE A5a

Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Alimentary System (continued)						
Pancreas	(8)	(7)	(8)	(8)	(8)	
Inflammation, chronic active			1 (13%)	2 (25%)	1 (13%)	
Acinus, atrophy		1 (14%)	1 (13%)	1 (13%)	1 (13%)	
Acinus, vacuolization cytoplasmic				6 (75%)	8 (100%)	
Stomach, forestomach	(8)				(8)	
Edema					1 (13%)	
Stomach, glandular	(8)				(8)	
Erosion					2 (25%)	
Endocrine System						
Adrenal cortex	(8)	(7)	(8)	(8)	(8)	
Angiectasis		2 (29%)	3 (38%)	4 (50%)	1 (13%)	
Atrophy					5 (63%)	
Degeneration, cystic			1 (13%)	2 (25%)		
Hyperplasia			1 (13%)	1 (13%)	1 (13%)	
Hypertrophy	5 (63%)	3 (43%)	6 (75%)	7 (88%)	5 (63%)	
Vacuolization cytoplasmic	1 (13%)	1 (14%)	1 (13%)	1 (13%)		
Pituitary gland	(8)				(8)	
Cyst					1 (13%)	
Thyroid gland	(8)	(7)	(8)	(8)	(8)	
C-cell, hyperplasia	1 (13%)				1 (13%)	
Follicular cell, hypertrophy	3 (38%)	4 (57%)	6 (75%)	7 (88%)	7 (88%)	
Genital System						
Ovary	(8)	(7)	(8)	(8)	(8)	
Atrophy	7 (88%)	6 (86%)	6 (75%)	5 (63%)		
Cyst		1 (14%)				
Uterus	(8)	(7)	(8)	(8)	(8)	
Inflammation, suppurative			2 (25%)			
Metaplasia, squamous	6 (75%)	5 (71%)	5 (63%)	5 (63%)		
Endometrium, hyperplasia, cystic	6 (75%)	6 (86%)	5 (63%)	5 (63%)		
Hematopoietic System						
Spleen	(8)				(8)	
Hematopoietic cell proliferation	1 (13%)					
Pigmentation	8 (100%)				8 (100%)	
Thymus	(8)	(7)	(8)	(8)	(6)	
Atrophy	5 (63%)	7 (100%)	7 (88%)	8 (100%)	6 (100%)	
Integumentary System						
Mammary gland	(8)	(2)	(1)	(1)	(8)	
Cyst	1 (13%)					
Hyperplasia	1 (13%)					

TABLE A5a

Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
Respiratory System						
Lung	(8)	(7)	(8)	(8)	(8)	
Infiltration cellular, histiocyte	7 (88%)	5 (71%)	7 (88%)	6 (75%)	8 (100%)	
Inflammation				1 (13%)		
Pigmentation				1 (13%)		
Alveolar epithelium, hyperplasia				1 (13%)		
Alveolar epithelium, metaplasia, bronchiolar			3 (38%)	3 (38%)	6 (75%)	
Interstitialium, fibrosis				1 (13%)		

Systems Examined at 53 Weeks with No Nonneoplastic Lesions Observed

Cardiovascular System

General Body System

Musculoskeletal System

Nervous System

Special Senses System

Urinary System

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Disposition Summary							
Animals initially in study	53	54	53	53	53	66	50
Early deaths							
Accidental deaths				2			
Moribund	21	20	21	23	43	44	28
Natural deaths	5	14	8	13	10	22	12
Survivors							
Terminal sacrifice	27	17	24	15			10
Other		3					
Animals examined microscopically	53	51	53	53	53	66	50
Alimentary System							
Esophagus	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Degeneration		1 (2%)					
Muscularis, inflammation	3 (6%)		2 (4%)	3 (6%)	3 (6%)	2 (3%)	
Intestine large, colon	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Parasite metazoan	2 (4%)	1 (2%)				2 (3%)	1 (2%)
Artery, inflammation, chronic active			1 (2%)	1 (2%)			
Intestine large, rectum	(52)	(51)	(53)	(53)	(52)	(66)	(50)
Hyperplasia, cystic						1 (2%)	
Parasite metazoan	3 (6%)	2 (4%)	2 (4%)	2 (4%)	1 (2%)		1 (2%)
Artery, inflammation, chronic active		1 (2%)	1 (2%)	3 (6%)	1 (2%)		4 (8%)
Intestine large, cecum	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Edema						2 (3%)	1 (2%)
Inflammation						2 (3%)	1 (2%)
Artery, inflammation, chronic active				2 (4%)			1 (2%)
Intestine small, duodenum	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Dilatation				1 (2%)			
Erosion				1 (2%)			
Hyperplasia				1 (2%)			
Artery, inflammation, chronic active			1 (2%)				
Intestine small, ileum	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Parasite metazoan					1 (2%)		1 (2%)
Liver	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Angiectasis		1 (2%)	2 (4%)	1 (2%)	3 (6%)	2 (3%)	4 (8%)
Basophilic focus	14 (26%)	8 (16%)	6 (11%)	5 (9%)	4 (8%)		2 (4%)
Basophilic focus, multiple	13 (25%)	8 (16%)	4 (8%)	3 (6%)			3 (6%)
Cholangiofibrosis	1 (2%)	2 (4%)	3 (6%)	21 (40%)	42 (79%)	29 (45%)	27 (54%)
Clear cell focus	4 (8%)	1 (2%)					1 (2%)
Clear cell focus, multiple	1 (2%)			1 (2%)			
Degeneration, cystic			1 (2%)		1 (2%)		1 (2%)
Eosinophilic focus	5 (9%)	5 (10%)	11 (21%)	1 (2%)	2 (4%)		1 (2%)
Eosinophilic focus, multiple	5 (9%)	16 (31%)	12 (23%)	14 (26%)	4 (8%)	10 (15%)	14 (28%)
Fatty change, diffuse	3 (6%)	14 (27%)	27 (51%)	45 (85%)	49 (92%)	56 (86%)	35 (70%)

^a Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Alimentary System (continued)							
Liver (continued)	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Fatty change, focal	10 (19%)	10 (20%)	6 (11%)	1 (2%)	10 (19%)	6 (9%)	14 (28%)
Hematopoietic cell proliferation	27 (51%)	27 (53%)	30 (57%)	32 (60%)	22 (42%)	11 (17%)	22 (44%)
Hemorrhage							1 (2%)
Hepatodiaphragmatic nodule	2 (4%)	2 (4%)		2 (4%)			1 (2%)
Hyperplasia, nodular			10 (19%)	45 (85%)	50 (94%)	61 (94%)	34 (68%)
Inflammation	49 (92%)	47 (92%)	50 (94%)	52 (98%)	51 (96%)	64 (98%)	48 (96%)
Metaplasia					4 (8%)	4 (6%)	2 (4%)
Mineralization						1 (2%)	
Mixed cell focus	5 (9%)	5 (10%)	4 (8%)		1 (2%)	3 (5%)	1 (2%)
Mixed cell focus, multiple	33 (62%)	23 (45%)	28 (53%)	16 (30%)	1 (2%)		19 (38%)
Necrosis	3 (6%)	13 (25%)	6 (11%)	26 (49%)	17 (32%)	27 (42%)	6 (12%)
Pigmentation	5 (9%)	18 (35%)	38 (72%)	52 (98%)	53 (100%)	60 (92%)	50 (100%)
Thrombosis						1 (2%)	
Toxic hepatopathy		8 (16%)	32 (60%)	51 (96%)	52 (98%)	64 (98%)	50 (100%)
Artery, inflammation		1 (2%)		1 (2%)			
Bile duct, cyst	4 (8%)	3 (6%)	3 (6%)	14 (26%)	25 (47%)	14 (22%)	21 (42%)
Bile duct, dilatation						1 (2%)	
Bile duct, fibrosis	6 (11%)	2 (4%)	3 (6%)	10 (19%)	3 (6%)	4 (6%)	12 (24%)
Bile duct, hyperplasia	5 (9%)	5 (10%)	7 (13%)	46 (87%)	52 (98%)	60 (92%)	37 (74%)
Centrilobular, degeneration	2 (4%)	3 (6%)	8 (15%)	8 (15%)	3 (6%)	8 (12%)	6 (12%)
Centrilobular, fibrosis			7 (13%)	36 (68%)	52 (98%)	57 (88%)	43 (86%)
Hepatocyte, glandular structures				1 (2%)	5 (9%)	25 (38%)	9 (18%)
Hepatocyte, hypertrophy		16 (31%)	22 (42%)	47 (89%)	52 (98%)	65 (100%)	36 (72%)
Hepatocyte, multinucleated		9 (18%)	21 (40%)	48 (91%)	52 (98%)	48 (74%)	38 (76%)
Oval cell, hyperplasia	4 (8%)	4 (8%)	23 (43%)	49 (92%)	52 (98%)	62 (95%)	43 (86%)
Portal, fibrosis			2 (4%)	37 (70%)	52 (98%)	58 (89%)	42 (84%)
Serosa, inflammation		2 (4%)					1 (2%)
Mesentery	(53)	(35)	(45)	(49)	(52)	(63)	(43)
Inflammation		1 (3%)					
Necrosis			1 (2%)	1 (2%)			1 (2%)
Artery, inflammation, chronic active		1 (3%)	1 (2%)	9 (18%)	9 (17%)	3 (5%)	7 (16%)
Fat, necrosis	1 (2%)			3 (6%)		3 (5%)	
Oral mucosa	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Cyst						1 (2%)	
Gingival, hyperplasia, squamous	11 (21%)	10 (20%)	20 (38%)	24 (45%)	27 (51%)	18 (27%)	18 (36%)
Pancreas	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Edema			1 (2%)		4 (8%)	19 (29%)	12 (24%)
Hemorrhage						2 (3%)	1 (2%)
Inflammation, chronic active	1 (2%)	5 (10%)	4 (8%)	3 (6%)	2 (4%)	6 (9%)	5 (10%)
Lipomatosis		1 (2%)	1 (2%)				
Acinus, atrophy	1 (2%)	5 (10%)	3 (6%)	5 (9%)	9 (17%)	8 (12%)	8 (16%)
Acinus, hyperplasia	2 (4%)						
Acinus, hyperplasia, focal	1 (2%)						
Acinus, vacuolization cytoplasmic		1 (2%)	8 (15%)	39 (74%)	49 (92%)	43 (66%)	41 (82%)
Artery, inflammation, chronic active		2 (4%)	2 (4%)	21 (40%)	14 (26%)	4 (6%)	10 (20%)
Duct, dilatation				1 (2%)	3 (6%)		
Salivary glands	(53)	(51)	(53)	(52)	(52)	(66)	(49)
Atrophy	1 (2%)						

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Alimentary System (continued)							
Stomach, forestomach	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Edema				2 (4%)	1 (2%)		1 (2%)
Erosion		1 (2%)		1 (2%)			
Hemorrhage						1 (2%)	
Hyperplasia, squamous	4 (8%)	1 (2%)	3 (6%)	11 (21%)	4 (8%)	9 (14%)	2 (4%)
Inflammation	2 (4%)		2 (4%)	4 (8%)		1 (2%)	2 (4%)
Mineralization				1 (2%)		2 (3%)	1 (2%)
Ulcer	2 (4%)		1 (2%)	1 (2%)		1 (2%)	2 (4%)
Artery, inflammation, chronic active				2 (4%)			
Stomach, glandular	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Cyst					1 (2%)		
Erosion		1 (2%)			1 (2%)	2 (3%)	
Hemorrhage					1 (2%)	3 (5%)	
Inflammation						1 (2%)	
Mineralization	12 (23%)	6 (12%)	6 (11%)	1 (2%)	6 (11%)	5 (8%)	1 (2%)
Glands, cyst						1 (2%)	
Artery, inflammation, chronic active							1 (2%)
Tongue			(1)				
Cyst			1 (100%)				
Tooth	(29)	(26)	(33)	(34)	(29)	(25)	(23)
Peridental tissue, inflammation	29 (100%)	26 (100%)	33 (100%)	34 (100%)	29 (100%)	25 (100%)	23 (100%)
Cardiovascular System							
Blood vessel	(53)	(51)	(52)	(53)	(53)	(66)	(50)
Mineralization	1 (2%)						
Aorta, adventitia, hemorrhage					1 (2%)	1 (2%)	2 (4%)
Aorta, adventitia, inflammation					1 (2%)	2 (3%)	2 (4%)
Heart	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Cardiomyopathy	20 (38%)	13 (25%)	17 (32%)	34 (64%)	14 (26%)	19 (29%)	15 (30%)
Atrium, thrombosis						1 (2%)	1 (2%)
Coronary artery, inflammation, chronic active	1 (2%)	1 (2%)		7 (13%)	10 (19%)	9 (14%)	6 (12%)
Coronary artery, thrombosis					1 (2%)		
Endocardium, hyperplasia				1 (2%)			
Epicardium, hemorrhage						1 (2%)	
Epicardium, inflammation	1 (2%)	1 (2%)		6 (11%)	13 (25%)	6 (9%)	1 (2%)
Myocardium, hemorrhage						3 (5%)	1 (2%)
Myocardium, inflammation						1 (2%)	
Myocardium, necrosis						2 (3%)	
Pericardium, inflammation, chronic active		1 (2%)					
Valve, fibrosis					1 (2%)		
Valve, hyperplasia			1 (2%)				
Ventricle, thrombosis						1 (2%)	

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Endocrine System							
Adrenal cortex	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Angiectasis	42 (79%)	35 (69%)	39 (74%)	25 (47%)	4 (8%)	6 (9%)	21 (42%)
Atrophy	2 (4%)	1 (2%)		15 (28%)	44 (83%)	40 (62%)	12 (24%)
Degeneration, cystic	12 (23%)	12 (24%)	13 (25%)	11 (21%)	6 (11%)	2 (3%)	9 (18%)
Hematopoietic cell proliferation	1 (2%)						
Hyperplasia	13 (25%)	7 (14%)	13 (25%)	12 (23%)	10 (19%)	5 (8%)	10 (20%)
Hypertrophy	45 (85%)	40 (78%)	47 (89%)	44 (83%)	45 (85%)	27 (42%)	29 (58%)
Inflammation				1 (2%)			2 (4%)
Necrosis	1 (2%)		2 (4%)	4 (8%)	1 (2%)	4 (6%)	2 (4%)
Vacuolization cytoplasmic	14 (26%)	16 (31%)	13 (25%)	24 (45%)	27 (51%)	12 (18%)	16 (32%)
Adrenal medulla	(53)	(51)	(53)	(53)	(53)	(64)	(49)
Hyperplasia	13 (25%)	4 (8%)	9 (17%)	12 (23%)	2 (4%)		8 (16%)
Islets, pancreatic	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Hyperplasia			1 (2%)				
Pituitary gland	(53)	(51)	(52)	(53)	(53)	(65)	(50)
Angiectasis	23 (43%)	13 (25%)	19 (37%)	7 (13%)			3 (6%)
Cyst	1 (2%)						3 (6%)
Cytoplasmic alteration		1 (2%)	1 (2%)				2 (4%)
Necrosis						1 (2%)	
Vacuolization cytoplasmic	2 (4%)	1 (2%)		5 (9%)			
Pars distalis, hyperplasia	11 (21%)	14 (27%)	19 (37%)	18 (34%)	4 (8%)	1 (2%)	8 (16%)
Pars intermedia, hyperplasia							1 (2%)
Thyroid gland	(53)	(51)	(53)	(52)	(52)	(66)	(49)
Hemorrhage						1 (2%)	
Infiltration cellular, lymphoid							2 (4%)
Inflammation					1 (2%)		
Artery, inflammation, chronic active					1 (2%)		
C-cell, hyperplasia	7 (13%)	11 (22%)	13 (25%)	10 (19%)	15 (29%)	3 (5%)	6 (12%)
Follicle, cyst	1 (2%)						
Follicular cell, hyperplasia					2 (4%)	1 (2%)	
Follicular cell, hypertrophy	22 (42%)	35 (69%)	34 (64%)	38 (73%)	44 (85%)	37 (56%)	29 (59%)
General Body System							
None							
Genital System							
Clitoral gland	(53)	(51)	(51)	(52)	(52)	(66)	(50)
Inflammation	49 (92%)	44 (86%)	46 (90%)	34 (65%)	21 (40%)	31 (47%)	30 (60%)
Artery, inflammation, chronic active				1 (2%)			
Duct, cyst	40 (75%)	41 (80%)	43 (84%)	45 (87%)	48 (92%)	61 (92%)	40 (80%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Genital System (continued)							
Ovary	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Atrophy	46 (87%)	34 (67%)	43 (81%)	42 (79%)	7 (13%)	9 (14%)	15 (30%)
Cyst	19 (36%)	12 (24%)	15 (28%)	19 (36%)	8 (15%)	13 (20%)	7 (14%)
Inflammation			3 (6%)	3 (6%)			
Inflammation, chronic active		1 (2%)	3 (6%)	3 (6%)			
Artery, inflammation				1 (2%)			
Periovarian tissue, inflammation, chronic active		1 (2%)					
Oviduct					(1)		
Cyst					1 (100%)		
Inflammation					1 (100%)		
Uterus	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Adenomyosis		1 (2%)		3 (6%)		1 (2%)	
Congestion	1 (2%)						1 (2%)
Hemorrhage				2 (4%)		1 (2%)	1 (2%)
Hyperplasia, cystic	1 (2%)						
Inflammation, chronic active	2 (4%)	5 (10%)	2 (4%)	5 (9%)			1 (2%)
Inflammation, suppurative	4 (8%)	3 (6%)	5 (9%)	7 (13%)		2 (3%)	4 (8%)
Metaplasia, squamous	23 (43%)	24 (47%)	28 (53%)	24 (45%)			8 (16%)
Thrombosis						1 (2%)	
Cervix, stromal hyperplasia							1 (2%)
Endometrium, hyperplasia, cystic	32 (60%)	23 (45%)	25 (47%)	30 (57%)	7 (13%)	8 (12%)	17 (34%)
Serosa, inflammation, chronic active		1 (2%)					
Hematopoietic System							
Bone marrow	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Atrophy					2 (4%)	12 (18%)	4 (8%)
Hyperplasia	37 (70%)	41 (80%)	36 (68%)	48 (91%)	47 (89%)	49 (74%)	40 (80%)
Lymph node	(28)	(16)	(16)	(25)	(20)	(45)	(22)
Hemorrhage				1 (4%)			
Deep cervical, congestion				1 (4%)			
Deep cervical, ectasia				1 (4%)			
Deep cervical, hemorrhage				1 (4%)			
Deep cervical, hyperplasia, histiocytic				1 (4%)			
Deep cervical, hyperplasia, plasma cell	1 (4%)						
Lumbar, ectasia		1 (6%)		1 (4%)			
Lumbar, hemorrhage	1 (4%)						1 (5%)
Lumbar, hyperplasia, histiocytic							1 (5%)
Lumbar, hyperplasia, plasma cell		1 (6%)					

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Hematopoietic System (continued)							
Lymph node (continued)	(28)	(16)	(16)	(25)	(20)	(45)	(22)
Mediastinal, congestion				1 (4%)			
Mediastinal, ectasia	7 (25%)	7 (44%)	7 (44%)	7 (28%)	7 (35%)	27 (60%)	3 (14%)
Mediastinal, hemorrhage	2 (7%)	2 (13%)	4 (25%)	15 (60%)	7 (35%)	25 (56%)	11 (50%)
Mediastinal, hyperplasia, histiocytic				2 (8%)	2 (10%)	2 (4%)	4 (18%)
Mediastinal, hyperplasia, lymphoid			2 (13%)				1 (5%)
Mediastinal, hyperplasia, plasma cell		3 (19%)		1 (4%)			1 (5%)
Mediastinal, pigmentation						2 (4%)	
Pancreatic, ectasia					1 (5%)	1 (2%)	
Pancreatic, hemorrhage					1 (5%)	4 (9%)	3 (14%)
Pancreatic, hyperplasia, histiocytic				1 (4%)	4 (20%)	3 (7%)	3 (14%)
Pancreatic, hyperplasia, lymphoid				2 (8%)		1 (2%)	1 (5%)
Renal, ectasia		1 (6%)		1 (4%)	1 (5%)		1 (5%)
Renal, hemorrhage					1 (5%)		2 (9%)
Renal, hyperplasia					1 (5%)		
Renal, hyperplasia, histiocytic					1 (5%)	2 (4%)	3 (14%)
Lymph node, mandibular	(53)	(51)	(53)	(52)	(52)	(66)	(49)
Atrophy					1 (2%)		
Ectasia	3 (6%)	2 (4%)	3 (6%)	4 (8%)	4 (8%)	1 (2%)	11 (22%)
Hemorrhage			1 (2%)		2 (4%)	7 (11%)	4 (8%)
Hyperplasia, histiocytic				1 (2%)			
Hyperplasia, lymphoid		1 (2%)	3 (6%)		2 (4%)	1 (2%)	
Hyperplasia, plasma cell	45 (85%)	35 (69%)	34 (64%)	27 (52%)	22 (42%)	20 (30%)	28 (57%)
Inflammation							1 (2%)
Necrosis						1 (2%)	
Necrosis, lymphoid						1 (2%)	
Pigmentation						1 (2%)	
Lymph node, mesenteric	(53)	(51)	(53)	(53)	(52)	(65)	(50)
Ectasia	1 (2%)	1 (2%)		2 (4%)	1 (2%)	2 (3%)	1 (2%)
Hemorrhage		2 (4%)	2 (4%)	2 (4%)	4 (8%)	14 (22%)	10 (20%)
Hyperplasia, histiocytic	2 (4%)		1 (2%)	6 (11%)	5 (10%)		1 (2%)
Hyperplasia, lymphoid					1 (2%)	1 (2%)	
Hyperplasia, plasma cell	1 (2%)						1 (2%)
Spleen	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Hematopoietic cell proliferation	49 (92%)	44 (86%)	49 (92%)	52 (98%)	50 (94%)	43 (66%)	45 (90%)
Pigmentation	52 (98%)	50 (98%)	52 (98%)	50 (94%)	50 (94%)	59 (91%)	41 (82%)
Artery, inflammation, chronic active						1 (2%)	
Capsule, fibrosis	1 (2%)						
Lymphoid follicle, atrophy	4 (8%)	5 (10%)	2 (4%)	5 (9%)	3 (6%)	9 (14%)	4 (8%)
Red pulp, atrophy					1 (2%)	2 (3%)	
Thymus	(53)	(51)	(51)	(51)	(51)	(59)	(46)
Atrophy	40 (75%)	44 (86%)	44 (86%)	49 (96%)	50 (98%)	57 (97%)	45 (98%)
Hemorrhage			2 (4%)				1 (2%)
Necrosis				1 (2%)			
Artery, inflammation, chronic active		1 (2%)			1 (2%)	1 (2%)	1 (2%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Integumentary System							
Mammary gland	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Cyst	1 (2%)	2 (4%)	2 (4%)	2 (4%)			1 (2%)
Hyperplasia	26 (49%)	17 (33%)	13 (25%)	6 (11%)	6 (11%)		11 (22%)
Inflammation		1 (2%)					
Inflammation, granulomatous	3 (6%)	2 (4%)	1 (2%)				1 (2%)
Skin	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Cyst epithelial inclusion		1 (2%)		2 (4%)	1 (2%)		
Hemorrhage						1 (2%)	
Inflammation					1 (2%)	1 (2%)	
Ulcer					1 (2%)		
Subcutaneous tissue, inflammation, chronic active					1 (2%)		
Musculoskeletal System							
Bone	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Tarsal, inflammation							1 (2%)
Nervous System							
Brain	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Hemorrhage		1 (2%)	1 (2%)	4 (8%)	5 (9%)	14 (21%)	8 (16%)
Hydrocephalus			1 (2%)				
Inflammation				2 (4%)	1 (2%)		
Mineralization						1 (2%)	1 (2%)
Necrosis				2 (4%)	1 (2%)		
Artery, inflammation, chronic active					1 (2%)		
Cerebrum, degeneration				1 (2%)			
Meninges, inflammation					1 (2%)		
Meninges, vein, thrombosis					1 (2%)		
Respiratory System							
Lung	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Congestion				2 (4%)			
Hemorrhage				1 (2%)	2 (4%)	4 (6%)	3 (6%)
Infiltration cellular, histiocyte	40 (75%)	33 (65%)	46 (87%)	48 (91%)	46 (87%)	33 (50%)	24 (48%)
Inflammation	5 (9%)	9 (18%)	7 (13%)	3 (6%)	2 (4%)	2 (3%)	6 (12%)
Inflammation, granulomatous							4 (8%)
Keratin cyst							9 (18%)
Metaplasia, squamous		1 (2%)	2 (4%)	14 (26%)	16 (30%)	7 (11%)	8 (16%)
Necrosis			1 (2%)				
Alveolar epithelium, hyperplasia	18 (34%)	10 (20%)	2 (4%)	1 (2%)	1 (2%)	4 (6%)	5 (10%)
Alveolar epithelium, metaplasia, bronchiolar	1 (2%)	14 (27%)	39 (74%)	46 (87%)	35 (66%)	8 (12%)	15 (30%)
Artery, thrombosis						1 (2%)	
Artery, mediastinum, inflammation, chronic active						1 (2%)	
Mediastinum, inflammation					1 (2%)		
Serosa, fibrosis	3 (6%)			1 (2%)	16 (30%)	8 (12%)	1 (2%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Respiratory System (continued)							
Nose	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Inflammation	17 (32%)	15 (29%)	16 (30%)	10 (19%)	22 (42%)	11 (17%)	11 (22%)
Necrosis					1 (2%)		
Ulcer					1 (2%)		
Glands, hyperplasia					2 (4%)		
Olfactory epithelium, degeneration				1 (2%)			
Olfactory epithelium, metaplasia		2 (4%)	1 (2%)	3 (6%)	8 (15%)	1 (2%)	5 (10%)
Respiratory epithelium, hyperplasia	8 (15%)	11 (22%)	5 (9%)	8 (15%)	16 (30%)	9 (14%)	10 (20%)
Squamous epithelium, hyperplasia			1 (2%)		2 (4%)	1 (2%)	
Special Senses System							
Eye	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Cataract	1 (2%)	3 (6%)					
Cornea, inflammation		1 (2%)		1 (2%)	1 (2%)		
Iris, inflammation	1 (2%)						
Retina, atrophy	1 (2%)	3 (6%)		1 (2%)		1 (2%)	1 (2%)
Retina, degeneration						1 (2%)	
Harderian gland	(53)	(51)	(53)	(53)	(53)	(66)	(50)
Inflammation	24 (45%)	7 (14%)	20 (38%)	13 (25%)	7 (13%)	9 (14%)	12 (24%)
Urinary System							
Kidney	(53)	(51)	(53)	(53)	(53)	(65)	(50)
Accumulation, hyaline droplet							1 (2%)
Calculus microscopic							
observation only	6 (11%)	6 (12%)	6 (11%)				
Cyst		2 (4%)					1 (2%)
Infarct	2 (4%)					1 (2%)	
Inflammation, chronic active	1 (2%)						
Inflammation, suppurative		1 (2%)	4 (8%)	3 (6%)		1 (2%)	
Mineralization	46 (87%)	34 (67%)	38 (72%)	44 (83%)	34 (64%)	37 (57%)	32 (64%)
Necrosis						1 (2%)	
Nephropathy	41 (77%)	37 (73%)	37 (70%)	48 (91%)	50 (94%)	43 (66%)	34 (68%)
Pigmentation					1 (2%)		
Artery, inflammation, chronic active				1 (2%)			
Pelvis, dilatation			1 (2%)				
Pelvis, inflammation	4 (8%)	6 (12%)	1 (2%)	6 (11%)			
Renal tubule, hyperplasia	2 (4%)						
Transitional epithelium, hyperplasia	4 (8%)	6 (12%)	6 (11%)	16 (30%)	1 (2%)		
Transitional epithelium, metaplasia, squamous				1 (2%)			
Ureter	(1)						
Transitional epithelium, hyperplasia	1 (100%)						

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg	360 ng TEQ/kg (Stop- Exposure)
Urinary System (continued)							
Urinary bladder	(53)	(51)	(53)	(53)	(52)	(66)	(50)
Calculus gross observation	1 (2%)						
Edema	1 (2%)						
Inflammation	8 (15%)	3 (6%)	2 (4%)	4 (8%)	1 (2%)		1 (2%)
Metaplasia, squamous				1 (2%)			
Artery, inflammation, chronic active				1 (2%)			
Transitional epithelium, hyperplasia	4 (8%)	2 (4%)		4 (8%)	1 (2%)		

APPENDIX B
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE B1	Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 118	162
-----------------	--	------------

TABLE B1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats
at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118^a

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	7	8	8	8	0
Necropsy body wt						
Week 14	296 ± 8	284 ± 8	276 ± 8*	258 ± 5**	255 ± 5**	245 ± 7**
Week 31	317 ± 9	322 ± 7	289 ± 6**	292 ± 8**	261 ± 3**	240 ± 4**
Week 53	340 ± 9	354 ± 16	326 ± 10	298 ± 12*	273 ± 8**	
L. Kidney						
Week 14						
Absolute	0.787 ± 0.026	0.774 ± 0.024	0.759 ± 0.024	0.736 ± 0.018 ^b	0.777 ± 0.023	0.749 ± 0.015
Relative	2.654 ± 0.045	2.724 ± 0.049	2.765 ± 0.081	2.853 ± 0.070 ^b	3.045 ± 0.061**	3.067 ± 0.064**
Week 31						
Absolute	0.880 ± 0.031	0.935 ± 0.016	0.881 ± 0.017	0.856 ± 0.022	0.773 ± 0.014**	0.741 ± 0.028**
Relative	2.779 ± 0.064	2.911 ± 0.062	3.055 ± 0.047*	2.942 ± 0.058	2.963 ± 0.036	3.081 ± 0.110*
Week 53						
Absolute	0.948 ± 0.028	1.063 ± 0.041*	0.977 ± 0.030	0.915 ± 0.027	0.887 ± 0.020	
Relative	2.792 ± 0.076	3.018 ± 0.077	3.004 ± 0.081	3.086 ± 0.068*	3.258 ± 0.085**	
Liver						
Week 14						
Absolute	8.805 ± 0.390	9.278 ± 0.310	9.627 ± 0.298	9.672 ± 0.138	10.792 ± 0.418**	10.628 ± 0.419**
Relative	29.692 ± 0.952	32.643 ± 0.736**	34.941 ± 0.304**	37.594 ± 0.547**	42.212 ± 0.966**	43.285 ± 0.767**
Week 31						
Absolute	9.052 ± 0.333	10.279 ± 0.265*	10.343 ± 0.315*	11.514 ± 0.389**	11.295 ± 0.275**	11.395 ± 0.421**
Relative	28.586 ± 0.701	32.010 ± 0.928*	35.819 ± 0.748**	39.523 ± 0.854**	43.314 ± 0.833**	47.643 ± 2.276**
Week 53						
Absolute	10.28 ± 0.36	12.31 ± 0.54	11.33 ± 0.40 ^c	12.56 ± 0.69*	18.75 ± 1.27**	
Relative	30.293 ± 1.065	35.032 ± 1.566	35.748 ± 1.071 ^c	42.072 ± 0.968**	68.526 ± 3.906**	
Lung						
Week 14						
Absolute	1.809 ± 0.062	1.829 ± 0.062	1.846 ± 0.075	1.745 ± 0.054	2.063 ± 0.097	2.001 ± 0.107
Relative	6.126 ± 0.198	6.442 ± 0.182	6.713 ± 0.234	6.765 ± 0.148	8.103 ± 0.394**	8.139 ± 0.283**
Week 31						
Absolute	2.114 ± 0.140	1.960 ± 0.050	1.865 ± 0.097	2.093 ± 0.051	2.322 ± 0.085	2.323 ± 0.168
Relative	6.640 ± 0.324	6.091 ± 0.120	6.452 ± 0.271	7.205 ± 0.172	8.925 ± 0.361**	9.696 ± 0.758**
Week 53						
Absolute	1.873 ± 0.105	1.963 ± 0.083	2.080 ± 0.098	2.538 ± 0.146**	2.993 ± 0.155**	
Relative	5.578 ± 0.457	5.598 ± 0.281	6.429 ± 0.373	8.591 ± 0.581**	10.965 ± 0.497**	
L. Ovary						
Week 14						
Absolute	0.074 ± 0.004	0.073 ± 0.005	0.069 ± 0.005	0.065 ± 0.004	0.062 ± 0.003	0.057 ± 0.002**
Relative	0.249 ± 0.012	0.258 ± 0.021	0.252 ± 0.015	0.251 ± 0.016	0.246 ± 0.012	0.235 ± 0.011
Week 31						
Absolute	0.061 ± 0.004	0.061 ± 0.005	0.056 ± 0.004	0.055 ± 0.004	0.056 ± 0.003	0.046 ± 0.003**
Relative	0.191 ± 0.007	0.189 ± 0.014	0.193 ± 0.013	0.188 ± 0.010	0.216 ± 0.013	0.189 ± 0.010
Week 53						
Absolute	0.056 ± 0.004	0.060 ± 0.004	0.065 ± 0.007	0.060 ± 0.004	0.067 ± 0.004	
Relative	0.164 ± 0.009	0.172 ± 0.011	0.195 ± 0.015	0.199 ± 0.008*	0.246 ± 0.013**	

TABLE B1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats
at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study
of a Binary Mixture of PCB 126 and PCB 118

	Vehicle Control	7 ng TEQ/kg	22 ng TEQ/kg	72 ng TEQ/kg	216 ng TEQ/kg	360 ng TEQ/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	7	8	8	8	0
Necropsy body wt						
Week 14	296 ± 8	284 ± 8	276 ± 8*	258 ± 5**	255 ± 5**	245 ± 7**
Week 31	317 ± 9	322 ± 7	289 ± 6**	292 ± 8**	261 ± 3**	240 ± 4**
Week 53	340 ± 9	354 ± 16	326 ± 10	298 ± 12*	273 ± 8**	
Spleen						
Week 14						
Absolute	0.601 ± 0.028	0.604 ± 0.029	0.614 ± 0.033	0.542 ± 0.015	0.508 ± 0.027*	0.498 ± 0.030*
Relative	2.024 ± 0.067	2.123 ± 0.087	2.217 ± 0.067	2.105 ± 0.062	1.979 ± 0.079	2.022 ± 0.080
Week 31						
Absolute	0.598 ± 0.023	0.579 ± 0.020	0.508 ± 0.017**	0.521 ± 0.025**	0.470 ± 0.012**	0.466 ± 0.022**
Relative	1.892 ± 0.069	1.797 ± 0.043	1.761 ± 0.053	1.784 ± 0.051	1.802 ± 0.037	1.943 ± 0.101
Week 53						
Absolute	0.576 ± 0.023	0.611 ± 0.034	0.556 ± 0.024	0.477 ± 0.030*	0.526 ± 0.021	
Relative	1.693 ± 0.048	1.733 ± 0.076	1.716 ± 0.086	1.599 ± 0.069	1.934 ± 0.089	
Thymus						
Week 14						
Absolute	0.216 ± 0.021	0.213 ± 0.009	0.212 ± 0.011	0.191 ± 0.023	0.143 ± 0.013**	0.138 ± 0.014**
Relative	0.720 ± 0.054	0.754 ± 0.036	0.772 ± 0.040	0.745 ± 0.092	0.562 ± 0.051	0.564 ± 0.054
Thyroid gland						
Week 14						
Absolute	0.024 ± 0.002	0.020 ± 0.001	0.021 ± 0.001	0.021 ± 0.001	0.022 ± 0.002	0.019 ± 0.001*
Relative	0.081 ± 0.005	0.072 ± 0.005	0.078 ± 0.005	0.081 ± 0.006	0.086 ± 0.007	0.076 ± 0.005
Week 31						
Absolute	0.023 ± 0.001	0.023 ± 0.001	0.023 ± 0.001	0.023 ± 0.001	0.019 ± 0.001*	0.017 ± 0.002** ^b
Relative	0.072 ± 0.004	0.070 ± 0.004	0.079 ± 0.004	0.081 ± 0.006	0.073 ± 0.004	0.071 ± 0.005 ^b
Week 53						
Absolute	0.032 ± 0.002	0.035 ± 0.005	0.030 ± 0.002	0.025 ± 0.001*	0.023 ± 0.001*	
Relative	0.096 ± 0.008	0.098 ± 0.011	0.093 ± 0.007	0.086 ± 0.008	0.083 ± 0.004	

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

^b n=9

^c n=7

APPENDIX C

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION	166
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS	167
FIGURE C1 Infrared Absorption Spectrum of PCB 118	168
FIGURE C2 Proton Nuclear Magnetic Resonance Spectrum of PCB 118	169
FIGURE C3 Low Resolution Mass Spectrum of PCB 118	170
TABLE C1 Gas Chromatography Systems Used in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118	171
TABLE C2 Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118	172
TABLE C3 Results of Analyses of PCB 118 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118	173
TABLE C4 Results of Analyses of PCB 126 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118	175

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

PCB 118

PCB 118 was obtained from Radian International LLC (Austin, TX), in one lot (31542-46) that was used in the 2-year study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (Research Triangle Institute, Research Triangle Park, NC) and the study laboratory (Battelle Columbus Operations, Columbus, OH). Reports on analyses performed in support of the study of a mixture of PCB 126 and PCB 118 are on file at the National Institute of Environmental Health Sciences.

Lot 31542-46 of the chemical, a white powder, was identified as PCB 118 by the analytical chemistry laboratory using melting point determination and infrared (IR), ultraviolet/visible (UV/Vis), proton nuclear magnetic resonance (NMR), and low resolution mass spectroscopy (MS) and by the study laboratory using IR spectroscopy. The melting point agreed with the vendor's certificate of analysis; all spectra were consistent with the structure of PCB 118, and IR and NMR spectra matched reference spectra (Radian International; NBS Registry of Mass Spectral Data). Infrared, proton NMR, and low resolution MS spectra are presented in Figures C1, C2, and C3.

The moisture content of lot 31542-46 was determined by the analytical chemistry laboratory by gas chromatography moisture analysis (GCMA) purge and trap method by system A (Table C1). The purity of lot 31542-46 was determined by the analytical chemistry laboratory (systems B and C) and the study laboratory (system D) using gas chromatography.

GCMA indicated a water content of approximately 0.06% by weight. Gas chromatography using system B indicated one major peak and two impurity peaks with areas of 0.4% and 0.5% relative to the total integrated peak area. Gas chromatography using system C detected one major peak and three impurity peaks with areas of 0.2%, 0.8%, and 0.5% relative to the total integrated peak area. GC by system D indicated a purity of 97.4% relative to a frozen reference sample from the same lot. The overall purity of lot 31542-46 was determined to be greater than 98.5%.

The three impurity peaks observed during purity determination were identified by the analytical chemistry laboratory using GC/MS by system E and GC by system F. GC/MS indicated that the impurities were tetrachlorinated, pentachlorinated, and hexachlorinated biphenyls. Using GC by system F, the impurities were identified as PCB 77 (0.2%), PCB 126 (0.8%), and PCB 167 (0.5%) by relative retention time matching to selected congener standards.

Since PCB 126 was predicted to be a high contributor to the dioxin-like activity of the bulk material, it was further analyzed to obtain a more accurate assessment of its level in the bulk material. The concentration of PCB 126 in lot 31542-46 of PCB 118 was determined by the study laboratory by standard addition of PCB 126 using GC by a system similar to system D. The concentration of PCB 126 in lot 31542-46 of PCB 118 was determined to be $0.622\% \pm 0.061\%$.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC by system G. Aliquots of the test chemical were stored at -80° , -20° , 3° , 23° , and 60° C in amber glass vials for 14 days. Samples stored protected from light were stable for at least 2 weeks at temperatures up to 60° C. Stability of the bulk chemical was monitored by the study laboratory using GC by systems similar to system D. No degradation of the bulk chemical was detected during the study. To ensure stability, the bulk chemical was stored at room temperature (23° to 25° C) and protected from light.

Formulation Materials

USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) in three lots and was used with corn oil (Spectrum Quality Products) as the vehicle in the 2-year gavage study. The identity of each lot was confirmed by the study laboratory using infrared spectroscopy prior to its use. The purity of each lot was determined by the study laboratory using gas chromatography by system H prior to initial use and at intervals of no more than 6 months thereafter. All acetone lots showed a purity of at least 99.9%. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

For the 4 µg/mL PCB 118 dose formulation, a working stock solution was made, and an aliquot transferred and diluted in the corn oil vehicle (Table C2). Higher dose formulations were prepared by dissolving the appropriate amount of chemical in acetone and diluting in the corn oil vehicle for the concentration desired. The final dose formulations contained 1% acetone and were stored at room temperature in amber glass bottles sealed with Teflon[®]-lined lids for up to 35 days.

Homogeneity and stability studies of a 4 µg/mL PCB 118 formulation were performed by the study laboratory using GC by a system similar to system D. A gavageability study of an 1,840 µg/mL PCB 118 formulation was conducted by the study laboratory. Homogeneity was confirmed, stability was confirmed for at least 42 days for dose formulations stored in amber glass bottles with Teflon[®]-lined lids at temperatures up to room temperature and for up to 3 hours when exposed to air and light at room temperature, and gavageability was confirmed.

Analyses of the dose formulations for PCB 118 concentrations were conducted by the study laboratory using gas chromatography systems similar to system D at least every 3 months (Table C3). Of the dose formulations analyzed and used in the study, all 47 were within 10% of the target concentrations; all 16 animal room samples analyzed were within 10% of the target concentrations.

Analyses of the dose formulations for PCB 126 concentrations were conducted twice by the study laboratory using GC/MS. All (4/4) of the preadministration samples analyzed were within 10% of the expected concentration (Table C4). Of archived dose formulations prepared June 7, 2000, November 20, 2000, February 12, 2001, and May 7, 2001, and analyzed in June 2001, 45% (9/20) were within 10% of the expected concentrations; all were within 15% of expected concentrations.

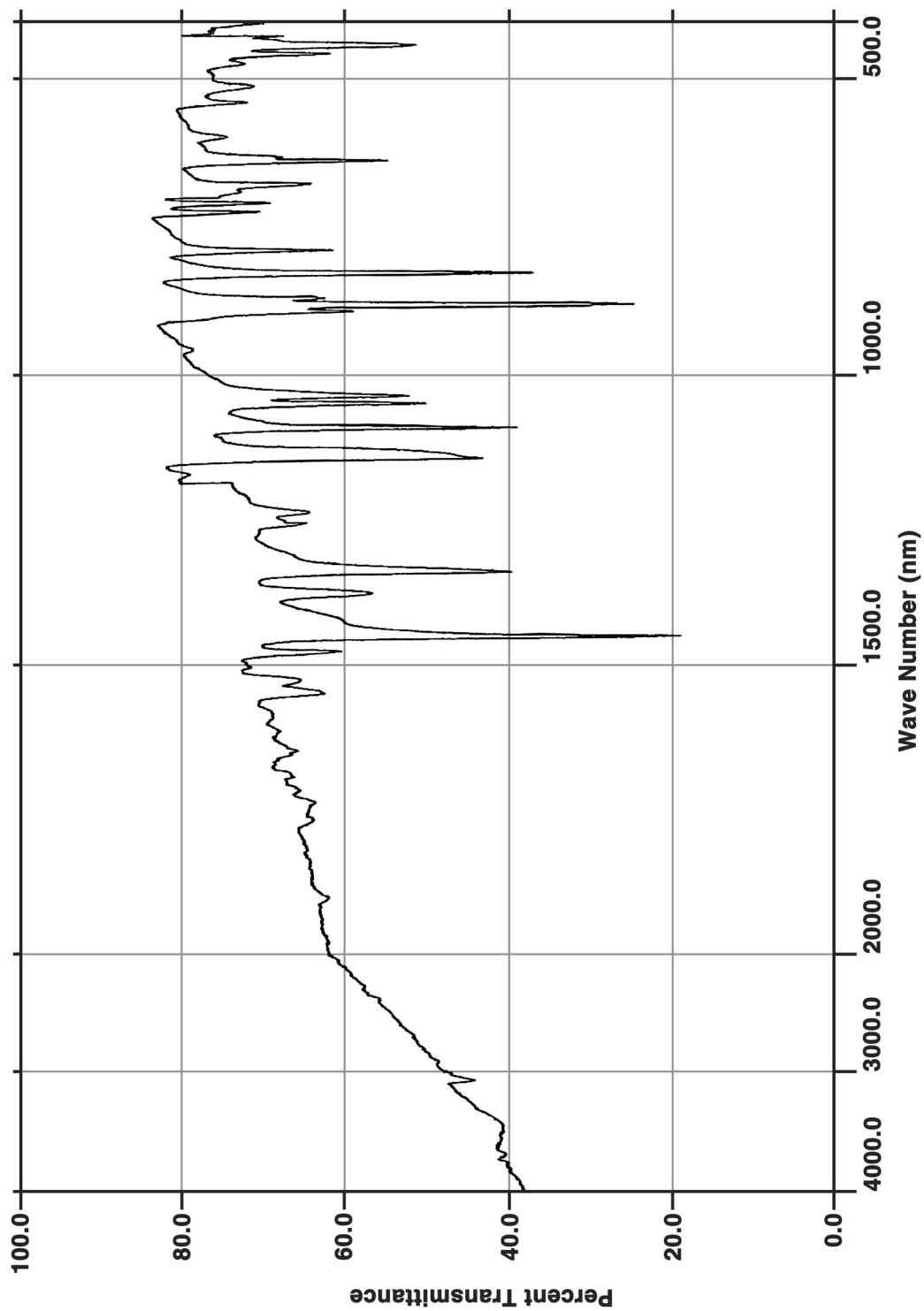


FIGURE C1
Infrared Absorption Spectrum of PCB 118

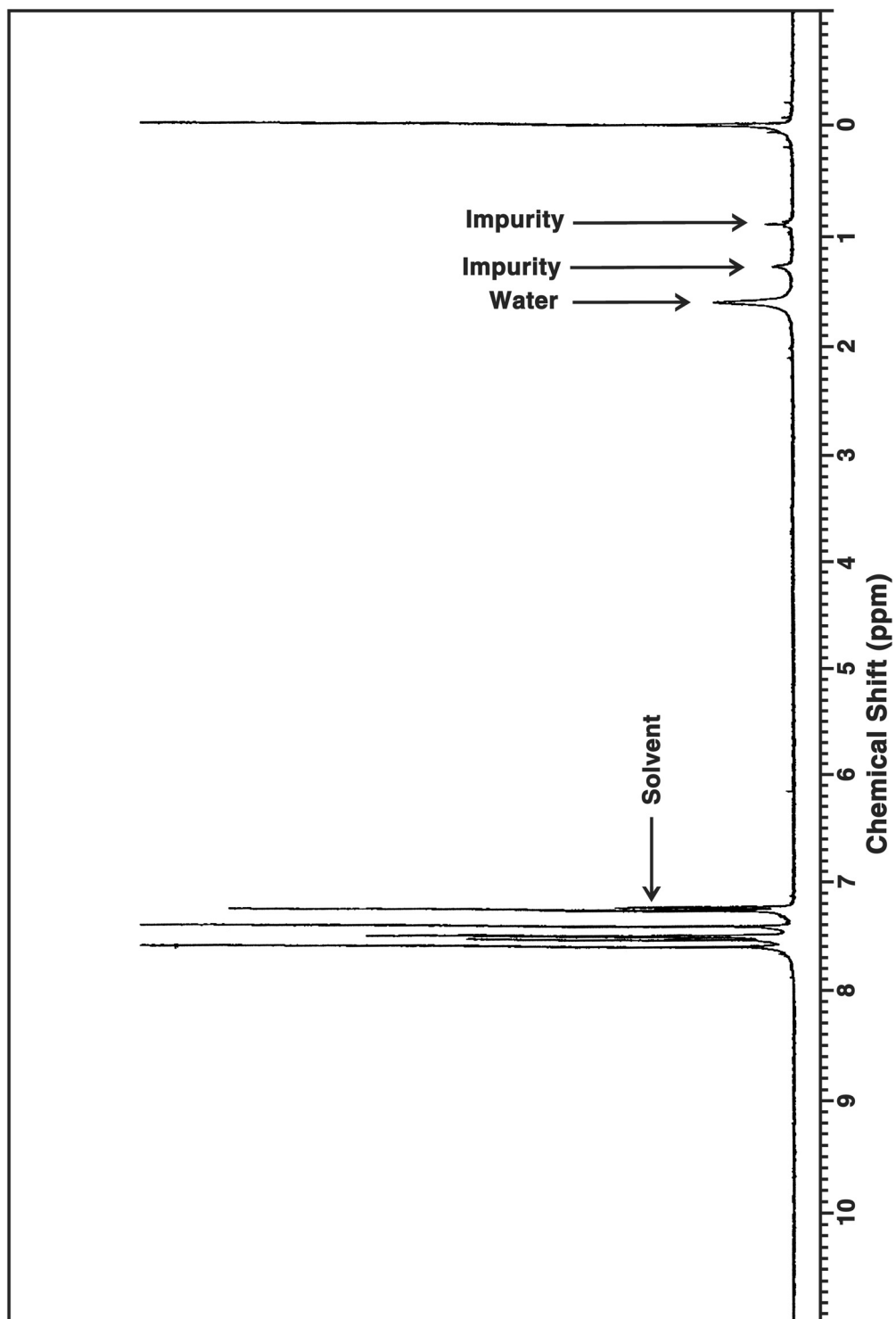


FIGURE C2
Proton Nuclear Magnetic Resonance Spectrum of PCB 118

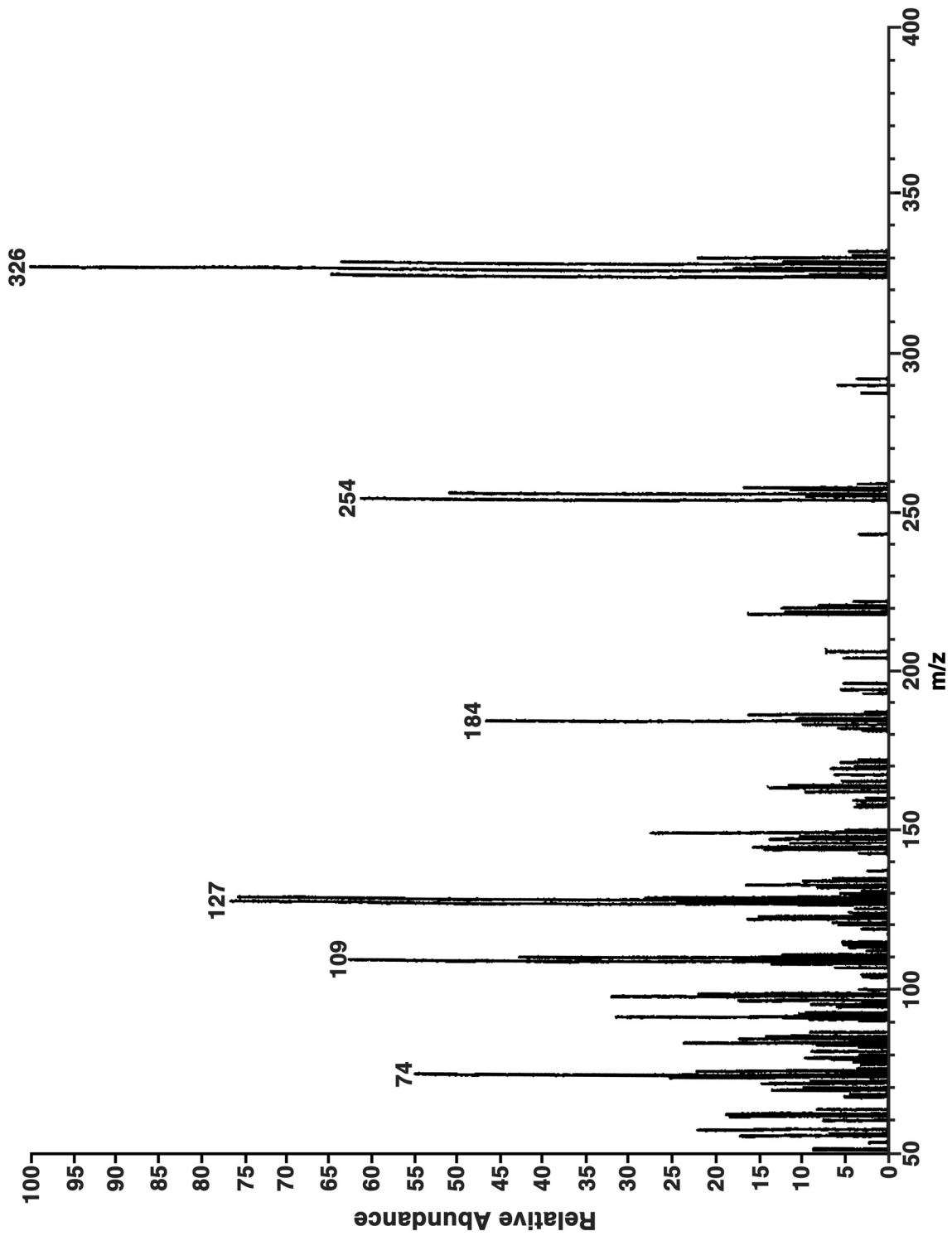


FIGURE C3
Low Resolution Mass Spectrum of PCB 118

TABLE C1
Gas Chromatography Systems Used in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118^a

Detection System	Column	Carrier Gas	Oven Temperature Program
System A Thermal conductivity	Porapak QS 80/100 packed column (6 feet × 4 mm) (laboratory prepared)	Helium at 100 mL/minute	40° C for 1 minute, then 15° C/minute to 120° C
System B Flame ionization	J&W DB-5MS, 30 m × 0.32 mm, 0.5- μ m film thickness (J&W Scientific, Folsom, CA)	Helium at 1.7 mL/minute	50° C, then 10° C/minute to 300° C, held for 10 minutes
System C Electron capture	J&W DB-5, 30 m × 0.25 mm, 0.25- μ m film thickness (J&W Scientific)	Helium at 1.0 mL/minute	50° C, then 10° C/minute to 300° C, held for 10 minutes
System D Electron capture	Supelco PTE-5 30 m × 0.32 mm 1.0- μ m film thickness (Supelco, Inc., Bellefonte, PA)	Helium at 1.5 mL/minute	220° C, then 5° C/minute to 255° C, then 1° C/minute to 270° C, then 15° C/minute to 300° C
System E Low resolution mass spectrometry	J&W DB-5MS, 30 m × 0.32 mm, 0.5- μ m film thickness (J&W Scientific)	Helium at 2.2 mL/minute 8.1 psi head pressure	100° C, then 10° C/minute to 300° C, held for 15 minutes
System F Electron capture	J&W DB-5MS 30 m × 0.32 mm, 0.5- μ m film thickness (J&W Scientific)	Helium at 1.7 mL/minute	50° C, then 10° C/minute to 300° C, held for 15 minutes
System G Electron capture	J&W DB-5MS, 30 m × 0.32 mm, 0.5- μ m film thickness (J&W Scientific)	Helium at 1.5 mL/minute	220° C, then 5° C/minute to 300° C, held for 4 minutes
System H Flame ionization	20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcort 2.4 m × 2 mm (Supelco, Inc.)	Helium at 30 mL/minute	40° C for 4 minutes, then 10° C/minute to 170° C

^a The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA) (systems A, B, C, D, E, F, and G or Carlo Erba/Fisons, Ltd. (Valencia, CA) (system H). The mass spectrometer used in system E was manufactured by Hewlett-Packard (Palo Alto, CA).

TABLE C2**Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118**

Preparation

Dose formulations were prepared every 5 weeks. A single dose formulation working stock of 8 mg/mL PCB 118 was prepared by adding the appropriate amount of chemical, ground to a fine powder with mortar and pestle, to a 10-mL volumetric flask; 5 mL of acetone was added, and the flask was capped and shaken well. The contents were diluted to volume with acetone, vortexed for approximately 2 minutes, and sonicated for approximately 30 minutes in an ice-cooled water bath.

To prepare the 4 µg/mL PCB 118 dose formulation, 1 mL of the 8 mg/mL PCB 118 dose formulation working stock solution was pipetted into a 5-L volumetric flask containing approximately 1 L of corn oil, 19 mL of acetone was added, and the flask was sealed and shaken vigorously. The contents of the volumetric flask were diluted to 2 L with corn oil and the flask was capped, shaken, and stirred on a stirplate for approximately 24 hours, with vigorous shaking at least eight times over the stirring period.

The four higher dose formulations were prepared by adding the appropriate quantity of the test chemical, ground to a fine powder with mortar and pestle, to a 5-L volumetric flask containing approximately 1 L of corn oil; 20 mL of acetone was added, and the flask was sealed and shaken vigorously. The contents of the volumetric flask were diluted to 2 L with corn oil and the flask was capped, shaken, and stirred on a stirplate for approximately 24 hours, with vigorous shaking at least eight times over the stirring period.

All dose formulations contained a final concentration of 1% acetone in corn oil.

Chemical Lot Number

PCB 118: 31542-46

Maximum Storage Time

35 days

Storage Conditions

Dose formulations were stored in 120 or 250 mL amber glass screw-cap bottles with Teflon[®]-lined lids at room temperature (approximately 25° C).

Study Laboratory

Battelle Columbus Operations (Columbus, OH)

TABLE C3
Results of Analyses of PCB 118 Concentrations in Dose Formulations Administered to Female Rats
in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118^a

Date Prepared	Date Analyzed	Target PCB 118 Concentration (µg/mL)	Determined PCB 118 Concentration (µg/mL)	Difference from Target (%)
October 1, 1999	October 6-7, 1999	4	4.068	+2
		12	11.71	-2
		40	40.60	+2
		120	121.1	+1
		200	201.5	+1
		200	199.8	0
	November 15-16, 1999 ^b	4	4.071	+2
		12	11.56	-4
		40	40.67	+2
		200	204.2	+2
November 22, 1999 ^b	200	199.4	0	
December 21, 1999	December 27-28, 1999	4	4.174	+4
		12	12.23	+2
		40	41.15	+3
		120	119.0	-1
		200	204.4	+2
		200	205.8	+3
March 13, 2000	March 20-21, 2000	4	4.133 ± 0.016	+3
		12	11.90 ± 0.07	-1
		40	39.57 ± 0.48	-1
		120	122.0 ± 0.5	+2
		200	201.8 ± 0.9	+1
		200	206.5 ± 2.0	+3
June 7, 2000	June 12-13, 2000	4	4.165 ± 0.033	+4
		12	11.85 ± 0.01	-1
		40	40.37 ± 0.48	+1
		120	123.5 ± 0.5	+3
		200	200.5 ± 1.6	0
	July 19-20, 2000 ^b	4	4.087 ± 0.019	+2
		12	11.56 ± 0.03	-4
		40	39.81 ± 0.10	0
		120	121.3 ± 1.2	+1
		200	202.2 ± 2.5	+1
August 28, 2000	August 31-September 1, 2000	4	3.808 ± 0.027	-5
		12	11.10 ± 0.59	-7
		40	36.96 ± 0.29	-8
		120	121.7 ± 8.1	+1
		200	184.5 ± 0.8	-8
November 20, 2000	November 27-28, 2000	4	4.252 ± 0.028	+6
		12	12.10 ± 0.08	+1
		40	40.41 ± 0.76	+1
		120	121.6 ± 1.1	+1
		200	201.6 ± 5.8	+1

TABLE C3
Results of Analyses of PCB 118 Concentrations in Dose Formulations Administered to Female Rats
in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118

Date Prepared	Date Analyzed	Target PCB 118 Concentration (µg/mL)	Determined PCB 118 Concentration (µg/mL)	Difference from Target (%)
February 12, 2001	February 15-16, 2001	4	4.095 ± 0.012	+2
		12	11.61 ± 0.20	-3
		40	40.53 ± 0.38	+1
		120	121.1 ± 1.0	+1
		200	201.8 ± 5.3	+1
	March 20-21, 2001 ^b	4	4.356 ± 0.035	+9
		12	12.70 ± 0.11	+6
		40	43.45 ± 0.48	+9
		120	128.2 ± 2.7	+7
		200	213.8 ± 8.1	+7
May 7, 2001	May 9-10, 2001	4	4.022 ± 0.037	+1
		12	12.15 ± 0.10	+1
		40	39.74 ± 0.20	-1
		120	119.5 ± 0.5	0
		200	202.6 ± 1.5	+1
July 30, 2001	August 2-3, 2001	4	3.844 ± 0.070	-4
		12	11.20 ± 0.10	-7
		40	39.55 ± 0.61	-1
		120	118.6 ± 1.5	-1

^a Determined concentration is the average of duplicate analyses or the average of quadruplicate analyses ± standard deviation.
Dosing volume = 2.5 mL/kg: 4 µg PCB 118/mL = 10 µg PCB 118/kg; 12.0 µg/mL = 30 µg/kg; 40 µg/mL = 100 µg/kg;
120 µg/mL = 300 µg/kg, and 200 µg/mL = 500 µg/kg.

^b Animal room samples

TABLE C4
Results of Analyses of PCB 126 Concentrations in Dose Formulations Administered to Female Rats
in the 2-Year Gavage Study of a Mixture of PCB 126 and PCB 118^a

Date Prepared	Date Analyzed	Expected PCB 126 Concentration ^b (ng/mL)	Determined PCB 126 Concentration (ng/mL)	Difference from Target (%)
June 7, 2000	June 11-13, 2001 ^c	24.8	23.03	-7
		74.8	64.52	-14
		249	214.5	-14
		748	668.0	-11
		1,240	1,102 ^d	-11
November 20, 2000	June 11-13, 2001 ^c	24.8	22.88	-8
		74.8	67.06	-10
		249	219.2	-12
		748	663.7	-11
		1,240	1,101	-11
February 12, 2001	June 11-13, 2001 ^c	24.8	22.90	-8
		74.8	66.41	-11
		249	225.9	-9
		748	682.6	-9
		1,240	1,117	-10
May 7, 2001	June 11-13, 2001 ^c	24.8	22.33	-10
		74.8	67.94	-9
		249	215.4	-13
		748	666.0	-11
		1,240	1,058	-15
July 30, 2001	August 6, 2001	24.8	23.15	-7
		74.8	67.64	-10
		249	233.2	-6
		748	686.5	-8

^a Determined concentration is the average of duplicate analyses. Dosing volume = 2.5 mL/kg: 24.8 ng PCB 126/mL = 62 ng PCB 126/kg; 74.8 ng/mL = 187 ng/kg; 249 ng/mL = 622 ng/kg; 748 ng/mL = 1,866 ng/kg, and 1,240 ng/mL = 3,110 ng/kg.

^b Based on a PCB 126 level of 0.622% of the bulk synthesized PCB 118

^c Archived samples

^d Replicate lost during analysis.

APPENDIX D
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NTP-2000 RAT AND MOUSE RATION

TABLE D1	Ingredients of NTP-2000 Rat and Mouse Ration	178
TABLE D2	Vitamins and Minerals in NTP-2000 Rat and Mouse Ration	178
TABLE D3	Nutrient Composition of NTP-2000 Rat and Mouse Ration	179
TABLE D4	Contaminant Levels in NTP-2000 Rat and Mouse Ration	180
TABLE D5	Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration	181

TABLE D1
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 D2
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	
<i>d</i> -Pantothenic acid	10 mg	<i>d</i> -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	<i>d</i> -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 D3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.1 ± 0.66	13.2 – 15.7	25
Crude fat (% by weight)	8.1 ± 0.26	7.6 – 8.5	25
Crude fiber (% by weight)	9.1 ± 0.55	8.0 – 10.5	25
Ash (% by weight)	5.2 ± 0.28	4.7 – 5.8	25
Amino Acids (% of total diet)			
Arginine	0.748 ± 0.053	0.670 – 0.850	12
Cystine	0.223 ± 0.027	0.150 – 0.250	12
Glycine	0.702 ± 0.043	0.620 – 0.750	12
Histidine	0.343 ± 0.023	0.310 – 0.390	12
Isoleucine	0.534 ± 0.041	0.430 – 0.590	12
Leucine	1.078 ± 0.059	0.960 – 1.140	12
Lysine	0.729 ± 0.065	0.620 – 0.830	12
Methionine	0.396 ± 0.053	0.260 – 0.460	12
Phenylalanine	0.611 ± 0.038	0.540 – 0.660	12
Threonine	0.492 ± 0.045	0.430 – 0.590	12
Tryptophan	0.129 ± 0.016	0.110 – 0.160	12
Tyrosine	0.378 ± 0.054	0.280 – 0.460	12
Valine	0.658 ± 0.049	0.550 – 0.710	12
Essential Fatty Acids (% of total diet)			
Linoleic	3.89 ± 0.278	3.49 – 4.54	12
Linolenic	0.30 ± 0.038	0.21 – 0.35	12
Vitamins			
Vitamin A (IU/kg)	4,879 ± 891	3,060 – 6,810	25
Vitamin D (IU/kg)	1,000 ^a		
α-Tocopherol (ppm)	84.3 ± 17.06	52.0 – 110.0	12
Thiamine (ppm)	7.3 ± 0.81	6.0 – 8.8	25
Riboflavin (ppm)	6.4 ± 2.11	4.20 – 11.20	12
Niacin (ppm)	78.6 ± 10.86	66.4 – 98.2	12
Pantothenic acid (ppm)	23.1 ± 3.61	17.4 – 29.1	12
Pyridoxine (ppm)	8.88 ± 2.05	6.4 – 12.4	12
Folic acid (ppm)	1.84 ± 0.56	1.26 – 3.27	12
Biotin (ppm)	0.337 ± 0.13	0.225 – 0.704	12
Vitamin B ₁₂ (ppb)	64.8 ± 50.9	18.3 – 174.0	12
Choline (ppm)	3,094 ± 292	2,700 – 3,790	12
Minerals			
Calcium (%)	1.037 ± 0.045	0.964 – 1.140	25
Phosphorus (%)	0.600 ± 0.038	0.552 – 0.701	25
Potassium (%)	0.668 ± 0.023	0.627 – 0.694	12
Chloride (%)	0.368 ± 0.033	0.300 – 0.423	12
Sodium (%)	0.189 ± 0.016	0.160 – 0.212	12
Magnesium (%)	0.200 ± 0.009	0.185 – 0.217	12
Sulfur (%)	0.176 ± 0.026	0.116 – 0.209	12
Iron (ppm)	177 ± 46.2	135 – 311	12
Manganese (ppm)	53.4 ± 6.42	42.1 – 63.1	12
Zinc (ppm)	52.5 ± 6.95	43.3 – 66.0	12
Copper (ppm)	6.64 ± 1.283	5.08 – 9.92	12
Iodine (ppm)	0.535 ± 0.242	0.233 – 0.972	12
Chromium (ppm)	0.545 ± 0.125	0.330 – 0.751	12
Cobalt (ppm)	0.23 ± 0.041	0.20 – 0.30	12

^a From formulation^b As hydrochloride^c As chloride

TABLE D4
Contaminant Levels in NTP-2000 Rat and Mouse Ration^a

	Mean ± Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.21 ± 0.046	0.10 – 0.37	25
Cadmium (ppm)	0.04 ± 0.005	0.04 – 0.06	25
Lead (ppm)	0.10 ± 0.100	0.05 – 0.54	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.22 ± 0.054	0.14 – 0.36	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm) ^c	11.2 ± 3.37	6.85 – 21.1	25
Nitrite nitrogen (ppm) ^c	<0.61		25
BHA (ppm) ^d	<1.0		25
BHT (ppm) ^d	<1.0		25
Aerobic plate count (CFU/g)	14 ± 13	10 – 70	25
Coliform (MPN/g)	2.3 ± 1.6	0.0 – 3.6	25
<i>Escherichia coli</i> (MPN/g)	<10		25
<i>Salmonella</i> (MPN/g)	Negative		25
Total nitrosoamines (ppb) ^e	4.7 ± 1.19	2.3 – 7.5	25
<i>N</i> -Nitrosodimethylamine (ppb) ^e	2.0 ± 0.60	1.0 – 3.2	25
<i>N</i> -Nitrosopyrrolidine (ppb) ^e	2.7 ± 1.03	1.0 – 5.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.169 ± 0.112	0.020 – 0.499	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.186 ± 0.144	0.020 – 0.557	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

^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.

TABLE D5
Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration^a

Analyte	Mean Concentration ^b	Standard Deviation	Mean LOQ	Standard Deviation
2,3,7,8-TCDD			0.0592	0.0106
1,2,3,7,8-PeCDD			0.119	0.0498
1,2,3,4,7,8-HxCDD			0.124	0.0366
1,2,3,6,7,8-HxCDD			0.120	0.0345
1,2,3,7,8,9-HxCDD			0.124	0.0387
1,2,3,4,6,7,8-HpCDD	0.573	0.417	0.573	0.417
OCDD	3.47	2.00	3.47	2.00
2,3,4,7,8-PeCDF	0.0413	0.0821	0.0934	0.0545
2,3,7,8-TCDF	0.0102		0.0692	0.0187
1,2,3,4,7,8-HxCDF	0.00753		0.0492	0.0213
1,2,3,6,7,8-HxCDF			0.0445	0.0155
1,2,3,7,8,9-HxCDF			0.0712	0.0259
2,3,4,6,7,8-HxCDF			0.0485	0.0176
1,2,3,7,8-PeCDF	0.00707		0.0871	0.0275
1,2,3,4,6,7,8-HpCDF	0.115	0.425	0.162	0.254
1,2,3,4,7,8,9-HpCDF			0.0870	0.0212
OCDF	0.207	0.272	0.330	0.211
2-Chlorobiphenyl	19.2	11.0	19.2	11.0
3-Chlorobiphenyl	1.73	0.465	4.99	0.893
4-Chlorobiphenyl	15.6	8.68	15.6	8.68
2,2'-Dichlorobiphenyl	62.0	54.3	62.0	54.3
2,3-Dichlorobiphenyl	267	244	267	244
2,3'-Dichlorobiphenyl	46.5	41.7	46.5	41.7
2,4-Dichlorobiphenyl/2,5-Dichlorobiphenyl	26.9	24.6	28.5	24.1
3,3'-Dichlorobiphenyl	101	108	101	108
3,4-Dichlorobiphenyl/3,4'-Dichlorobiphenyl	11.7	9.48	16.5	10.6
3,5-Dichlorobiphenyl			8.96	0.314
4,4'-Dichlorobiphenyl	63.5	64.8	78.5	67.8
2,2',3-Trichlorobiphenyl/2,4',6-Trichlorobiphenyl	112	102	112	103
2,2',4-Trichlorobiphenyl	82.4	75.3	82.4	75.3
2,2',5-Trichlorobiphenyl	202	183	202	183
2,2',6-Trichlorobiphenyl	13.7	14.8	14.9	14.1
2,3,3'-Trichlorobiphenyl/2,3,4-Trichlorobiphenyl/2',3,4-Trichlorobiphenyl	157	150	157	150
2,3,4'-Trichlorobiphenyl	80.5	76.3	80.5	76.3
2,3,5-Trichlorobiphenyl			4.48	0.158
2,3,6-Trichlorobiphenyl/2,3',6-Trichlorobiphenyl	13.3	12.9	14.1	12.5
2,3',4-Trichlorobiphenyl	21.4	20.2	21.8	20.0
2,3',5-Trichlorobiphenyl	44.9	39.1	44.9	39.1
2,4,4'-Trichlorobiphenyl	222	215	222	215
2,4,5-Trichlorobiphenyl	1.11	2.14	4.78	0.945
2,4,6-Trichlorobiphenyl			4.48	0.158
2,4',5-Trichlorobiphenyl	223	195	223	195
2',3,5-Trichlorobiphenyl			4.48	0.158
3,3',4-Trichlorobiphenyl	4.29	2.71	6.32	2.62
3,3',5-Trichlorobiphenyl			4.48	0.158
3,4,4'-Trichlorobiphenyl	30.1	25.9	30.1	25.9
3,4,5-Trichlorobiphenyl			4.48	0.158
3,4',5-Trichlorobiphenyl			4.48	0.158
2,2',3,3'-TeCB	14.4	15.4	19.2	15.4
2,2',3,4-TeCB/2,3,4',6-TeCB/2,3',4',6-TeCB/2,3',5,5'-TeCB	108	106	108	106
2,2',3,4'-TeCB/2,3,3',6-TeCB	35.7	35.5	37.3	34.8
2,2',3,5-TeCB/2,2',4,5'-TeCB	141	142	141	142
2,2',3,5'-TeCB	173	192	173	192

TABLE D5
Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration

Analyte	Mean Concentration	Standard Deviation	Mean LOQ	Standard Deviation
2,2',3,6-TeCB	17.7	18.1	21.7	17.8
2,2',3,6'-TeCB	5.75	3.36	11.4	3.97
2,2',4,4'-TeCB	45.1	39.3	45.1	39.3
2,2',4,5-TeCB/2,4,4',6-TeCB	26.1	27.2	29.4	26.6
2,2',4,6-TeCB			8.96	0.314
2,2',4,6'-TeCB	6.15	3.60	11.8	4.51
2,2',5,5'-TeCB/2,3',4,6-TeCB	371	441	371	441
2,2',5,6'-TeCB	20.0	19.3	24.1	19.9
2,2',6,6'-TeCB			8.96	0.314
2,3,3',4-TeCB			8.96	0.314
2,3,3',4'-TeCB/2,3,4,4'-TeCB	70.4	80.9	70.4	80.9
2,3,3',5-TeCB			8.96	0.314
2,3,3',5'-TeCB			8.96	0.314
2,3,4,5-TeCB			8.96	0.314
2,3,4,6-TeCB			8.96	0.314
2,3,4',5-TeCB	1.25		9.40	1.49
2,3,5,6-TeCB			8.96	0.314
2,3',4,4'-TeCB	104	116	104	116
2,3',4,5-TeCB			8.96	0.314
2,3',4,5'-TeCB			8.96	0.314
2,3',4',5-TeCB	197	238	197	238
2,3',5',6-TeCB			8.96	0.314
2,4,4',5-TeCB	67.2	80.3	68.0	78.7
2',3,4,5-TeCB			8.96	0.314
3,3',4,4'-TeCB	6.95	3.92	12.6	5.59
3,3',4,5-TeCB			8.96	0.314
3,3',4,5'-TeCB			8.96	0.314
3,3',5,5'-TeCB			8.96	0.314
3,4,4',5-TeCB			8.96	0.314
2,2',3,3',4-PeCB	16.7	24.2	20.8	20.5
2,2',3,3',5-PeCB			8.96	0.314
2,2',3,3',6-PeCB/2,2',3,5,5'-PeCB	106	124	106	124
2,2',3,4,4'-PeCB	27.6	38.1	30.9	34.3
2,2',3,4,5-PeCB			8.96	0.314
2,2',3,4,5'-PeCB/2,3,4',5,6-PeCB/2',3,4,5,6'-PeCB	66.5	79.2	66.5	79.2
2,2',3,4,6-PeCB/2,2',3,4',6-PeCB	38.1	47.7	41.4	45.0
2,2',3,4,6'-PeCB	0.882		9.03	0.385
2,2',3,4',5-PeCB/2,2',4,5,5'-PeCB	233	252	233	252
2,2',3,5,6-PeCB			8.96	0.314
2,2',3,5,6'-PeCB			8.96	0.314
2,2',3,5',6-PeCB/2,2',3',4,6-PeCB/2,2',4,5,6'-PeCB	237	287	237	287
2,2',3,6,6'-PeCB			8.96	0.314
2,2',3',4,5-PeCB	61.3	77.5	62.9	74.3
2,2',4,4',5-PeCB	109	116	109	116
2,2',4,4',6-PeCB			8.96	0.314
2,2',4,5',6-PeCB			8.96	0.314
2,2',4,6,6'-PeCB			8.96	0.314
2,3,3',4,4'-PeCB	32.4	31.4	32.4	31.4
2,3,3',4,5-PeCB	142	187	142	187
2,3,3',4',5-PeCB/2,3,3',4,6-PeCB	7.59	6.23	13.2	6.96
2,3,3',4,5'PeCB/2,3,3',5,6-PeCB	6.10	7.90	12.5	7.23
2,3,3',4',6-PeCB	127	142	127	142
2,3,3',5,5'-PeCB/2,3,4,4',6-PeCB	3.88	6.58	10.3	3.86
2,3,3',5',6-PeCB			8.96	0.314

TABLE D5
Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration

Analyte	Mean Concentration	Standard Deviation	Mean LOQ	Standard Deviation
2,3,4,4',5-PeCB	0.927		9.08	0.487
2,3',4,4',5-PeCB	130	198	131	192
2,3',4,4',6-PeCB	1.26		9.40	1.49
2,3',4,5,5'-PeCB			8.96	0.314
2,3',4,5',6-PeCB			8.96	0.314
2',3,3',4,5-PeCB			8.96	0.314
2',3,4,4',5-PeCB			8.96	0.314
2',3,4,5,5'-PeCB	1.49		9.64	2.26
3,3',4,4',5-PeCB			8.96	0.314
3,3',4,4,5'-PeCB			8.96	0.314
2,2',3,3',4,4'-HxCB/2,3,3',4',5,5'-HxCB	7.48	7.04	13.1	7.06
2,2',3,3',4,5-HxCB			8.96	0.314
2,2',3,3',4,5'-HxCB	2.52	0.495	9.86	2.00
2,2',3,3',4,6-HxCB			8.96	0.314
2,2',3,3',4,6'-HxCB/2,3,3',4,5',6-HxCB	18.9	18.6	21.3	17.5
2,2',3,3',5,5'-HxCB/2,2',3,4,5,6-HxCB	3.45	1.45	9.90	1.88
2,2',3,3',5,6-HxCB/2,2',3,4,5,6'-HxCB	2.79	2.62	10.1	2.75
2,2',3,3',5,6'-HxCB	14.0	12.9	18.0	12.6
2,2',3,3',6,6'-HxCB	16.1	18.9	20.9	18.3
2,2',3,4,4',5-HxCB			8.96	0.314
2,2',3,4,4',5'-HxCB/2,3,3',4',5,6-HxCB/2,3,3',4',5',6-HxCB	88.3	65.5	88.3	65.5
2,2',3,4,4',6-HxCB	89.2	68.4	89.2	68.4
2,2',3,4,4',6'-HxCB			8.96	0.314
2,2',3,4,5,5'-HxCB	6.01	4.88	11.7	4.70
2,2',3,4,5',6-HxCB	1.31		9.46	1.67
2,2',3,4,6,6'-HxCB			8.96	0.314
2,2',3,4',5,5'-HxCB/2,3,3',4',5',6-HxCB	25.0	21.5	25.8	21.2
2,2',3,4',5,6-HxCB	1.03		9.18	0.768
2,2',3,4',5,6'-HxCB			8.96	0.314
2,2',3,4',6,6'-HxCB			8.96	0.314
2,2',3,5,5',6-HxCB	21.9	18.2	24.3	18.1
2,2',3,5,6,6'-HxCB			8.96	0.314
2,2',4,4',5,5'-HxCB	587	1,513	587	1,514
2,2',4,4',5,6'-HxCB	1.59		9.75	2.59
2,2',4,4',6,6'-HxCB			8.96	0.314
2,3,3',4,4',5-HxCB	1.79	0.382	9.05	0.423
2,3,3',4,4',5'-HxCB			8.96	0.314
2,3,3',4,4',6-HxCB/2,3,3',4,5,6-HxCB	3.79	2.82	10.2	2.67
2,3,3',4,5,5'-HxCB			8.96	0.314
2,3,4,4',5,6-HxCB			8.96	0.314
2,3',4,4',5,5'-HxCB	0.865		9.02	0.352
2,3',4,4',5',6-HxCB			8.96	0.314
3,3',4,4',5,5'-HxCB			8.96	0.314
2,2',3,3',4,4',5-HpCB	10.9	9.25	14.1	8.29
2,2',3,3',4,4',6-HpCB	0.945		9.10	0.532
2,2',3,3',4,5,5'-HpCB			8.96	0.314
2,2',3,3',4,5,6-HpCB			8.96	0.314
2,2',3,3',4,5,6'-HpCB	9.18	8.79	13.2	7.48
2,2',3,3',4,5',6-HpCB			8.96	0.314
2,2',3,3',4,6,6'-HpCB			8.96	0.314
2,2',3,3',4',5,6-HpCB	8.07	9.24	12.9	7.46
2,2',3,3',5,5',6-HpCB	4.98	7.90	11.4	5.64
2,2',3,3',5,6,6'-HpCB	4.77	8.51	11.3	5.51
2,2',3,4,4',5,5'-HpCB	33.4	21.9	33.4	21.9
2,2',3,4,4',5,6-HpCB			8.96	0.314

TABLE D5
Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration

Analyte	Mean Concentration	Standard Deviation	Mean LOQ	Standard Deviation
2,2',3,4,4',5,6'-HpCB/2,2',3,4',5,5',6-HpCB	38.1	34.0	38.1	34.0
2,2',3,4,4',5',6-HpCB	7.49	9.53	12.3	7.22
2,2',3,4,4',6,6'-HpCB			8.96	0.314
2,2',3,4,5,5',6-HpCB			8.96	0.314
2,2',3,4,5,6,6'-HpCB			8.96	0.314
2,2',3,4',5,6,6'-HpCB			8.96	0.314
2,3,3',4,4',5,5'-HpCB			8.96	0.314
2,3,3',4,4',5,6-HpCB			8.96	0.314
2,3,3',4,4',5',6-HpCB			8.96	0.314
2,3,3',4,5,5',6-HpCB			8.96	0.314
2,3,3',4',5,5',6-HpCB			8.96	0.314
2,2',3,3',4,4',5,5'-OCB	2.41		14.2	4.22
2,2',3,3',4,4',5,6-OCB			13.0	1.07
2,2',3,3',4,4',5,6'-OCB/2,2',3,4,4',5,5',6-OCB	6.94	15.4	16.6	8.94
2,2',3,3',4,4',6,6'-OCB			13.0	1.07
2,2',3,3',4,5,5',6-OCB			13.0	1.07
2,2',3,3',4,5,6,6'-OCB	7.65	17.5	17.3	10.4
2,2',3,3',4,5',6,6'-OCB			13.0	1.07
2,2',3,3',4,5,5',6'-OCB	1.64		13.4	1.85
2,2',3,3',5,5',6,6'-OCB	3.18		15.0	6.73
2,2',3,4,4',5,6,6'-OCB			13.0	1.07
2,3,3',4,4',5,5',6-OCB			13.0	1.07
2,2',3,3',4,4',5,5',6-NCB	6.15		18.0	16.5
2,2',3,3',4,4',5,6,6'-NCB	1.65		13.4	1.90
2,2',3,3',4,5,5',6,6'-NCB	4.36		16.1	10.6
DeCB	6.17		18.0	16.6

^a Data presented as pg analyte/g feed; LOQ=Limit of quantitation. Dioxin and dibenzofuran congeners were analyzed by EPA Method 1613, using GC with high resolution mass spectrometry and isotope dilution. PCB congeners were analyzed by EPA Method 1668, using GC with high resolution mass spectrometry.

^b Mean concentration of samples with measurable concentrations; blanks indicate concentrations below the limit of detection in all samples.

APPENDIX E

SENTINEL ANIMAL PROGRAM

METHODS	186
RESULTS	186

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from five male and five female sentinel rats at 1 month; five sentinel male rats at 6 and 18 months; six sentinel male rats at 12 months; and five females at the end of the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corp. (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test

Time of Analysis

RATS

2-Year Study

ELISA

<i>Mycoplasma arthritidis</i>	Study termination
<i>Mycoplasma pulmonis</i>	Study termination
PVM (pneumonia virus of mice)	1, 6, 12, and 18 months, study termination
RCV/SDA	
(rat coronavirus/sialodacryodenitis virus)	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination

Immunofluorescence Assay

<i>M. arthritidis</i>	Study termination
Parvovirus	1, 6, 12, and 18 months, study termination
PVM	12 months
RCV/SDA	Study termination

RESULTS

All serology tests were negative.

APPENDIX F

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION	189
MODEL DEVELOPMENT	190
RESULTS AND DISCUSSION	191
TABLE F1 Model Parameters for a Physiologically Based Pharmacokinetic Model of a Binary Mixture of PCB 126 and PCB 118	193
TABLE F2 Partition Coefficients for a Physiologically Based Pharmacokinetic Model of a Binary Mixture of PCB 126 and PCB 118	193
FIGURE F1 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the Vehicle Control Group in the 2-Year Gavage Study	194
FIGURE F2 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the 7 ng TEQ/kg Group in the 2-Year Gavage Study	195
FIGURE F3 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the 22 ng TEQ/kg Group in the 2-Year Gavage Study	196
FIGURE F4 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the 72 ng TEQ/kg Group in the 2-Year Gavage Study	197
FIGURE F5 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the 216 ng TEQ/kg Group in the 2-Year Gavage Study	198
FIGURE F6 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Group in the 2-Year Gavage Study	199
FIGURE F7 Model Predictions and Observed Data for PCB 126 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Stop-Exposure Group in the 2-Year Gavage Study	200
FIGURE F8 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the Vehicle Control Group in the 2-Year Gavage Study	201
FIGURE F9 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the 7 ng TEQ/kg Group in the 2-Year Gavage Study	202
FIGURE F10 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the 22 ng TEQ/kg Group in the 2-Year Gavage Study	203

FIGURE F11 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the 72 ng TEQ/kg Group in the 2-Year Gavage Study	204
FIGURE F12 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the 216 ng TEQ/kg Group in the 2-Year Gavage Study	205
FIGURE F13 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Group in the 2-Year Gavage Study	206
FIGURE F14 Model Predictions and Observed Data for PCB 118 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Stop-Exposure Group in the 2-Year Gavage Study	207

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model for the mixture of PCB 126 and PCB 118 was developed in support of the dioxin toxic equivalency factor (TEF) evaluation studies. The model is based on a PBPK model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The PCB 126 data in the mixture was also compared to the predictions of a PBPK model for PCB 126 alone. A goal for the PBPK modeling of the disposition data from the TEF studies is a general model for the tissue distribution of dioxin-like compounds (DLCs) and mixtures of compounds that interact with the aryl hydrocarbon receptor (AhR) in the Sprague-Dawley rat.

One key aspect to understanding the toxicity of an agent is how dose is related to the toxicity of concern. The utility of a PBPK model is its ability to predict alternate measures of “dose” other than those that are readily measured (such as administered dose or tissue concentrations). In addition, the kinetics of tissue distribution of a compound can be compared between different routes and patterns of exposure. Also, an understanding of the factors that govern the tissue distribution of a compound and its metabolites and subsequent molecular/biochemical responses may provide insights into the factors governing the dose response of toxicity, site specificity, and mode of action of the compound under study.

In general, PBPK models have been validated in the observable response range for numerous compounds in both animals and humans, making them useful for risk assessment, especially for cross-species extrapolation. They also aid in extrapolation from one chemical to other structurally related chemicals because many of the components of the model are the same or can be deduced for related compounds.

The time course of behavior in each compartment of a PBPK model is defined by equations and model parameters for input and loss of chemical. The specific structure of a PBPK model and the assumptions used to develop the model are encoded in the equations. The model’s physiological parameters are, in many cases, compound independent, well established, and available in the literature (e.g., rates of blood flow, blood volume, tissue volumes, etc.). Physicochemical parameters are used that are often specific to a given compound but can be measured experimentally and may be available in the literature. Some of these parameters may not be available *a priori* and so have to be determined within the framework of the model by an iterative process of changing the parameter, fitting the model to a given dataset and evaluating the goodness of the fit of the model to the data. Careful evaluation of any PBPK model must involve the adequacy of its fit to the data, the relationship of its structure to the underlying biology, and the mathematical details linking these two. In addition, the biological plausibility of optimized parameters needs to be considered. Validation of the model using datasets that were not used in its construction lends more credence to the predictive power of a model.

The disposition of a chemical within the body is governed by the absorption of an administered chemical and its distribution among tissues, metabolism, and elimination from the body (ADME). These processes for TCDD and related DLCs in part depend upon their physicochemical properties (e.g., tissue permeation constants, partition coefficients, kinetic constants, and biochemical parameters) and physiological parameters (e.g., organ volumes and blood flow rates). The PBPK model for TCDD describes the pharmacokinetics of TCDD by a series of mass-balance differential equations in which the state variables represent the concentration of TCDD in anatomically distinct regions or compartments of the body. These tissue “compartments” are linked by a physiologically realistic pattern of blood perfusion and flow through the different tissue compartments.

A model for the mixture of PCB 126 and PCB 118 was built from the model for TCDD. Separate models for PCB 118 and PCB 126 are linked as a mixture model by having both chemicals bind to AhR and cytochrome

P450 1A2 (CYP1A2). Data and a model for the chronic exposure of female Sprague-Dawley rats to PCB 126 alone were available to aid the model development. However, only data for PCB 118 as part of the mixture were available and no PCB 118 model was available. Because data was not available for PCB 118 administered alone, the mixture model was used to deduce parameters for PCB 118. The PCB 126 data in the mixture is predicted by a PCB 126-only model, suggesting that the pharmacokinetics of PCB 126 are not affected by the presence of PCB 118. The mixture model could not fit the tissue disposition data of both PCB 126 and PCB 118 without the addition of another protein to bind PCB 118.

MODEL DEVELOPMENT

The same basic model structure was used for all compounds studied in the dioxin TEF evaluation, with some of the model parameters, such as those parameters involved in metabolism or binding to AhR, unique to each compound. The common model for individual compounds was based upon the model of Kohn *et al.* (2001). The Kohn model is an extension of earlier PBPK models for TCDD in rats (Kohn *et al.*, 1993, 1996, 2001) that with each iteration has gone through further rounds of refinement and inclusion of increased biological complexity. A thorough summary of PBPK modeling for TCDD, including the basic model used in this study, can be found elsewhere (USEPA, 2000c).

Kohn's model includes compartments for fat, liver, kidney, gastrointestinal tract, muscle, and viscera with blood distributed among arterial, venous, and tissue capillary spaces. The model includes equations for the amounts of AhR, CYP1A1, CYP1A2, and CYP1B1 in the liver as well as equations describing the basal expression, induction by TCDD, and degradation of the mRNA for each of these. The amount of each enzyme depends on the time-lagged concentration of the corresponding mRNA. TCDD in the liver may bind to CYP1A2 and AhR. A key to the model is that the induction rates of all four represented mRNAs depend on the time-lagged concentration of AhR bound to TCDD. Induction increases from zero to a maximum rate as the concentration of the AhR-TCDD complex increases. The model also includes a blood protein that can bind TCDD. Because transthyretin (also known as prealbumin) can bind hydroxylated polychlorinated dibenzodioxins, and single doses of TCDD can cause prolonged decreases in this protein, a dose-dependent decrease of blood protein was included in the model. This protein-bound TCDD cannot enter the tissues in the model but may become free in the blood by dissociation or proteolysis. To fit data at both low and high doses, this model includes loss of TCDD from the liver by lysis of dead cells (as a result of hepatotoxicity) where the rate of cell death was assumed to increase as a hyperbolic function of the cumulative amount of unbound hepatic TCDD.

There were several steps to building a PBPK model for the dioxin TEF evaluation studies: addition of a lung compartment, conversion of the body weight function, functionally linking model protein levels and activity data, and linking the mixtures together. A lung compartment was added to the model because the NTP data for the TEF studies include lung tissue concentrations. The lung compartment is diffusion limited and includes the same equations used in the liver for AhR, CYP1A1, and CYP1B1. The lung and liver compartments use the same gene expression parameters on a per liter basis. Values of the lung partition coefficient and the lung permeability factor were estimated by optimization, fitting the model predictions to TCDD tissue data (liver, lung, fat, blood).

Kohn's model has a specific time-dependent function for the body weight. This function does not apply to female Sprague-Dawley rats. Body weights were available weekly for the first 12 weeks of the study and then monthly for the remainder of the study, so these weights were used in the model. For each dose group, the interpolated mean body weights were used as the time-dependent body weight function.

Functional relationships linking CYP1A1 to 7-ethoxyresorufin-*O*-deethylase (EROD) activity and CYP1A2 to acetanilide-4-hydroxylase (A4H) activity were added to the model. The Kohn *et al.* (2001) TCDD model was used to set up these relationships. The model was run for the dioxin TEF evaluation TCDD doses (0, 3, 10, 22, 46, 100 ng/kg per day) to get model predicted CYP1A1 and CYP1A2 at 14, 31, and 53 weeks for each dose. EROD

data were fit as a Hill function of model predicted CYP1A1 while A4H activity was fit as a linear function of CYP1A2.

Partition coefficients for the chemicals in the mixture were based on the partition coefficients in Kohn's TCDD model. Kohn fit the TCDD partition coefficients along with the tissue permeability. Assuming that the permeability is the same for TCDD, PCB 126, and PCB 118, the values from Kohn's model can be used, and only partition coefficients are needed for PCB 126 and PCB 118. The ratios of partition coefficients (*n*-octanol:water; log P) were used to scale the TCDD partition coefficients to the partition coefficients of the binary mixture chemicals. Tissue partition coefficients (PC) of TCDD were multiplied by the ratio of log P values, i.e.,

$$PC_{PCB\ 126} = PC_{TCDD} \cdot \frac{\log P_{PCB\ 126}}{\log P_{TCDD}}$$

While many model parameters might be different for each dioxin-like chemical, the procedure was to start with a small set of the most likely parameters. The parameters for binding to AhR, CYP1A2, and blood protein were the first group. In turn, parameters for metabolism, absorption, and hepatotoxicity were added to the list of chemical specific parameters. The binding, metabolic, hepatotoxic, and absorption parameters were estimated by fitting the model predictions to logarithmic values of liver EROD and A4H activities and tissue concentration data (liver, fat, blood, lung). Two parameters describing hepatotoxicity, k_{lysis} and $k_{recovery}$, are included in the optimizations because they are multipliers of the chemical concentration in the cytotoxicity equations (Kohn *et al.*, 2001). Thus, the model can represent the differences in the amount of chemical causing liver tissue damage among the dioxin-like chemicals.

The mixture model was constructed by modifying Kohn's model to include the appropriate number of compounds. Each compound in the mixture has unique binding constants. Binding to blood protein, the AhR, and CYP1A2 is represented as noncompetitive binding. The metabolism of each compound is assumed to occur independently. Hepatotoxicity constants and partition coefficients are unique for each compound. All of the other model parameters are kept as constants from Kohn's model. Background concentrations for PCB 126 and PCB 118 were computed from measured concentrations in the NTP feed. The model was written in Simulink and all optimizations were run in Matlab.

One potentially important difference between modeling PCBs and TCDD not included in the present model is a rat liver cytosolic protein different from the AhR and CYP1A2 that binds PCBs but not dioxin (Buff and Brundl, 1992; Brundl and Buff, 1993). While little is known about this PCB binding protein, its effects may need to be added in applications of the model involving multiple PCBs in a mixture. A model was set up with a PCB binding protein to explore this possibility.

RESULTS AND DISCUSSION

PCB 126 parameters were previously estimated by fitting PCB 126 tissue and activity data when PCB 126 was administered alone (Table F1). PCB 118 has not been previously administered alone for a chronic study; therefore, several attempts were made to optimize parameters for PCB 118 to fit the PCB mixture data.

For the first analysis, the model was run as though PCB 126 was administered in the absence of PCB 118. When the data for PCB 126 is modeled in the absence of PCB 118, the model gives predictions that are consistent with the PCB mixture data of PCB 126 concentrations in the liver, fat, blood, and lung (Figures F1 to F7). The highest two PCB 126 doses are higher than the highest dose of PCB 126 that was used to estimate the PCB 126 parameters (1,000 ng/kg per day). This serves to validate the previous model estimates describing the toxicokinetics of PCB 126. The model also predicts liver EROD and A4H activity levels that are consistent with the PCB mixture data. These results suggest that the toxicokinetics of PCB 126 in the PCB mixture are not significantly affected by

the presence of PCB 118. These results also suggest that the liver EROD and A4H activity in the PCB mixture study is primarily a result of the binding of PCB 126 to the AhR.

For the second analysis, many attempts were made to fit the PCB 118 tissue data by changing PCB 118 parameters. Some fits were able to describe the lowest dose, however none of the fits were able to represent all the data across all doses. One possibility is that we need to represent competitive binding of PCB 118 and PCB 126 in the model. Another possibility is that PCB 118 and/or PCB 126 binds to the PCB binding protein in the liver.

For the third analysis, the model was modified to include the liver PCB binding protein that has been shown to bind PCBs in the liver but not TCDD. Because little information is known about this protein, only the basic structure of binding, dissociation, and degradation was added into the model. The four additional parameters were then estimated along with the chemical-dependent parameters for PCB 118 (Table F1). This model prediction was able to fit to fat and liver data but was unable to fit to lung and blood data for PCB 118 (Figures F8 to F14). This optimization did not change the model prediction for PCB 126 tissue data compared to modeling PCB 126 alone (not shown). The EROD and A4H activity predictions were also unchanged compared with modeling PCB 126 alone (Figures F1 to F7). This suggests that PCB 118 is binding to something in the liver, possibly PCB binding protein, and is not binding to the AhR in any significant amount in the presence of PCB 126.

While DLCs are found ubiquitously as mixtures, only limited analyses have previously been performed on dioxin mixtures. Acute effects of a TCDD/PCB 153 mixture were determined with interactive pharmacokinetic effects only occurring at high doses (Van Birgelen *et al.*, 1996). Another acute study demonstrated that a PCB 126/PCB 153 mixture caused an increase in liver concentration of PCB 153 and a decrease in fat concentration of PCB 153 (Lee *et al.*, 2002). To describe the dynamics in this study, the PBPK model of PCB 153 was modified to include a time-dependent increase of the liver partition coefficient and a decrease in the diffusion permeation constant in the fat. These modeling changes were to represent the increase in liver lipid content caused by PCB 126 and inhibition of lipoprotein lipase activity in fat by PCB 126, respectively.

TABLE F1
Model Parameters for a Physiologically Based Pharmacokinetic Model of a Binary Mixture of PCB 126 and PCB 118

	TCDD	PCB 126	PCB 118	Unit
<i>Background</i>	0.082	0.88	15.15	ng/kg per day
$K_{d_{protein}}$	10	572	6.5	nM
K_{AhR}	0.27	5.47	84	nM
K_{CYP1A2}	30	19.88	208	nM
$V_{metabolism}$	9.12	1.85	2.33	nmole/L per day
$K_{metabolism}$	0.968	31.45	2.98	nM
$n_{metabolism}$	1.12	—	—	—
$k_{absorption}$	4.8	1.08	0.84	kg ^{0.75} /day
$k_{binding}$	1,000	38.96	0.0000106	/nmole per day
k_{lysis}	200	20.86	2.46	/day
$k_{critical_{accumulation}}$	0.6	0.16	6.51	nmole
$k_{recovery}$	0.01	0.13	0.27	/day
$k_{critical_{concentration}}$	2	101.8	36.37	nM
$k_{PCBprot_{chem_{degradation}}}$	—	—	0.32	/day
$k_{liverInitPCBprot_{chem}}$	—	—	0.0000568	nmole
$K_{PCB_{protein}}$	—	—	59.67	nM
<i>PCB binding protein</i>	—	—	51,412	nmole

TABLE F2
Partition Coefficients for a Physiologically Based Pharmacokinetic Model of a Binary Mixture of PCB 126 and PCB 118

	TCDD	PCB 126	PCB 118
Fat	187.0	188.62	188.62
Muscle	4.48	4.52	4.52
Viscera	3.35	3.38	3.38
Liver	4.60	4.64	4.64
Kidney	3.35	3.38	3.38
Gastrointestinal tract	3.35	3.38	3.38
Lung	4.57	4.64	4.64

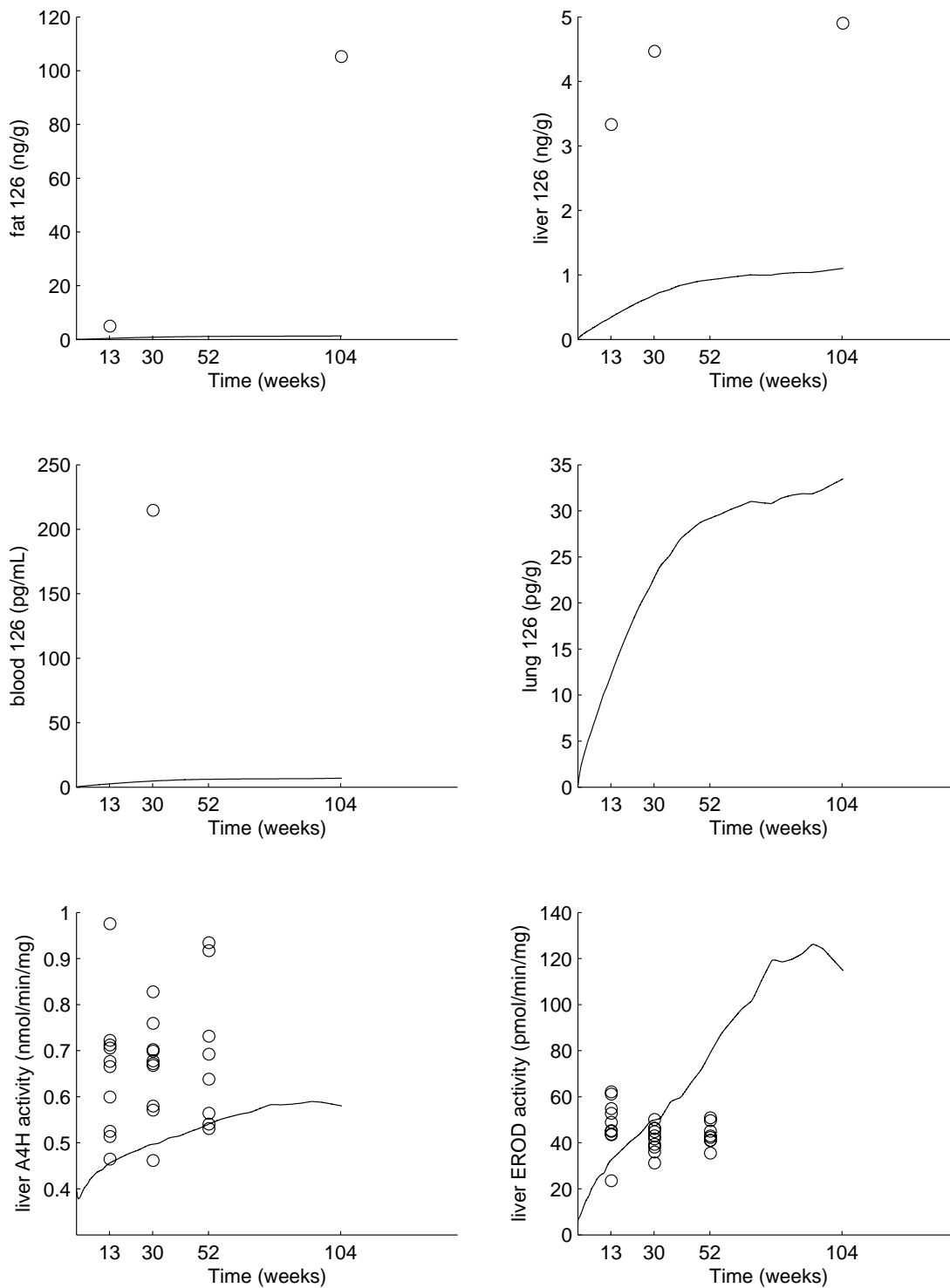


FIGURE F1
Model Predictions (-) and Observed Data (O) for PCB 126 Concentrations and Tissue Enzyme Activities for the Vehicle Control Group in the 2-Year Gavage Study

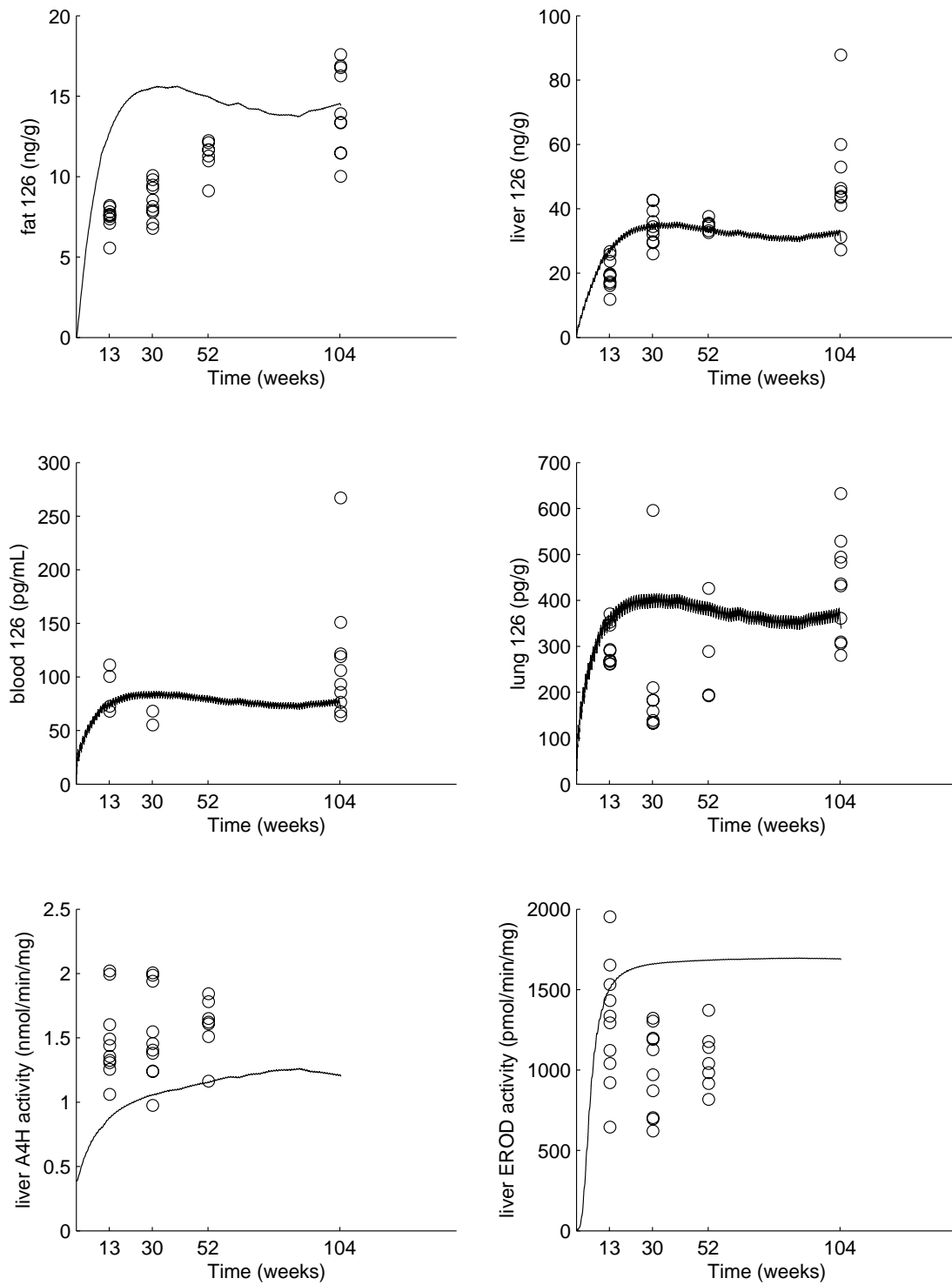


FIGURE F2
Model Predictions (–) and Observed Data (○) for PCB 126 Concentrations and Tissue Enzyme Activities for the 7 ng TEQ/kg Group in the 2-Year Gavage Study

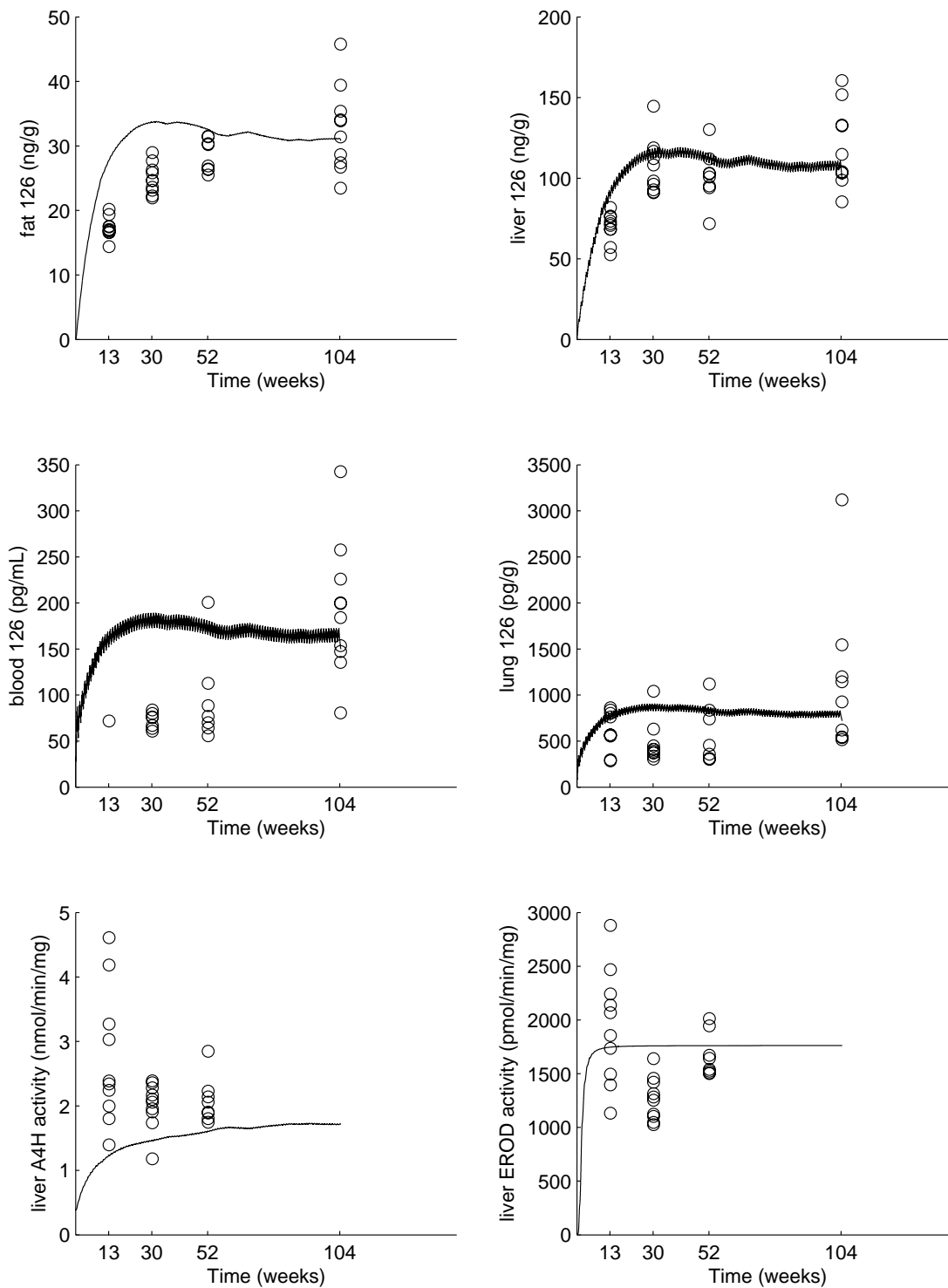


FIGURE F3
Model Predictions (-) and Observed Data (O) for PCB 126 Concentrations and Tissue Enzyme Activities for the 22 ng TEQ/kg Group in the 2-Year Gavage Study

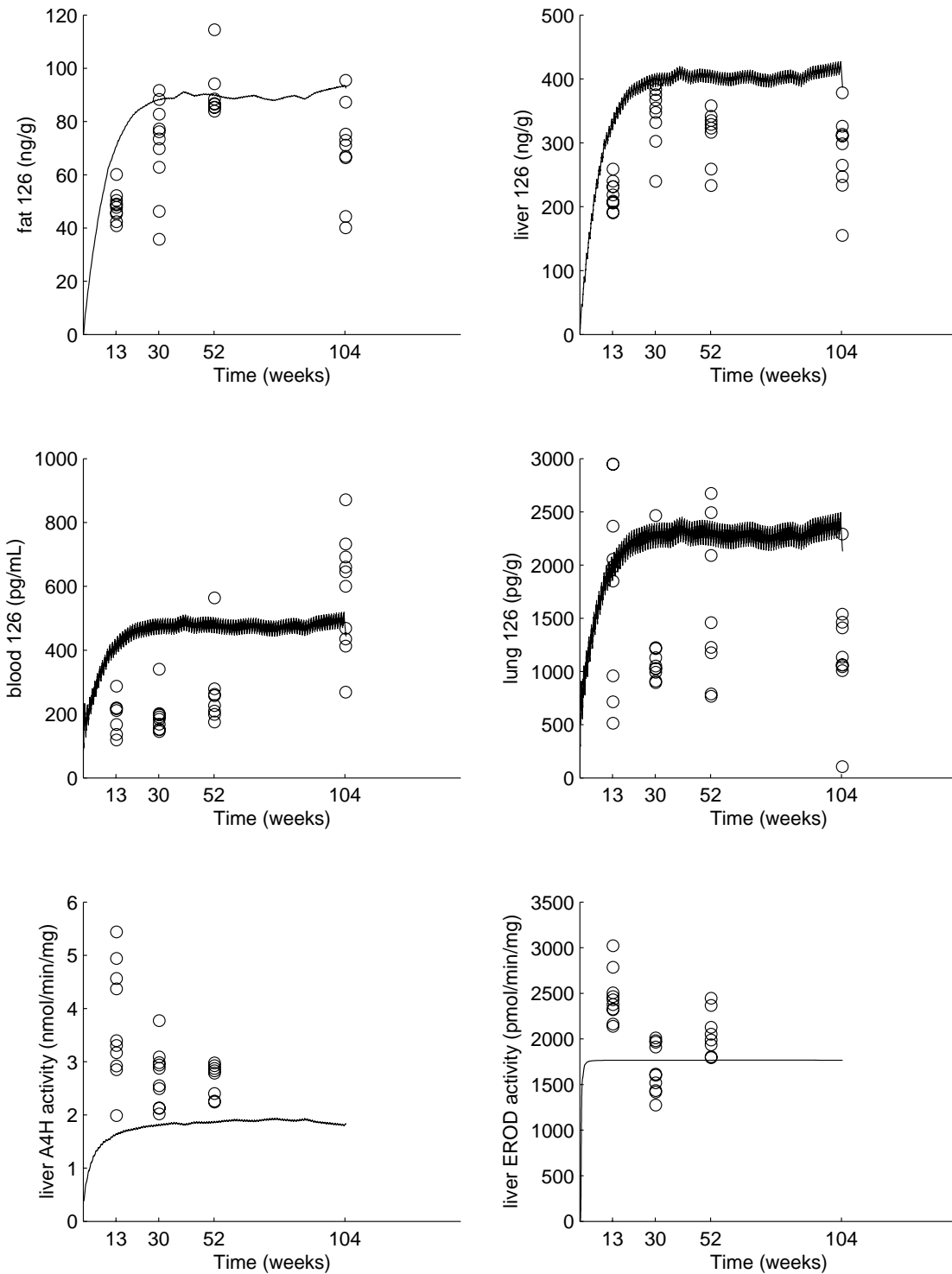


FIGURE F4
Model Predictions (-) and Observed Data (O) for PCB 126 Concentrations and Tissue Enzyme Activities for the 72 ng TEQ/kg Group in the 2-Year Gavage Study

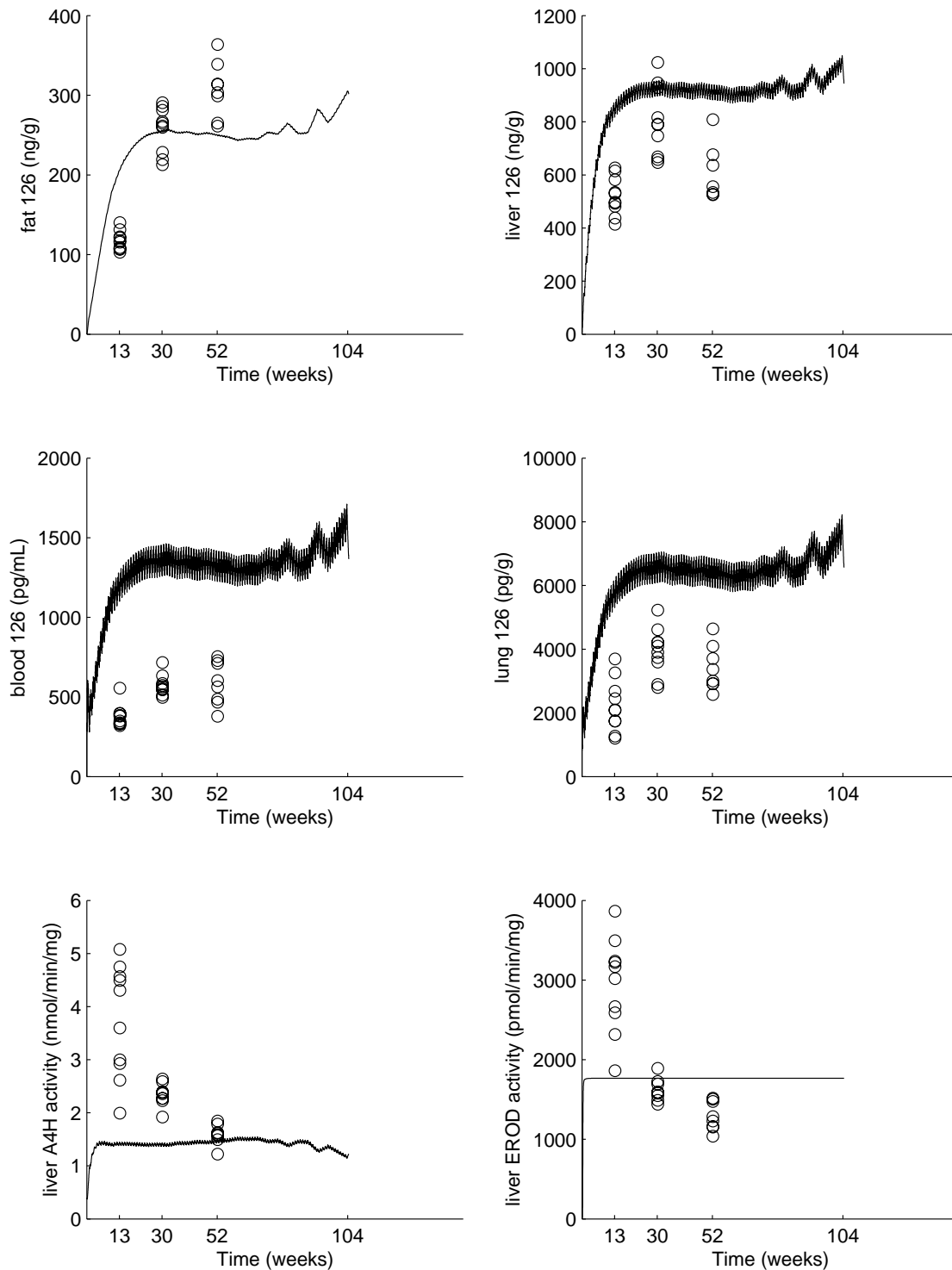


FIGURE F5
Model Predictions (-) and Observed Data (O) for PCB 126 Concentrations and Tissue Enzyme Activities for the 216 ng TEQ/kg Group in the 2-Year Gavage Study

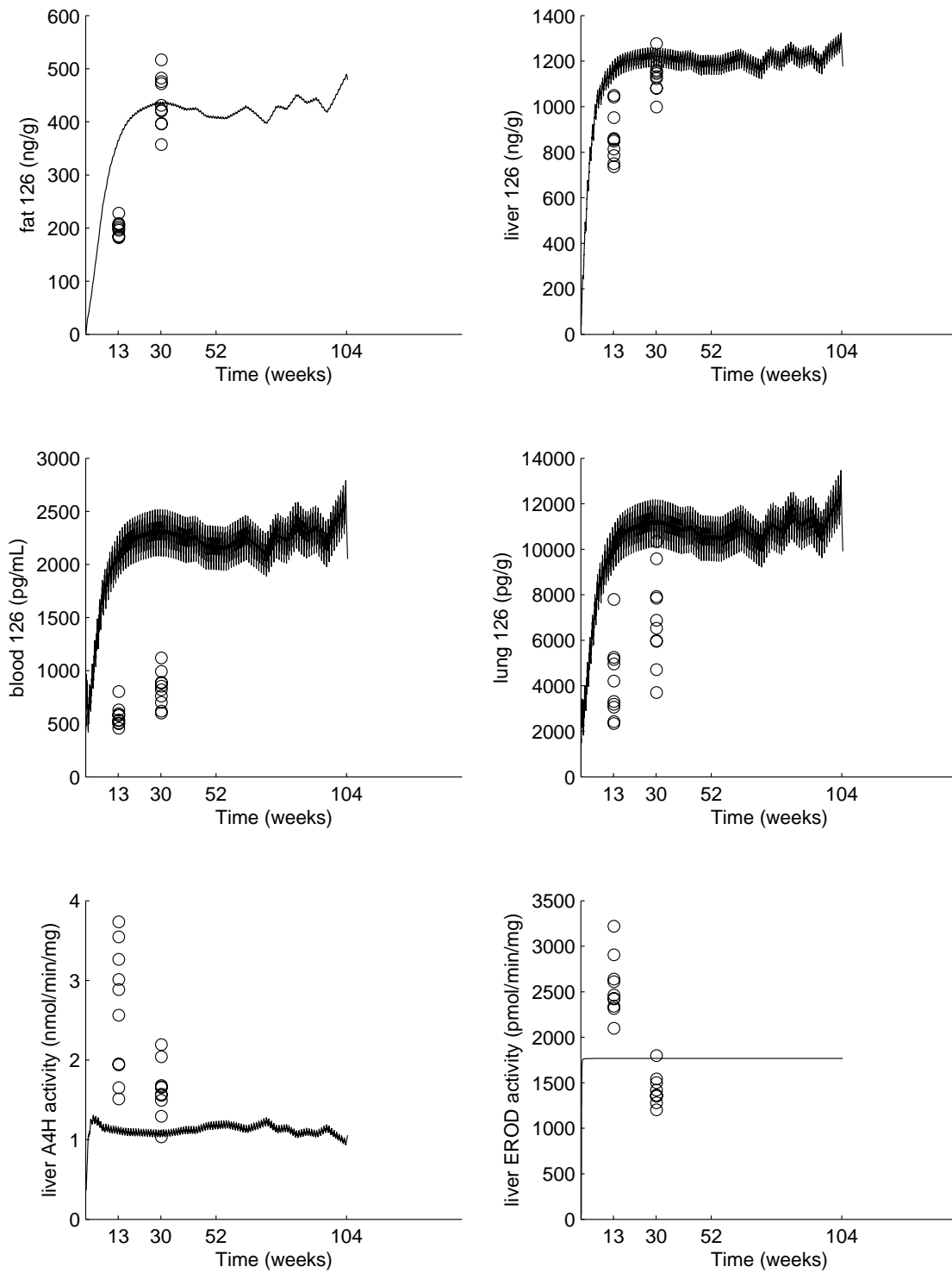


FIGURE F6
Model Predictions (–) and Observed Data (○) for PCB 126 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Group in the 2-Year Gavage Study

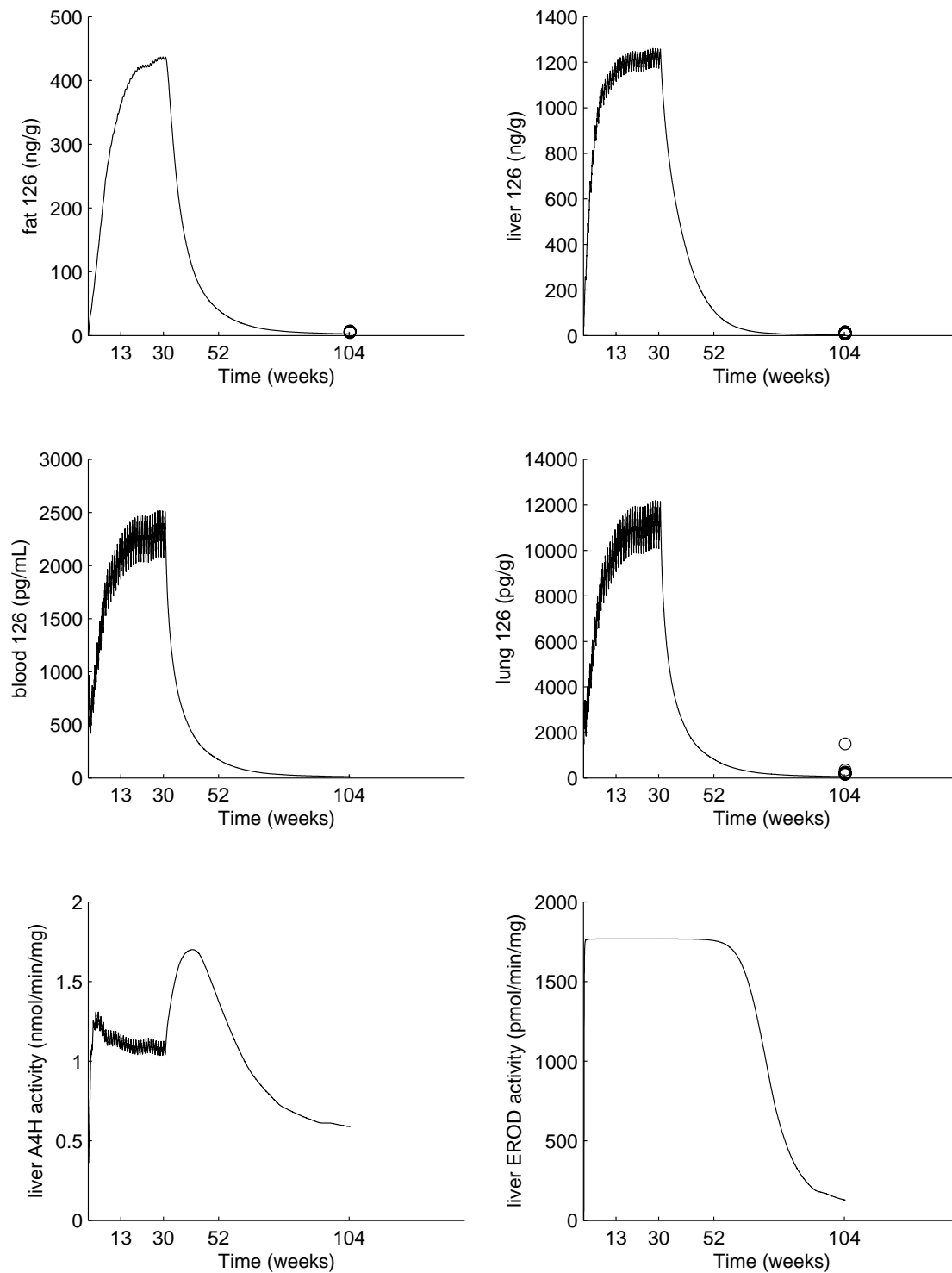


FIGURE F7
Model Predictions (—) and Observed Data (O) for PCB 126 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Stop-Exposure Group in the 2-Year Gavage Study

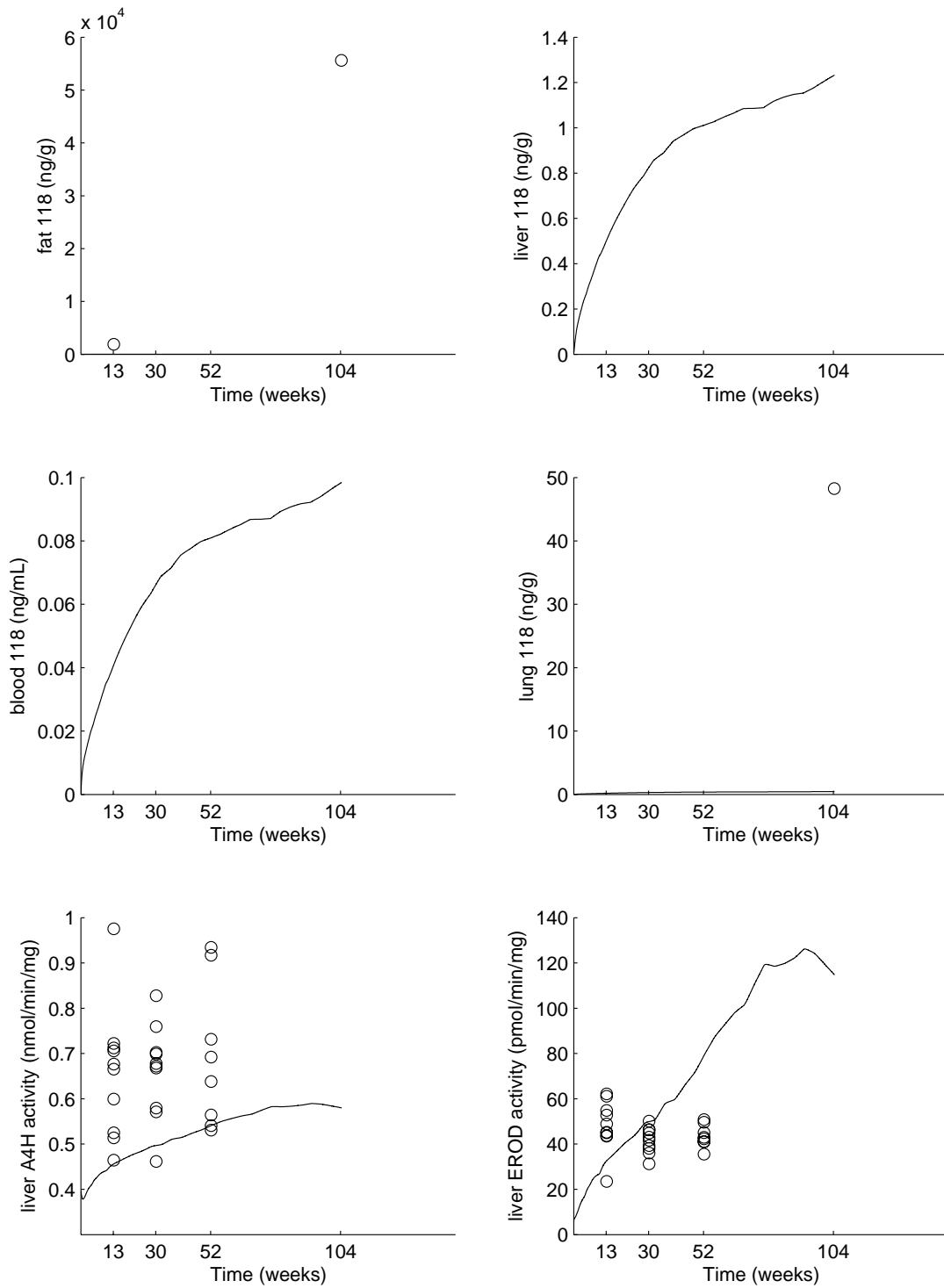


FIGURE F8
Model Predictions (–) and Observed Data (○) for PCB 118 Concentrations and Tissue Enzyme Activities for the Vehicle Control Group in the 2-Year Gavage Study

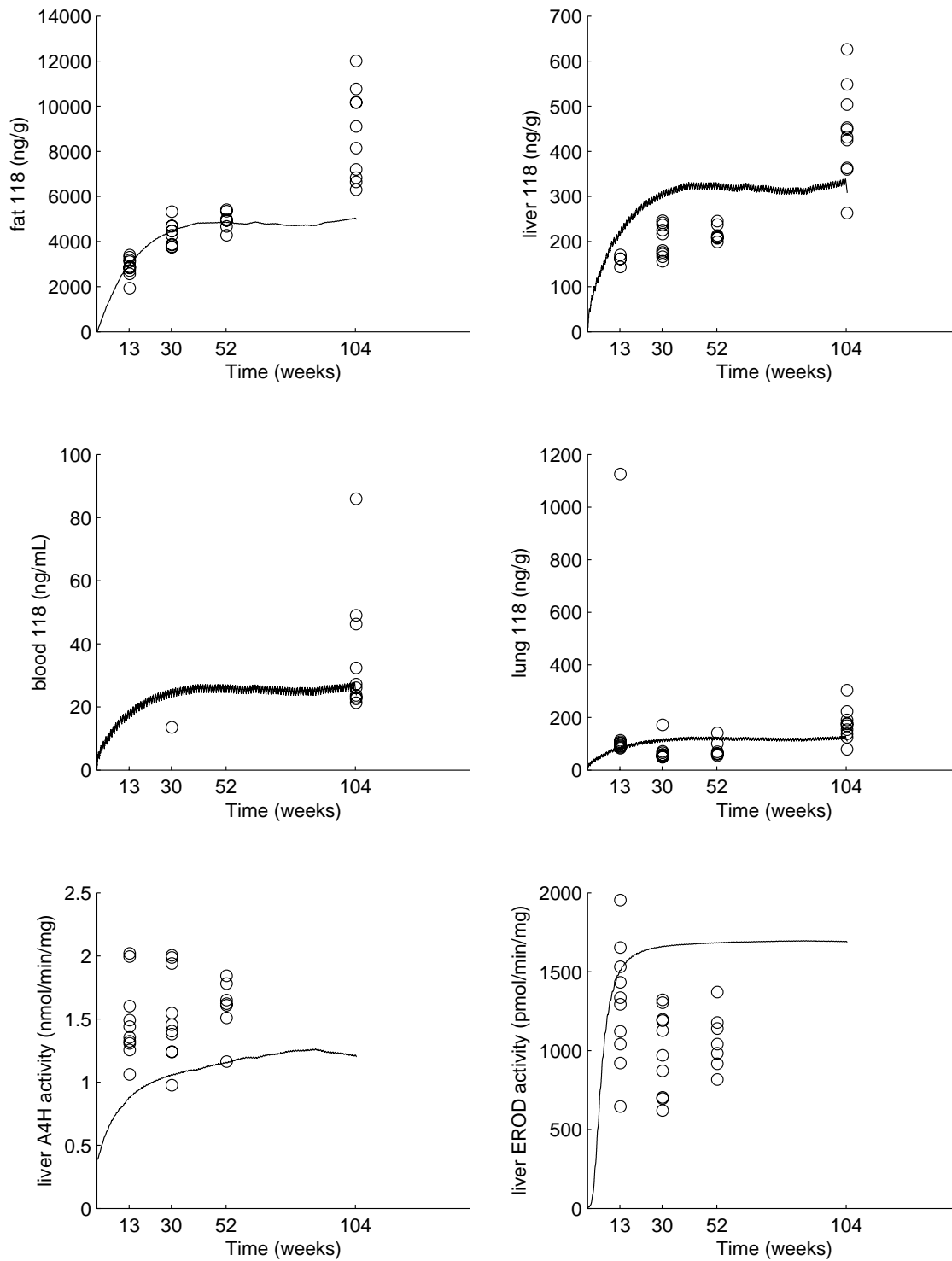


FIGURE F9
Model Predictions (-) and Observed Data (O) for PCB 118 Concentrations and Tissue Enzyme Activities for the 7 ng TEQ/kg Group in the 2-Year Gavage Study

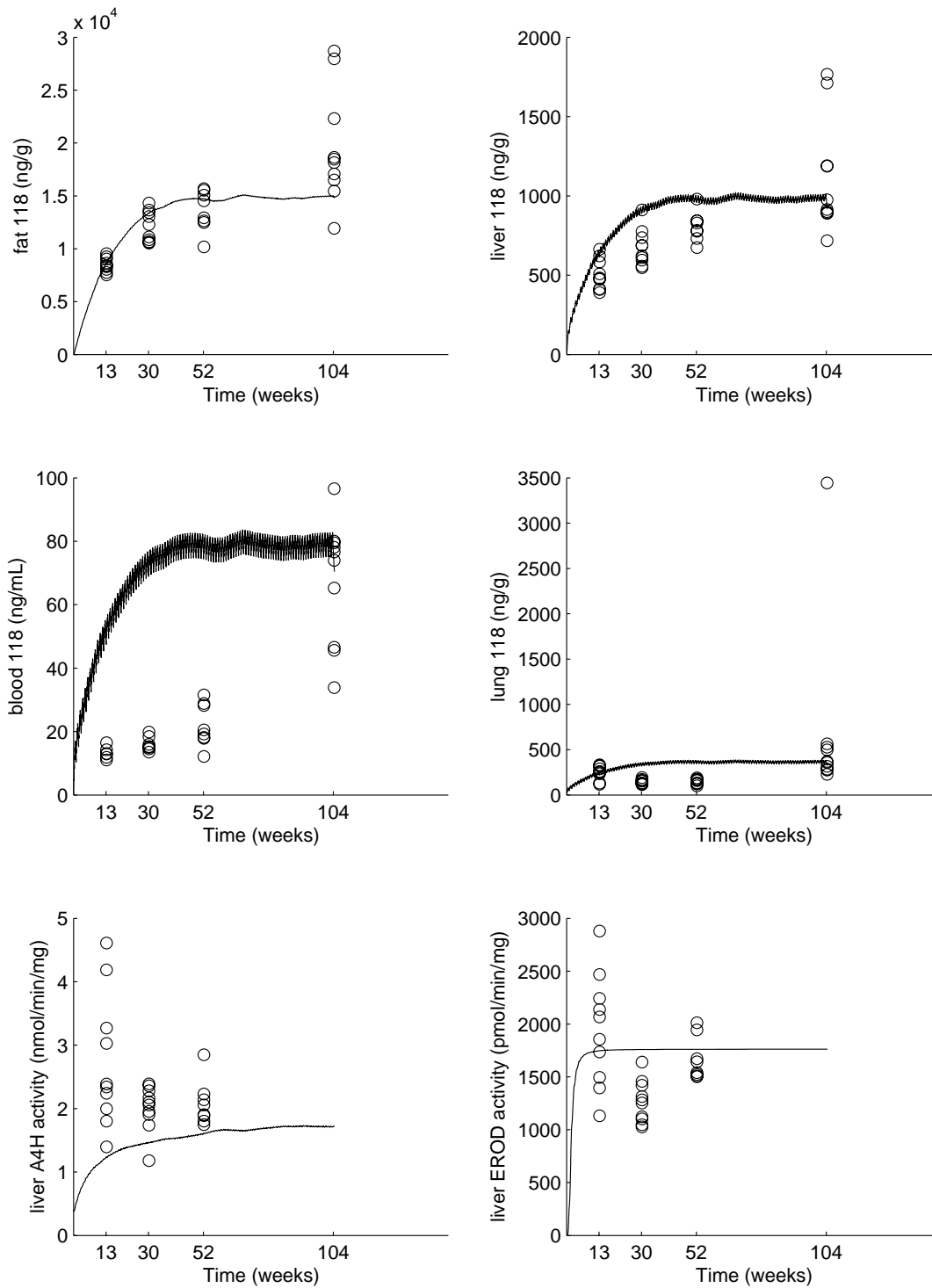


FIGURE F10
Model Predictions (–) and Observed Data (○) for PCB 118 Concentrations and Tissue Enzyme Activities for the 22 ng TEQ/kg Group in the 2-Year Gavage Study

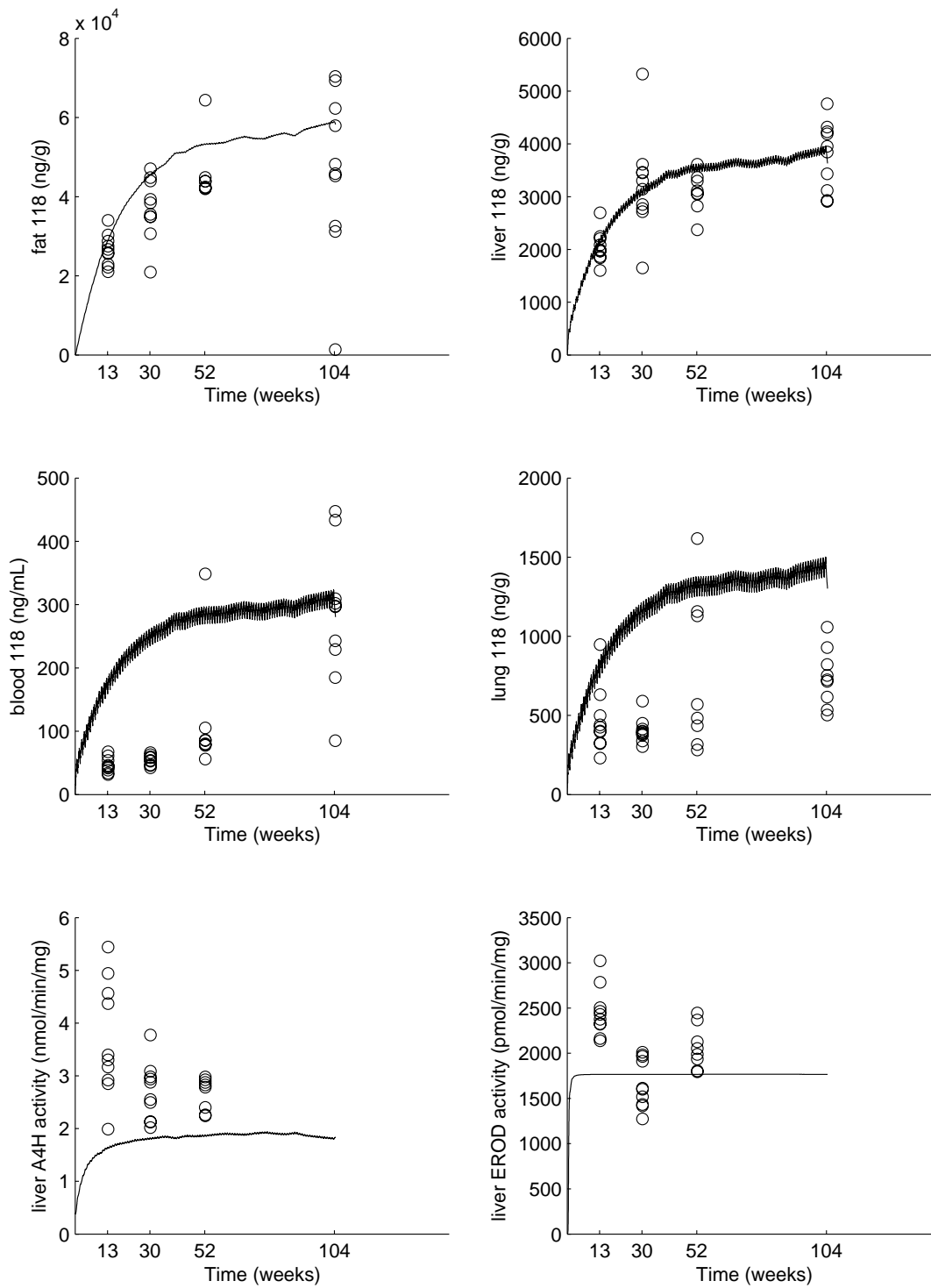


FIGURE F11
Model Predictions (-) and Observed Data (O) for PCB 118 Concentrations and Tissue Enzyme Activities for the 72 ng TEQ/kg Group in the 2-Year Gavage Study

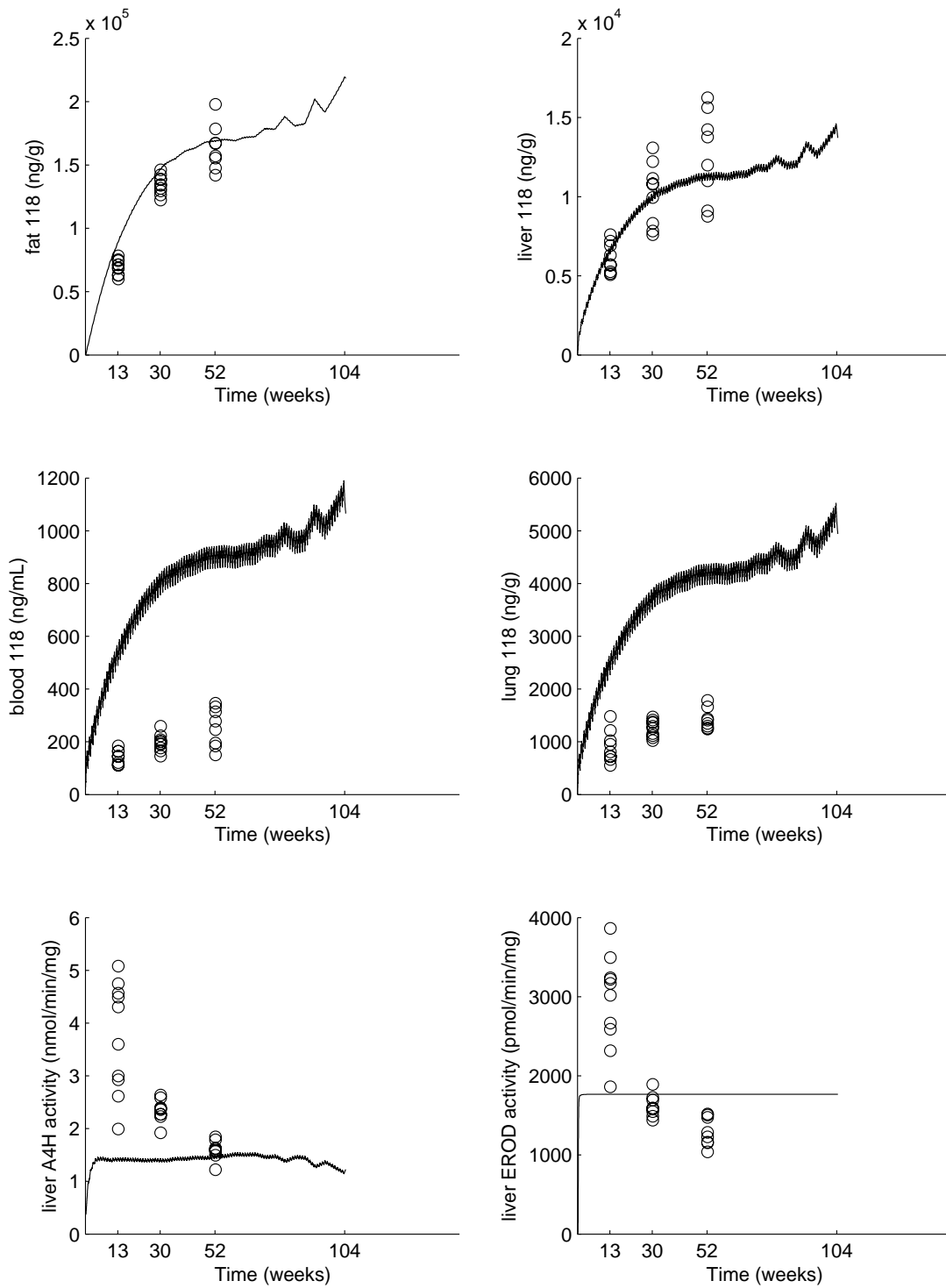


FIGURE F12
Model Predictions (–) and Observed Data (○) for PCB 118 Concentrations and Tissue Enzyme Activities for the 216 ng TEQ/kg Group in the 2-Year Gavage Study

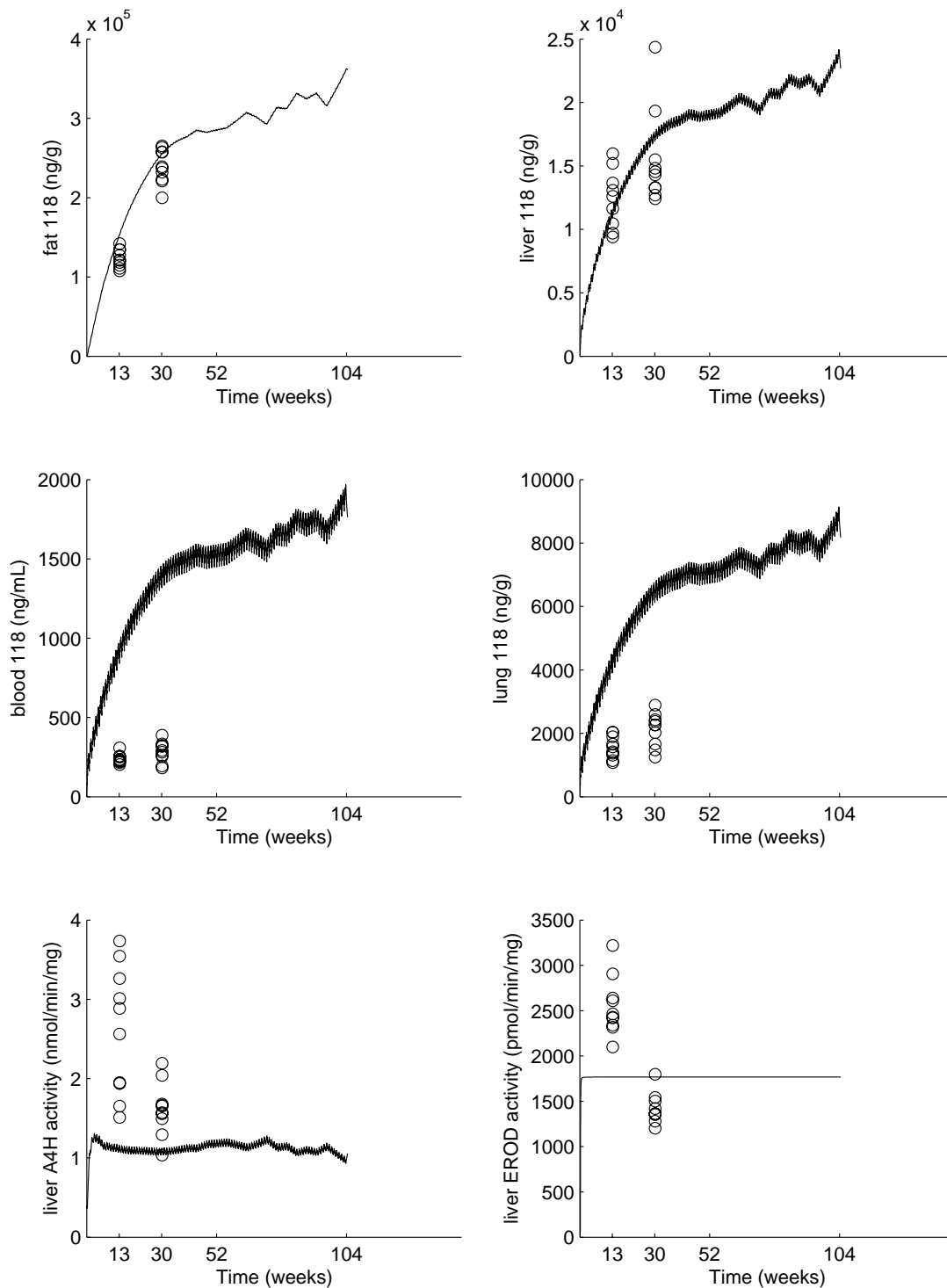


FIGURE F13
Model Predictions (-) and Observed Data (O) for PCB 118 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Group in the 2-Year Gavage Study

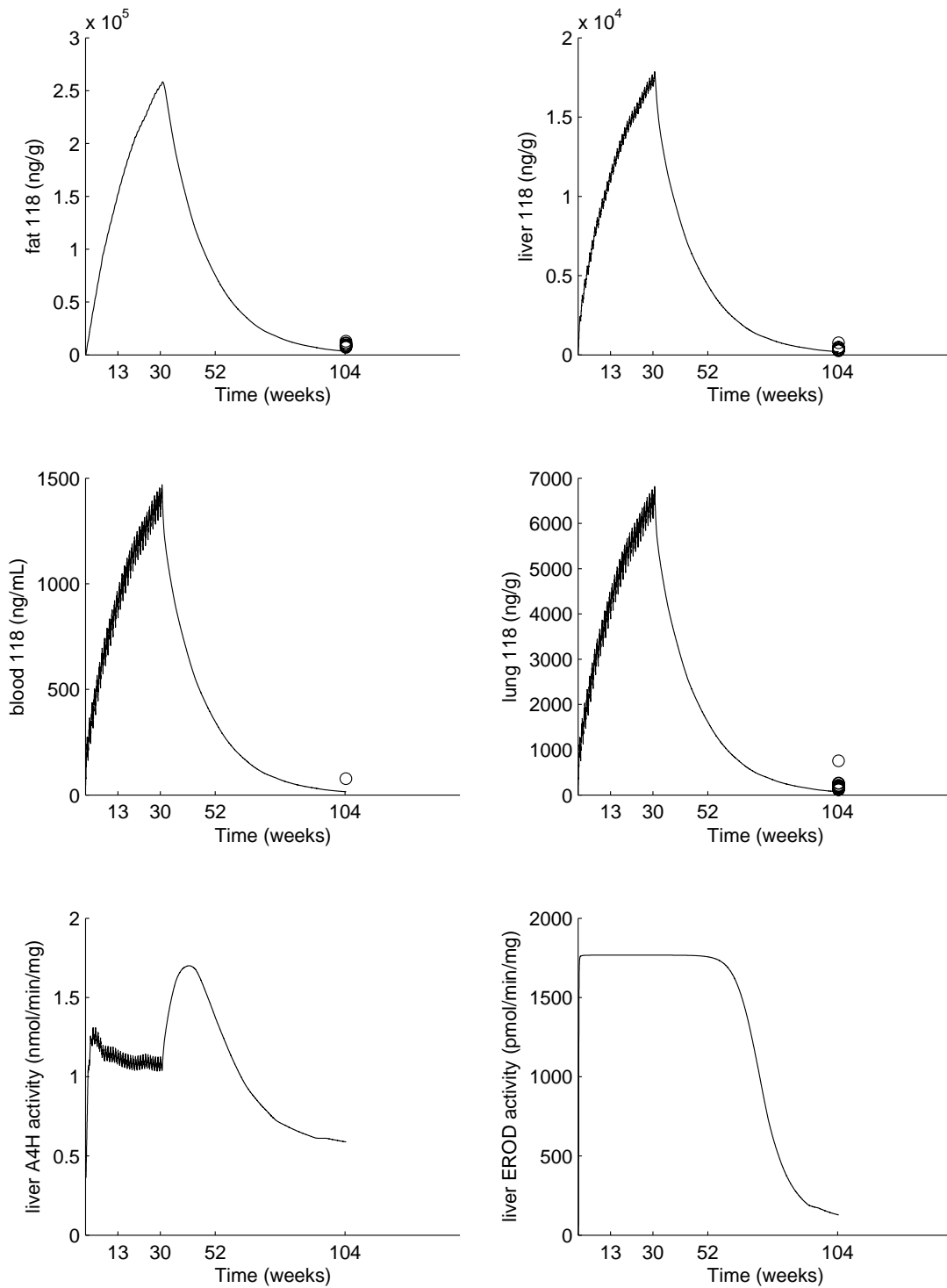


FIGURE F14
Model Predictions (–) and Observed Data (○) for PCB 118 Concentrations and Tissue Enzyme Activities for the 360 ng TEQ/kg Stop-Exposure Group in the 2-Year Gavage Study

APPENDIX G

TRANSPLANTATION OF LIVER AND LUNG NEOPLASMS

INTRODUCTION	210
MATERIALS AND METHODS	210
RESULTS	210
DISCUSSION	211
REFERENCES	212
TABLE G1 Findings from the Tumor Transplant Study in NCr-nu/nu Recipient Mice during the Chronic Gavage Study of the Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats	213
TABLE G2 Findings from the Tumor Transplant Study in SCID Recipient Mice during the Chronic Gavage Study of the Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats	215

TRANSPLANTATION OF LIVER AND LUNG NEOPLASMS

INTRODUCTION

Implantation experiments were performed using fragments of neoplasms diagnosed as cholangiocarcinoma of the liver and cystic keratinizing epithelioma of the lung. The purpose of this study was to evaluate the biological nature of these neoplasms and their ability for independent growth in the absence of exposure to the mixture of PCB 126 and PCB 118. Such transplantation protocols have been used previously to investigate the malignant nature of cholangiocarcinomas and other types of neoplasms derived from rodent chemical carcinogenesis studies or neoplasms originating from humans (Murakami *et al.*, 1987; Ulland *et al.*, 1989; Fidler, 1991; Maronpot *et al.*, 1991).

MATERIALS AND METHODS

Athymic NCr-nu/nu outbred and severe combined immunodeficiency (SCID) mice were used for the transplantation studies. All mice were examined and determined to be free of any evidence of overt disease or parasitism. Animals used for the transplantation studies were housed in a specially assigned room, and no other animals or chemicals on test were present in that area. Animals were housed five per cage. Feed and water were supplied *ad libitum*. Animal husbandry conditions were those routinely used in NTP experiments.

The neoplasms were excised from moribund female Harlan Sprague-Dawley rats (necropsied on days 672 to 709 of the 2-year gavage study) in the 216 ng TEQ/kg group (1,866 ng/kg PCB 126:300 µg/kg PCB 118). Each neoplasm was at least 2 mm in diameter. For the cholangiocarcinomas, neoplasms were taken from seven rats. For the cystic keratinizing epitheliomas, neoplasms were taken from five rats. Each neoplasm was transplanted into each strain of mice, up to eight per strain.

For each neoplasm selected for transplantation, the following data were recorded: donor identification number, organ of origin, trace gross lesion number, neoplasm size, block number, and removal date and time. Each neoplasm was assigned a unique identification number. The neoplasm diagnosis was confirmed by a pathologist before implantation. While the prosector conducted the necropsy procedure, the neoplasm transplant team proceeded with the liver fragmentation procedure. Transplantation was accomplished using a trocar, injecting the neoplasm fragments into the subcutis of the right hind leg.

Mice were followed for up to 3 months after implantation; the neoplasm implants were measured weekly and their dimensions recorded. At necropsy, the implant (if still present) was measured, harvested, and prepared for histopathologic evaluation. Slides of the originally selected neoplasms and of the transplantation sites were checked by Dr. Don M. Sells (Battelle Columbus Operations pathologist) and the diagnoses were confirmed by Dr. Abraham Nyska (NTP pathologist).

RESULTS

Transplantation of neoplasm fragments diagnosed as cholangiocarcinoma of the liver or cystic keratinizing epithelioma of the lungs into NCr-nu/nu and SCID mice did not result in proliferation of the masses. The transplanted fragments were either too small to be identified or appeared to be undergoing regression when examined microscopically. Approximately 30% to 43% of the transplanted neoplasms showed some degree of regression and no fragment of the transplanted neoplasm was identified in 48% to 60% of the mice (Tables G1 and G2).

In the case of cholangiocarcinoma, evaluation of transplanted sites indicated marked regression of the ducts with no or some biliary epithelial remnants (Tables G1 and G2). Collections of apparently preexisting remains of mucinous secretion were still present (Plates G1 and G2). When bile ducts were identified, they were lined by cuboidal epithelium, with no signs of atypia (Plates G3 and G4). The ducts were embedded within abundant, mature connective tissues, associated with aggregates of mononuclear inflammatory cells. No mitotic figures or local invasion were noted.

In the case of cystic keratinizing epithelioma, evaluation of transplanted sites indicated marked regression of the original tumor with no evidence of remnants or some cystic structures containing keratin remnants (Tables G1 and G2).

DISCUSSION

No neoplastic growth occurred in any of the recipients in this transplantation study. The negative results in the current study are in contrast to those of a previous study of the growth of transplanted cholangiocarcinomas induced by furan. In the furan study, the use of syngeneic recipients (Fischer 344 rats) resulted in transplanted site tumor growth for four of the 21 donor cholangiocarcinomas (Maronpot *et al.*, 1991). The four transplanted lines were successfully transferred through eight serial passages and resulted in metastases in the recipients. Cholangiocarcinomas failed to grow in syngeneic recipients in a transplantation study of cholangiocarcinomas that occurred in Fischer F344 rats exposed to methapyrilene hydrochloride in feed (Ohshima *et al.*, 1984).

The negative findings from the current study suggest that cholangiocarcinoma of the liver and cystic keratinizing epithelioma of the lung do not have the capacity for autonomous growth. However, interpretation of these results is somewhat confounded by lack of inclusion of a positive control. Furthermore, results from the furan study were positive (Maronpot *et al.*, 1991). It is uncertain whether the disparate results are due to differences in the experiments, the neoplasms being transplanted, or both. Morphologically, the lesions in these studies are qualitatively similar, however the process does appear to be more advanced in the furan study. The lesions tended to comprise a greater portion of the liver in the furan study, and albeit a relatively rare occurrence (around 1%), there were instances of metastasis in the furan study; no metastases were observed in the current study.

In the current study of the binary mixture of PCB 126 and PCB 118, a 360 ng TEQ/kg stop-exposure group (3,110 ng/kg PCB126:500 µg/kg PCB 118) was included in which animals were treated for 30 weeks and held without further treatment to the end of the 2-year study. Cholangiofibrosis, the precursor lesion to cholangiocarcinoma, was observed in the three highest dose groups of rats at the 31-week interim evaluation. Similar, but more advanced lesions were diagnosed as cholangiocarcinoma in the stop-exposure group at 2 years (38% incidence), albeit less than the 53% incidence observed in the sustained treatment group (also 216 ng TEQ/kg). In contrast to the transplant studies, this progression does suggest autonomy of growth.

Solid data on the biological behavior of lesions are preferable to morphologic evidence alone; however, the available biological data are somewhat conflicting. Morphologically, these lesions appear to have malignant potential. The lesions diagnosed as cholangiocarcinoma in the 2-year gavage study are composed of atypical appearing cells and there is apparent localized invasion by these cells. These lesions resemble the lesions observed in the furan study in which tumor growth was observed after transplantation.

Further work is necessary to fully understand the biological behavior of the group of lesions diagnosed in this and other dioxin toxic equivalency factor evaluation studies as cholangiofibrosis and cholangiocarcinoma.

REFERENCES

Fidler, I.J. (1991). Orthotopic implantation of human colon carcinomas into nude mice provides a valuable model for the biology and therapy of metastasis. *Cancer Metastasis Rev.* **10**, 229-243.

Maronpot, R.R., Giles, H.D., Dykes, D.J., and Irwin, R.D. (1991). Furan-induced hepatic cholangiocarcinomas in Fischer 344 rats. *Toxicol. Pathol.* **19**, 561-570.

Murakami, T., Yano, H., Maruiwa, M., Sugihara, S., and Kojiro, M. (1987). Establishment and characterization of a human combined hepatocholangiocarcinoma cell line and its heterologous transplantation in nude mice. *Hepatology* **7**, 551-556.

Ohshima, M., Ward, J.M., Brennan, L.M., and Creasia, D.A. (1984). A sequential study of methapyrilene hydrochloride-induced liver carcinogenesis in male F344 rats. *J. Natl. Cancer Inst.* **72**, 759-768.

Ulland, B.M., Maronpot, R.R., Lemen, J.K., and Mennear J.H. (1989). Transplantation studies of preputial gland and epithelial skin neoplasms derived from benzidine-based dye carcinogenicity assays in Fischer 344 male rats. *Toxicol. Pathol.* **17**, 50-56.

TABLE G1
Findings from the Tumor Transplant Study in NCr-nu/nu Recipient Mice during the Chronic Gavage Study of the Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats^a

Mouse ID	Tumor Recipient Mouse			Fragment	Donor Rat (216 ng TEQ/kg)		
	Tumor Implant Site Finding ^b	TGL A-B	Days on Test		Rat ID	Organ	Diagnosis
1	Inflammation - marked	1-1	91	A	449	Liver	Cholangiocarcinoma
2	No lesion	—	91	B	449	Liver	Cholangiocarcinoma
3	No lesion	—	91	C	449	Liver	Cholangiocarcinoma
4	Inflammation - marked	1-1	91	A	449	Lung	Cystic keratinizing epithelioma
5	Necrosis with inflammation - marked	1-1	4	B	449	Lung	Cystic keratinizing epithelioma
6	No lesion	—	5	C	449	Lung	Cystic keratinizing epithelioma
7	No lesion	1-NCL	89	A	475	Liver	Cholangiocarcinoma
8	No lesion	—	89	B	475	Liver	Cholangiocarcinoma
9	No lesion	—	89	C	475	Liver	Cholangiocarcinoma
10	Regression - moderate	1-1	89	D	475	Liver	Cholangiocarcinoma
11	No lesion	—	89	E	475	Liver	Cholangiocarcinoma
12	No lesion	—	68	A	475	Lung	Cystic keratinizing epithelioma
13	No lesion - normal fat	1-NCL	89	B	475	Lung	Cystic keratinizing epithelioma
14	No lesion	—	89	C	475	Lung	Cystic keratinizing epithelioma
15	Regression - moderate	—	89	D	475	Lung	Cystic keratinizing epithelioma
16	No lesion - normal fat	1-NCL	89	E	475	Lung	Cystic keratinizing epithelioma
17	No lesion	—	89	A	447	Liver	Cholangiocarcinoma
18	Regression - marked	1-1	89	B	447	Liver	Cholangiocarcinoma
19	Regression - moderate	—	89	C	447	Liver	Cholangiocarcinoma
20	No lesion	—	89	A	447	Liver	Cholangiocarcinoma
21	Regression - marked	1-1	89	B	447	Liver	Cholangiocarcinoma
22	Regression - marked	1-1	89	C	447	Liver	Cholangiocarcinoma
23	Regression - moderate	1-1	89	A	447	Lung	Cystic keratinizing epithelioma
24	Lymph node - fat	1-NCL	89	B	447	Lung	Cystic keratinizing epithelioma
25	No lesion	—	89	C	447	Lung	Cystic keratinizing epithelioma
26	No lesion	—	89	D	447	Lung	Cystic keratinizing epithelioma
27	Regression - marked	1-1	89	E	447	Lung	Cystic keratinizing epithelioma
28	No lesion	—	89	A	488	Liver	Cholangiocarcinoma
29	Lymph node - fat	1-NCL	89	A	488	Liver	Cholangiocarcinoma
30	Regression - marked	1-1	89	B	488	Liver	Cholangiocarcinoma
31	No lesion	—	89	A	488	Lung	Cystic keratinizing epithelioma
32	Regression - moderate	1-1	89	B	488	Lung	Cystic keratinizing epithelioma
33	No lesion - normal fat	1-NCL	89	C	488	Lung	Cystic keratinizing epithelioma
34	Regression - marked	1-1	89	D	488	Lung	Cystic keratinizing epithelioma
35	No lesion	—	89	E	488	Lung	Cystic keratinizing epithelioma
36	No lesion	—	77	A	412	Liver	Cholangiocarcinoma
37	Regression - marked	1-1	77	B	412	Liver	Cholangiocarcinoma
38	No lesion	1-NCL	77	C	412	Liver	Cholangiocarcinoma
39	No lesion	—	77	A	412	Liver	Cholangiocarcinoma
40	No lesion - lymph node	1-NCL	77	B	412	Liver	Cholangiocarcinoma
41	Regression - marked	1-1	77	C	412	Liver	Cholangiocarcinoma
42	No lesion	—	77	D	412	Liver	Cholangiocarcinoma
43	No lesion	—	77	E	412	Liver	Cholangiocarcinoma
44	Regression - moderate	1-1	77	A	412	Lung	Cystic keratinizing epithelioma
45	No lesion	—	77	B	412	Lung	Cystic keratinizing epithelioma
46	Regression - moderate	—	77	C	412	Lung	Cystic keratinizing epithelioma
47	No lesion	—	77	A	412	Lung	Cystic keratinizing epithelioma
48	No lesion	1-NCL	77	B	412	Lung	Cystic keratinizing epithelioma
49	Regression - marked	1-1	54	A	409	Liver	Cholangiocarcinoma
50	Regression - marked	—	54	B	409	Liver	Cholangiocarcinoma

TABLE G1
Findings from the Tumor Transplant Study in NCr-nu/nu Recipient Mice during the Chronic Gavage Study of the Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats

Mouse ID	Tumor Recipient Mouse				Donor Rat (216 ng TEQ/kg)		
	Tumor Implant Site Finding	TGL A-B	Days on Test	Fragment	Rat ID	Organ	Diagnosis
51	Lymph node - fat	1-NCL	54	A	409	Lung	No tumor in sample saved
52	Lymph node - fat	—	54	B	409	Lung	No tumor in sample saved
53	Regression - marked	—	54	C	409	Lung	No tumor in sample saved
54	Regression - marked	—	54	A	409	Lung	No tumor in sample saved
55	Inflammation - marked Hemorrhage - moderate	—	54	B	409	Lung	No tumor in sample saved
56	Inflammation - marked	1-1	54	A	465	Liver	Cholangiocarcinoma
57	Regression - marked	1-1	54	B	465	Liver	Cholangiocarcinoma
58	Inflammation - marked	1-1	54	A	465	Liver	Cholangiocarcinoma
59	Inflammation - mild	1-1	54	B	465	Liver	Cholangiocarcinoma
60	Regression - marked	1-1	54	C	465	Liver	Cholangiocarcinoma
61	Regression - marked	—	54	D	465	Liver	Cholangiocarcinoma

^a NCL = No corresponding lesions; TGL = Trace gross lesion; Fragment = Identification of fragment of rat tumor transplanted into the recipient mouse.

^b Regression of cholangiocarcinoma:

Moderate = Fibrous connective tissue remains and ductal structures remain that are lined by cuboidal or flattened epithelial cells.

Cyst-like structures containing a mucinous material with no cells or attenuated cells lining cyst-like structures.

Marked = Small amount of fibrous connective tissue remains with cyst-like structures containing a mucinous material with no cells or attenuated cells lining cyst-like structures.

Regression of cystic keratinizing epithelioma:

Moderate = Fibrous connective tissue surrounds a cyst lined by a very thin layer of squamous epithelium. In some instances the lining epithelium is of a respiratory type.

Marked = Fibrous connective tissue and inflammatory cells with keratin that may be mineralized. Squamous epithelium mostly absent.

TABLE G2
Findings from the Tumor Transplant Study in SCID Recipient Mice during the Chronic Gavage Study of the Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats^a

Mouse ID	Tumor Recipient Mouse				Donor Rat (216 ng TEQ/kg)		
	Tumor Implant Site Finding ^b	TGL A-B	Days on Test	Fragment	Rat ID	Organ	Diagnosis
101	No lesion - normal fat	1-NCL	91	D	449	Liver	Cholangiocarcinoma
102	Inflammation - mild	1-1	91	E	449	Liver	Cholangiocarcinoma
103	Inflammation - mild	1-1	91	F	449	Liver	Cholangiocarcinoma
104	No lesion	1-NCL	13	D	449	Lung	Cystic keratinizing epithelioma
105	Regression - marked	1-1	13	E	449	Lung	Cystic keratinizing epithelioma
106	No lesion	1-NCL	14	F	449	Lung	Cystic keratinizing epithelioma
107	No lesion	—	89	F	475	Liver	Cholangiocarcinoma
108	No lesion	—	89	G	475	Liver	Cholangiocarcinoma
109	Regression - moderate	1-1	89	H	475	Liver	Cholangiocarcinoma
110	No lesion	—	89	I	475	Liver	Cholangiocarcinoma
111	No lesion	1-NCL	89	J	475	Liver	Cholangiocarcinoma
112	No lesion	1-NCL	89	F	475	Lung	Cystic keratinizing epithelioma
113	No lesion	—	89	G	475	Lung	Cystic keratinizing epithelioma
114	No lesion	1-NCL	89	H	475	Lung	Cystic keratinizing epithelioma
115	No lesion	—	89	I	475	Lung	Cystic keratinizing epithelioma
116	No lesion	—	89	J	475	Lung	Cystic keratinizing epithelioma
117	Regression - marked	1-1	89	D	447	Liver	Cholangiocarcinoma
118	No lesion	1-NCL	89	E	447	Liver	Cholangiocarcinoma
119	No lesion	—	89	F	447	Liver	Cholangiocarcinoma
120	No lesion	—	89	D	447	Liver	Cholangiocarcinoma
121	Regression - moderate	—	89	E	447	Liver	Cholangiocarcinoma
122	No lesion	1-NCL	89	F	447	Liver	Cholangiocarcinoma
123	Regression - marked Inflammation - moderate	1-1	11	F	447	Lung	Cystic keratinizing epithelioma
124	Regression - marked	1-1	65	G	447	Lung	Cystic keratinizing epithelioma
125	Regression - marked	1-1	89	H	447	Lung	Cystic keratinizing epithelioma
126	No lesion	—	89	I	447	Lung	Cystic keratinizing epithelioma
127	No lesion	1-NCL	89	J	447	Lung	Cystic keratinizing epithelioma
128	No lesion - fat	1-NCL	89	B	488	Liver	Cholangiocarcinoma
129	Regression - marked	1-1	89	C	488	Liver	Cholangiocarcinoma
130	No lesion	1-NCL	89	D	488	Liver	Cholangiocarcinoma
131	Regression - marked	1-1	89	F	488	Lung	Cystic keratinizing epithelioma
132	Regression - marked	—	89	G	488	Lung	Cystic keratinizing epithelioma
133	Regression - marked	—	89	H	488	Lung	Cystic keratinizing epithelioma
134	No lesion	—	89	I	488	Lung	Cystic keratinizing epithelioma
135	Regression - marked	1-1	89	J	488	Lung	Cystic keratinizing epithelioma
136	No lesion	—	77	D	412	Liver	Cholangiocarcinoma
137	Regression - marked	1-1	77	E	412	Liver	Cholangiocarcinoma
138	No lesion	1-NCL	77	F	412	Liver	Cholangiocarcinoma
139	No lesion	—	77	F	412	Liver	Cholangiocarcinoma
140	Regression - marked	—	77	G	412	Liver	Cholangiocarcinoma
141	No lesion	1-NCL	77	H	412	Liver	Cholangiocarcinoma
142	Regression - marked	—	77	I	412	Liver	Cholangiocarcinoma
143	Regression - moderate	—	77	J	412	Liver	Cholangiocarcinoma
144	No lesion	1-NCL	77	D	412	Lung	Cystic keratinizing epithelioma
145	No lesion	1-NCL	77	E	412	Lung	Cystic keratinizing epithelioma
146	Regression - marked	—	77	F	412	Lung	Cystic keratinizing epithelioma
147	No lesion	—	77	C	412	Lung	Cystic keratinizing epithelioma
148	Regression - marked	—	77	D	412	Lung	Cystic keratinizing epithelioma
149	Regression - marked	—	54	C	409	Liver	Cholangiocarcinoma
150	No lesion	—	54	D	409	Liver	Cholangiocarcinoma

TABLE G2
Findings from the Tumor Transplant Study in SCID Recipient Mice during the Chronic Gavage Study of the Binary Mixture of PCB 126 and PCB 118 in Female Harlan Sprague-Dawley Rats

Mouse ID	Tumor Recipient Mouse			Fragment	Donor Rat (216 ng TEQ/kg)		
	Tumor Implant Site Finding ^b	TGL A-B	Days on Test		Rat ID	Organ	Diagnosis
151	Regression - moderated	—	54	D	409	Lung	No tumor in sample saved
152	Regression - marked	1-1	54	E	409	Lung	No tumor in sample saved
153	No lesion	1-NCL	54	F	409	Lung	No tumor in sample saved
154	No lesion	1-NCL	54	C	409	Lung	No tumor in sample saved
155	Regression - marked	—	54	D	409	Lung	No tumor in sample saved
156	Regression - marked	—	54	C	465	Liver	Cholangiocarcinoma
157	No lesion	—	54	D	465	Liver	Cholangiocarcinoma
158	No lesion	—	54	E	465	Liver	Cholangiocarcinoma
159	Normal fat	1-NCL	54	F	465	Liver	Cholangiocarcinoma
160	Regression - marked	1-1	54	G	465	Liver	Cholangiocarcinoma
161	Regression - marked	1-1	54	H	465	Liver	Cholangiocarcinoma

^a NCL = No corresponding lesions; TGL = Trace gross lesion; Fragment = Identification of fragment of rat tumor transplanted into the recipient mouse.

^b Regression of cholangiocarcinoma:

Moderate = Fibrous connective tissue remains and ductal structures remain that are lined by cuboidal or flattened epithelial cells. Cyst-like structures containing a mucinous material with no cells or attenuated cells lining cyst-like structures.

Marked = Small amount of fibrous connective tissue remains with cyst-like structures containing a mucinous material with no cells or attenuated cells lining cyst-like structures.

Regression of cystic keratinizing epithelioma:

Moderate = Fibrous connective tissue surrounds a cyst lined by a very thin layer of squamous epithelium. In some instances the lining epithelium is of a respiratory type.

Marked = Fibrous connective tissue and inflammatory cells with keratin that may be mineralized. Squamous epithelium mostly absent.

APPENDIX H

ASSOCIATED PUBLICATIONS

The following peer reviewed journal publications have been published using data or special study samples obtained from this study and other studies carried out as part of the dioxin TEF evaluation.

- Brix, A.E., Jokinen, M.P., Walker, N.J., Sells, D.M., and Nyska, A. (2004). Characterization of bronchiolar metaplasia of the alveolar epithelium in female Sprague-Dawley rats exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB126). *Toxicol. Pathol.* **32**, 333-337.
- Brix, A.E., Nyska, A., Haseman, J.K., Sells, D.M., Jokinen, M.P., and Walker, N.J. (2005). Incidences of selected lesions in control female Harlan Sprague-Dawley rats from two-year studies performed by the National Toxicology Program. *Toxicol. Pathol.* **33**, 477-483.
- Hailey, J.R., Walker, N.J., Sells, D.M., Brix, A.E., Jokinen, M.P., and Nyska, A. (2005). Classification of proliferative hepatocellular lesions in Harlan Sprague-Dawley rats chronically exposed to dioxin-like compounds. *Toxicol. Pathol.* **33**, 165-174.
- Hassoun, E.A., Li, F., Abushaban, A., and Stohs, S.J. (2000). The relative abilities of TCDD and its congeners to induce oxidative stress in the hepatic and brain tissues of rats after subchronic exposure. *Toxicology* **145**, 103-113.
- Hassoun, E.A., Li, F., Abushaban, A., and Stohs, S.J. (2001). Production of superoxide anion, lipid peroxidation and DNA damage in the hepatic and brain tissues of rats after subchronic exposure to mixtures of TCDD and its congeners. *J. Appl. Toxicol.* **21**, 211-219.
- Hassoun, E.A., Wang, H., Abushaban, A., and Stohs, S.J. (2002). Induction of oxidative stress in the tissues of rats after chronic exposure to TCDD, 2,3,4,7,8-pentachlorodibenzofuran, and 3,3',4,4',5-pentachlorobiphenyl. *J. Toxicol. Environ. Health A* **65**, 825-842.
- Jokinen, M.P., Walker, N.J., Brix, A.E., Sells, D.M., Haseman, J.K., and Nyska, A. (2003). Increase in cardiovascular pathology in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3,3',4,4',5-pentachlorobiphenyl. *Cardiovasc. Toxicol.* **3**, 299-310.
- Lee, H.M., He, Q., Englander, E.W., and Greeley, G.H., Jr. (2000). Endocrine disruptive effects of polychlorinated aromatic hydrocarbons on intestinal cholecystokinin in rats. *Endocrinology* **141**, 2938-2944.
- Nyska, A., Jokinen, M.P., Brix, A.E., Sells, D.M., Wyde, M.E., Orzech, D., Haseman, J.K., Flake, G., and Walker, N.J. (2004). Exocrine pancreatic pathology in female Harlan Sprague-Dawley rats after chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and dioxin-like compounds. *Environ. Health Perspect.* **112**, 903-909.
- Nyska, A., Yoshizawa, K., Jokinen, M.P., Brix, A.E., Sells, D.M., Wyde, M.E., Orzech, D.P., Kissling, G.E., and Walker, N.J. (2005). Olfactory epithelial metaplasia and hyperplasia in female Harlan Sprague-Dawley rats following chronic treatment with polychlorinated biphenyls. *Toxicol. Pathol.* **33**, 371-377.

Tani, Y., Maronpot, R.R., Foley, J.F., Haseman, J.K., Walker, N.J., and Nyska, A. (2004). Follicular epithelial cell hypertrophy induced by chronic oral administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Harlan Sprague-Dawley rats. *Toxicol. Pathol.* **32**, 41-49.

Toyoshiba, H., Walker, N.J., Bailer, A.J., and Portier, C.J. (2004). Evaluation of toxic equivalency factors for induction of cytochromes P450 CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. *Toxicol. Appl. Pharmacol.* **194**, 156-168.

Vezina, C.M., Walker, N.J., and Olson, J.R. (2004). Subchronic exposure to TCDD, PeCDF, PCB126, and PCB153: Effect on hepatic gene expression. *Environ. Health Perspect.* **112**, 1636-1644.

Walker, N.J., Crockett, P.W., Nyska, A., Brix, A.E., Jokinen, M.P., Sells, D.M., Hailey, J.R., Easterling, M., Haseman, J.K., Yin, M., Wyde, M.E., Bucher, J.R., and Portier, C.J. (2005). Dose-additive carcinogenicity of a defined mixture of "dioxin-like compounds." *Environ. Health Perspect.* **113**, 43-48.

Yoshizawa, K., Marsh, T., Foley, J.F., Cai, B., Peddada, S., Walker, N.J., and Nyska, A. (2005). Mechanisms of exocrine pancreatic toxicity induced by oral treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Harlan Sprague-Dawley rats. *Toxicol. Sci.* **85**, 594-606.

Yoshizawa, K., Walker, N.J., Jokinen, M.P., Brix, A.E., Sells, D.M., Marsh, T., Wyde, M.E., Orzech, D., Haseman, J.K., and Nyska, A. (2005). Gingival carcinogenicity in female Harlan Sprague-Dawley rats following two-year oral treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and dioxin-like compounds. *Toxicol. Sci.* **83**, 64-77.