



NTP

National Toxicology Program

U.S. Department of Health and Human Services

NTP TECHNICAL REPORT ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF 2,3',4,4',5- PENTACHLOROBIPHENYL (PCB 118) (CAS No. 31508-00-6) IN FEMALE HARLAN SPRAGUE-DAWLEY RATS (GAVAGE STUDIES)

NTP TR 559

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 2,3',4,4',5-PENTACHLOROBIPHENYL

(PCB 118)

(CAS NO. 31508-00-6)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)



National Toxicology Program
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FOREWORD

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

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

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

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (<http://ntp.niehs.nih.gov>) or in hardcopy upon request from the NTP Central Data Management group at cdm@niehs.nih.gov or (919) 541-3419.

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SUMMARY

Background

2,3',4,4',5-Pentachlorobiphenyl (PCB 118) is one of a large family of hydrocarbons containing chlorine (PCBs) that are similar in structure to dioxins. Some dioxins or dioxin-like compounds are highly toxic and cause cancer, and usually contaminated sites 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 some of the more prevalent of these compounds, both alone and in mixtures. This study evaluated the effects of PCB 118 on female rats for comparison with the potency of other chemicals in that family.

Methods

We exposed groups of 50 female rats by depositing solutions of PCB 118 dissolved in corn oil through a tube directly into their stomachs 5 days a week for 2 years. Daily doses of PCB 118 were 100, 220, 460, 1,000, or 4,600 micrograms (µg) per kilogram of body weight. Tissues from more than 40 sites were examined for every animal.

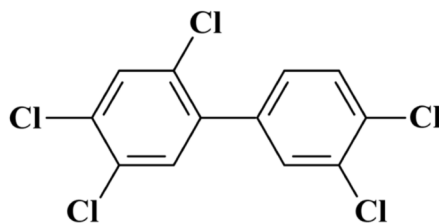
Results

Exposure to PCB 118 caused a variety of diseases in several organs. Cancers of the liver, lung, uterus, and pancreas were seen in female rats exposed to PCB 118. A variety of other toxic lesions observed in exposed animals included hypertrophy, hyperplasia, fibrosis, and toxic hepatopathy of the liver, metaplasia of the lung, atrophy of the adrenal cortex, inflammation and cytoplasmic vacuolization of the pancreas, and hypertrophy of the thyroid gland.

Conclusions

We conclude that PCB 118 caused cancer of the liver, lung, and uterus and possibly of the pancreas plus a variety of other toxic effects at several sites in female rats.

ABSTRACT



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

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 bioconcentrate through the food chain. Due to their lipophilicity and persistence, once internalized they accumulate in adipose tissue 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.

Polychlorinated biphenyls (PCBs) and their mixtures including 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 118 study was conducted as part of the dioxin TEF evaluation that included multiple 2-year rat bioassays to evaluate the relative chronic toxicity and carcinogenicity of DLCs, structurally related PCBs, and mixtures of these compounds. Female Harlan Sprague-Dawley rats were administered PCB 118 (at least 99% pure) in corn oil:acetone (99:1) by gavage for 14, 31, or 53 weeks or 2 years.

2-YEAR STUDY

Groups of 80 female rats were administered 100, 220, 460, 1,000, or 4,600 µg PCB 118/kg body weight in corn oil:acetone (99:1) by gavage, 5 days per week, for up to 105 weeks; a group of 80 vehicle control female rats received the corn oil/acetone vehicle alone. Groups of 30 female rats received 10 or 30 µg/kg for up to 53 weeks only. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. A stop-exposure group of 50 female rats was administered 4,600 µg/kg PCB 118 in corn oil:acetone (99:1) by gavage for 30 weeks then the vehicle for the remainder of the study.

Survival of all dosed groups of rats was similar to that of the vehicle control group. Mean body weights of 1,000 µg/kg rats were 7% less than those of the vehicle controls after week 36, and those of the 4,600 µg/kg core study and stop-exposure groups were 7% less than those of the vehicle controls after week 7. Following cessation of treatment, the body weight gain in the stop-exposure group was similar to that of the vehicle control group.

In general, exposure to PCB 118 lead to dose-dependent decreases in the concentrations of serum total thyroxine (T_4) and free T_4 in all dosed groups. There were no effects on triiodothyronine or thyroid stimulating hormone levels in any dosed groups evaluated at the 14-, 31-, and 53-week interim evaluations. There were increases in hepatic cell proliferation in the 4,600 µg/kg group at 14, 31, and 53 weeks. Administration of PCB 118 led to dose-dependent increases in CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase, CYP1A2-associated acetanilide-4-hydroxylase, and CYP2B-associated pent-

oxyresorufin-*O*-deethylase activities at the 14-, 31-, and 53-week interim evaluations. Analysis of PCB 118 concentrations in dosed groups showed dose- and duration of dosing-dependent increases in fat, liver, lung, and blood. The highest concentrations were seen in fat at 2 years with lower concentrations observed in the liver, lung, and blood.

At the 53-week interim evaluation, three 4,600 µg/kg rats had liver cholangiocarcinoma and one had hepatocellular adenoma. At 2 years, there were significant treatment-related increases in the incidences of cholangiocarcinoma and hepatocellular adenoma. Four incidences of hepatocholangioma occurred in the 4,600 µg/kg core study group.

At 2 years, a significant dose-related increase in hepatic toxicity was observed and was characterized by increased incidences of numerous lesions including hepatocyte hypertrophy, inflammation, oval cell hyperplasia, pigmentation, multinucleated hepatocyte, eosinophilic and mixed cell foci, diffuse fatty change, toxic hepatopathy, nodular hyperplasia, necrosis, bile duct hyperplasia and cyst, and cholangiofibrosis. The incidences of these lesions were often decreased in the 4,600 µg/kg stop-exposure group compared to the 4,600 µg/kg core study group.

In the lung at 2 years, a significantly increased incidence of cystic keratinizing epithelioma occurred in the 4,600 µg/kg core study group compared to the vehicle control group incidence. Incidences of bronchiolar metaplasia of the alveolar epithelium were significantly increased in the groups administered 460 µg/kg or greater, and the incidence of squamous metaplasia was significantly increased in the 4,600 µg/kg core study group.

The incidence of carcinoma of the uterus in the 4,600 µg/kg stop-exposure group was significantly greater than those in the vehicle control and 4,600 µg/kg core study groups at 2 years. A marginal increase in squamous cell carcinoma occurred in the 220 µg/kg group.

At 2 years, there were marginally increased incidences of exocrine pancreatic adenoma or carcinoma in the 460, 1,000, and 4,600 µg/kg core study groups.

Numerous nonneoplastic effects were seen in other organs including: adrenal cortical atrophy and cytoplas-

mic vacuolization, pancreatic acinar cell cytoplasmic vacuolization and arterial chronic active inflammation, follicular cell hypertrophy of the thyroid gland, inflammation and respiratory epithelial hyperplasia of the nose, and kidney pigmentation.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of PCB 118 in female Harlan Sprague-Dawley rats based on

increased incidences of neoplasms of the liver (cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma) and cystic keratinizing epithelioma of the lung. Occurrences of carcinoma in the uterus were considered to be related to the administration of PCB 118. Occurrences of squamous cell carcinoma of the uterus and acinar neoplasms of the pancreas may have been related to administration of PCB 118.

Administration of PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, thyroid gland, nose, and kidney.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10.

Summary of the 2-Year Carcinogenesis Study of PCB 118 in Female Sprague-Dawley Rats

Doses in corn oil/acetone by gavage

0, 100, 220, 460, 1,000, 4,600 µg/kg, and 4,600 µg/kg (stop-exposure)

Body weights

1,000 µg/kg group 7% less than the vehicle control group after week 36; 4,600 µg/kg core and stop-exposure groups 7% less than the vehicle control group after week 7

Survival rates

21/52, 20/52, 25/52, 30/52, 28/52, 25/52, 25/50

Nonneoplastic effects

Liver:

hepatocyte hypertrophy (0/52, 12/51, 15/52, 20/52, 44/52, 48/49, 30/49);
 multinucleated hepatocyte (0/52, 1/51, 3/52, 21/52, 40/52, 43/49, 32/49);
 eosinophilic focus (5/52, 8/51, 9/52, 15/52, 25/52, 41/49, 20/49);
 mixed cell focus (21/52, 19/51, 29/52, 36/52, 31/52, 7/49, 36/49);
 hyperplasia, nodular (0/52, 0/51, 0/52, 0/52, 12/52, 43/49, 4/49);
 inflammation (21/52, 30/51, 35/52, 36/52, 43/52, 44/49, 47/49);
 necrosis (1/52, 2/51, 1/52, 2/52, 20/52, 22/49, 14/49);
 fatty change, diffuse (1/52, 2/51, 1/52, 9/52, 39/52, 48/49, 8/49);
 bile duct, hyperplasia (5/52, 6/51, 7/52, 8/52, 21/52, 40/49, 25/49);
 oval cell, hyperplasia (0/52, 12/51, 9/52, 29/52, 40/52, 46/49, 29/49);
 bile duct, cyst (2/52, 3/51, 5/52, 6/52, 6/52, 6/52, 21/49, 14/49);
 pigmentation (1/52, 5/51, 12/52, 41/52, 50/52, 48/49, 43/49);
 cholangiofibrosis (0/52, 2/51, 2/52, 3/52, 2/52, 22/49, 10/49);
 toxic hepatopathy (0/52, 0/51, 3/52, 14/52, 33/52, 46/49, 36/49)

Lung:

alveolar epithelium, metaplasia, bronchiolar (6/51, 7/52, 14/52, 18/52, 24/52, 40/50, 32/50);
 squamous metaplasia (1/51, 0/52, 0/52, 1/52, 1/52, 13/50, 0/50)

Adrenal Cortex:

atrophy (1/52, 0/52, 0/52, 2/51, 9/52, 35/49, 4/49);
 vacuolization cytoplasmic (10/52, 12/52, 13/52, 12/51, 12/52, 18/49, 21/49)

Pancreas:

acinus, cytoplasmic vacuolization (0/52, 0/52, 0/52, 0/52, 4/52, 42/47, 10/49);
 artery, inflammation, chronic active (1/52, 2/52, 1/52, 7/52, 7/52, 12/47, 5/49)

Thyroid Gland:

follicular cell, hypertrophy (6/51, 7/51, 13/51, 18/51, 21/52, 23/49, 12/50)

Nose:

inflammation (1/52, 5/52, 5/52, 3/52, 5/52, 23/52, 8/50);
 respiratory epithelium, hyperplasia (5/52, 5/52, 7/52, 7/52, 14/52, 27/52, 11/50)

Kidney:

pigmentation (2/52, 3/52, 3/52, 4/52, 6/52, 42/50, 6/49)

Neoplastic effects

Liver:

cholangiocarcinoma (0/52, 0/51, 0/52, 0/52, 3/52, 36/49, 29/49);
 hepatocellular adenoma (0/52, 1/51, 1/52, 4/52, 12/52, 24/49, 1/49);
 hepatocholangioma (0/52, 0/51, 0/52, 0/52, 0/52, 4/49, 0/49)

Lung:

cystic keratinizing epithelioma (0/51, 0/52, 0/52, 0/52, 0/52, 20/50, 0/50)

Uterus:

carcinoma (2/52, 2/52, 1/52, 3/52, 4/52, 3/52, 11/50)

Summary of the 2-Year Carcinogenesis Study of PCB 118 in Female Sprague-Dawley Rats

Equivocal findings

Pancreas:

acinar adenoma (0/52, 0/52, 0/52, 2/52, 3/52, 1/47, 0/49);

acinar adenoma or carcinoma (0/52, 0/52, 0/52, 2/52, 3/52, 2/47, 0/49)

Uterus:

squamous cell carcinoma (0/52, 0/52, 3/52, 1/52, 1/52, 0/52, 1/50)

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 PCB 118 on February 25, 2009, 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.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 25, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of PCB 118 received public review by the National Toxicology Program's Board of Scientific Counselors Technical Report Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. Nigel Walker, NIEHS, introduced the technical report on PCB 118 by giving an overview of the series of NTP studies on dioxin-like compounds evaluating the concept of the toxic equivalency factor (TEF). He then described the study design and dose selection for the long-term study on PCB 118, and the body weight, tissue disposition, and histopathologic findings in the study. The proposed conclusions were:

Under the condition of this 2-year gavage study, there was clear evidence of carcinogenic activity of PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of neoplasms of the liver (cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma) and cystic keratinizing epithelioma of the lung. Occurrences of carcinoma in the uterus were considered to be related to the administration of PCB 118. Occurrences of squamous cell carcinoma of the uterus and acinar neoplasms of the pancreas may have been related to administration of PCB 118.

Administration of PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, thyroid gland, nose, and kidney.

Dr. Jim Riviere, the first primary reviewer, felt that portions of the discussion were speculative, but he agreed with the overall conclusions.

Dr. Pino, the second primary reviewer, felt the occurrence of uterine carcinomas was a strong response and should be included in the category of clear evidence. He also inquired whether the four hepatocholangiomas should be considered clear evidence of carcinogenicity.

Dr. Portier, the third primary reviewer, had no additional comments.

Dr. Walker explained that the liver hepatocholangiomas were considered with the other liver neoplasms to give an overall conclusion of clear evidence at that site. Regarding the uterine carcinomas, Dr. Walker explained that the question of whether variations in body weight may have modulated the tumor response led to the lower level of evidence of carcinogenic activity of the chemical at that site.

Dr. Riviere moved and Dr. Eastmond seconded that the conclusions be accepted as written. The motion was approved with 7 yes votes, 1 no vote (Dr. Pino), and 0 abstentions. Dr. Pino voted no as he thought that the uterine carcinomas should be included in the category of clear evidence of carcinogenicity.

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 adipose tissue, 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 translocat-

tor 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 indicates 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 dif-

ferentiation; 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, 2006; 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 (WHO). 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 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 (Van den Berg *et al.*, 2006). 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 acti-

vation 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.

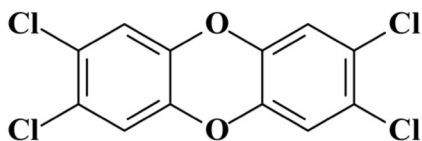
The Dioxin Toxic Equivalency Factor Evaluation Studies

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

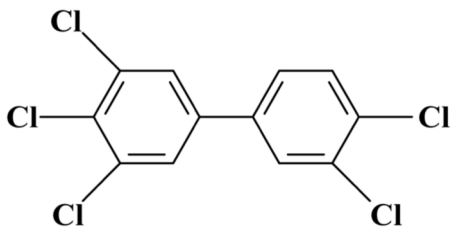


2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCDD

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 (IARC Group 1).

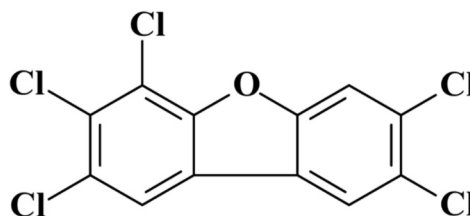


3,3',4,4',5-Pentachlorobiphenyl
PCB 126

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.

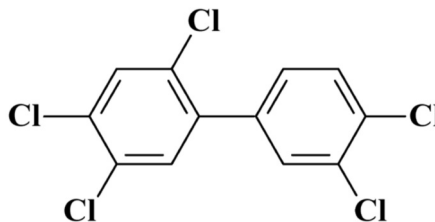


2,3,4,7,8-Pentachlorodibenzofuran
PeCDF

CAS No. 57117-31-4

Chemical Formula: $C_{12}H_3Cl_5O$ Molecular weight: 340.42

PeCDF is a dioxin-like PHAH with high bioaccumulation in the food chain. When the dioxin TEF initiative was initially designed, PeCDF had a WHO TEF value of 0.5. In the recent reevaluation of the WHO TEFs, the TEF for PeCDF was changed to 0.3. This change in the TEF value was based on a reevaluation of the available information on the potency of PeCDF and the use of a half-log potency scale by the WHO expert panel (Van den Berg *et al.*, 2006). This compound represents the most potent PCDF present in human tissues.



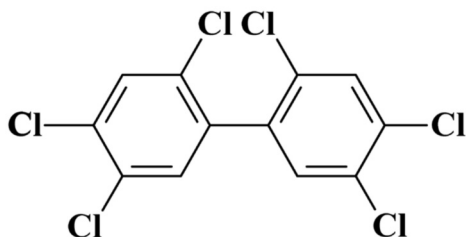
2,3',4,4',5-Pentachlorobiphenyl
PCB 118

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. The WHO₁₉₉₈ TEF value for PCB 118 is 0.0001 (Van den Berg *et al.*, 1998) although there is controversy over whether mono-*ortho*-substituted PCBs should be included in the TEF methodology. In the most recent reevaluation of the WHO TEFs, all mono-*ortho*-substituted PCBs, including PCB 118, were assigned TEFs of 0.00003 based on a reevaluation of the

available information and the use of a half-log potency scale by the WHO expert panel (Van den Berg *et al.*, 2006).



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 of PCB 126 and PCB 153 consisted of two parts (Table 1):

1. Dosing with a mixture of PCB 126 and PCB 153 together at the environmentally relevant ratio of 1:1,000. The dosage levels of PCB 126 in the mixture (10 to 1,000 ng/kg) were chosen to span the dosage range used in the 2-year study of PCB 126 alone.
2. Dosing of three mixtures that contained PCB 126 at a mid-dose of 300 ng/kg together with one of three different dose levels of PCB 153 (100, 300, or 3,000 µg/kg) to assess the impact of varying levels of PCB 153 on the effect of PCB 126.

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 (formerly 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; the results of that study are presented in this Technical Report.

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

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 Toxic Equivalency Factor (TEF) values used for the original design of the TEF evaluation studies were the WHO₁₉₉₈ TEFs based on Van den Berg et al. (1998).

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

^c 10, 22, 46, 100 ng Toxic Equivalents (TEQ)/kg based on the WHO₁₉₉₈ TEF values for PCB 126 and PeCDF of 0.1 and 0.5, respectively.

^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. TEQ/kg values are based on the WHO₁₉₉₈ TEF values for PCB 126 and PCB 118 of 0.1 and 0.0001, respectively.

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 (Goodman and Sauer, 1992).

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 fat, liver, lung, and blood were included at each interim necropsy and at terminal necropsy for dose response analysis using administered dose, total body burden, and target tissue dose as the dose metrics.

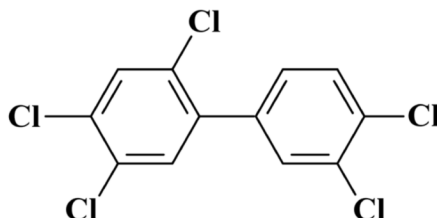
Additional “special study” animals were included at each interim necropsy, except in the current study. Tissues

from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies (Appendix G). These animals were not evaluated as part of the core studies.

During the conduct of this study, the TEF values for all DLCs were reevaluated at a WHO expert panel meeting. This led to a change in the official WHO TEF values for several congeners (Van den Berg *et*

al., 2006) evaluated as part of the NTP dioxin TEF evaluation. Notably, the TEF value for PCB 118 decreased from 0.0001 to 0.00003. Also the TEF value for PeCDF was reduced from 0.5 to 0.3. To maintain continuity with the previous seven dioxin TEF evaluation technical reports, the TEF and TEQ values shown in Table 1 are reflective of the original study design and are based on the 1998 WHO TEF values.

INTRODUCTION



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

CHEMICAL AND PHYSICAL PROPERTIES

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 118 has a melting point of 108° C, is sparingly soluble in water (0.0134 mg/L at 20° C), has a vapor pressure of 8.97×10^{-6} mmHg at 25° C, and has a log octanol:water partition coefficient of 7.12.

PRODUCTION, USE, AND HUMAN EXPOSURE

PCB mixtures containing 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).

Most 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 118 in various

food groups range from 14 to 1,900 pg/g. Estimated daily exposure from food to PCB 118 is 31 ng/day (USEPA, 2000b).

PCB 118 is classed as a dioxin-like compound (DLC) and is included in the World Health Organization (WHO) toxic equivalency factor (TEF) scheme for DLCs (Van den Berg *et al.*, 1998, 2006). The WHO₁₉₉₈ TEF value for PCB 118 was 0.0001. More recently there was a reevaluation of the TEFs for all DLCs and all mono-*ortho*-substituted PCBs, including PCB 118, were assigned TEF values (WHO₂₀₀₆ TEF) of 0.00003 (Van den Berg *et al.*, 2006). Exposure to DLCs is usually calculated in terms of total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs). For this report TEQ values for human exposure are based mainly on published findings prior to the TEF reevaluation, and so are based on the 1998 WHO values (WHO₁₉₉₈ TEQ), unless otherwise stated. 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 118. Of this, PCB 118 accounts for 3.1 pg TEQ/day. By comparison, intake of TCDD from food is estimated to be 5 pg/day (USEPA, 2000b). Bioaccumulation of PCB 118 results in persistent levels of this PCB in human tissues. The average human tissue concentration of PCB 118 is 32,000 pg/g lipid. PCB 118 accounts for 14% 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 (USEPA, 2000c). As a lipophilic DLC, it is expected that PCB 118 will have similar toxicokinetic properties to that of TCDD and other PCBs such as PCB 126. 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 to 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 (CYP1A2) (Gillner *et al.*, 1987; Diliberto *et al.*, 1997). CYP1A2 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 Bründl, 1992). More recently it has been suggested that binding PCB 126 to MRP-2 in the liver may be involved in the hepatic sequestration of PCB 126 (Lohitnavy *et al.*, 2008).

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 and skin, and fluids such as breast milk (ATSDR, 2000).

TOXICITY

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 histo-

logical changes in the thyroid gland 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; Knerr and Schrenk, 2006). In general, these studies indicate that PCB mixtures (e.g., Aroclors) 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) until the recent NTP studies of PCB congeners and mixtures as part of the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f,g), there had been no individual studies on the carcinogenicity of specific PCB congeners.

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 (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.

Similarly, in the binary mixture study of PCB 126 and PCB 118 in female Harlan Sprague-Dawley rats that was conducted as part of the dioxin TEF evaluation, there was clear evidence of carcinogenicity of the mixture based on increased incidences of cholangiocarcinoma and hepatocellular neoplasms (predominantly hepatocellular adenomas) of the liver and cystic keratinizing epithelioma of the lung (NTP, 2006d). 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.

In the NTP study of the binary mixture of the dioxin-like PCB 126 and the non-dioxin-like PCB 153, there was clear evidence of carcinogenic activity of a constant ratio binary mixture of PCB 126 and PCB 153 in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma, hepatocholangioma, and hepatocellular neoplasms (predominantly adenomas) of the liver, squamous neoplasms of the lung (predominantly cystic keratinizing epithelioma), and gingival squamous cell carcinoma of the oral mucosa (NTP, 2006c). Increased incidences of pancreatic acinar neoplasms were also considered to be related to administration of the binary mixture of PCB 126 and PCB 153. Increased incidences of uterine squamous cell carcinoma may have been related to administration of the binary mixture of PCB 126 and PCB 153. Administration of the binary mixture of PCB 126 and PCB 153 also caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, pancreas, adrenal cortex, thyroid gland, thymus, kidney, nose, and forestomach.

In contrast to the effects seen with these dioxin-like PCBs, in the NTP study of the non-dioxin-like di-*ortho*-substituted PCB 153, at doses up to 3 mg/kg, there was equivocal evidence of carcinogenic activity of PCB 153 in female Harlan Sprague-Dawley rats based on the occurrences of cholangioma of the liver. PCB 153 administration caused increased incidences of nonneoplastic lesions of the liver, thyroid gland, ovary, oviduct, and uterus in female rats (NTP, 2006b).

A comparative carcinogenicity study of PCB mixtures 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 dioxin-like PCBs (including PCB 118) 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 up to 100 ng/kg 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 (NTP, 2006e). 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 118 alone. Exposures to PCB 118 occur in a mixture combined with other structurally related compounds such as PCDDs, PCDFs, and PCBs. Retrospective cohort studies examining the possible carcinogenicity in humans of PCB mixtures from both occupational and environmental exposures have been extensively reviewed elsewhere (Knerr and Schrenk, 2006). While no conclusions can be made about individual PCB congeners from these studies, in general the findings show that there are some possible associations between PCB exposure and cancer in certain organs, notably the liver, biliary tract, intestines, and skin. Two well-documented 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 follow-up studies do 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.

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 expan-

sion 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.*, 1980, 1991).

There have been numerous studies demonstrating the ability of PCBs to enhance the development of preneoplastic liver lesions (Silberhorn *et al.*, 1990; Knerr and Schrenk, 2006). Numerous studies have examined the promotion of putative preneoplastic liver lesions by TCDD and DLCs within the framework of a two-stage initiation-promotion protocol (Wærn *et al.*, 1991; 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).

The induction of preneoplastic lesions in the liver 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 AHF data and 0.0001 based on increased 7-ethoxyresorufin-*O*-deethylase activity (Haag-Grönlund *et al.*, 1997, 2000).

Tests of the tumor initiating and promoting capacity of DLCs have been conducted in two-stage (initiation-TCDD promotion) models of mouse skin tumorigenesis (Hebert *et al.*, 1990; IARC, 1997; Dragan and Schrenk, 2000; USEPA, 2000c). 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). 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 oncogenic constitutively active *v-Ha-ras* gene (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 the NTP showed that the induction of papillomas and squa-

mous 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. While skin tumor promotion by PCB 118 has not been evaluated, based on the similarity of action of dioxin-like PCBs and TCDD, it would be predicted that PCB 118 would have weak activity in the Tg.AC model.

In addition to the liver and skin, DLCs 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 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 OF DIOXIN-LIKE COMPOUNDS

DLCs are generally classified as nongenotoxic and non-mutagenic. 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 TCDD and DLC effects on hormone and growth factor systems, cytokines, and other signal transducer pathways indicates that they are powerful growth dysregulators (Birnbbaum, 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 CYP1A1 gene, 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-hydroxyla-

tion 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 thyrotropin 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 (Sutter and Greenlee, 1992; Birnbaum, 1994b). 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, gonadotropin 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; Vezina *et al.*, 2004). 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 also induce non-AhR mediated PCB-specific effects. These include increased expression of cytochromes P450 of the 2B family, and associated pentoxoresorufin-*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 Harlan 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 doses used in the present study are based on the WHO₁₉₉₈ TEF value for PCB 118 of 0.0001. Based on this TEF, assuming that the TEF values are predictive of the carcinogenic potency of PCB 118, the 1,000 µg PCB 118/kg body weight dose would be expected to be equivalent to doses of 100 ng TCDD/kg, or 1,000 ng PCB 126/kg. The 4,600 µg/kg dose was included in case the true potency of PCB 118 was lower.

During the conduct of this study, the TEF values for all DLCs were reevaluated at a WHO expert panel meeting leading to a decrease in the official WHO TEF for PCB 118 from 0.0001 to 0.00003 (Van den Berg *et al.*, 2006). Based on this new TEF value, the doses used in this PCB 118 study correspond to TCDD equivalent doses of 0.3, 0.9, 3, 6.6, 13.8, 30, and 138 ng TEQ/kg.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

PCB 118 was obtained from Cerilliant Corp. (Round Rock, TX) in one lot (35108-72) that was used for the 2-year study. Identity and purity analyses were conducted by the analytical chemistry laboratory (Research Triangle Institute, Research Triangle Park, NC) and the study laboratory (Battelle Columbus Operations, Columbus, OH), and stability analyses were conducted by the analytical chemistry laboratory (Appendix C). Reports on analyses performed in support of the PCB 118 study are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a white powder, was identified as PCB 118 by infrared, ultraviolet/visible, proton nuclear magnetic resonance, and low resolution mass spectroscopy. The moisture content was determined using a gas chromatography (GC) moisture analysis purge and trap method, and the purity was determined using three GC systems. No moisture was detected. The first GC purity system detected one major peak and three impurity peaks with areas of 0.023%, 0.15%, and 0.077% relative to the total integrated peak area. A second GC system detected one major peak and four impurity peaks with areas of 0.15%, 0.047%, 0.25%, and 0.13% relative to the total integrated peak area. The overall purity was determined to be 99% or greater with trace quantities of other dioxins.

Stability studies of the bulk chemical were performed using gas chromatography/mass spectrometry. These studies indicated that PCB 118 was stable as a bulk chemical for 14 days when stored protected from light at temperatures up to 25° C. To ensure stability, the bulk chemical was stored at room temperature (approximately 25° C) in an amber glass bottle.

Stability was monitored during the 2-year study using GC, and no degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared approximately every 5 weeks by mixing PCB 118 with acetone and corn oil such that the final formulation contained 1.0% acetone (Table C3). Homogeneity and stability studies of a 4 µg/mL dose formulation were performed by the study laboratory using GC. Homogeneity was confirmed, and 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 under simulated animal room conditions.

Periodic analyses of the dose formulations of PCB 118 were conducted by the study laboratory using GC. During the 2-year study, the dose formulations were analyzed every 2 to 3 months (Table C4). Of the dose formulations analyzed and used in the study, all 63 were within 10% of the target concentrations; all 25 animal room samples were within 10% of the target concentrations. Periodic analyses of the corn oil vehicle performed by the study laboratory demonstrated peroxide concentrations less than 3 mEq/kg.

2-YEAR STUDY

Study Design

Groups of 80 female rats received PCB 118 in corn oil:acetone (99:1) by gavage at doses of 100, 220, 460, 1,000, or 4,600 µg/kg 5 days per week for up to 105 weeks; a group of 80 female rats received the corn oil:acetone (99:1) vehicle alone. Groups of 30 female rats received 10 or 30 µg/kg for up to 53 weeks only. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. For stop-exposure evaluation, a group of 50 female rats was administered 4,600 µg/kg for 30 weeks then the vehicle alone for the remainder of the 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 the female rats. Male rats were not administered the test compound. Rats were quarantined for 9 days before the beginning of the study. Female rats were 7 to 8 weeks old at the beginning of the study. Five males and five females were randomly selected for parasite evaluation and gross observation of disease. The health of the rats was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix E). Sentinel rats included five males and five females at 1 month, five males at 6, 12, and 18 months, and five 4,600 µg/kg females at the end of the study.

Animal Maintenance

Male rats were housed 2 per cage and female rats were housed 5 per cage. Feed and water were available *ad libitum*. Cages were changed twice weekly; racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 2. 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 thereafter, and at the end of the study. Body weights were recorded on the first day of the study, weekly for 13 weeks, every 4 weeks thereafter, and at the end of the study.

At 14, 31, and 53 weeks, blood was taken from the retroorbital sinus of up to 10 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 0.4 mg BrdU/mL Milli-Q water for 5 days. BrdU solutions were administered in amber glass water bottles (Allentown Caging Equipment Company, Inc., Allentown, NJ) equipped with Teflon[®]-lined lids and stainless steel sipper tubes. BrdU solutions were changed after 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. Samples of duodenum (positive control) and liver were 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 least 2,000 labeled or unlabeled hepatocyte nuclei were counted using a 20× 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 -60° to -80° C. Microsomal suspensions were prepared using the Pearce Method (Pearce *et al.*, 1996). The concentration of protein in each suspension was determined using the microtiter 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/minute per mg (EROD and PROD) or nmol/minute per mg (A4H) microsomal protein.

For analysis of tissue concentrations of PCB 118 and PCB 156, 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 hexane, and sample extract cleanup using automated solid phase extraction with hexane elution of silica gel columns.

Complete necropsies and microscopic examinations were performed on all female 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, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

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 gland, bone marrow, clitoral gland, esophagus, heart, kidney, liver, lung, mammary gland, mesentery, nose, oral mucosa, ovary, pancreas, forestomach, thymus, thyroid gland, tooth, and uterus.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists.

Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the study laboratory pathologist, quality assessment 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 principal pathologists (study pathologist, NTP pathologist, and quality assessment pathologist) were used. Within many of the studies conducted as part of the dioxin TEF evaluation, 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 special PWG was held to ensure that these important proliferative lesions were sufficiently and consistently categorized; additionally, a review panel utilizing a different group of pathologists was convened to provide additional guidance on the most appropriate classification of the hepatocellular proliferative lesions from these studies of DLCs. Final diagnoses for the hepatocellular proliferative lesions in the PCB 118 study reflect the diagnostic criteria developed in this review process (Hailey *et al.*, 2005).

TABLE 2
Experimental Design and Materials and Methods in the 2-Year Gavage Study of 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

9 days

Average Age When Study Began

7 to 8 weeks

Date of First Dose (female rats only)

March 26, 2004

Duration of Dosing

5 days/week for 14, 31, or 53 (interim evaluations), 30 (stop-exposure), or 105 (core study) weeks

Date of Last Dose

March 29, 2006 (core study)

October 21, 2004 (stop-exposure)

Necropsy Dates

March 28-30, 2006

Average Age at Necropsy

112 to 113 weeks

Size of Study Groups

80 (vehicle control, 100, 220, 460, 1,000, and 4,600 µg/kg), 30 (10 and 30 µg/kg), or 50 (4,600 µg/kg stop-exposure)

Method of Distribution

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

Animals per Cage

Male rats: 2

Female rats: 5

Method of Animal Identification

Tail tattoo

Diet

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

Water

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

Cages

Solid polycarbonate (Lab Products, Inc., Maywood, NJ), changed twice weekly

Bedding

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

TABLE 2
Experimental Design and Materials and Methods in the 2-Year Gavage Study of PCB 118

Cage Filters

Spun-bonded polyester (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: $72^{\circ} \pm 3^{\circ}$ F

Relative humidity: $50\% \pm 15\%$

Room fluorescent light: 12 hours/day

Room air changes: 10/hour

Doses

0, 10, 30, 100, 220, 460, 1,000, and 4,600 $\mu\text{g/kg}$ (dosing volume = 2.5 mL/kg body weight)

Type and Frequency of Observation

Observed twice daily; animals were weighed initially, weekly for 13 weeks, every 4 weeks thereafter, and at the end of the study. Clinical findings were recorded on day 29, monthly thereafter, and at the end of the study. Water consumption was measured during BrdU administration.

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, and 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 female rats per group (except for stop-exposure) for total and free thyroxine, total triiodothyronine, and thyroid stimulating hormone determinations.

Cell Proliferation

At 14, 31, and 53 weeks, up to 10 female rats per group (except for stop-exposure) 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 female rats per group (except for stop-exposure) for 7-ethoxyresorufin-O-deethylase, acetanilide-4-hydroxylase, and 7-pentoxeresorufin-O-deethylase 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 fat, liver, lung, and blood were taken from up to 10 female rats per group (except for stop-exposure) for analysis of PCB 118 and PCB 156 concentrations.

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, 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. For interim evaluations the adrenal gland, liver, lung, pancreas, thymus, thyroid gland, and uterus were examined; other tissues were examined in vehicle control and 4,600 $\mu\text{g/kg}$ rats.

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, A4a, and A4b 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 A2a and A2b) 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 A2a and A2b 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 evaluations, 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 dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). 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 current study of PCB 118, the seven gavage studies previously conducted as part of the dioxin

TEF evaluation (PCB 126, PCB 153, Binary Mixture of PCB 126 and PCB 153, Binary Mixture of PCB 126 and PCB 118, TCDD, PeCDF, and the TEF Dioxin Mixture; NTP, 2006a,b,c,d,e,f,g), and the gavage study of 3,3',4,4'-tetrachloroazobenzene (NTP, 2010).

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 the female rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 1). Survival of all dosed groups of rats was similar to that of the vehicle control group. Thirteen deaths in the 4,600 µg/kg core study group were attributed to toxic hepatopathy, and five deaths in the 4,600 µg/kg stop-exposure group were attributed to uterine carcinoma; except in one other rat with uterine carcinoma, these lesions were not the cause of death in any other dose group.

Body Weights and Clinical Findings

Mean body weights of 100, 220, and 460 µg/kg rats were similar to those of the vehicle controls during most of the 2-year study; mean body weights of 1,000 µg/kg rats were less than those of the vehicle controls after week 29, and those of the 4,600 µg/kg core study and stop-exposure groups were less than those of the vehicle controls after week 6 (Table 4 and Figure 2). While the terminal weight of animals in the 4,600 µg/kg stop-exposure group was less than the vehicle controls, the rate of body weight gain of these animals was comparable to the vehicle controls after cessation of exposure (Figure 2). The mean body weights of 10 and 30 µg/kg rats were similar to those of the vehicle controls until they were sacrificed at week 14, 31, or 53 (data not shown). No clinical findings were attributed to administration of PCB 118.

Thyroid Hormone Concentrations

Assays for total thyroxine (T_4), free T_4 , total triiodothyronine (T_3), and thyroid stimulating hormone (TSH), were conducted at the 14-, 31-, and 53-week interim evaluations (Table 5). At 14 weeks, serum total T_4 and free T_4 concentrations in the 220 µg/kg or greater groups were significantly lower than those in the vehicle controls. No significant differences were observed in total T_3 or TSH concentrations at 14 weeks.

At the 31-week interim evaluation, serum total T_4 and free T_4 concentrations in the 220 µg/kg or greater groups were significantly lower than those in the vehicle controls. No significant differences were observed in total T_3 or TSH concentrations at 31 weeks.

At the 53-week interim evaluation, serum total T_4 concentrations in the 220 µg/kg or greater groups were significantly lower than those in the vehicle controls. Serum free T_4 concentrations in the 460, 1,000, and 4,600 µg/kg groups were significantly lower than those in the vehicle controls. No significant differences were observed in serum T_3 or TSH concentrations at 53 weeks.

Hepatic Cell Proliferation Data

BrdU labeling indexes were measured in the liver at the 14-, 31-, and 53-week interim evaluations and are presented in Table 6. At 14, 31, and 53 weeks, the labeling indexes in the 4,600 µg/kg group were significantly higher than those in the vehicle controls.

Cytochrome P450 Enzyme Activities

At each interim evaluation, liver and lung samples were collected for determinations of P450 enzyme activity (Table 7). Microsomal suspensions were prepared from liver samples and were assayed for 7-ethoxyresorufin-O-deethylase (EROD) activity (a marker for CYP1A1 activity), acetanilide-4-hydroxylase (A4H) activity (a marker for CYP1A2 activity), and 7-pentoxoresorufin-O-deethylase (PROD) activity (a marker for CYP2B activity). Microsomal samples from lung were analyzed for EROD activity only.

Hepatic P450 activity generally increased with increasing dose at the 14-, 31-, and 53-week interim evaluations. Significant induction of hepatic EROD, A4H, and PROD activities was observed in all groups administered 100 µg/kg or greater at 14, 31, and 53 weeks. Hepatic EROD and A4H activities were induced more than 40- and 7-fold, respectively, at all time points in the 4,600 µg/kg group. Similarly, hepatic CYP2B-associated PROD activity was induced more than 23-fold at all time points in the 4,600 µg/kg group.

EROD activities in the lung were in general significantly higher in groups administered 220 µg/kg or greater compared to the vehicle controls at 14, 31, and 53 weeks and were increased more than 77-fold in the 4,600 µg/kg group.

Determinations of PCB 118 and PCB 156 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 (105 weeks) (Table 8). Given the presence of PCB 156 at a concentration of 0.06% in the bulk PCB 118 test article (Table C2), PCB 156 was also measured in tissues during the course of the study (Table 9). Samples were analyzed using gas chromatography with electron capture detection (for PCB 118) and gas chromatography with high

resolution mass spectrometry (for PCB 156) by systems similar to those described in Appendix C following saponification and extraction with hexane.

The highest concentrations of PCB 118 were observed in the fat with lower concentrations seen in the liver, lung, and blood. PCB 118 concentrations increased in dosed groups in a dose-dependent manner. Concentrations in fat in the 4,600 µg/kg group were 6.4-, 7.1-, 5.8-, and 4.3-fold higher than those seen in liver at 14, 31, 53, and 105 weeks, respectively.

Similarly, the highest concentrations of PCB 156 were observed in fat, with lower concentrations seen in the liver, lung, and blood. PCB 156 concentrations increased in dosed groups in a dose-dependent manner. The concentrations of PCB 156 in fat were more than 1,000-fold lower than PCB 118 concentrations at all time points.

TABLE 3
Survival of Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Animals initially in study	80	30	30	80	80	80	80	80	50
14-Week interim evaluation ^a	10	10	10	10	10	10	10	10	
31-Week interim evaluation ^a	10	10	10	10	10	10	10	10	
53-Week interim evaluation ^a	8	8	10	8	8	8	8	8	
Accidental deaths ^a	0	0	0	1	0	0	0	0	1
Moribund	27	0	0	22	22	17	16	16	18
Natural deaths	4	2 ^a	0	9	5	5	8	11	6
Animals surviving to study termination	21			20	25	30	28	25	25 ^b
Percent probability of survival at end of study ^c	40			40	48	58	54	48	51
Mean survival (days) ^d	616			607	626	669	648	628	658
Survival analysis ^e	P=0.377N			P=1.000N	P=0.474N	P=0.061N	P=0.188N	P=0.321N	P=0.149N

^a Censored from survival analyses

^b Includes one animal that was a moribund sacrifice (MSAC) during the last week of the study.

^c Kaplan-Meier determinations

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

^e The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dosed group is indicated by N. The trend test does not include the 10 or 30 µg/kg or 4,600 µg/kg stop-exposure groups.

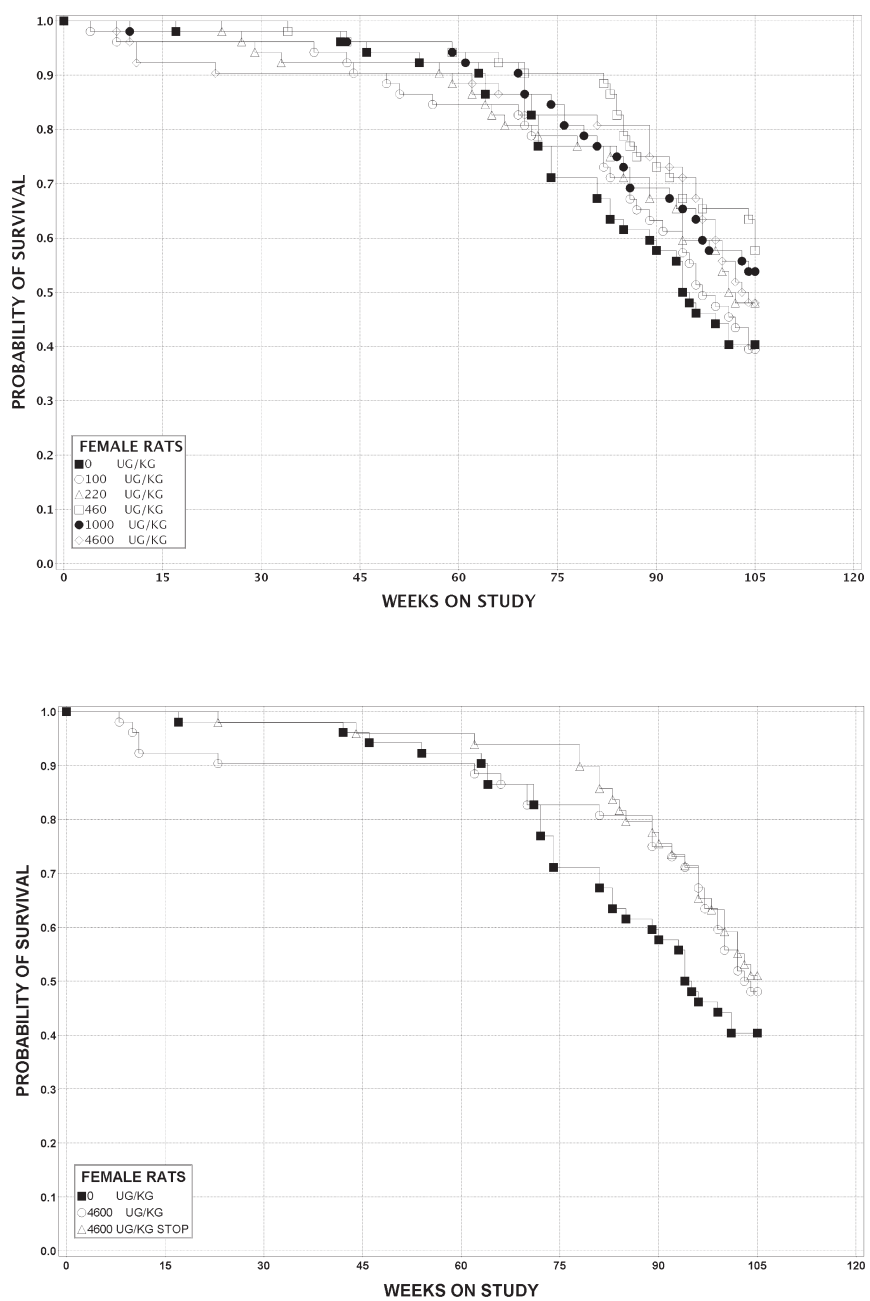


FIGURE 1
Kaplan-Meier Survival Curves for Female Rats Administered PCB 118 by Gavage for 2 Years

TABLE 4
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of PCB 118^a

Days on Study	Vehicle Control		100 µg/kg			220 µg/kg			460 µg/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	155	80	155	100	80	155	100	80	157	101	80
8	176	80	176	100	80	176	100	80	178	101	80
15	195	80	195	100	80	193	99	80	196	101	80
22	209	80	210	100	80	211	101	80	210	101	80
29	220	80	223	101	79	220	100	80	224	102	80
36	232	80	232	100	79	232	100	80	234	101	80
43	237	80	240	101	79	239	101	80	242	102	80
50	241	80	245	102	79	243	101	80	245	102	80
57	247	80	251	101	78	249	101	80	252	102	80
64	252	80	255	101	78	253	101	80	255	101	80
71	256	80	260	102	78	257	100	80	259	101	80
78	259	80	262	101	78	260	100	80	260	101	80
85	264	80	265	101	78	264	100	80	267	101	80
113	274	70	273	100	68	275	101	70	275	100	70
141	282	69	283	100	68	284	101	70	282	100	70
169	289	69	287	99	68	288	100	69	286	99	70
197	294	69	291	99	68	296	101	67	293	100	70
225	303	59	297	98	58	306	101	57	300	99	60
253	307	59	302	98	58	310	101	56	301	98	59
281	309	59	303	98	57	312	101	56	303	98	59
309	316	58	310	98	55	319	101	56	309	98	58
337	323	57	318	98	55	327	101	56	316	98	58
365	329	57	324	98	53	332	101	56	322	98	58
393	335	48	326	97	44	337	101	47	328	98	50
421	343	48	334	97	44	344	100	46	333	97	49
449	355	45	343	97	44	345	97	44	341	96	49
477	359	45	348	97	44	349	97	42	345	96	48
505	359	40	346	96	40	351	98	41	347	97	47
533	366	37	354	97	40	359	98	41	348	95	47
561	379	35	362	96	40	365	97	40	356	94	47
589	374	33	363	97	36	365	98	38	355	95	43
617	378	31	362	96	33	372	98	35	357	95	39
645	384	30	369	96	31	376	98	34	356	93	37
673	372	24	364	98	26	377	101	31	352	95	35
701	373	21	368	98	24	370	99	27	353	95	34
729	364	21	374	103	20	371	102	25	353	97	32
Mean for weeks											
1-13	226		228	101		227	100		229	101	
14-52	300		296	99		302	101		296	99	
53-105	362		353	98		358	99		346	96	

TABLE 4
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of PCB 118

Days on Study	1,000 µg/kg			4,600 µg/kg			4,600 µg/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	154	100	80	156	101	80	154	100	50
8	175	100	80	177	101	80	177	101	50
15	194	100	80	194	100	80	193	99	50
22	211	101	80	205	98	80	204	98	50
29	220	100	80	215	98	80	213	97	50
36	231	100	80	218	94	80	219	95	50
43	237	100	80	225	95	80	224	94	50
50	241	100	80	226	94	80	223	93	50
57	247	100	80	229	93	79	226	92	50
64	249	99	79	231	92	79	229	91	50
71	253	99	79	237	93	77	232	91	50
78	254	98	79	237	92	76	233	90	50
85	256	97	79	242	92	76	238	90	50
113	266	97	69	247	90	66	245	89	50
141	272	96	69	252	89	66	250	89	50
169	274	95	69	257	89	65	255	88	49
197	279	95	69	261	89	65	259	88	49
225	285	94	59	266	88	55	265	88	48
253	287	94	59	269	88	55	267	87	48
281	288	93	59	268	87	55	268	87	48
309	295	93	58	271	86	55	273	86	47
337	302	94	58	274	85	55	278	86	47
365	304	92	58	275	84	55	281	85	47
393	309	92	50	276	82	47	289	86	47
421	316	92	48	275	80	47	295	86	47
449	321	90	48	277	78	46	300	85	46
477	323	90	48	273	76	45	306	85	46
505	326	91	45	271	75	43	308	86	46
533	327	89	42	270	74	43	314	86	46
561	330	87	40	273	72	43	321	85	44
589	333	89	39	275	74	42	318	85	39
617	332	88	36	271	72	41	319	85	38
645	332	87	35	274	71	38	321	84	36
673	326	88	33	270	73	34	322	87	32
701	327	88	30	269	72	29	319	86	29
729	325	89	28	266	73	25	318	88	25
Mean for weeks									
1-13	225	100	80	215	95		213	94	
14-52	283	94	63	263	88		262	87	
53-105	324	90	41	273	75		309	85	

^a Interim evaluations occurred during weeks 14, 31, and 53 (except stop-exposure group)

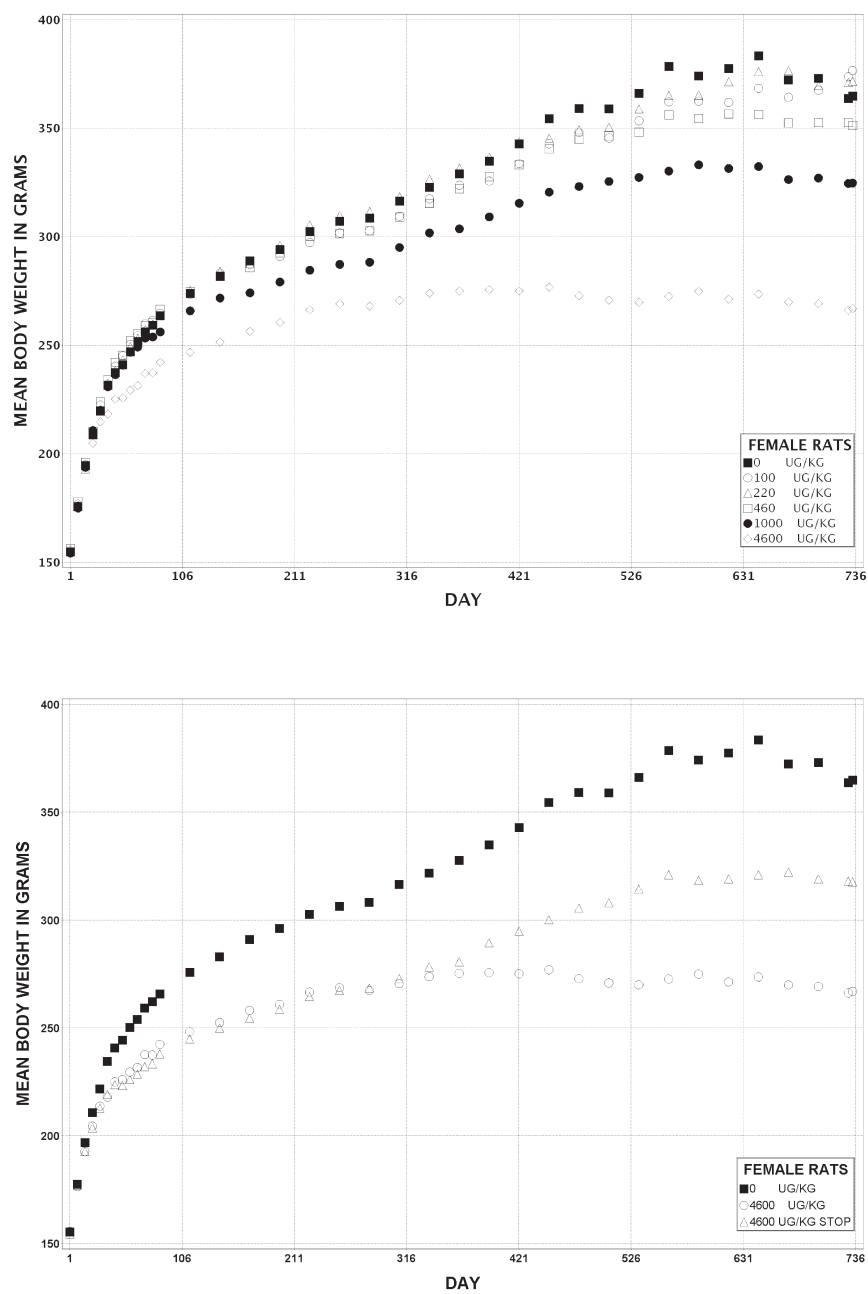


FIGURE 2
Growth Curves for Female Rats Administered PCB 118 by Gavage for 2 Years

TABLE 5
Serum Concentrations of Thyroid Hormones in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations
in the 2-Year Gavage Study of PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
Week 14								
n	10	10	10	10	10	10	10	10
Total T ₄ (µg/dL)	5.02 ± 0.25	5.37 ± 0.41	5.24 ± 0.24	4.93 ± 0.23	3.39 ± 0.31**	1.78 ± 0.22**	0.83 ± 0.14**	0.68 ± 0.09**
Free T ₄ (ng/dL)	2.28 ± 0.11	2.70 ± 0.24	2.70 ± 0.19	2.41 ± 0.11	1.67 ± 0.14*	0.98 ± 0.09**	0.70 ± 0.07**	0.45 ± 0.04**
Total T ₃ (ng/dL)	140.50 ± 9.57	132.00 ± 14.35	137.61 ± 13.35	145.69 ± 8.47	133.14 ± 13.20	117.73 ± 10.89	120.13 ± 14.13	125.80 ± 13.05
TSH (ng/mL)	12.22 ± 1.37	10.53 ± 1.17	10.50 ± 1.09	15.82 ± 3.04 ^b	12.08 ± 1.28	8.59 ± 1.02	12.70 ± 1.27	11.44 ± 1.39
Week 31								
n	10	10	10	10	10	10	10	10
Total T ₄ (µg/dL)	4.17 ± 0.14	3.99 ± 0.24	3.69 ± 0.17	4.05 ± 0.17	2.37 ± 0.20**	1.35 ± 0.19**	1.11 ± 0.37**	0.32 ± 0.06**
Free T ₄ (ng/dL)	2.23 ± 0.11	2.13 ± 0.10	2.10 ± 0.15	2.42 ± 0.10	1.48 ± 0.11**	1.10 ± 0.13**	0.80 ± 0.06**	0.46 ± 0.04**
Total T ₃ (ng/dL)	145.42 ± 6.83	147.44 ± 7.11	122.96 ± 6.73	134.53 ± 7.39	124.54 ± 6.68	135.62 ± 9.02	138.08 ± 6.24	118.10 ± 10.39
TSH (ng/mL)	16.72 ± 2.07 ^b	14.23 ± 1.46	15.06 ± 2.24	15.24 ± 1.82	13.98 ± 1.27	17.71 ± 1.89	16.79 ± 1.79	18.74 ± 1.58
Week 53								
n	8	8	10	8	8	8	8	8
Total T ₄ (µg/dL)	2.91 ± 0.27	2.58 ± 0.19	2.73 ± 0.16	2.25 ± 0.14	2.20 ± 0.29*	1.19 ± 0.15**	0.54 ± 0.05**	0.06 ± 0.04**
Free T ₄ (ng/dL)	1.61 ± 0.14	1.56 ± 0.07	1.65 ± 0.10	1.28 ± 0.08	1.48 ± 0.14	0.84 ± 0.10**	0.38 ± 0.02**	0.34 ± 0.00**
Total T ₃ (ng/dL)	122.38 ± 4.62	123.00 ± 6.59	118.40 ± 5.53	122.13 ± 6.54	119.88 ± 5.75	123.63 ± 5.25	120.13 ± 5.80	133.25 ± 13.87
TSH (ng/mL)	19.50 ± 2.37	18.00 ± 2.71	19.10 ± 2.06	16.50 ± 2.58	15.88 ± 1.49	20.88 ± 2.64	23.50 ± 2.86	16.75 ± 2.06

*Significantly different (P≤0.05) from the vehicle control group by Shirley's test

** P≤0.01

^a Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. T₄=thyroxine; T₃=triiodothyronine; TSH=thyroid stimulating hormone

^b n=9

TABLE 6
Hepatic Cell Proliferation Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Labeling index (%)								
Week 14	2.36 ± 0.25	3.18 ± 1.15	1.72 ± 0.30	1.93 ± 0.27	2.12 ± 0.22	2.82 ± 0.52	2.07 ± 0.29	7.83 ± 1.37*
Week 31	2.07 ± 0.54	1.50 ± 0.24	1.76 ± 0.51	1.35 ± 0.21	1.67 ± 0.27	1.95 ± 0.19	1.65 ± 0.25	9.35 ± 1.47**
Week 53	1.07 ± 0.19	1.31 ± 0.28	1.55 ± 0.42	1.24 ± 0.29	2.47 ± 1.09	1.53 ± 0.24	1.09 ± 0.29	5.60 ± 0.93**

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

** $P \leq 0.01$

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

TABLE 7
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 PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Liver Microsomes								
7-Ethoxyresorufin- <i>O</i> -deethylase (EROD) (pmol/minute per mg microsomal protein)								
Week 14	42.89 ± 3.91	38.47 ± 3.92	53.53 ± 3.37*	183.26 ± 14.64**	557.62 ± 67.72**	990.89 ± 79.85**	1,709.60 ± 75.36**	1,730.00 ± 59.21**
Week 31	43.80 ± 2.10	42.85 ± 3.44	52.81 ± 3.18	213.08 ± 21.14**	599.49 ± 47.17**	1,255.70 ± 98.13**	1,444.70 ± 68.38**	1,910.10 ± 83.72**
Week 53	35.53 ± 2.10	32.50 ± 1.84	53.94 ± 6.27**	164.60 ± 16.16**	399.30 ± 90.63**	1,151.60 ± 155.14**	1,837.90 ± 100.01**	2,109.10 ± 103.51**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)								
Week 14	0.453 ± 0.020	0.554 ± 0.032*	0.575 ± 0.024**	0.585 ± 0.025**	0.987 ± 0.073**	1.383 ± 0.103**	2.323 ± 0.148**	3.024 ± 0.191**
Week 31	0.420 ± 0.021	0.427 ± 0.023	0.586 ± 0.028**	0.725 ± 0.046**	1.015 ± 0.069**	1.806 ± 0.141**	2.593 ± 0.114**	3.264 ± 0.273**
Week 53	0.404 ± 0.024	0.341 ± 0.021	0.414 ± 0.024	0.549 ± 0.022**	0.718 ± 0.110**	1.628 ± 0.209**	2.517 ± 0.143**	3.485 ± 0.169**
7-Pentoxoresorufin- <i>O</i> -deethylase (PROD) (pmol/minute per mg microsomal protein)								
Week 14	1.508 ± 0.099	1.583 ± 0.120	2.177 ± 0.124**	4.264 ± 0.161**	7.162 ± 0.245**	20.806 ± 0.835**	30.969 ± 1.761**	43.002 ± 2.747**
Week 31	2.302 ± 0.093	2.660 ± 0.205*	3.146 ± 0.170**	5.728 ± 0.224**	14.721 ± 1.067**	28.981 ± 1.590**	34.854 ± 1.378**	53.856 ± 3.073**
Week 53	1.748 ± 0.096	1.676 ± 0.120	2.374 ± 0.202*	5.415 ± 0.371**	10.078 ± 1.541**	29.898 ± 3.063**	38.127 ± 1.598**	48.232 ± 1.845**
Lung Microsomes								
7-Ethoxyresorufin- <i>O</i> -deethylase (EROD) (pmol/minute per mg microsomal protein)								
Week 14	0.845 ± 0.347	0.423 ± 0.042	1.086 ± 0.653	0.636 ± 0.061	1.070 ± 0.106*	4.371 ± 0.755**	29.385 ± 2.642**	65.616 ± 3.531**
Week 31	0.359 ± 0.054	0.337 ± 0.074	0.231 ± 0.043	0.277 ± 0.056	0.378 ± 0.065** ^b	2.205 ± 0.339**	16.427 ± 1.101** ^b	45.620 ± 3.268** ^c
Week 53	0.462 ± 0.241	0.241 ± 0.040**	0.076 ± 0.034	0.122 ± 0.033	0.118 ± 0.070	1.667 ± 0.220*	8.570 ± 1.269**	53.057 ± 3.458**

* Significantly different (P≤0.05) from the vehicle control group by Shirley's test

** P≤0.01

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

^b n=9

^c n=8

TABLE 8
Tissue Concentrations of PCB 118 in Female Rats in the 2-Year Gavage Study of PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Week 105	10			10	10	10	10	10
Fat (µg/g)								
Week 14	BLOQ	3.9 ± 0.2	10.7 ± 0.5	35.1 ± 1.6	79.9 ± 2.6	143.7 ± 8.7	288.1 ± 13.5	1,409.4 ± 93.0
Week 31	BLOQ	5.0 ± 0.2	14.4 ± 0.9	41.7 ± 3.2	88.0 ± 3.5	162.0 ± 8.4	311.9 ± 13.0	1,915.1 ± 111.5
Week 53	BLOQ	4.8 ± 0.3	15.3 ± 1.2	33.1 ± 3.4	68.1 ± 4.9	150.2 ± 5.9	283.7 ± 12.9	1,314.3 ± 149.0
Week 105	BLOQ			117.1 ± 11.3	220.3 ± 18.2	467.4 ± 49.3	875.6 ± 98.8	4,654.5 ± 411.9
Liver (ng/g)								
Week 14	BLOQ	117.0 ± 5.6	341.9 ± 23.4	1,146.0 ± 70.7	2,973.7 ± 152.0	6,259.5 ± 488.3	16,269.1 ± 660.3	219,659 ± 20,502.8
Week 31	BLOQ	126.1 ± 10.0	404.1 ± 29.1	1,539.0 ± 306.0	3,149.0 ± 232.4	6,196.5 ± 359.5	14,142.2 ± 828.2	270,740 ± 23,526.8
Week 53	BLOQ	123.0 ± 10.5	392.4 ± 26.6	966.2 ± 42.5	2,121.1 ± 195.4	5,687.1 ± 730.4	11,781.1 ± 528.3	228,981 ± 29,509.1
Week 105	BLOQ			3,415.6 ± 306.5	7,147.7 ± 439.8	32,004.1 ± 8,035.5	85,330.1 ± 13,811.3	1,087,019 ± 358,068
Lung (ng/g)								
Week 14	BLOQ	113.0 ± 14.8	424.1 ± 78.6	993.8 ± 110.3	2,341.2 ± 385.2	3,736.6 ± 275.6	5,447.9 ± 476.2	22,505.0 ± 1,759.6
Week 31	BLOQ	111.5 ± 7.3	276.1 ± 23.8	757.2 ± 66.1	1,654.8 ± 129.3	2,514.3 ± 175.6	4,624.0 ± 355.3	20,167.8 ± 1,666.3
Week 53	BLOQ	60.5 ± 6.1	168.6 ± 8.6	453.5 ± 48.2	986.9 ± 132.5	1,866.6 ± 216.0	2,593.2 ± 117.6	14,185.9 ± 1,208.2
Week 105	BLOQ			1,663.2 ± 196.3	2,641.5 ± 253.0	5,822.2 ± 895.3	9,266.3 ± 1,230.4	40,576.9 ± 6,372.8
Blood (ng/mL)								
Week 14	BLOQ	2.7 ± 0.4	8.2 ± 0.7	28.4 ± 1.2	54.3 ± 2.8	113.3 ± 10.6	292.7 ± 19.6	1,764.3 ± 120.6
Week 31	BLOQ	4.4 ± 0.5	15.4 ± 2.0	37.3 ± 3.8	75.4 ± 3.4	137.0 ± 8.5	284.5 ± 13.5	1,898.5 ± 86.8
Week 53	BLOQ	6.0 ± 0.4	15.8 ± 1.3	43.3 ± 3.4	78.4 ± 11.8	165.4 ± 10.3	307.1 ± 14.0	2,815.2 ± 347.0
Week 105	BLOQ			204.2 ± 39.2	487.8 ± 48.0	1,090.1 ± 212.4	2,934.2 ± 469.3	11,385.1 ± 1,936.4

^a Data are given as the mean ± standard error. BLOQ=below the limit of quantitation. LOQ_{fat}=1.152 µg/g, LOQ_{liver}=76.80 ng/g, LOQ_{lung}=33.60 ng/g, LOQ_{blood}=3.36 ng/mL

TABLE 9
Tissue Concentrations of PCB 156 in Female Rats in the 2-Year Gavage Study of PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Week 105	10			10	10	10	10	10
Fat (ng/g)								
Week 14	BLOQ	2.0 ± 0.1	5.0 ± 0.3	15.7 ± 0.8	36.6 ± 1.2	69.8 ± 5.0	148.9 ± 8.9	708.9 ± 48.7
Week 31	BLOQ	3.3 ± 0.2	8.0 ± 0.5	19.3 ± 2.9	42.2 ± 2.2	81.8 ± 5.4	202.5 ± 26.2	1,133.9 ± 77.2
Week 53	BLOQ	3.4 ± 0.2	8.7 ± 0.5	17.9 ± 1.8	37.2 ± 4.1	99.0 ± 10.1	215.3 ± 12.4	937.5 ± 114.8
Week 105	BLOQ			46.3 ± 6.7	91.2 ± 8.2	271.9 ± 34.1	423.9 ± 54.9	2,643.3 ± 172.7
Liver (pg/g)								
Week 14	BLOQ	38.4 ± 0.0	144.0 ± 11.0	488.8 ± 33.8	1,337.9 ± 62.5	3,057.3 ± 298.4	8,754.3 ± 466.4	160,974 ± 5,245.0
Week 31	BLOQ	43.7 ± 5.3	179.0 ± 11.4	752.9 ± 183.7	1,572.1 ± 119.6	3,552.8 ± 292.1	8,769.7 ± 705.6	173,800 ± 16,739.1
Week 53	BLOQ	59.3 ± 11.2	177.2 ± 17.6	442.9 ± 26.3	1,009.3 ± 115.5	3,374.7 ± 591.7	8,161.8 ± 409.1	168,157 ± 22,400.5
Week 105	BLOQ			1,969.2 ± 231.5	4,282.7 ± 321.8	27,115.2 ± 8,556.5	62,544.4 ± 11,082.7	668,956 ± 255,904
Lung (pg/g)								
Week 14	BLOQ	16.8 ± 0.0	71.4 ± 27.0	230.2 ± 29.1	602.5 ± 111.6	979.1 ± 57.0	1,818.7 ± 178.2	9,346.5 ± 793.3
Week 31	BLOQ	16.8 ± 0.0	69.3 ± 6.6	172.2 ± 24.8	436.2 ± 36.5	856.9 ± 62.2	1,707.4 ± 159.9	9,746.9 ± 987.7
Week 53	BLOQ	19.6 ± 2.8	86.5 ± 25.9	118.2 ± 14.2	513.1 ± 133.6	978.8 ± 204.4	1,863.5 ± 641.5	8,006.1 ± 544.9
Week 105	BLOQ			845.5 ± 133.5	1,411.6 ± 216.9	4,482.0 ± 1,139.2	5,997.6 ± 940.1	34,287.2 ± 6,871.9
Blood (pg/mL)								
Week 14	BLOQ	10.4 ± 8.2	2.8 ± 0.6	19.9 ± 4.6	28.6 ± 1.9	63.5 ± 6.9	194.6 ± 16.5	1,178.5 ± 91.7
Week 31	BLOQ	5.7 ± 2.3	7.6 ± 2.4	59.5 ± 18.3	50.3 ± 3.7	96.1 ± 7.6	203.1 ± 14.2	1,384.6 ± 71.2
Week 53	BLOQ	4.2 ± 1.1	9.7 ± 0.8	25.4 ± 2.5	45.6 ± 8.6	118.1 ± 12.6	284.0 ± 9.9	2,393.5 ± 300.8
Week 105	BLOQ			208.6 ± 37.5	312.1 ± 42.8	935.1 ± 213.9 ^b	2,350.7 ± 458.1	11,287.9 ± 1,992.7

^a Data are given as the mean ± standard error. BLOQ=below the limit of quantitation. LOQ_{fat}=1.152 ng/g, LOQ_{liver}=76.80 pg/g, LOQ_{lung}=33.60 pg/g, LOQ_{blood}=3.36 pg/mL

^b n=9

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, lung, uterus, pancreas, adrenal cortex, mesentery, thyroid gland, thymus, clitoral gland, bone marrow, nose, kidney, mammary gland, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A.

Liver: At 14 weeks, the absolute and relative liver weights of all dosed groups (except the relative liver weight of the 220 µg/kg group) were significantly greater than those of the vehicle controls (Table B1). At 31 weeks, the absolute and relative liver weights of the 1,000 and 4,600 µg/kg groups were significantly greater than those of the vehicle controls. At 53 weeks, the absolute and relative liver weights of the 4,600 µg/kg group and the relative liver weight of the 1,000 µg/kg group were significantly greater than those of the vehicle controls.

At 14 weeks, the incidences of hepatocyte hypertrophy were significantly increased in the 220 µg/kg or greater groups and tended to be accompanied by increased liver weight; severities increased in the 1,000 and 4,600 µg/kg groups (Tables 10 and A4a). Significantly increased incidences of diffuse fatty change, toxic hepatopathy, and multinucleated hepatocytes occurred in the 4,600 µg/kg group. The severities of inflammation increased with increasing dose in the 460 µg/kg or greater groups. The incidences of pigmentation were significantly increased in the 1,000 and 4,600 µg/kg groups, and the incidence of necrosis was significantly increased in the 4,600 µg/kg group.

At 31 weeks, the incidences of hepatocyte hypertrophy were significantly increased in the 460 µg/kg or greater groups and tended to correlate with increased liver weight; severities also increased in the 1,000 and 4,600 µg/kg groups (Tables 10 and A4a). Significantly increased incidences of diffuse fatty change, multinucleated hepatocytes, toxic hepatopathy, and oval cell hyperplasia occurred in the 4,600 µg/kg group. The severities of inflammation were increased in the 1,000 and 4,600 µg/kg groups. The incidences of pigmentation were significantly increased in the 1,000 and 4,600 µg/kg groups with the severity increased at 4,600 µg/kg. One

1,000 µg/kg rat had necrosis, one 4,600 µg/kg rat had cholangiofibrosis, and two 4,600 µg/kg rats had bile duct hyperplasia.

At 53 weeks, three cholangiocarcinomas and one hepatocellular adenoma occurred in the 4,600 µg/kg group (Tables 10 and A1a). The incidences of hepatocyte hypertrophy were significantly increased in the 460 µg/kg or greater groups and the severity was increased in the 4,600 µg/kg group (Tables 10 and A4a). Significantly increased incidences of diffuse fatty change, eosinophilic focus, toxic hepatopathy, bile duct hyperplasia, oval cell hyperplasia, cholangiofibrosis, mixed cell focus, and multinucleated hepatocytes occurred in the 4,600 µg/kg group. Significantly increased incidences of pigmentation occurred in the 460 µg/kg or greater groups and necrosis occurred in a few rats in these groups. The severity of inflammation increased in the 4,600 µg/kg group.

At 2 years, the incidences of multiple cholangiocarcinoma and single or multiple cholangiocarcinoma (combined) in the 4,600 µg/kg core and stop-exposure groups were significantly greater than those in the vehicle control group; the combined incidence was also increased in the 1,000 µg/kg group (Tables 11, A1b, A2a, and A2b). Four incidences of hepatocholangioma occurred in the 4,600 µg/kg core study group. The incidences of multiple hepatocellular adenoma in the 4,600 µg/kg core study group and single or multiple hepatocellular adenoma (combined) in the 1,000 µg/kg or greater core study groups were significantly greater than those in the vehicle control group. A single incidence of hepatocellular carcinoma occurred in the 4,600 µg/kg core study group. The incidences of hepatocellular adenoma and cholangiocarcinoma in the stop-exposure group were significantly decreased compared to those in the 4,600 µg/kg core study group.

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 (Plates 1 and 2).

TABLE 10

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
14-Week Interim Evaluation								
Number Examined Microscopically	10	10	10	10	10	10	10	10
Hepatocyte, Hypertrophy ^a	0	0	1 (1.0) ^b	1 (1.0)	5* (1.0)	6**(1.0)	9**(1.4)	10**(2.9)
Fatty Change, Diffuse	0	0	0	0	0	0	0	10**(2.6)
Toxic Hepatopathy	0	0	0	0	0	0	0	10**(1.0)
Hepatocyte, Multinucleated	0	0	0	0	0	0	0	10**(2.0)
Inflammation	9 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.3)	10 (2.0)	10 (2.4)
Pigmentation	0	0	0	0	0	0	5* (1.0)	8**(1.0)
Necrosis	0	0	0	0	0	0	3 (1.0)	9**(1.1)
31-Week Interim Evaluation								
Number Examined Microscopically	10	10	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	0	1 (1.0)	1 (1.0)	3 (1.0)	8**(1.1)	10**(1.4)	10**(3.0)
Fatty Change, Diffuse	0	0	0	0	0	0	0	10**(2.8)
Hepatocyte, Multinucleated	0	0	0	0	0	0	0	10**(1.1)
Toxic Hepatopathy	0	0	0	0	0	0	0	10**(1.0)
Oval Cell, Hyperplasia	0	0	0	0	0	0	1 (1.0)	9**(1.1)
Inflammation	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.1)	9 (1.0)	10 (1.5)	10 (2.1)
Pigmentation	0	0	0	0	0	1 (1.0)	6**(1.0)	10**(1.6)
Necrosis	0	0	0	0	0	0	1 (1.0)	0
Cholangiofibrosis	0	0	0	0	0	0	0	1 (2.0)
Bile Duct, Hyperplasia	0	0	0	0	0	0	0	2 (1.0)

TABLE 10

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PCB 118 (continued)

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
53-Week Interim Evaluation								
Number Examined Microscopically	8	8	10	8	8	8	8	8
Hepatocyte, Hypertrophy	2 (1.0)	0	4 (1.3)	5 (1.0)	6 (1.0)	7* (1.0)	8** (1.1)	8** (2.9)
Fatty Change, Diffuse	0	0	0	0	0	0	0	8** (2.8)
Eosinophilic Focus	0	0	0	0	0	0	0	4*
Toxic Hepatopathy	0	0	0	0	0	0	0	8** (2.0)
Bile Duct, Hyperplasia	1 (1.0)	0	0	0	0	0	0	6** (1.2)
Oval Cell, Hyperplasia	0	0	0	0	0	0	0	8** (1.4)
Cholangiofibrosis	0	1 (1.0)	0	0	0	0	0	4* (1.8)
Mixed Cell Focus	1	0	0	0	0	1	1	7**
Hepatocyte, Multinucleated	0	0	0	0	0	0	1 (1.0)	7** (1.1)
Pigmentation	0	0	0	0	0	4* (1.0)	7** (1.3)	8** (1.8)
Necrosis	0	0	0	0	0	1 (1.0)	2 (1.0)	1 (1.0)
Inflammation	8 (1.1)	7 (1.0)	8 (1.0)	5 (1.0)	8 (1.0)	8 (1.1)	8 (1.3)	8 (1.8)
Cholangiocarcinoma, Multiple	0	0	0	0	0	0	0	2
Cholangiocarcinoma (includes multiple)	0	0	0	0	0	0	0	3
Hepatocellular Adenoma	0	0	0	0	0	0	0	1

* 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

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 (Plates 3 and 4).

Hepatocellular adenoma was a nodular mass that usually was larger than a focus, had a distinct border, and produced more compression of surrounding normal parenchyma (Plate 5). Adenoma was composed of a rather uniform population of mildly to moderately pleomorphic hepatocytes that generally were normal in 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.

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 the core study groups at 2 years, there were several significantly increased, dose-related incidences of minimal to moderate nonneoplastic lesions in the liver, which

tended to increase in severity with increasing dose (Tables 11 and A4b). Significantly increased incidences included hepatocyte hypertrophy, inflammation, and oval cell hyperplasia in all dosed groups; pigmentation in the 220 µg/kg or greater groups; multinucleated hepatocytes, eosinophilic focus, diffuse fatty change, and toxic hepatopathy in the 460 µg/kg or greater groups; mixed cell focus in the 460 and 4,600 µg/kg groups; nodular hyperplasia, necrosis, and bile duct hyperplasia in the 1,000 and 4,600 µg/kg groups; and bile duct cyst and cholangiofibrosis in the 4,600 µg/kg group. Significantly decreased incidences of basophilic cell foci occurred in the 100, 460, and 4,600 µg/kg groups compared to that in the vehicle controls. Significantly decreased incidences of clear cell foci occurred in the 220, 1,000, and 4,600 µg/kg groups compared to that in the vehicle controls.

In the 4,600 µg/kg stop-exposure group, incidences of lesions significantly increased compared to those in the vehicle control group included hepatocyte hypertrophy, multinucleated hepatocytes, eosinophilic and mixed cell foci, inflammation, necrosis, diffuse fatty change, bile duct hyperplasia and cyst, oval cell hyperplasia, pigmentation, cholangiofibrosis, toxic hepatopathy, and focal fatty change (Tables 11 and A4b). Compared to the 4,600 µg/kg core study group, only the incidences of mixed cell, basophilic, and clear cell foci and focal fatty change were significantly increased in the stop-exposure group. The incidences of the following lesions were significantly decreased in the stop-exposure group compared to those in the 4,600 µg/kg core study group: hepatocyte hypertrophy, multinucleated hepatocytes, eosinophilic focus, nodular hyperplasia, diffuse fatty change, bile duct and oval cell hyperplasia, cholangiofibrosis, and toxic hepatopathy.

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.

TABLE 11

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Number Examined							
Microscopically	52	51	52	52	52	49	49
Hepatocyte, Hypertrophy ^a	0	12** (1.3) ^b	15** (1.6)	20** (1.6)	44** (2.0)	48** (3.5)	30***▲▲ (2.0)
Hepatocyte, Multinucleated	0	1 (1.0)	3 (1.0)	21** (1.2)	40** (1.3)	43** (1.7)	32***▲▲ (1.3)
Eosinophilic Focus	5	8	9	15*	25**	41**	20***▲▲
Mixed Cell Focus	21	19	29	36**	31	7**	36***▲▲
Basophilic Focus	15	7*	11	6**	9	1**	9▲▲
Clear Cell Focus	15	10	7*	14	5**	0**	13▲▲
Hyperplasia, Nodular	0	0	0	0	12**	43**	4▲▲
Inflammation	21 (1.0)	30* (1.0)	35** (1.1)	36** (1.1)	43** (1.3)	44** (1.3)	47** (1.1)
Necrosis	1 (1.0)	2 (3.0)	1 (1.0)	2 (2.5)	20** (1.5)	22** (1.7)	14** (1.8)
Fatty Change, Diffuse	1 (1.0)	2 (1.5)	1 (1.0)	9* (1.7)	39** (2.0)	48** (2.5)	8***▲▲ (1.8)
Bile Duct, Hyperplasia	5 (1.0)	6 (1.2)	7 (1.3)	8 (1.4)	21** (1.5)	40** (1.9)	25***▲▲ (1.9)
Oval Cell, Hyperplasia	0	12** (1.1)	9** (1.2)	29** (1.2)	40** (1.6)	46** (3.0)	29***▲▲ (1.7)
Bile Duct, Cyst	2 (2.5)	3 (2.3)	5 (2.8)	6 (2.3)	6 (2.2)	21** (2.1)	14** (2.3)
Pigmentation	1 (1.0)	5 (1.2)	12** (1.3)	41** (1.4)	50** (2.2)	48** (1.7)	43** (1.4)
Cholangiofibrosis	0	2 (1.0)	2 (1.5)	3 (1.7)	2 (1.5)	22** (2.5)	10***▲▲ (2.0)
Toxic Hepatopathy	0	0	3 (1.0)	14** (1.0)	33** (1.4)	46** (3.4)	36***▲▲ (1.7)
Fatty Change, Focal	2 (1.0)	1 (1.0)	6 (1.0)	4 (1.0)	3 (1.7)	0	9***▲▲ (1.2)
Cholangiocarcinoma, Multiple	0	0	0	0	0	30**	17***▲▲
Cholangiocarcinoma (includes multiple) ^c							
Overall rate ^d	0/52 (0%)	0/51 (0%)	0/52 (0%)	0/52 (0%)	3/52 (6%)	36/49 (73%)	29/49 (59%)
Adjusted rate ^e	0.0%	0.0%	0.0%	0.0%	7.5%	84.6%	68.1%
Terminal rate ^f	0/21 (0%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	1/28 (4%)	22/25 (88%)	17/25 (68%)
First incidence (days)	— ⁱ	—	—	—	642	433	567
Poly-3 test ^g	P<0.001	— ^j	—	—	P=0.144	P<0.001	P<0.001
Poly-3 test ^h			P=0.048N				
Hepatocellular Adenoma, Multiple	0	0	0	0	4	14**	1▲▲
Hepatocellular Adenoma (includes multiple) ^k							
Overall rate	0/52 (0%)	1/51 (2%)	1/52 (2%)	4/52 (8%)	12/52 (23%)	24/49 (49%)	1/49 (2%)
Adjusted rate	0.0%	2.8%	2.6%	9.3%	29.5%	59.3%	2.5%
Terminal rate	0/21 (0%)	0/20 (0%)	1/25 (4%)	2/30 (7%)	8/28 (29%)	17/25 (68%)	1/25 (4%)
First incidence (days)	—	602	733 (T)	593	602	617	733 (T)
Poly-3 test	P<0.001	P=0.506	P=0.515	P=0.091	P<0.001	P<0.001	P=0.525
Poly-3 test							P<0.001N

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Number Examined							
Microscopically	52	51	52	52	52	49	49
Hepatocellular Carcinoma ^l	0	0	0	0	0	1	0
Cholangioma ^m	1	0	0	0	0	0	0
Hepatocholangioma ^d							
Overall rate	0/52 (0%)	0/51 (0%)	0/52 (0%)	0/52 (0%)	0/52 (0%)	4/49 (8%)	0/49 (0%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	10.1%	0.0%
Terminal rate	0/21 (0%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	0/28 (0%)	3/25 (12%)	0/25 (0%)
First incidence (days)	—	—	—	—	—	687	—
Poly-3 test	P<0.001	—	—	—	—	P=0.076	—
Poly-3 test							—

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

** $P \leq 0.01$

▲▲ Significantly different ($P \leq 0.01$) from the 4,600 µg/kg core study group by the Poly-3 test

(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):
 1/473 (0.2% $\pm$ 0.7%), range 0%-2%

^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.

^h Pairwise comparison between the 4,600 µg/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by N.

ⁱ Not applicable; no neoplasms in animal group

^j Value of statistic cannot be computed

^k Historical incidence: 6/473 (1.3% $\pm$ 1.7%), range 0%-4%

^l Historical incidence: 0/473

^m Historical incidence: 1/473 (0.2% $\pm$ 0.6%), range 0%-2%

Eosinophilic, mixed, basophilic, and clear cell foci were characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic focus was composed of cells with eosinophilic cytoplasm (Plate 6). Mixed cell focus was composed of a mixture of cells with different staining properties, generally a mixture of eosinophilic cells and cells with clear cytoplasm (clear cells). To be classified as an eosinophilic focus, at least 80% of the cells within the focus had to be eosinophilic cells, otherwise the focus was classified as a mixed cell focus. Basophilic focus consisted of hepatocytes with basophilic cytoplasm, occasionally with basophilic linear intracytoplasmic aggregates. Clear cell focus was composed of cells having clear cytoplasm. If two or more foci of a given type were present in a liver, it was diagnosed as multiple. The hepatic cords at the periphery of these foci generally merged imperceptibly with the surrounding normal liver resulting in an indistinct border and little or no compression of the adjacent liver parenchyma. 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 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.

Nodular hyperplasia was characterized by small to large nodular foci generally composed of hepatocytes that were considerably larger than normal hepatocytes (hepatocytic hypertrophy) and sometimes mixed with areas of increased numbers of small hepatocytes (hepatocytic hyperplasia). Areas of nodular hyperplasia blended with the surrounding parenchyma, although they often had a distinct border. Large, focal to multifocal areas of nodular hyperplasia were sometimes seen that caused compression of surrounding tissue or bulging of the capsular surface (Plate 7). 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 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 a useful diagnostic criterion.

Inflammation was generally a minor change consisting of accumulation of mononuclear cells (predominantly lymphocytes and plasma cells with occasional macrophages) most often within portal areas but also sometimes scattered randomly throughout the liver. Necrosis consisted of scattered necrotic areas of hepatic parenchyma that were often randomly distributed, but occasionally, in more severe cases, were distributed more widely. Focal and diffuse fatty change were generally minimal to mild changes consisting of discrete clear vacuoles (consistent with lipid) in the cytoplasm of hepatocytes involving singular foci of hepatocytes or widely scattered foci throughout the liver. Bile duct hyperplasia consisted of increased numbers of bile duct nuclei within portal areas (Plate 8). Oval cell hyperplasia consisted of small ovoid cells with basophilic cytoplasm and a round to ovoid nucleus 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. 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. Cholangiofibrosis appeared relatively small in size and well demarcated, and did not show invasion (Plates 9 and 10). Cholangiofibrosis is characterized by atypical bile ducts surrounded by abundant collagenous connective tissue. The ducts are often irregular, dilated, sometimes glandular, and contain mucinous material, cellular debris, and white blood cells in the lumen. The epithelium varies from low cuboidal to tall columnar with variable numbers of goblet cells. Connective tissue surrounding the ducts is dense.

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

TABLE 12

Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
53-Week Interim Evaluation								
Number Examined Microscopically	8	8	10	8	8	8	8	8
Alveolar Epithelium, Metaplasia, Bronchiolar ^a	0	0	0	0	0	0	0	3 (1.3) ^b
	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)	
2-Year Evaluation								
Number Examined Microscopically	51	52	52	52	52	50	50	
Alveolar Epithelium, Metaplasia, Bronchiolar	6 (1.2)	7 (1.4)	14 (1.3)	18* (1.4)	24**(1.4)	40**(2.0)	32**▲(1.8)	
Metaplasia, Squamous	1 (1.0)	0	0	1 (1.0)	1 (1.0)	13**(2.2)	0▲▲	
Artery, Mediastinum, Inflammation, Chronic Active	0	0	0	0	0	1 (2.0)	0	
Cystic Keratinizing Epithelioma, Multiple	0	0	0	0	0	8*	0	
Cystic Keratinizing Epithelioma, (includes multiple) ^c								
Overall rate ^d	0/51 (0%)	0/52 (0%)	0/52 (0%)	0/52 (0%)	0/52 (0%)	20/50 (40%)	0/50 (0%)	
Adjusted rate ^e	0.0%	0.0%	0.0%	0.0%	0.0%	49.2%	0.0%	
Terminal rate ^f	0/21 (0%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	0/28 (0%)	12/25 (48%)	0/25 (0%)	
First incidence (days)	— ⁱ	—	—	—	—	617	—	
Poly-3 test ^g	P<0.001	— ^j	—	—	—	P<0.001	—	
Poly-3 test ^h							P<0.001N	

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

** P≤0.01

▲ Significantly different (P≤0.05) from the 4,600 µg/kg core study group by the Poly-3 test

▲▲ P≤0.01

TABLE 12**Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats in the 2-Year Gavage Study of PCB 118**^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 ± standard deviation): 0/471^d Number of animals with neoplasm per number of animals with lung 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.^h Pairwise comparison between the 4,600 µg/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by N.ⁱ Not applicable; no neoplasms in animal group^j Value of statistic cannot be computed.

diagnosis included hepatocyte hypertrophy, pigmentation, inflammation, multinucleated hepatocytes, diffuse fatty change, bile duct hyperplasia, oval cell hyperplasia, nodular hyperplasia, focal cellular alteration, cholangiofibrosis, bile duct cyst, necrosis, and centrilobular degeneration. When only findings of hepatocyte hypertrophy, pigmentation, and slight fatty change were present, no diagnosis of toxic hepatopathy was made. Minimal toxic hepatopathy was diagnosed when additional changes indicative of a toxic effect, usually a slight degree of bile duct or oval cell hyperplasia, sometimes a few large prominent altered hepatocellular foci, and occasionally a small focus of 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.

Lung: At 14, 31, and 53 weeks, the absolute and relative lung weights of all core study groups were similar to those of the vehicle controls (Table B1).

At the 53-week interim evaluation, three 4,600 µg/kg rats had minimal bronchiolar metaplasia of the alveolar epithelium; this lesion was not seen in any other dose group (Tables 12 and A4a).

At 2 years, significantly increased incidences of multiple cystic keratinizing epithelioma and single or multiple cystic keratinizing epithelioma (combined) occurred in the 4,600 µg/kg core study group (Tables 12, A1b, and A2a). Significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium occurred in the 460 µg/kg or greater groups, including the stop-exposure group, and the severities were increased in both 4,600 µg/kg groups (Tables 12 and A4b). However, the incidence in the stop-exposure group was significantly less than that in the 4,600 µg/kg core study group. A significantly increased incidence of squamous metaplasia

occurred in the 4,600 µg/kg core study group compared to the vehicle controls. No incidences of squamous metaplasia occurred in the 4,600 µg/kg stop-exposure group, which was a significant decrease compared to the incidence in the 4,600 µg/kg core study group. A single incidence of chronic active inflammation of the artery located in the mediastinum occurred in the 4,600 µg/kg core study group.

Cystic keratinizing epithelioma 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. The outer portion of the lesion grew by expansion into the adjacent lung but evidence of invasion was not observed (Plate 11).

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 12). 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.

Squamous metaplasia 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 13).

Uterus: At 2 years, the incidence of carcinoma in the 4,600 µg/kg stop-exposure group was significantly greater than the vehicle control incidence and the historical vehicle control mean (Tables 13, A1b, A2b, and A3c). Squamous cell carcinomas occurred in the 220, 460, and 1,000 µg/kg core study groups and in the 4,600 µg/kg stop-exposure group (Tables 13 and A1b).

The incidences of squamous metaplasia in the 4,600 µg/kg core study group and cystic endometrial hyperplasia in the 1,000 and 4,600 µg/kg core study groups were significantly less than the vehicle control incidences (Tables 13 and A4b). In the 4,600 µg/kg stop-exposure group, the incidences of these two lesions were significantly increased compared to those in the 4,600 µg/kg core study group.

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Number Necropsied	52	52	52	52	52	52	50
Metaplasia, Squamous ^a	29 (1.9) ^b	26 (1.7)	27 (2.0)	34 (1.8)	35 (2.3)	5** (1.4)	23 ^{▲▲} (1.6)
Endometrium, Hyperplasia, Cystic	28 (1.6)	27 (1.7)	22 (1.5)	23 (2.0)	13** (1.6)	9** (2.0)	21 ^{▲▲} (2.7)
Carcinoma ^c							
Overall rate ^d	2/52 (4%)	2/52 (4%)	1/52 (2%)	3/52 (6%)	4/52 (8%)	3/52 (6%)	11/50 (22%)
Adjusted rate ^e	5.7%	5.5%	2.6%	7.1%	9.9%	7.5%	26.9%
Terminal rate ^f	2/21 (10%)	1/20 (5%)	0/25 (0%)	2/30 (7%)	2/28 (7%)	2/25 (8%)	6/25 (24%)
First incidence (days)	733 (T)	602	595	730	669	672	642
Poly-3 test ^g	P=0.427	P=0.685N	P=0.470N	P=0.585	P=0.402	P=0.561	P=0.014
Poly-3 test ^h				P=0.019			
Squamous Cell Carcinoma ⁱ							
Overall rate	0/52 (0%)	0/52 (0%)	3/52 (6%)	1/52 (2%)	1/52 (2%)	0/52 (0%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	7.9%	2.3%	2.5%	0.0%	2.5%
Terminal rate	0/21 (0%)	0/20 (0%)	2/25 (8%)	0/30 (0%)	1/28 (4%)	0/25 (0%)	1/25 (4%)
First incidence (days)	— ^j	—	707	627	733 (T)	—	733 (T)
Poly-3 test	P=0.315N	— ^k	P=0.132	P=0.539	P=0.525	—	P=0.525
Poly-3 test				P=0.499			

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Poly-3 test

^{▲▲} Significantly different ($P \leq 0.01$) from the 4,600 µg/kg core study group by the Poly-3 test

(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): 6/473 (1.3% $\pm$ 1.4%), range 0%-4%

^d Number of animals with neoplasm per number of animals necropsied

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g Beneath the vehicle control incidence is the P value associated with the trend test; 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 negative trend or a lower incidence in a dosed group is indicated by N.

^h Pairwise comparison between the 4,600 µg/kg core and stop-exposure groups.

ⁱ Historical incidence: 2/473 (0.4% $\pm$ 0.9%), range 0%-2%

^j Not applicable; no neoplasms in animal group

^k Value of statistic cannot be computed

Carcinoma consisted of sheets of poorly differentiated ovoid to spindle-shaped epithelial cells, often with invasion into the underlying myometrium (Plates 14 and 15). Squamous cell carcinoma occurred on the endometrial surface, caused dilatation of the uterus, and was characterized by irregular cords and clusters of atypical stratified squamous epithelial cells that invaded the underlying myometrium (Plates 16 and 17).

Squamous metaplasia was generally a minimal to mild, multifocal change consisting of replacement of the endometrium by stratified squamous epithelium (Plates 18 and 19). Endometrial cystic hyperplasia, a common age-associated change in female rats, was characterized by varying degrees of hyperplasia of the endometrial epithelium.

Pancreas: Acinar cytoplasmic vacuolization occurred only in the 4,600 µg/kg group in the interim evaluations and the incidences at 31 and 53 weeks were significantly increased compared to those in the vehicle control groups (Tables 14 and A4a). At 2 years, an incidence of carcinoma occurred in the 4,600 µg/kg core study group and incidences of adenoma occurred in the 460 µg/kg or greater core study groups (Tables 14 and A1b). Also, at 2 years, the incidences of acinar cytoplasmic vacuolization were significantly increased in the 4,600 µg/kg core and stop-exposure groups compared to that in the vehicle controls and the severity of the lesion was increased in the core study group (Tables 14 and A4b). The incidence in the 4,600 µg/kg stop-exposure group was significantly decreased compared to that in the 4,600 µg/kg core study group. Incidences of chronic active inflammation of the artery were significantly increased in the 1,000 and 4,600 µg/kg core study groups compared to the vehicle controls but significantly decreased in the stop-exposure group compared to the 4,600 µg/kg core study group. Incidences of duct dilatation and duct inflammation were observed in a few 4,600 µg/kg core study rats.

Cytoplasmic vacuolization consisted of small clear 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 thick-

ened. This inflammatory reaction sometimes extended into the surrounding parenchyma (Plate 20).

Adrenal Cortex: At 31 weeks, a single incidence of atrophy occurred in the 4,600 µg/kg group and at 53 weeks, two incidences of cortical atrophy occurred in this group (Tables 15 and A4a). At 2 years, the incidences of atrophy in the 1,000 and 4,600 µg/kg core study groups were significantly increased and the severities were also increased (Tables 15 and A4b). The incidence of atrophy in the 4,600 µg/kg stop-exposure group was significantly decreased compared to that in the 4,600 µg/kg core study group. Cytoplasmic vacuolization occurred in all groups at 2 years with a significantly increased incidence in the 4,600 µg/kg stop-exposure group; the severity of this lesion was decreased in this group compared to that in the 4,600 µg/kg core study group.

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 (Plates 21 and 22).

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.

Mesentery: At 2 years, a dose-related increase in the incidences of chronic active inflammation of the artery occurred although it was not statistically significant [vehicle control, 1/2; 100 µg/kg, 0/1; 220 µg/kg, 0/3; 460 µg/kg, 2/3; 1,000 µg/kg, 5/9; 4,600 µg/kg (core study), 8/9; 4,600 µg/kg (stop-exposure), 5/9; Table A4b]. The morphological characteristics of this lesion were comparable to the chronic active inflammation of the artery seen in the pancreas.

Thyroid Gland: At 14, 31, and 53 weeks, the absolute and relative thyroid gland weights of all core study groups were similar to those of the vehicle controls (Table B1).

TABLE 14

Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
14-Week Interim Evaluation								
Number Examined Microscopically	10	10	10	10	10	10	10	10
Acinus, Vacuolization Cytoplasmic ^a	0	0	0	0	0	0	0	1 (1.0) ^b
31-Week Interim Evaluation								
Number Examined Microscopically	10	10	10	10	10	10	10	10
Acinus, Vacuolization Cytoplasmic	0	0	0	0	0	0	0	6**(1.2)
53-Week Interim Evaluation								
Number Examined Microscopically	8	8	10	8	8	8	8	8
Acinus, Vacuolization Cytoplasmic	0	0	0	0	0	0	0	8**(1.4)
	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)	
2-Year Evaluation								
Number Examined Microscopically	52	52	52	52	52	47	49	
Acinus, Vacuolization Cytoplasmic	0	0	0	0	4 (1.0)	42**(2.2)	10**▲(1.0)	
Artery, Inflammation, Chronic Active	1 (3.0)	2 (2.0)	1 (2.0)	7 (2.4)	7* (2.4)	12**(2.1)	5▲ (1.8)	
Duct, Dilatation	0	0	0	0	0	3 (4.0)	0	
Duct, Inflammation	0	0	0	0	0	2 (3.0)	0	

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of PCB 118 (continued)

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
2-Year Evaluation (continued)							
Number Examined Microscopically	52	52	52	52	52	47	49
Acinus, Adenoma ^c	0	0	0	2	3	1	0
Acinus, Carcinoma ^c	0	0	0	0	0	1	0
Acinus, Adenoma or Carcinoma ^d							
Overall rate ^e	0/52 (0%)	0/52 (0%)	0/52 (0%)	2/52 (4%)	3/52 (6%)	2/47 (4%)	0/49 (0%)
Adjusted rate ^f	0.0%	0.0%	0.0%	4.7%	7.5%	5.3%	0.0%
Terminal rate ^g	0/21 (0%)	0/20 (0%)	0/25 (0%)	1/30 (3%)	3/28 (11%)	2/25 (8%)	0/25 (0%)
First incidence (days)	— ^j	—	—	658	733 (T)	733 (T)	—
Poly-3 test ^h	P=0.196	— ^k	—	P=0.283	P=0.142	P=0.252	—
Poly-3 test ⁱ							P=0.224N

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

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test (interim evaluations) or by the Poly-3 test (2-year evaluation)

▲ Significantly different ($P \leq 0.05$) from the 4,600 µg/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):

1/468 (0.2% $\pm$ 0.7%), range 0%-2%

^d Historical incidence: 2/468 (0.4% $\pm$ 0.9%), range 0%-2%

^e Number of animals with neoplasm per number of animals with pancreas 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 4,600 µg/kg core and stop-exposure groups.

^j Not applicable; no neoplasms in animal group

^k Value of statistic cannot be computed

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
14-Week Interim Evaluation								
Thyroid Gland ^a	10	10	10	10	10	10	10	10
Follicular Cell, Hypertrophy ^b	1 (1.0) ^c	0	1 (2.0)	0	1 (1.0)	2 (1.0)	2 (1.0)	4 (1.5)
31-Week Interim Evaluation								
Adrenal Cortex	10	10	10	10	10	10	10	10
Atrophy	0	0	0	0	0	0	0	1 (2.0)
Thyroid Gland	10	10	10	10	10	10	10	10
Follicular Cell, Hypertrophy	1 (2.0)	0	0	0	0	0	2 (2.0)	8**(1.8)
53-Week Interim Evaluation								
Adrenal Cortex	8	8	10	8	8	8	8	8
Atrophy	0	0	0	0	0	0	0	2 (2.0)
Thyroid Gland	8	8	10	8	8	8	8	8
Follicular Cell, Hypertrophy	1 (1.0)	0	1 (1.0)	1 (1.0)	1 (1.0)	5 (1.2)	5 (1.4)	7**(2.0)

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118 (continued)

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
2-Year Evaluation							
Adrenal Cortex	52	52	52	51	52	49	49
Atrophy	1 (2.0)	0	0	2 (2.0)	9* (2.3)	35** (2.5)	4▲▲ (2.0)
Vacuolization Cytoplasmic	10 (1.4)	12 (1.4)	13 (1.5)	12 (1.6)	12 (1.4)	18 (1.8)	21* (1.4)
Thyroid Gland	51	51	51	51	52	49	50
Follicular Cell, Hypertrophy	6 (1.5)	7 (1.9)	13 (1.6)	18** (1.4)	21** (1.8)	23** (2.0)	12▲▲ (2.3)
Clitoral Gland	52	52	51	52	51	49	48
Duct, Cyst	26 (2.0)	39* (2.0)	31 (1.9)	35 (2.2)	37 (2.2)	30 (1.9)	28 (1.8)
Inflammation	41 (1.6)	38 (1.8)	39 (1.7)	40 (1.5)	35 (1.2)	13** (1.2)	29*▲▲ (1.3)
Bone Marrow	52	52	52	52	52	52	50
Hyperplasia	31 (3.0)	30 (2.8)	30 (3.1)	32 (2.7)	34 (2.6)	47** (3.0)	43** (2.9)
Nose	52	52	52	52	52	52	50
Inflammation	1 (2.0)	5 (2.0)	5 (1.8)	3 (1.7)	5 (1.6)	23** (1.7)	8*▲▲ (1.5)
Respiratory Epithelium, Hyperplasia	5 (1.2)	5 (1.4)	7 (1.4)	7 (1.3)	14* (1.1)	27** (1.3)	11▲▲ (1.2)
Kidney	52	52	52	52	52	50	49
Pigmentation	2 (1.0)	3 (1.0)	3 (1.3)	4 (1.3)	6 (1.0)	42** (2.2)	6▲▲ (1.2)

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

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test (interim evaluations) or by the Poly-3 test (2-year evaluation)

▲▲ Significantly different ($P \leq 0.01$) from the 4,600 µg/kg core study group by the Poly-3 test

^a Number of animals with tissue examined microscopically

^b Number of animals with lesion

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

In the 4,600 µg/kg groups, the incidences of follicular cell hypertrophy were slightly increased at 14 weeks and significantly increased at 31 and 53 weeks; the severities were increased at 14 and 53 weeks (Tables 15 and A4a). At 2 years, the incidences of follicular cell hypertrophy were significantly increased in the 460 µg/kg or greater core study groups compared to the vehicle controls and severity was increased in the 1,000 and 4,600 µg/kg groups (Tables 15 and A4b); the incidence of this lesion in the 4,600 µg/kg stop-exposure group was significantly less than that in the 4,600 µg/kg core study group.

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 (Plates 23 and 24). Since some degree of 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.

Thymus: Thymic atrophy has often been observed in studies of TCDD and related dioxin-like compounds. At 14 weeks in the present study, the absolute and relative thymus weights of all core study groups were similar to those of the vehicle controls (Table B1). Neither the incidences nor the severities of atrophy were significantly affected in any dose group at 14, 31, or 53 weeks (Table A4a).

At 2 years, more than 80% of the vehicle controls exhibited thymic atrophy and slight, but statistically significant increases in the incidences of this lesion were observed in the 1,000 and 4,600 µg/kg core study groups compared to the vehicle controls [vehicle control, 41/51; 100 µg/kg, 38/51; 220 µg/kg, 44/51; 460 µg/kg, 44/50; 1,000 µg/kg, 46/50; 4,600 µg/kg (core study), 44/49; 4,600 µg/kg (stop-exposure), 46/50; Table A4b]. Atrophy consisted of varying degrees of loss of lymphoid cells from the cortex resulting in a reduction of cortical thickness.

Clitoral Gland: At 2 years, the incidence of cystic duct was significantly increased in the 100 µg/kg group (Tables 15 and A4b). Significantly decreased incidences

of inflammation occurred in the 4,600 µg/kg core and stop-exposure groups compared to the vehicle controls; the 4,600 µg/kg stop-exposure group incidence was significantly greater than the 4,600 µg/kg core study group incidence.

Cystic ducts consisted of dilated ducts that were filled with keratin and lined by attenuated epithelium. The severity varied from minimal to marked and was graded depending upon the size of the dilated ducts. Minimal lesions consisted of ducts dilated approximately 2 to 3 mm, and marked lesions consisted of ducts dilated approximately 1 cm or more in diameter.

Bone Marrow: At 2 years, significantly increased incidences of hyperplasia occurred in the 4,600 µg/kg core and stop-exposure groups compared to the vehicle controls; severities of the lesion were generally similar among all groups (Tables 15 and A4b).

Hyperplasia was graded from minimal to marked. Minimal hyperplasia was rarely recorded because of the normal variation in the amount of bone marrow; normal was when the distal end of the femur section contained 20% to 60% marrow. Mild hyperplasia was recorded when marrow elements filled approximately 60% to 90% of the marrow cavity. Moderate hyperplasia was recorded when marrow elements filled about 90% of the cavity (the remaining 10% was fat). Marked was used when the entire marrow cavity was filled with dense marrow.

Nose: At 2 years, the incidences of inflammation in the 4,600 µg/kg core and stop-exposure groups and respiratory epithelium hyperplasia in the 1,000 and 4,600 µg/kg core study groups were significantly greater than those in the vehicle controls; the incidences of these lesions in the stop-exposure group were significantly less than those in the 4,600 µg/kg core study group (Tables 15 and A4b).

Inflammation was usually seen in nasal Section III and was generally characterized by accumulation of varying numbers of neutrophils, with fewer lymphocytes, mixed with mucus and debris within the nasal cavity, or in the submucosa underlying the respiratory epithelium. Respiratory epithelium hyperplasia, consisting of varying degrees of thickening of the respiratory epithelium due to an increase in the number of epithelial cells, was generally seen in association with inflammation, and appeared to be secondary to the inflammation (Plates 25 and 26).

Kidney: At 14 weeks, the absolute and relative left kidney weights of all core study groups were similar to those of the vehicle controls (Table B1). At 31 weeks, the relative left kidney weight of the 4,600 µg/kg group was significantly greater than that of the vehicle controls. At 53 weeks, the relative left kidney weights of the 1,000 and 4,600 µg/kg groups were significantly greater than that of the vehicle controls.

At 2 years, a significantly increased incidence of pigmentation, with increased severity, occurred in the 4,600 µg/kg core study group compared to the vehicle control group, while a significantly decreased incidence of pigmentation occurred in the stop-exposure group compared to the 4,600 µg/kg core study group (Tables 15 and A4b).

Pigmentation was characterized by minimal to moderate amounts of yellow-brown granular material within the cytoplasm of renal tubule epithelial cells in the outer cortex. Slight amounts of similar appearing pigment scattered in the cortex of vehicle controls was considered to represent a normal background change. Pigmentation

was diagnosed when the amount of pigment present exceeded this normal background level, and the severity of pigmentation was graded based upon the increase in the amount of pigment over background levels.

Mammary Gland: At 2 years, significantly decreased incidences of multiple fibroadenoma [vehicle control, 9/52; 100 µg/kg, 11/51; 220 µg/kg, 10/52; 460 µg/kg, 14/52; 1,000 µg/kg, 5/52; 4,600 µg/kg (core study), 0/50; 4,600 µg/kg (stop-exposure), 5/50] and single or multiple fibroadenoma (combined) (25/52, 29/51, 27/52, 33/52, 20/52, 8/50, 17/50) occurred in the 4,600 µg/kg core study group compared to those in the vehicle control group (Tables A1b, A2a, and A2b).

Pituitary Gland: At 2 years, the incidence of pars distalis single or multiple adenoma (combined) was significantly decreased in the 4,600 µg/kg core study group compared to that in the vehicle control group (17/52, 24/52, 18/52, 24/52, 17/52, 4/52, 12/50; Tables A1b, A2a, and A2b).

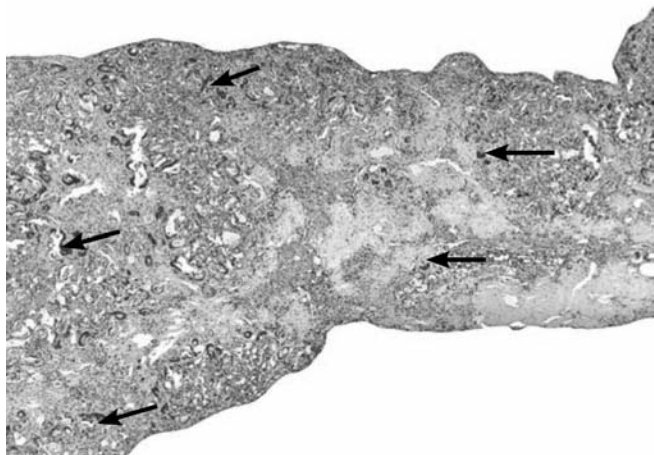


PLATE 1

Cholangiocarcinoma in the liver of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The cholangiocarcinoma is a noncircumscribed lesion, consisting of fibrous connective tissue stroma containing numerous atypical bile ducts that frequently contain mucinous material and cellular debris (arrows). The epithelium forming the atypical bile ducts is often discontinuous, consists of large atypical cells, and displays degenerative changes. Invasion of the adjacent liver parenchyma is observed. H&E

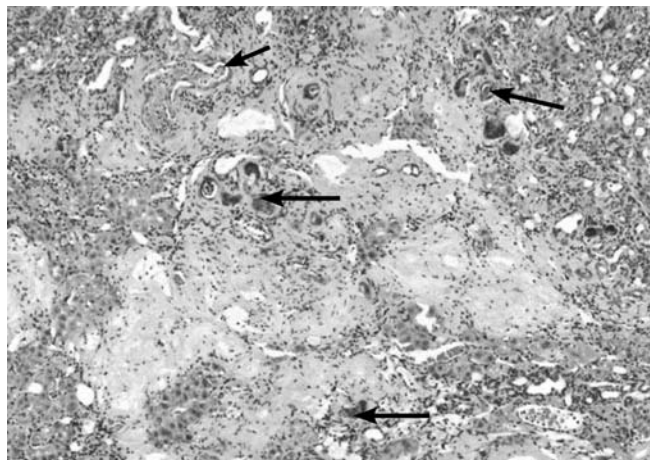


PLATE 2

Higher magnification of Plate 1. Arrows indicate the presence of atypical bile ducts that frequently contain mucinous material and cellular debris. H&E

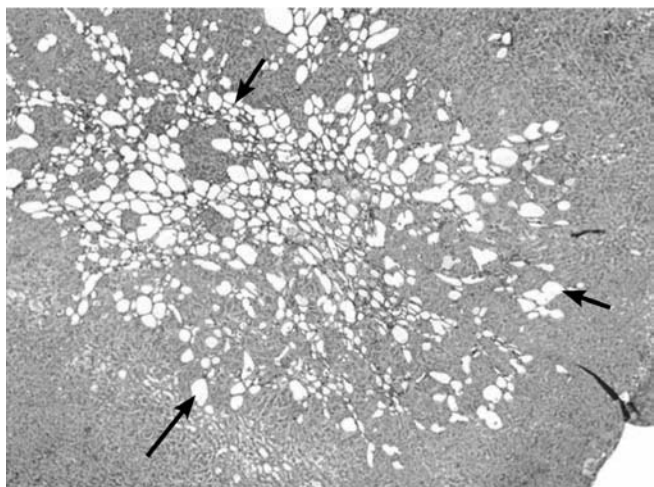


PLATE 3

Hepatocholangioma in the liver of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The neoplasm is composed of a mixture of proliferating hepatocellular and bile duct elements. The hepatocellular element appears similar to that seen in hepatocellular adenoma. Intermixed with the proliferating hepatocytes are numerous small and large biliary structures surrounded by small amounts of dense fibrous tissue stroma (arrows) that appear similar to the biliary structures seen within a cholangioma. H&E

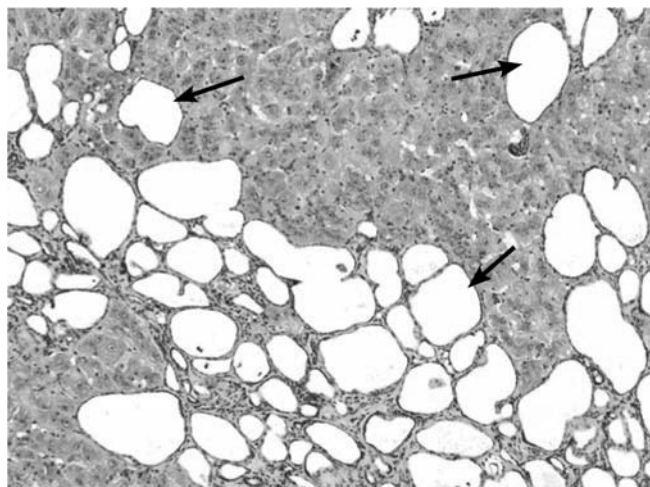


PLATE 4

Higher magnification of Plate 3. Arrows indicate the biliary structures of the hepatocholangioma. H&E

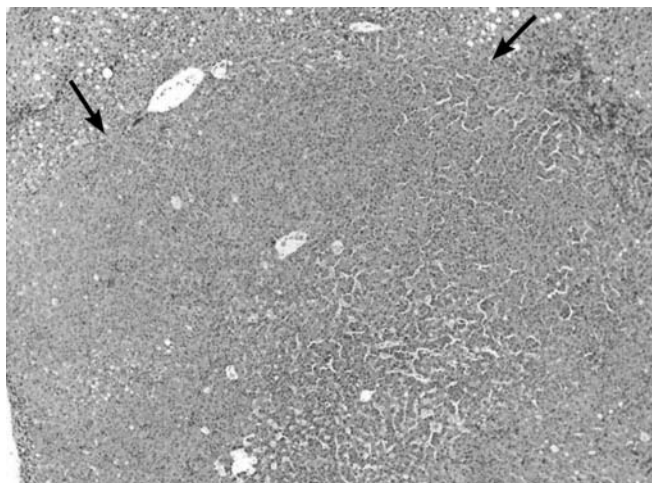


PLATE 5

Hepatocellular adenoma in the liver of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. Note the distinct border and compression of surrounding normal parenchyma (arrows). H&E

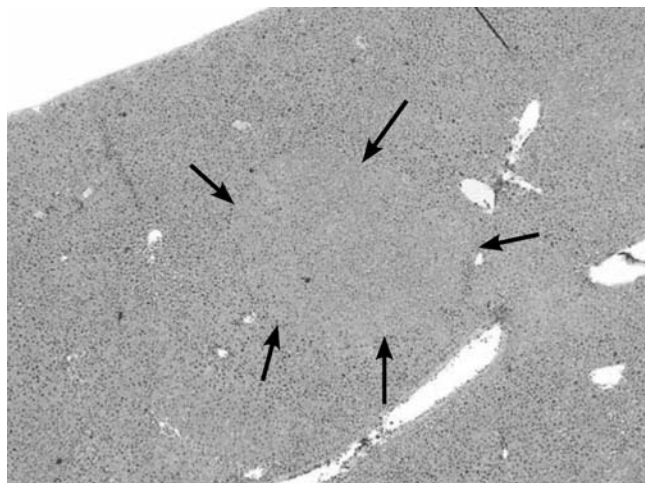


PLATE 6

Eosinophilic focus in the liver of a female rat in the 4,600 $\mu\text{g/kg}$ stop-exposure group in the 2-year gavage study of PCB 118. The focus consists of cells having eosinophilic cytoplasm, somewhat larger than normal, but appearing otherwise normal and arranged in a relatively normal lobular pattern. The hepatic cords at the periphery are merging imperceptibly with the surrounding normal liver resulting in an indistinct border and little or no compression of the adjacent liver parenchyma (arrows). H&E

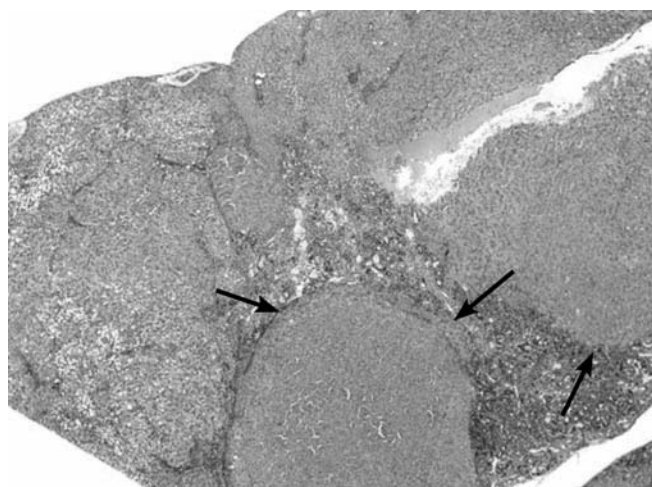


PLATE 7

Nodular hyperplasia in the liver of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The nodules are characterized by the presence of multiple small to large nodular foci that have a distinct border, cause compression of surrounding tissue, and cause bulging of the capsular surface (arrows). H&E

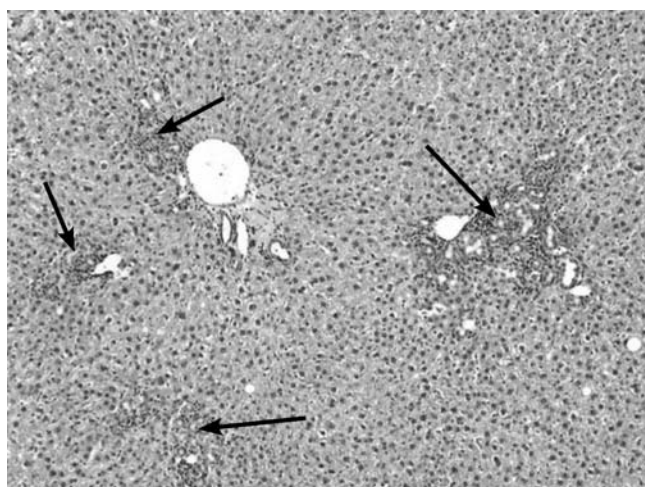


PLATE 8

Bile duct hyperplasia in the liver of a female rat administered 460 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. Note the increased number of regular bile ducts in several portal tracts (arrows). H&E

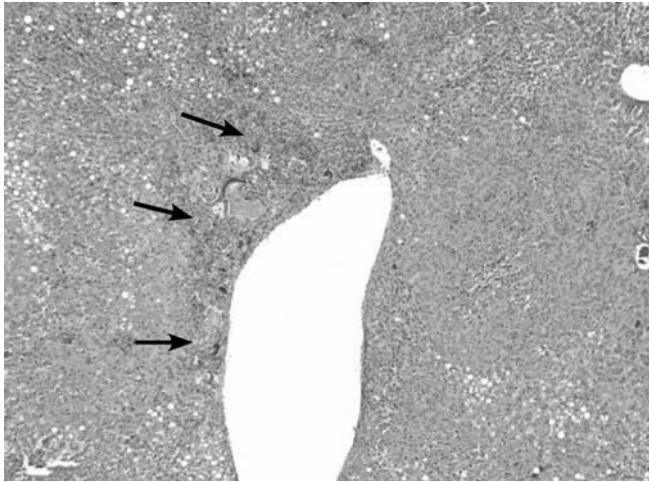


PLATE 9

Cholangiofibrosis in the liver of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The cholangiofibrosis is relatively small in size, well demarcated, and does not show invasion (arrows). H&E

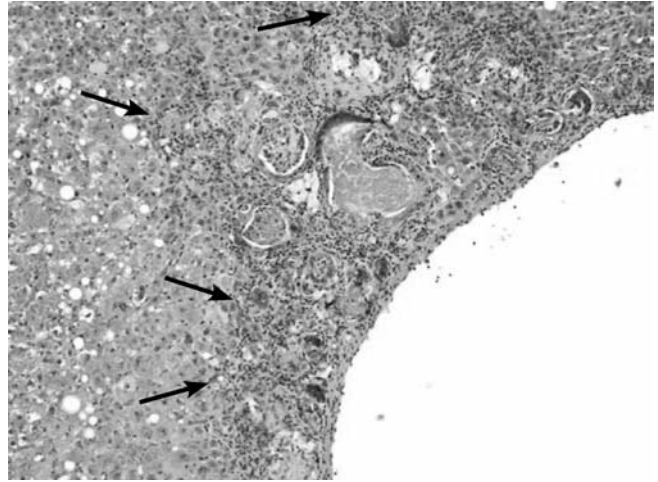


PLATE 10

Higher magnification of Plate 9. Arrows indicate the characteristic well-demarcated noninvasive growth of the cholangiofibrosis. H&E

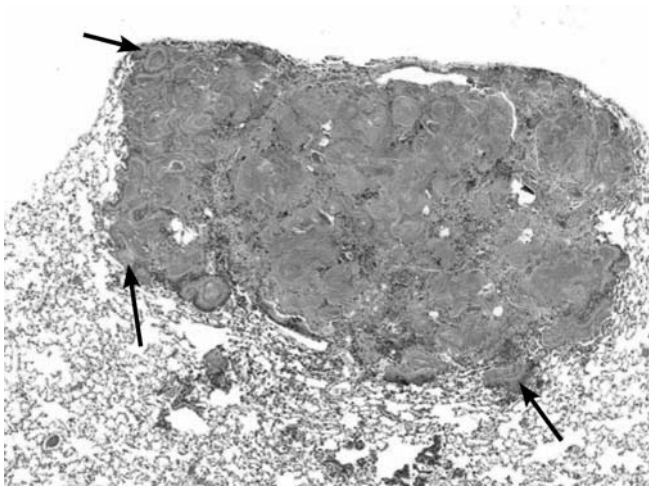


PLATE 11

Cystic keratinizing epithelioma in the lung of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The epithelioma is a cystic structure having an irregular wall (arrows) of highly keratinized stratified squamous epithelium and a center filled with keratin. The outer portion of the lesion grew by expansion into the adjacent lung but there is no evidence of invasion. H&E

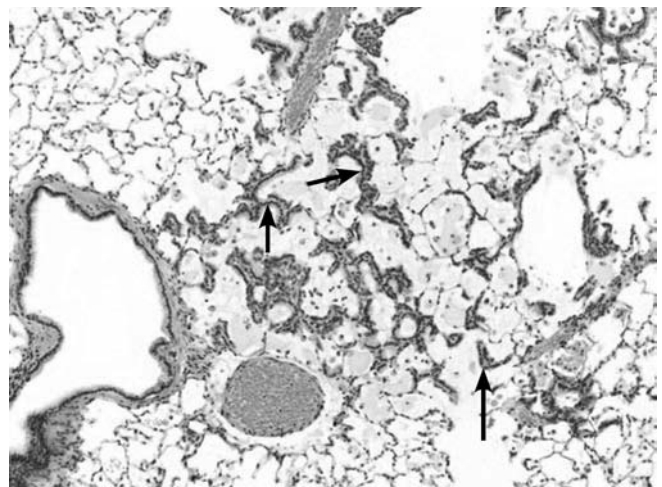


PLATE 12

Bronchiolar metaplasia of the alveolar epithelium in the lung of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The normal alveolar epithelium is replaced by cuboidal to columnar, sometimes ciliated cells, and accompanied by abundant mucus production in the affected area (arrows). H&E

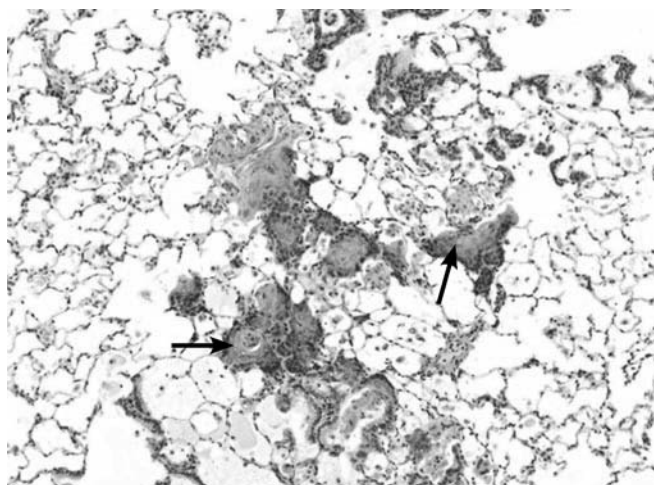


PLATE 13

Squamous metaplasia in the lung of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The normal alveolar epithelium is replaced by irregular foci of keratinizing stratified squamous epithelium (arrows). H&E



PLATE 14

Carcinoma in the uterus of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The neoplasm is characterized by anaplastic, formed solid sheets of cells that invade the underlying myometrium (arrow). H&E

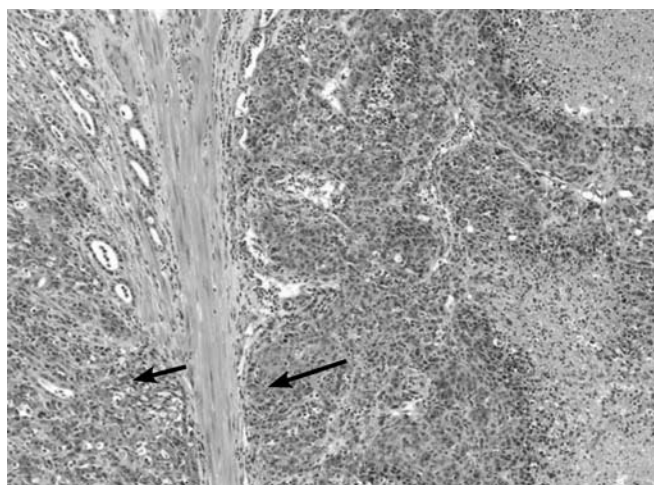


PLATE 15

Higher magnification of Plate 14. Arrows indicate the uterine carcinoma invasion into the myometrium. H&E

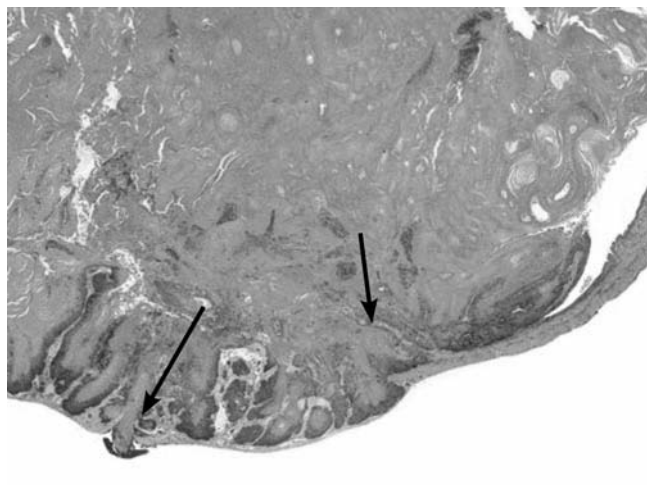


PLATE 16

Squamous cell carcinoma in the uterus of a female rat administered 220 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. The neoplasm is characterized by irregular cords and clusters of atypical, stratified squamous epithelial cells that invade the underlying myometrium (arrows). H&E

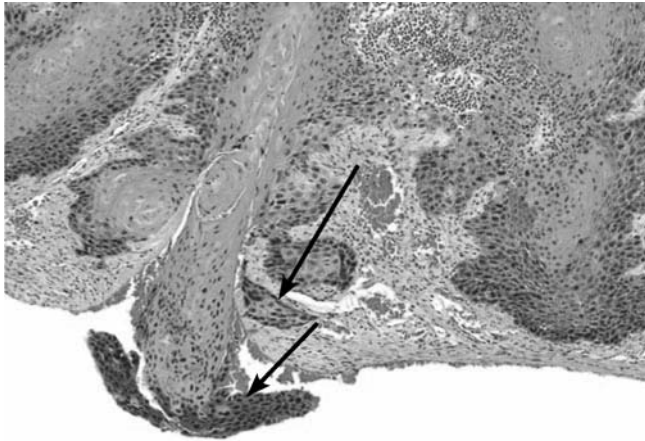


PLATE 17

Higher magnification of Plate 16. Arrows indicate the uterine squamous cell carcinoma invasion into the myometrium. H&E

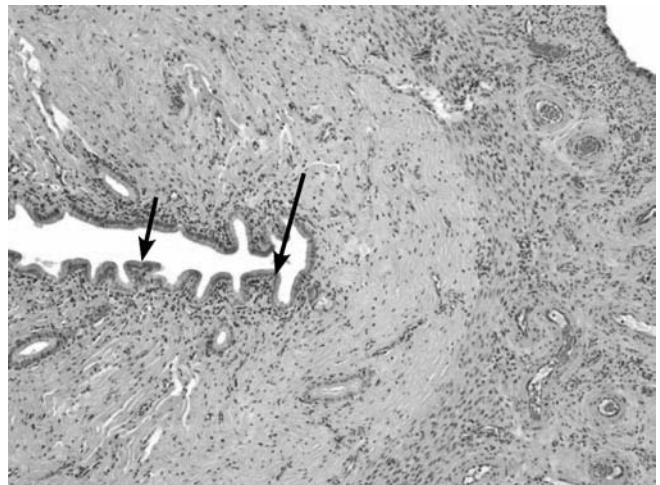


PLATE 18

Normal uterus in a female vehicle control rat at 2 years in the gavage study of PCB 118. Cuboidal epithelium lines the uterus (arrows). H&E

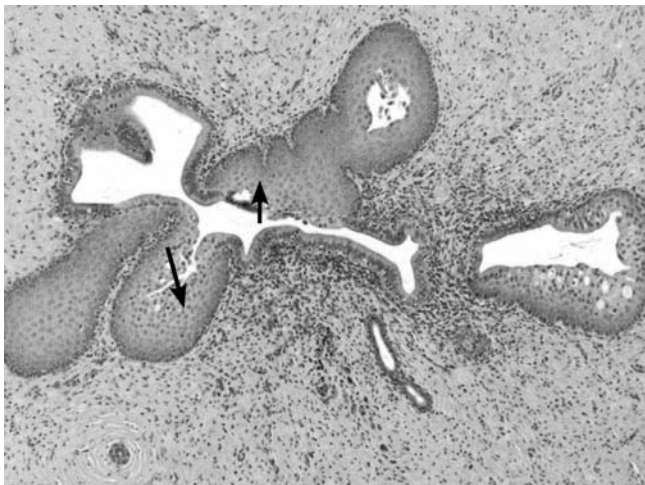


PLATE 19

Squamous metaplasia in the uterus of a female rat administered 1,000 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. Normal endometrial cuboidal epithelium is replaced by stratified squamous epithelium (arrows). Compare to Plate 18. H&E

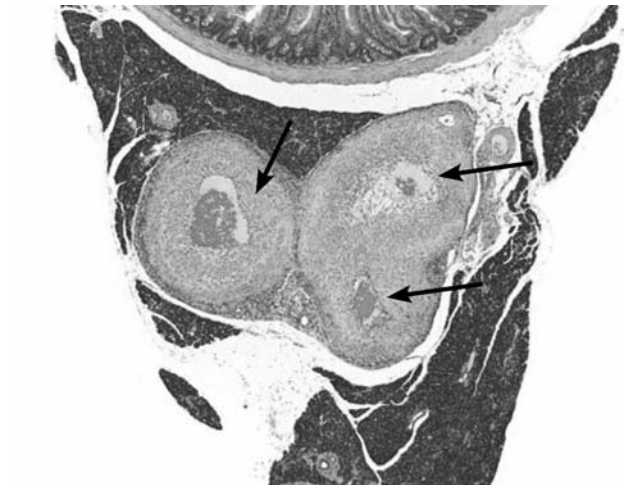


PLATE 20

Chronic active inflammation of an artery in the pancreas of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. Note the multifocal thickening of the arterial wall due to inflammatory cell infiltration into the muscular layers of the artery associated with medial smooth muscle cell proliferation and fibrosis (arrows). H&E

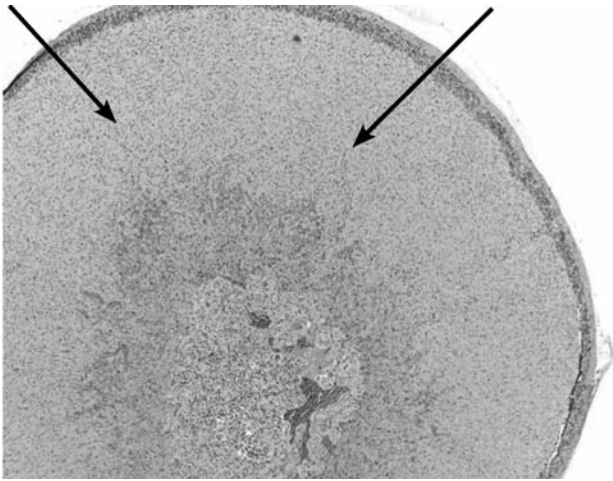


PLATE 21

Normal adrenal gland in a female vehicle control rat at 2 years in the gavage study of PCB 118. Arrows indicate the adrenal cortex. H&E

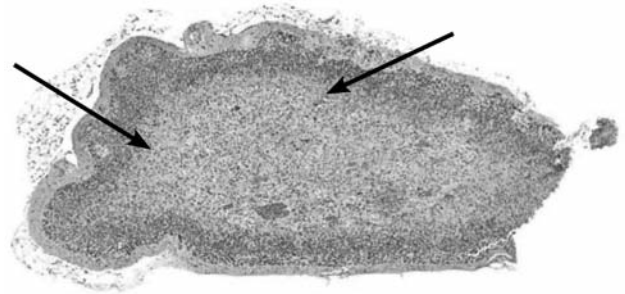


PLATE 22

Atrophy in the adrenal cortex of a female rat administered 4,600 µg/kg PCB 118 by gavage for 2 years. The diffuse change is characterized by loss of cortical epithelial cells within the zona fasciculata and zona reticularis with a subsequent reduction in cortical thickness (arrows). Compare to Plate 21. H&E

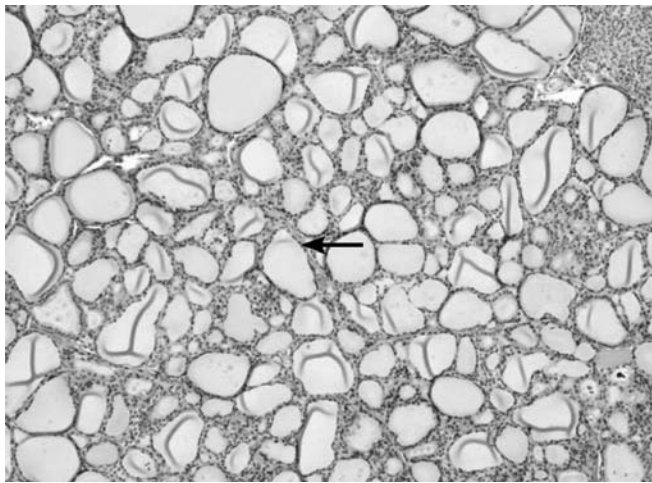


PLATE 23

Normal thyroid gland follicles in a female vehicle control rat at 2 years in the gavage study of PCB 118. Most of the follicles are lined by flattened epithelium (arrow). H&E

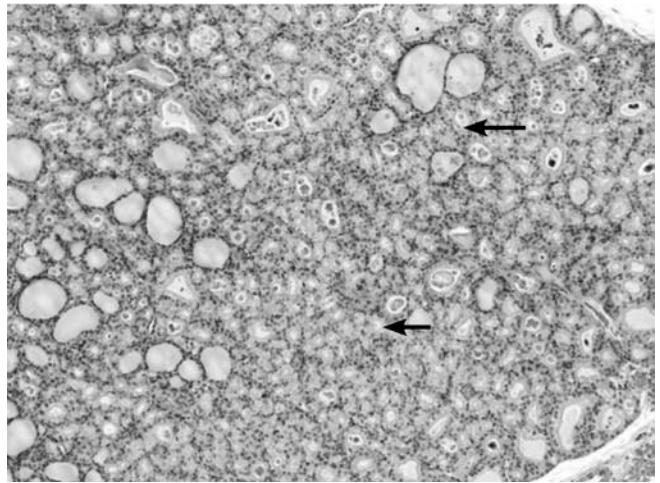


PLATE 24

Follicular cell hypertrophy in the thyroid gland of a female rat administered 220 µg/kg PCB 118 by gavage for 2 years. Most of the follicles are lined by cuboidal epithelium (arrows). Compare to Plate 23. H&E

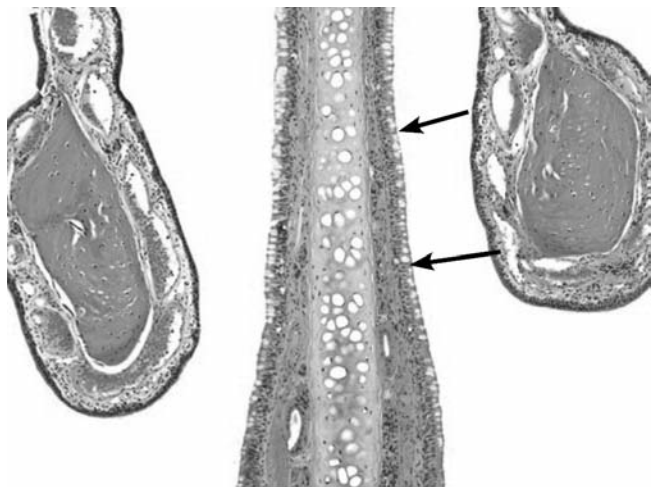


PLATE 25

Normal mucosa in Section III of the nasal cavity along the septum and ethmoid turbinates in the ventral nose of a female vehicle control rat at 2 years in the gavage study of PCB 118. Note the single layer of lining epithelium (arrows). H&E

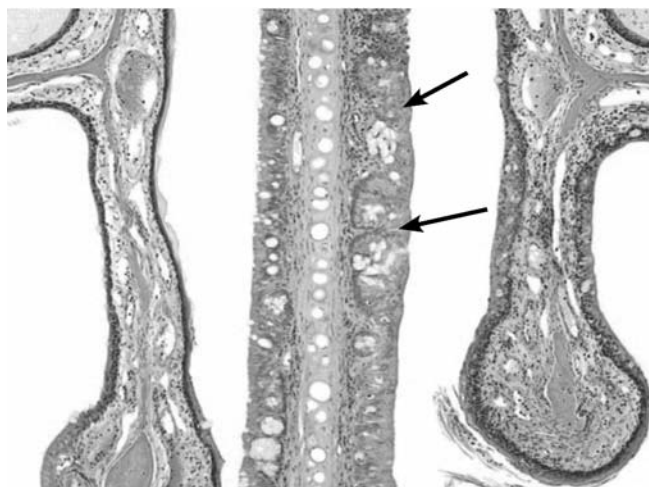


PLATE 26

Respiratory epithelium hyperplasia in Section III of the nasal cavity along the septum and ethmoid turbinates in the ventral nose of a female rat administered 4,600 $\mu\text{g/kg}$ PCB 118 by gavage for 2 years. There are increased numbers of goblet cells in these areas, and occasionally the cells pile up or form infoldings (arrows). Compare to Plate 25. H&E

DISCUSSION AND CONCLUSIONS

This 2-year study of the chronic toxicity and carcinogenicity of 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). In this Technical Report, only the results of the toxicology and carcinogenicity study of PCB 118 are described and, where appropriate, a qualitative comparison is made to neoplastic responses seen in the other studies conducted as part of the dioxin toxic equivalency factor (TEF) evaluation. A qualitative summary of the results of the current study of PCB 118 and the seven other studies conducted as part of the dioxin TEF evaluation series (NTP, 2006a,b,c,d,e,f,g) is presented in Table 16; a quantitative comparison of the potency of effects observed in the current study to responses observed in the other dioxin TEF evaluation studies will be presented elsewhere.

PCB 118 was included in the dioxin TEF evaluation due to the fact that it is the most potent of the mono-*ortho*-substituted PCBs that are included in the World Health Organization (WHO) TEF scheme. The WHO₁₉₉₈ value for PCB 118 is 0.0001 indicating that it should be 10,000-fold weaker than TCDD (TEF=1) and 1,000-fold weaker than PCB 126 (TEF=0.1) at inducing comparable effects (Van den Berg *et al.*, 1998). The initial design of the present study was such that the 1,000 µg/kg dose should have been comparable to the 1,000 ng/kg dose used in the NTP study of PCB 126 or the 100 ng/kg dose used in the NTP study of TCDD (NTP, 2006a,e). During the conduct of this study, the TEF values for all DLCs were reevaluated at a WHO expert panel meeting (Van den Berg *et al.*, 2006). This led to a change in the official WHO TEF values for several congeners evaluated as part of the NTP dioxin TEF evaluation. Notably, the TEF value for PCB 118 was reduced from 0.0001 to 0.00003 (Van den Berg *et al.*, 2006). Based on this new TEF value, the top two doses of 1,000 µg/kg and 4,600 µg/kg in the current study correspond to 30 ng TEQ/kg and 138 ng TEQ/kg, respectively, and should have comparable effects to those seen in the TCDD study at doses ranging from 3 to 100 ng TCDD/kg (NTP, 2006e).

Because of the low potency of PCB 118, any contaminants with high dioxin-like activity could potentially compromise the study. The presence of specific PCBs, PCDFs and PCDDs were measured in the bulk PCB 118 to ensure that the level of predicted dioxin-like activity, based on calculated TEQ levels, was acceptable (Table C2). Based on the WHO₁₉₉₈ TEF values, the total TEQ contribution of identified impurities in the PCB 118 bulk test article was 0.39 ng TEQ/1,000 µg bulk PCB 118. At the top dose of 4,600 µg/kg PCB 118 used in the current study, this corresponded to a maximum TEQ contribution from other DLCs of 1.79 ng TEQ/kg compared to the expected WHO₁₉₉₈ TEQ contribution from PCB 118 of 460 ng TEQ/kg. Consequently, the findings from this current study are due to PCB 118 and not due to minor contaminants with high dioxin-like activity.

The principal findings of the current PCB 118 study were significantly increased incidences of cholangiocarcinoma and hepatocellular adenoma in the liver and cystic keratinizing epithelioma (CKE) in the lung. Also, there were increased incidences of rare hepatocholangiomas in the 4,600 µg/kg core study group. In addition, an increased incidence of carcinoma of the uterus was also observed.

The increased incidences of liver and lung neoplasms were considered to be clear evidence of carcinogenicity because of clear increases in the incidences of hepatocellular adenoma and cholangiocarcinoma of the liver with increasing dose and a high incidence of CKE of the lung in the 4,600 µg/kg core study group; the rarity of hepatocholangioma, cholangiocarcinoma, and CKE; the invasive nature of cholangiocarcinoma and CKE; and the known ability of hepatocellular adenoma to progress to 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, thyroid gland, adrenal cortex, pancreas, kidney, and nose.

TABLE 16
Comparison of Treatment-Related Effects for NTP studies of Dioxin-Like Compounds^a

	TCDD (TR 521)	PCB 126 (TR 520)	PeCDF (TR 525)	Ternary Mixture (TR 526)	PCB 153 (TR 529)	PCB 126/ PCB 153 (TR 530)	PCB 126/ PCB 118 (TR 531)	PCB 118 (TR 559)
Evidence of Carcinogenic Activity	Clear	Clear	Some	Clear	Equivocal	Clear	Clear	Clear
Liver								
Cholangiocarcinoma	++	++	+	++		++	++	++
Hepatocellular Adenoma	++	+	+	++		++	++	++
Hepatocellular Carcinoma						++	++	
Cholangioma	±	±			±		±	
Hepatocholangioma	±	+				++	±	++
Lung								
Cystic Keratinizing Epithelioma	++	++	±	++		++	++	++
Squamous Cell Carcinoma		++				++		
Oral Mucosa								
Gingival, Squamous Cell Carcinoma	++	++	+			++	+	
Pancreas								
Acinar Adenoma or Carcinoma	±		±	±		+		±
Uterus								
Squamous Cell Carcinoma	+					±		±
Carcinoma			±					+
Adrenal Cortex								
Adenoma or Carcinoma		±						

++ Neoplastic effects related to treatment that were the basis for a “*Clear evidence of carcinogenic activity*” conclusion in the NTP Technical Report (TR).

+ Neoplastic effects also related to treatment or the basis for a “*Some evidence of carcinogenic activity*” conclusion in the NTP Technical Report.

± Equivocal findings that may have been related to treatment or the basis for an “*Equivocal evidence of carcinogenic activity*” conclusion in the NTP Technical Report.

^a TCDD=2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (NTP, 2006e); PCB 126=3,3',4,4',5-Pentachlorobiphenyl (NTP, 2006a); PeCDF=2,3,4,7,8-Pentachlorodibenzofuran (NTP, 2006f); Ternary Mixture=Tertiary Mixture of TCDD, PeCDF, and PCB 126 (NTP, 2006g); PCB 153=2,2',4,4',5,5'-Hexachlorobiphenyl (NTP, 2006b); PCB 126/PCB 153=Binary Mixture of PCB 126 and PCB 153 (NTP, 2006c); PCB 126/PCB 118=Binary Mixture of PCB 126 and PCB 118 (NTP, 2006d); PCB 118=2,3',4,4',5-Pentachlorobiphenyl (the current study)

While there were decreases in body weight gain in the 1,000 and 4,600 µg/kg core study groups and the 4,600 µg/kg stop-exposure group, there was no effect on survival in this study. Reduction in body weight gain is a characteristic toxic response to DLCs and was also seen in other studies conducted as part of the dioxin TEF evaluation.

Overall, the spectrum of both neoplasms and nonneoplastic lesions observed in this study of PCB 118 was comparable with that seen with TCDD and other DLCs studied as part of the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f,g). While PCB 118 has dioxin-like activity, it is also recognized as a “mixed” inducer, having also phenobarbital-like effects, as evidenced by its ability to induce CYP2B-associated pentoxoresorufin-*O*-deethylase (PROD) activity in the liver. That the spectrum of neoplastic and nonneoplastic effects is consistent with the other NTP studies of DLCs indicates that, while PCB 118 is a “mixed” inducer, the effects seen in the present study are likely primarily due to the dioxin-like activity of PCB 118. This is further supported by the prior observation that at comparable doses, the spectrum of responses induced by PCB 153, a non-dioxin-like PCB that has strong CYP2B inducing capacity, was markedly different from either PCB 118 or PCB 126. At similar doses to PCB 118, there was only equivocal evidence of carcinogenic activity of PCB 153 based on occurrences of cholangioma in the liver (NTP, 2006b).

Chronic exposure to PCB 118 led to significant accumulation of PCB 118 in fat, liver, and lung and detectable levels in blood. The significant accumulation in fat is consistent with the lipophilic nature of PCB 118. Previous studies of DLCs indicate that the liver and fat are the main depots for DLCs in rodents and together can comprise approximately 70% to 80% of the total body burden in rodents (DeVito *et al.*, 1995). For the more potent dioxin-like congeners such as TCDD and PCB 126, levels in liver are generally 2- to 4-fold higher than those in fat on a wet weight basis. This “hepatic sequestration” is a characteristic of some persistent dioxin-like compounds and is believed to be the result of binding of the compound to CYP1A2 whose expression is inducible by DLCs in the liver (Diliberto *et al.*, 1997). By comparison, in this study, PCB 118 levels in the liver were generally 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 seems to be determined by the lipophilic nature of the compound and fat content of the tissue of concern. Moreover,

these levels are consistent with those predicted by the physiologically based pharmacokinetic (PBPK) model that incorporated binding to the AHR and CYP1A2 (Appendix F). In the model, affinity of binding to CYP1A2 is linked to the affinity for the AhR. Since PCB 118 is a weak AhR ligand, the PBPK also models PCB 118 as a weak CYP1A2 ligand. In general, the predictions of the PBPK model were in the range of the data for fat and liver for the first year on study, although levels at 2 years were somewhat underpredicted. Moreover, the PBPK modeling and data are more consistent with the majority of PCB 118 in the liver being bound to a PCB-binding protein distinct from CYP1A2. The existence of a PCB-binding protein in the liver has been suggested (Bründl and Buff, 1993). It has been suggested that multidrug resistance-associated protein 2 (Mrp2) may be involved in the liver binding of PCB 126 (Lohitnavy *et al.*, 2008).

Using the WHO₁₉₉₈ TEF value for PCB 118 of 0.0001, on a TEQ basis, levels of PCB 118 in the liver in the 4,600 µg/kg core study group ranged from 22 to 109 ng TEQ_{WHO-1998}/g over the course of the study. During the conduct of this study, the TEF values for all DLCs were reevaluated at a WHO expert panel meeting leading to a change in the official WHO TEF values for several congeners (Van den Berg *et al.*, 2006). Notably, the assigned WHO₂₀₀₆ TEF value for PCB 118 decreased from 0.0001 to 0.00003. Using this newer TEF value, the levels of PCB 118 in the liver of the 1,000 and 4,600 µg/kg core study groups ranged from 0.5 to 2.6 and 7 to 33 ng TEQ_{WHO-2006}/g, respectively. By comparison, average terminal liver levels of TCDD in the TCDD study conducted as part of the dioxin TEF evaluation were 9 ng/g in the top 100 ng/kg group (NTP, 2006e) indicating that in the liver, the main target organ, similar tissue levels of dioxin-like activity were achieved.

While concentrations of PCB 118 in the fat, liver, lung, and blood of vehicle control animals in this study were below limits of quantitation, ingestion of very low levels of PCBs that are present in rodent chow does occur (Feeley and Jordan, 1998; Jordan and Feeley, 1999). NTP-2000 feed was analyzed for DLCs and contained an average of 130 pg PCB 118/g feed (Appendix D). Accumulation of PCDDs and PCDFs has also been observed in vehicle control animals in other studies (Vanden Heuvel *et al.*, 1994). Therefore, with respect to all the NTP TEF studies, in essence all experimental treatments are made on top of a background of prior exposure to DLCs normally present in feed and, there-

fore, the vehicle control group exposure is not strictly zero. However, the estimated intake from rodent chow (for example, 6.5 ng/kg for a 200 g rat ingesting 10 g feed per day) is at least three orders of magnitude lower than the doses where neoplastic responses were observed. Similarly, liver concentrations in the higher dose groups, where significant increases in incidences of neoplasms were observed were over 1,000× higher than the limits of quantitation. Consequently, the additional contribution of this background exposure rate to the observed neoplastic responses to the administered 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 used as a surrogate for the dioxin-like activity of a given compound and is used in the assignment of TEFs (Van den Berg *et al.*, 1998, 2006). In this study, significant dose-dependent increases in hepatic and pulmonary CYP1A1 and hepatic CYP1A2 activities as a result of exposure were observed at all time points. CYP2B-associated PROD activity was also significantly induced in all dosed groups. The data are consistent with the previous work showing that mono-*ortho*-substituted PCBs such as PCB 118 are “mixed” inducers and can exhibit both AhR-inducer and phenobarbital-like effects.

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 PCB 118, the principal hepatic neoplasms observed were cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma. In general, the spectrum of hepatic neoplasms seen in this study is consistent with those seen in the other dioxin TEF evaluation studies (Table 16; NTP, 2006a,b,c,d,e,f,g). In contrast, the non-dioxin-like PCB 153, at doses up to 3,000 µg/kg, only led to an equivocal increase in cholangioma (NTP, 2006b). These data indicate that the effects of PCB 118 in this study were likely due to the dioxin-like effects of this PCB.

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 was a significant increase in hepatic toxicity with increased severity occurring at higher doses and longer durations of treatment. The increased incidences and severities of

hepatotoxicity and increased incidences of hepatocellular adenoma are consistent with previously observed effects of TCDD and hexachlorodibenzodioxins 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 the increased incidences of cholangiocarcinoma. The increased incidences of cholangiocarcinoma and hepatocellular adenoma are consistent with the effects seen in the other NTP studies conducted as part of the dioxin TEF evaluation (Table 16; NTP, 2006a,b,c,d,e,f,g). Previous studies of DLCs and PCBs have only rarely seen cholangiocarcinomas despite data showing that bile ducts are targets for DLCs. In an initiation-promotion study, cholangiocarcinoma was seen in one of 14 diethylnitrosamine-initiated female rats exposed to 100 ng TCDD/kg body weight per day for 60 weeks (Walker *et al.*, 2000). In the 2-year bioassay of Aroclor 1254, a PCB mixture containing many dioxin-like PCBs, no cholangiocarcinomas were observed (Mayes *et al.*, 1998). In addition, there was no increased incidence of cholangiocarcinoma in the Kociba *et al.* (1978) TCDD feed study.

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 was 31% at the highest dose of 100 ng TCDD/kg body weight (Goodman and Sauer, 1992). By comparison, the survival-adjusted incidences of hepatocellular adenoma in the 1,000 and 4,600 µg/kg core study groups in this PCB 118 study were 30% and 59%, respectively. Using the WHO₂₀₀₆ TEF of 0.00003, doses of PCB 118 in the 1,000 and 4,600 µg/kg groups correspond to TCDD equivalent doses of 30 and 138 ng TEQ/kg, respectively.

The spectrum of hepatocellular proliferative lesions observed in this PCB 118 study is not common, 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 contin-

uum. 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 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 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 to carcinomas.

In the higher dose animals with 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. 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.

Dealing with the potential pathogenesis of the foci and nodular hyperplasia, the earliest and most sensitive 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 hyper-

trophic cells grew, giving rise to lesions diagnosed as foci, and that with continued dosing, aided by toxic changes, some instances 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 nuclear 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 increased incidences of cholangiocarcinoma following exposure to PCB 118 were consistent with observations made in other studies conducted as part of the NTP dioxin TEF evaluation (Table 16; NTP, 2006a,b,c,d,e,f,g). Spontaneous cholangioma and cholangiocarcinoma are apparently rare in Harlan Sprague-Dawley rats and were not observed in vehicle control animals from the other NTP dioxin TEF evaluation 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).

Cholangiocarcinoma, while different morphologically from spontaneous cholangiocarcinoma, was similar to chemically-induced cholangiocarcinoma in a study by Maronpot *et al.* (1991). In the present PCB 118 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 did expand 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. 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 nonneoplastic 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 occurrence 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.

It is of note that in the present study the incidence of cholangiocarcinoma in the 4,600 µg/kg stop-exposure group, while lower than that seen in the 4,600 µg/kg core study group, was still significantly greater than that in the vehicle control group. Cholangiocarcinoma was observed in three of eight animals (38% incidence) in the 4,600 µg/kg group at the 53-week time point. This high incidence at an interim time point was somewhat unusual and had not been seen before during the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f,g). The high incidence of cholangiocarcinoma in the 4,600 µg/kg stop-exposure group suggests that development of these lesions may have reached a "point of no return" such that cessation of exposure had little impact on their development. In con-

trast, the incidence of hepatocellular adenoma in the 4,600 µg/kg stop-exposure group was not different from that in the vehicle controls suggesting that their development required long-term, persistent exposure.

In the current PCB 118 study, there was also an increase in the incidence of hepatocholangioma. This neoplasm appears 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,g). 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 this study. Therefore, the occurrence of hepatocholangioma was related to the administration of PCB 118.

The mechanism underlying the increased incidences of the hepatocellular and biliary neoplasms is likely to be multifactorial. There clearly was 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 PCB 118 on the hepatocytes or due to a direct effect on the biliary cells themselves. 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. Data indicate that the acute toxic responses (including hepatotoxicity) to TCDD require AhR binding and nuclear localization (Bunger *et al.*, 2003) and binding to cognate dioxin response elements (Bunger *et al.*, 2008). 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 (Moennikes *et al.*, 2004).

The pattern of responses seen with PCB 118 in this study indicates that it is acting as a dioxin-like chemical, and the effects of PCB 118 are likely AhR-mediated. Due to the lack of direct genotoxicity, the action of PCB 118 in the liver is likely by promoting the development of spontaneously initiated cells. There are essentially three potential modes of action via the AhR: increases in the number of initiated cells capable of undergoing promotion, an increase in the 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 can promote the development of altered hepatic foci. Given that DLCs 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 pre-neoplastic 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 short 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.*, 2001b). Increases in BrdU labeling indexes were observed in the present study of 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; Wärngård *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 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). More recently Jeong *et al.* (2008) showed that chronic exposure to PCB 126 or the binary mixture of PCB 126 and PCB 153, but not PCB 153 alone, leads to an accumulation of M1G (3-(2'-deoxy-beta-D-erythro-pentofuranosyl)-pyrimido[1,2-a]-purin-10(3H)-one) DNA adducts, a marker of oxidative DNA damage, lending further support to the hypothesis that oxidative DNA damage plays an important role in the toxicity and carcinogenicity associated with chronic exposure to DLCs.

In the present study, there was also a clear increase in the incidence of 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,g). Significantly increased incidences of lung CKE were also observed in other studies carried out as part of the dioxin TEF evaluation studies (Table 16; Walker *et al.*, 2007). 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 current study,

squamous cell carcinomas were not observed. While no direct comparison has 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. CKE was not a diagnostic term consistently used at the time of the Kociba *et al.* (1978) evaluation. In contrast to the present study, a 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 PCB 118.

In addition to the increased incidences of CKE in the current study, there were significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium. This finding is 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 damage to the type I cells, is a proliferation of the type II cells. This is often diagnosed as alveolar epithelial hyperplasia. PCB 118 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. Histochemical analyses of mucin and glutathione S-transferase pi 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 and DLCs 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. Induction of lung EROD activity was seen in the other NTP studies of DLCs. 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, 1995a; 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.

In the current study of PCB 118, there was a significant increase in the incidence of uterine carcinoma and sporadic increases in the incidences of squamous cell carcinoma. The observation of uterine tumors (carcinoma and squamous cell carcinoma) is one that has also been seen in the recent NTP studies on DLCs (Table 16). In the studies of TCDD and PeCDF, there were increases in uterine squamous cell carcinoma and uterine carcinoma, respectively, at the lower dose group rather than at the highest dose (NTP, 2006e,f). In the present study, the highest incidence of uterine carcinoma was in the 4,600 µg/kg stop-exposure group (27% survival-adjusted incidence). In contrast, there were no significant increases in incidence in the core study groups.

The increased incidences of uterine carcinoma in the 4,600 µg/kg stop-exposure group may be related to endocrine changes during the study as a result of PCB 118 exposure. The decrease in body weight gain seen in the higher dose groups clearly led to a suppression of development of endocrine-related neoplasms in the

mammary and pituitary glands. Moreover, the cessation of exposure led to a recovery of normal body weight gain, resulting in the reestablishment of “normal” endocrine effects on the mammary and pituitary glands, as evidenced by the observation that the incidence of mammary gland fibroadenoma in the 4,600 µg/kg stop-exposure group was significantly greater than that in the 4,600 µg/kg core study group, but not significantly different from the vehicle controls.

It is speculated that it is the exposure to high doses of PCB 118 early in the study that leads to the development of hormonally responsive uterine carcinoma and that the cessation of exposure reestablishes a hormonal milieu that allows development of these neoplasms that would otherwise have been suppressed had exposure been maintained.

Partial estrogen agonists such as tamoxifen can induce uterine carcinoma in the mouse (Newbold *et al.*, 1997). In addition, PCB 118 can be metabolized to hydroxylated metabolites and it is known that some hydroxylated PCBs do have partial estrogenic activity *in vivo* (Martinez *et al.*, 2005). It has been reported that 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl can be formed from PCB 118 and that *in utero* exposure to this metabolite can have endocrine disrupting effects, especially in female offspring (Meerts *et al.*, 2004). Consequently, it is speculated that the induction of uterine carcinoma by PCB 118 may be mediated in part by an estrogenic action of hydroxylated PCB 118 metabolites. The metabolite profile of PCB 118 was not measured in the present study.

The incidence of uterine carcinoma in the 4,600 µg/kg stop-exposure group was clearly significantly increased over that in the vehicle controls. Uterine carcinoma only has been seen in 6 of 473 (1.2%) vehicle control animals in the historical database and thus the incidence of 11 of 50 animals seen in the current study is considered to be due to PCB 118 exposure. However, the mechanism by which this neoplasm is induced by exposure remains uncertain. While it is concluded that the increase is treatment-related, because the increase in incidence was seen only in the 4,600 µg/kg stop-exposure group it was not included as part of the evidence used for the overall assessment of clear evidence of carcinogenicity in the PCB 118 study.

In addition to uterine carcinoma, sporadic incidences of uterine squamous cell carcinoma occurred, and three animals were affected in the 200 µg/kg group. None of the incidences in the dosed groups were statistically different from the vehicle controls. However, this neoplasm is relatively rare, being seen in only 2 of 473 animals in the NTP historical control database. Given the observations of other effects in the uterus, the known ability of DLCs to alter squamous differentiation in multiple tissues, and an incidence higher than that in the historical controls, it is concluded that this increase may be related to treatment.

In several studies conducted as part of the dioxin TEF evaluation there were significantly increased incidences of gingival squamous cell carcinoma of the oral mucosa (Table 16; Yoshizawa *et al.*, 2005a). In the TCDD feed study by Kociba *et al.* (1978), there were also increased incidences of stratified squamous cell carcinoma of the hard palate/nasal turbinates in both male and female rats. In the current study at 2 years, one and two squamous cell carcinomas occurred in the 220 and 4,600 µg/kg core study groups, respectively (Table A1b). Given that sporadic occurrences often occur in control animals (the historical occurrence of this neoplasm is 4 in 473), it was concluded that the occurrences of these neoplasms in the current study were unlikely to have been related to treatment with PCB 118.

In the present study, there were increased incidences of adrenal cortical atrophy. These are consistent with effects seen in the NTP studies of PCB 126 and TCDD (NTP, 2006a,e). 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, 2006e), there were sporadic cases of adenoma of the adrenal cortex both in 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 study, the incidences of pancreatic acinar cytoplasmic vacuolization were significantly increased in dosed animals compared to vehicle controls. Similarly treatment-related increased incidences of these lesions were also observed in the NTP study of PCB 126 (NTP,

2006a). Acinar atrophy of the pancreas may be related to the down-regulation of cholecystokinin (CCK). As shown by Lee *et al.* (2000), 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 addition to acinar atrophy, there were sporadic incidences of pancreatic acinar neoplasms in dosed groups. None of the incidences were statistically different from controls. However, pancreatic acinar neoplasms (adenoma or carcinoma) are relatively rare, being seen in only 2/468 animals in the NTP historical control database. The observation of multiple affected animals in the higher dosed groups suggests that these may be related to treatment. Moreover, it is known that the pancreas is directly responsive to PCBs and dioxins (Yoshizawa *et al.*, 2005b), and low incidences of pancreatic neoplasms have been seen in other NTP studies of DLCs (Table 16).

In the current study, there were increased incidences of thyroid gland follicular cell hypertrophy in the higher dose groups at the 14-, 31-, and 53-week interim evaluations and at the end of the 2-year study. However, there was no significant effect on the incidences of 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 adenoma in male rats and nonsignificantly increased incidences in females (NTP, 1982a). Alteration in thyroid hormone homeostasis by DLCs such as PCB 126 and TCDD is well established (Van Birgelen *et al.*, 1994, 1995b; Schmidt *et al.*, 2003; Tani *et al.*, 2004). Analyses of thyroid hormones in the present study confirmed there were some alterations in thyroid hormones, notably a decrease in thyroxine (T_4) levels. The decrease in T_4 is due to the increase in T_4 glucuronidation as a result of increased expression of uridine diphosphate glucuronosyltransferase (UDPGT) by DLCs. This generally is believed to result in a decreased negative feedback inhibition of the thyroid gland leading to overexpression of thyroid stimulating hormone (TSH) (Curran and DeGroot, 1991). Hill *et al.* (1989) hypothesized that overstimulation of the thyroid gland by TSH may be involved in the mechanism

of follicular cell carcinogenesis. Kohn *et al.* (1996) developed a mathematical model of the effects of TCDD on UDPGT expression and thyroid hormone homeostasis that is consistent with this mechanism. In the present study, it was observed that, despite alterations in T_4 at all time points, there were no effects on TSH though there was a significant increase in thyroid gland follicular cell hypertrophy.

In the current study, there was a decreasing trend in the incidences of mammary gland fibroadenoma. Fibroadenoma is a spontaneous lesion in female Sprague-Dawley rats and occurred at high incidence in the vehicle control group in the current study. In addition, there was a significantly lower incidence of spontaneous pituitary gland (pars distalis) adenoma in the 4,600 $\mu\text{g/kg}$ core study group. 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 incidences 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 studies of PCB 126 and TCDD (NTP, 2006a,e). Reductions in insulin-like growth factor-1 (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.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of neoplasms of the liver (cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma) and cystic keratinizing epithelioma of the lung. Occurrences of carcinoma in the

uterus were considered to be related to the administration of PCB 118. Occurrences of squamous cell carcinoma of the uterus and acinar neoplasms of the pancreas may have been related to administration of PCB 118.

Administration of PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, thyroid gland, nose, and kidney.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10.

REFERENCES

- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Bründl, 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, M.J., 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 Bründl, 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.
- Bunger, M.K., Glover, E., Moran, S.M., Walisser, J.A., Lahvis, G.P., Hsu, E.L., and Bradfield, C.A. (2008). Abnormal liver development and resistance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity in mice carrying a mutation in the DNA-binding domain of the aryl hydrocarbon receptor. *Toxicol. Sci.* **106**, 83-92.
- 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., Håkansson, 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 multigeneration 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., 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 aroclors 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.
- 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., Flodstrom, 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., Conolly, R., Scheu, G., Wärngård, L., and Fransson-Steen, R. (2000). Analysis of rat liver foci growth with a quantitative two-cell model after treatment with 2,4,5,3',4'-pentachlorobiphenyl. *Toxicol. Sci.* **57**, 32-42.
- 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.
- 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 the 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 Dibenzo-para-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.
- Jeong, Y.C., Walker, N.J., Burgin, D.E., Kissling, G., Gupta, M., Kupper, L., Birnbaum, L.S., and Swenberg, J.A. (2008). Accumulation of M1dG DNA adducts after chronic exposure to PCBs, but not from acute exposure to polychlorinated aromatic hydrocarbons. *Free Radic. Biol. Med.* **45**, 585-591.
- 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.
- 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.
- Knerr, S., and Schrenk, D. (2006). Carcinogenicity of "non-dioxinlike" polychlorinated biphenyls. *Crit. Rev. Toxicol.* **36**, 663-694.
- 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.

- Lohitnavy, M., Lu, Y., Lohitnavy, O., Chubb, L.S., Hirono, S., and Yang, R.S. (2008). A possible role of multidrug resistance-associated protein 2 (Mrp2) in hepatic excretion of PCB 126, an environmental contaminant: PBPK/PD modeling. *Toxicol. Sci.* **104**, 27-39.
- Lotan, R. (1994). Suppression of squamous cell carcinoma growth and differentiation by retinoids. *Cancer Res.* **54**, 1987s-1990s.
- Lucier, G.W., Tritscher, A., Goldsworthy, 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.
- 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.
- Martinez, J.M., Stephens, L.C., and Jones, L.A. (2005). Long-term effects of neonatal exposure to hydroxylated polychlorinated biphenyls in the BALB/cCrgl mouse. *Environ. Health Perspect.* **113**, 1022-1026.
- 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.
- Meerts, I.A., Lilienthal, H., Hoving, S., van den Berg, J.H., Weijers, B.M., Bergman, A., Koeman, J.H., and Brouwer, A. (2004). Developmental exposure to 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl (4-OH-CB107): Long-term effects on brain development, behavior, and brain stem auditory evoked potentials in rats. *Toxicol. Sci.* **82**, 207-218.
- Moennikes, O., Loeppen, S., Buchmann, A., Andersson, P., Ittrich, C., Poellinger, L., and Schwarz, M. (2004). A constitutively active dioxin/aryl hydrocarbon receptor promotes hepatocarcinogenesis in mice. *Cancer Res.* **64**, 4707-4710.
- 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.

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 a Binary Mixture of PCB 126 and PCB 153 (CAS Nos. 57465-28-8 and 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.

National Toxicology Program (NTP) (2006d). Toxicology and Carcinogenesis Studies of a Binary Mixture of PCB 126 and PCB 118 (CAS Nos. 57465-28-8 and 31508-00-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Technical Report Series No. 531. NIH Publication No. 07-4467. 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 2,3,7,8-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) (2006f). 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) (2006g). Toxicology and Carcinogenesis Studies of a Mixture of TCDD, PeCDF, and PCB 126 (CAS Nos. 1746-01-6, 57117-31-4, and 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) (2010). Toxicology and Carcinogenesis Studies of 3,3',4,4'-Tetrachloroazobenzene (TCAB) (CAS No. 14047-09-7) in Harlan Sprague-Dawley Rats and B6C3F1 Mice (Gavage Studies). Technical Report Series No. 558. NIH Publication No. 11-5899. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC. (in press)
- Newbold, R.R., Jefferson, W.N., Padilla-Burgos, E., and Bullock, B.C. (1997). Uterine carcinoma in mice treated neonatally with tamoxifen. *Carcinogenesis* **18**, 2293-2298.
- 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.
- 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.
- 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.

- 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.
- Sánchez-Alonso, 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.
- Schecter, A., Fürst, P., Fürst, C., Pöpke, O., Ball, M., Ryan, J.J., Cau, H.D., Dai, L.C., Quynh, H.T., Cuong, H.Q., Phuong, 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 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-heptachlorodibenzo-*p*-dioxin and a defined mixture of 49 polychlorinated dibenzo-*p*-dioxins. *Carcinogenesis* **15**, 509-515.
- 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.
- 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.
- 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.
- 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.
- 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.
- Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

- Teeguarden, 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.
- 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-00/001 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-00/001 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.J.M., Van der Kolk, J., Fase, K.M., Bol, I., Poiger, H., Brouwer, A., and Van den Berg, M. (1995a). 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., 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. (1995b). 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., 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.
- Van den Berg, M., Birnbaum, L.S., Denison, M., DeVito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., and Peterson, R.E. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* **93**, 223-241.
- 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.

- 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.
- 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.
- 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, 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., Yoshizawa, K., Miller, R.A., Brix, A.E., Sells, D.M., Jokinen, M.P., Wyde, M.E., Easterling, M., and Nyska, A. (2007). Pulmonary lesions in female Harlan Sprague-Dawley rats following two-year oral treatment with dioxin-like compounds. *Toxicol. Pathol.* **35**, 880-889.
- Wärngård, 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-beta 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 beta-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-oxo-deoxyguanosine 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 beta-estradiol in male Sprague-Dawley rats. *Toxicol. Sci.* **68**, 295-303.

- Wyde, M.E., Braen, A.P., 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.
- 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. (2005a). 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.
- Yoshizawa, K., Marsh, T., Foley, J.F., Cai, B., Peddada, S., Walker, N.J., and Nyska, A. (2005b). 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.
- 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 PCB 118

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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 PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
Disposition Summary				
Animals initially in study	28	30	30	28
Natural deaths		2 ^b		
<i>14-Week interim evaluation</i>	10	10	10	10
<i>31-Week interim evaluation</i>	10	10	10	10
<i>53-Week interim evaluation</i>	8	8	10	8
Animals examined microscopically	28	28	30	28

14-Week Interim Evaluation

Systems Examined at 14 Weeks with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

31-Week Interim Evaluation

Integumentary System

Mammary gland	(10)	(1)
Carcinoma		1 (100%)

Systems Examined at 31 Weeks with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory 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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
Disposition Summary				
Animals initially in study	28	28	28	28
Natural deaths				
<i>14-Week interim evaluation</i>	10	10	10	10
<i>31-Week interim evaluation</i>	10	10	10	10
<i>53-Week interim evaluation</i>	8	8	8	8
Animals examined microscopically	28	28	28	28

14-Week Interim Evaluation

Systems Examined at 14 Weeks with No Neoplasms Observed

Alimentary System
 Cardiovascular System
 Endocrine System
 General Body System
 Genital System
 Hematopoietic System
 Integumentary System
 Musculoskeletal System
 Nervous System
 Respiratory System
 Special Senses System
 Urinary System

31-Week Interim Evaluation

Integumentary System

Mammary gland

(10)

Carcinoma

Systems Examined at 31 Weeks with No Neoplasms Observed

Alimentary System
 Cardiovascular System
 Endocrine System
 General Body System
 Genital System
 Hematopoietic System
 Musculoskeletal System
 Nervous System
 Respiratory 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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
53-Week Interim Evaluation				
Alimentary System				
Liver	(8)	(8)	(10)	(8)
Cholangiocarcinoma				
Cholangiocarcinoma, multiple				
Hepatocellular adenoma				
Genital System				
Uterus	(8)	(8)	(10)	(8)
Polyp stromal	1 (13%)			
Integumentary System				
Mammary gland	(8)	(2)	(2)	(1)
Fibroadenoma			2 (100%)	1 (100%)
Systems Examined at 53 Weeks with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms ^c				
31-Week interim evaluation			1	
53-Week interim evaluation	1		2	1
Total primary neoplasms				
31-Week interim evaluation			1	
53-Week interim evaluation	1		2	1
Total animals with benign neoplasms				
53-Week interim evaluation	1		2	1
Total benign neoplasms				
53-Week interim evaluation	1		2	1
Total animals with malignant neoplasms				
31-Week interim evaluation			1	
53-Week interim evaluation				
Total malignant neoplasms				
31-Week interim evaluation			1	
53-Week interim evaluation				

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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
53-Week Interim Evaluation				
Alimentary System				
Liver	(8)	(8)	(8)	(8)
Cholangiocarcinoma				1 (13%)
Cholangiocarcinoma, multiple			2	(25%)
Hepatocellular adenoma				1 (13%)
Genital System				
Uterus	(8)	(8)	(8)	(8)
Polyp stromal				
Integumentary System				
Mammary gland	(2)		(1)	(8)
Fibroadenoma	2 (100%)		1 (100%)	
Systems Examined at 53 Weeks with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms				
31-Week interim evaluation				
53-Week interim evaluation	2		1	4
Total primary neoplasms				
31-Week interim evaluation				
53-Week interim evaluation	2		1	4
Total animals with benign neoplasms				
31-Week interim evaluation				
53-Week interim evaluation	2		1	1
Total benign neoplasms				
31-Week interim evaluation				
53-Week interim evaluation	2		1	1
Total animals with malignant neoplasms				
31-Week interim evaluation				
53-Week interim evaluation				3
Total malignant neoplasms				
31-Week interim evaluation				
53-Week interim evaluation				3

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

^b No neoplasms were found in the two animals that died early (data not presented)

^c 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 PCB 118^a

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Disposition Summary							
Animals initially in study	52	52	52	52	52	52	50
Early deaths							
Accidental deaths		1					1
Moribund	27	22	22	17	16	16	18
Natural deaths	4	9	5	5	8	11	6
Survivors							
MSAC last week of study							1
Terminal sacrifice	21	20	25	30	28	25	24
Animals examined microscopically	52	52	52	52	52	52	50
Alimentary System							
Esophagus	(51)	(52)	(52)	(52)	(52)	(52)	(50)
Intestine large, cecum	(52)	(51)	(51)	(52)	(52)	(48)	(49)
Intestine large, colon	(52)	(52)	(52)	(52)	(52)	(48)	(49)
Schwannoma malignant, metastatic, uterus	1 (2%)						
Intestine large, rectum	(52)	(52)	(52)	(52)	(52)	(50)	(49)
Carcinoma, metastatic, ovary					1 (2%)		
Intestine small, duodenum	(52)	(52)	(52)	(52)	(52)	(48)	(49)
Carcinoma, metastatic, uterus							1 (2%)
Intestine small, ileum	(52)	(51)	(50)	(52)	(52)	(47)	(49)
Carcinoma, metastatic, uterus							2 (4%)
Intestine small, jejunum	(52)	(52)	(50)	(52)	(52)	(48)	(49)
Liver	(52)	(51)	(52)	(52)	(52)	(49)	(49)
Carcinoma, metastatic, uterus			1 (2%)		1 (2%)		2 (4%)
Cholangiocarcinoma					3 (6%)	6 (12%)	12 (24%)
Cholangiocarcinoma, multiple						30 (61%)	17 (35%)
Cholangioma	1 (2%)						
Hepatocellular adenoma		1 (2%)	1 (2%)	4 (8%)	8 (15%)	10 (20%)	
Hepatocellular adenoma, multiple					4 (8%)	14 (29%)	1 (2%)
Hepatocellular carcinoma						1 (2%)	
Hepatocholangioma						4 (8%)	
Schwannoma malignant, metastatic, uterus	1 (2%)						
Mesentery	(2)	(1)	(3)	(3)	(9)	(9)	(9)
Carcinoma, metastatic, ovary					1 (11%)		
Carcinoma, metastatic, uterus			2 (67%)		2 (22%)		4 (44%)
Fibroma		1 (100%)					
Schwannoma malignant			1 (33%)				
Oral mucosa	(1)		(1)	(1)	(1)	(3)	
Squamous cell carcinoma			1 (100%)			2 (67%)	
Pancreas	(52)	(52)	(52)	(52)	(52)	(47)	(49)
Carcinoma, metastatic, ovary					1 (2%)		
Carcinoma, metastatic, uterus			2 (4%)		1 (2%)		4 (8%)
Schwannoma malignant, metastatic, uterus	1 (2%)						
Acinus, adenoma				2 (4%)	3 (6%)	1 (2%)	
Acinus, carcinoma						1 (2%)	
Salivary glands	(51)	(51)	(52)	(51)	(52)	(51)	(50)

TABLE A1b

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Alimentary System (continued)							
Stomach, forestomach	(52)	(52)	(52)	(52)	(52)	(51)	(49)
Carcinoma, metastatic, ovary					1 (2%)		
Squamous cell carcinoma							1 (2%)
Squamous cell papilloma							1 (2%)
Stomach, glandular	(52)	(52)	(52)	(52)	(52)	(51)	(49)
Adenoma		1 (2%)					
Carcinoma, metastatic, ovary					1 (2%)		
Carcinoma, metastatic, uterus							1 (2%)
Sarcoma		1 (2%)					
Tongue							(1)
Tooth	(10)	(5)	(5)	(5)	(4)	(7)	(7)
Odontoma	1 (10%)						
Cardiovascular System							
Blood vessel	(52)	(52)	(52)	(52)	(52)	(51)	(50)
Heart	(52)	(52)	(52)	(52)	(52)	(50)	(50)
Carcinoma, metastatic, ovary					1 (2%)		
Schwannoma benign					1 (2%)		
Endocrine System							
Adrenal cortex	(52)	(52)	(52)	(51)	(52)	(49)	(49)
Adenoma	2 (4%)	2 (4%)		2 (4%)		4 (8%)	1 (2%)
Carcinoma	1 (2%)						
Carcinoma, metastatic, ovary					1 (2%)		
Carcinoma, metastatic, thyroid gland	1 (2%)						
Carcinoma, metastatic, uterus							2 (4%)
Granulosa cell tumor malignant, metastatic, ovary							1 (2%)
Adrenal medulla	(52)	(52)	(52)	(52)	(52)	(49)	(49)
Pheochromocytoma benign	3 (6%)	1 (2%)	3 (6%)	2 (4%)	1 (2%)		2 (4%)
Pheochromocytoma complex			1 (2%)				
Pheochromocytoma malignant			1 (2%)	1 (2%)	1 (2%)		
Bilateral, pheochromocytoma benign					1 (2%)		
Islets, pancreatic	(52)	(52)	(52)	(52)	(52)	(47)	(49)
Adenoma		2 (4%)	1 (2%)	3 (6%)			
Carcinoma			1 (2%)				
Parathyroid gland	(47)	(46)	(47)	(50)	(50)	(47)	(49)
Adenoma						1 (2%)	
Carcinoma, metastatic, thyroid gland		1 (2%)					
Pituitary gland	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Pars distalis, adenoma	16 (31%)	24 (46%)	18 (35%)	24 (46%)	17 (33%)	4 (8%)	12 (24%)
Pars distalis, adenoma, multiple	1 (2%)						
Pars distalis, carcinoma	2 (4%)	1 (2%)					1 (2%)

TABLE A1b

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Endocrine System (continued)							
Thyroid gland	(51)	(51)	(51)	(51)	(52)	(49)	(50)
Bilateral, adenoma			1 (2%)				
Bilateral, C-cell, adenoma				1 (2%)	1 (2%)		
C-cell, adenoma	9 (18%)	5 (10%)	5 (10%)	11 (22%)	4 (8%)	6 (12%)	5 (10%)
C-cell, adenoma, multiple				1 (2%)			
C-cell, carcinoma	2 (4%)	1 (2%)		1 (2%)			
Follicular cell, adenoma	1 (2%)	1 (2%)			1 (2%)		
Follicular cell, carcinoma							1 (2%)
General Body System							
None							
Genital System							
Clitoral gland	(52)	(52)	(51)	(52)	(51)	(49)	(48)
Adenoma				1 (2%)			
Carcinoma		1 (2%)			1 (2%)		
Ovary	(52)	(52)	(52)	(52)	(52)	(48)	(49)
Carcinoma, metastatic, uterus			1 (2%)		1 (2%)		3 (6%)
Granulosa cell tumor malignant							1 (2%)
Granulosa-theca tumor malignant			2 (4%)				
Luteoma	1 (2%)						
Thecoma benign					1 (2%)		
Bilateral, adamantinoma malignant, metastatic, ovary					1 (2%)		
Bilateral, carcinoma, metastatic, ovary					1 (2%)		
Uterus	(52)	(52)	(52)	(52)	(52)	(49)	(49)
Carcinoma	2 (4%)	2 (4%)	1 (2%)	3 (6%)	4 (8%)	3 (6%)	11 (22%)
Hemangiosarcoma					1 (2%)		
Polyp stromal	3 (6%)	6 (12%)	5 (10%)	10 (19%)	6 (12%)	5 (10%)	3 (6%)
Sarcoma				1 (2%)			
Schwannoma malignant	1 (2%)	1 (2%)		1 (2%)		1 (2%)	2 (4%)
Squamous cell carcinoma			3 (6%)	1 (2%)	1 (2%)		1 (2%)
Bilateral, polyp stromal			1 (2%)		2 (4%)	1 (2%)	1 (2%)
Vagina	(7)			(1)	(1)		
Polyp				1 (100%)			
Polyp, multiple	1 (14%)						
Schwannoma malignant					1 (100%)		
Squamous cell carcinoma	1 (14%)						
Hematopoietic System							
Bone marrow	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Lymph node		(2)	(1)	(1)		(2)	(1)
Renal, carcinoma, metastatic, uterus							1 (100%)
Lymph node, mandibular	(51)	(51)	(52)	(51)	(52)	(51)	(50)
Lymph node, mesenteric	(52)	(51)	(52)	(52)	(52)	(47)	(49)
Carcinoma, metastatic, ovary					1 (2%)		
Carcinoma, metastatic, uterus					1 (2%)		

TABLE A1b

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Hematopoietic System (continued)							
Spleen	(52)	(52)	(52)	(52)	(52)	(47)	(49)
Carcinoma, metastatic, uterus			1 (2%)		1 (2%)		3 (6%)
Thymus	(51)	(51)	(51)	(50)	(50)	(49)	(50)
Thymoma malignant		1 (2%)					
Integumentary System							
Mammary gland	(52)	(51)	(52)	(52)	(52)	(50)	(50)
Adenoma			1 (2%)	2 (4%)	1 (2%)		
Carcinoma	2 (4%)	6 (12%)	7 (13%)	4 (8%)	1 (2%)	2 (4%)	2 (4%)
Fibroadenoma	16 (31%)	18 (35%)	17 (33%)	19 (37%)	15 (29%)	8 (16%)	12 (24%)
Fibroadenoma, multiple	9 (17%)	11 (22%)	10 (19%)	14 (27%)	5 (10%)		5 (10%)
Skin	(52)	(51)	(52)	(52)	(52)	(51)	(50)
Fibroma	2 (4%)		1 (2%)		1 (2%)		1 (2%)
Hemangiosarcoma	1 (2%)						
Keratoacanthoma	1 (2%)	1 (2%)					
Schwannoma malignant	1 (2%)	1 (2%)		1 (2%)		1 (2%)	
Squamous cell papilloma				2 (4%)			
Pinna, neural crest tumor			1 (2%)				
Subcutaneous tissue, schwannoma malignant, metastatic, uterus	1 (2%)						
Musculoskeletal System							
Bone	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Skeletal muscle		(1)			(1)		(2)
Carcinoma, metastatic, uterus					1 (100%)		1 (50%)
Granulosa cell tumor malignant, metastatic, ovary							1 (50%)
Schwannoma malignant		1 (100%)					
Nervous System							
Brain	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Carcinoma, metastatic, pituitary gland	2 (4%)						1 (2%)
Ependymoma malignant			1 (2%)				
Glioma malignant					1 (2%)		
Meningioma malignant					1 (2%)		
Oligodendroglioma malignant		1 (2%)					
Spinal cord			(1)			(1)	
Schwannoma malignant			1 (100%)				

TABLE A1b

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Respiratory System							
Lung	(51)	(52)	(52)	(52)	(52)	(50)	(50)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)			
Carcinoma, metastatic, mammary gland			1 (2%)				
Carcinoma, metastatic, ovary					1 (2%)		
Carcinoma, metastatic, thyroid gland	1 (2%)						1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)			
Carcinoma, metastatic, uterus			1 (2%)		1 (2%)		2 (4%)
Cystic keratinizing epithelioma						12 (24%)	
Cystic keratinizing epithelioma, multiple						8 (16%)	
Nose	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Trachea	(51)	(52)	(52)	(52)	(52)	(52)	(50)
Special Senses System							
Eye	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Harderian gland	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Urinary System							
Kidney	(52)	(52)	(52)	(52)	(52)	(50)	(49)
Carcinoma, metastatic, uterus			1 (2%)		1 (2%)		1 (2%)
Granulosa cell tumor malignant, metastatic, ovary							1 (2%)
Mesenchymal tumor malignant		1 (2%)					
Nephroblastoma			1 (2%)				
Renal tubule, carcinoma							1 (2%)
Ureter					(1)		(2)
Carcinoma, metastatic, ovary					1 (100%)		
Urinary bladder	(52)	(52)	(52)	(52)	(52)	(50)	(49)
Carcinoma, metastatic, uterus			1 (2%)				1 (2%)
Sarcoma, metastatic, uterus				1 (2%)			
Systemic Lesions							
Multiple organs ^b	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Adenolipoma				1 (2%)			
Histiocytic sarcoma	1 (2%)						
Lymphoma malignant	2 (4%)	1 (2%)	2 (4%)	2 (4%)	1 (2%)	3 (6%)	

TABLE A1b

Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Neoplasm Summary							
Total animals with							
primary neoplasms ^c	48	43	47	47	45	45	44
Total primary neoplasms	83	93	88	116	88	128	94
Total animals with							
benign neoplasms	43	39	37	45	41	36	30
Total benign neoplasms	67	74	64	99	72	78	44
Total animals with							
malignant neoplasms	15	18	23	13	16	39	37
Total malignant neoplasms	16	19	23	16	16	50	50
Total animals with							
metastatic neoplasms	4	1	3	2	4		9
Total metastatic neoplasms	8	1	11	2	22		33
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 A2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
Adrenal Cortex: Adenoma						
Overall rate ^a	2/52 (4%)	2/52 (4%)	0/52 (0%)	2/51 (4%)	0/52 (0%)	4/49 (8%)
Adjusted rate ^b	5.6%	5.6%	0.0%	4.8%	0.0%	10.1%
Terminal rate ^c	0/21 (0%)	2/20 (10%)	0/25 (0%)	1/30 (3%)	0/28 (0%)	2/25 (8%)
First incidence (days)	658	733 (T)	— ^e	731	—	673
Poly-3 test ^d	P=0.079	P=0.695N	P=0.223N	P=0.635N	P=0.213N	P=0.385
Adrenal Medulla: Benign Pheochromocytoma						
Overall rate	3/52 (6%)	1/52 (2%)	3/52 (6%)	2/52 (4%)	2/52 (4%)	0/49 (0%)
Adjusted rate	8.3%	2.8%	7.9%	4.7%	5.0%	0.0%
Terminal rate	1/21 (5%)	1/20 (5%)	3/25 (12%)	2/30 (7%)	1/28 (4%)	0/25 (0%)
First incidence	512	733 (T)	733 (T)	733 (T)	718	—
Poly-3 test	P=0.105N	P=0.308N	P=0.643N	P=0.428N	P=0.455N	P=0.104N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma						
Overall rate	3/52 (6%)	1/52 (2%)	4/52 (8%)	3/52 (6%)	3/52 (6%)	0/49 (0%)
Adjusted rate	8.3%	2.8%	10.6%	7.1%	7.4%	0.0%
Terminal rate	1/21 (5%)	1/20 (5%)	4/25 (16%)	3/30 (10%)	1/28 (4%)	0/25 (0%)
First incidence (days)	512	733 (T)	733 (T)	733 (T)	298	—
Poly-3 test	P=0.077N	P=0.308N	P=0.525	P=0.587N	P=0.606N	P=0.104N
Liver: Hepatocolangioma						
Overall rate	0/52 (0%)	0/51 (0%)	0/52 (0%)	0/52 (0%)	0/52 (0%)	4/49 (8%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	10.1%
Terminal rate	0/21 (0%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	0/28 (0%)	3/25 (12%)
First incidence (days)	—	—	—	—	—	687
Poly-3 test	P<0.001	— ^f	—	—	—	P=0.076
Liver: Cholangiocarcinoma						
Overall rate	0/52 (0%)	0/51 (0%)	0/52 (0%)	0/52 (0%)	3/52 (6%)	36/49 (73%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.5%	84.6%
Terminal rate	0/21 (0%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	1/28 (4%)	22/25 (88%)
First incidence (days)	—	—	—	—	642	433
Poly-3 test	P<0.001	—	—	—	P=0.144	P<0.001
Liver: Hepatocellular Adenoma						
Overall rate	0/52 (0%)	1/51 (2%)	1/52 (2%)	4/52 (8%)	12/52 (23%)	24/49 (49%) ^g
Adjusted rate	0.0%	2.8%	2.6%	9.3%	29.5%	59.3%
Terminal rate	0/21 (0%)	0/20 (0%)	1/25 (4%)	2/30 (7%)	8/28 (29%)	17/25 (68%)
First incidence (days)	—	602	733 (T)	593	602	617
Poly-3 test	P<0.001	P=0.506	P=0.515	P=0.091	P<0.001	P<0.001
Lung: Cystic Keratinizing Epithelioma						
Overall rate	0/51 (0%)	0/52 (0%)	0/52 (0%)	0/52 (0%)	0/52 (0%)	20/50 (40%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	49.2%
Terminal rate	0/21 (0%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	0/28 (0%)	12/25 (48%)
First incidence (days)	—	—	—	—	—	617
Poly-3 test	P<0.001	—	—	—	—	P<0.001
Mammary Gland: Fibroadenoma						
Overall rate	25/52 (48%)	29/52 (56%)	27/52 (52%)	33/52 (63%)	20/52 (38%)	8/52 (15%)
Adjusted rate	56.9%	70.0%	63.3%	68.8%	48.5%	19.4%
Terminal rate	8/21 (38%)	14/20 (70%)	15/25 (60%)	20/30 (67%)	16/28 (57%)	5/25 (20%)
First incidence (days)	320	386	412	238	593	433
Poly-3 test	P<0.001N	P=0.140	P=0.346	P=0.158	P=0.281N	P<0.001N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
Mammary Gland: Fibroadenoma or Adenoma						
Overall rate	25/52 (48%)	29/52 (56%)	27/52 (52%)	34/52 (65%)	21/52 (40%)	8/52 (15%)
Adjusted rate	56.9%	70.0%	63.3%	70.9%	50.4%	19.4%
Terminal rate	8/21 (38%)	14/20 (70%)	15/25 (60%)	21/30 (70%)	16/28 (57%)	5/25 (20%)
First incidence (days)	320	386	412	238	593	433
Poly-3 test	P<0.001N	P=0.140	P=0.346	P=0.109	P=0.344N	P<0.001N
Mammary Gland: Carcinoma						
Overall rate	2/52 (4%)	6/52 (12%)	7/52 (13%)	4/52 (8%)	1/52 (2%)	2/52 (4%)
Adjusted rate	5.6%	16.3%	17.6%	9.4%	2.5%	4.9%
Terminal rate	1/21 (5%)	3/20 (15%)	3/25 (12%)	4/30 (13%)	1/28 (4%)	1/25 (4%)
First incidence (days)	503	571	393	733 (T)	733 (T)	484
Poly-3 test	P=0.125N	P=0.137	P=0.104	P=0.418	P=0.463N	P=0.648N
Mammary Gland: Adenoma or Carcinoma						
Overall rate	2/52 (4%)	6/52 (12%)	7/52 (13%)	5/52 (10%)	2/52 (4%)	2/52 (4%)
Adjusted rate	5.6%	16.3%	17.6%	11.8%	5.0%	4.9%
Terminal rate	1/21 (5%)	3/20 (15%)	3/25 (12%)	5/30 (17%)	1/28 (4%)	1/25 (4%)
First incidence (days)	503	571	393	733 (T)	602	484
Poly-3 test	P=0.112N	P=0.137	P=0.104	P=0.289	P=0.652N	P=0.648N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma						
Overall rate	27/52 (52%)	32/52 (62%)	30/52 (58%)	35/52 (67%)	21/52 (40%)	10/52 (19%)
Adjusted rate	60.5%	76.3%	67.8%	73.0%	50.4%	23.9%
Terminal rate	9/21 (43%)	16/20 (80%)	16/25 (64%)	22/30 (73%)	16/28 (57%)	6/25 (24%)
First incidence (days)	320	386	393	238	593	433
Poly-3 test	P<0.001N	P=0.077	P=0.306	P=0.136	P=0.224N	P<0.001N
Pancreas: Adenoma						
Overall rate	0/52 (0%)	0/52 (0%)	0/52 (0%)	2/52 (4%)	3/52 (6%)	1/47 (2%)
Adjusted rate	0.0%	0.0%	0.0%	4.7%	7.5%	2.7%
Terminal rate	0/21 (0%)	0/20 (0%)	0/25 (0%)	1/30 (3%)	3/28 (11%)	1/25 (4%)
First incidence (days)	—	—	—	658	733 (T)	733 (T)
Poly-3 test	P=0.508	—	—	P=0.283	P=0.142	P=0.514
Pancreas: Adenoma or Carcinoma						
Overall rate	0/52 (0%)	0/52 (0%)	0/52 (0%)	2/52 (4%)	3/52 (6%)	2/47 (4%)
Adjusted rate	0.0%	0.0%	0.0%	4.7%	7.5%	5.3%
Terminal rate	0/21 (0%)	0/20 (0%)	0/25 (0%)	1/30 (3%)	3/28 (11%)	2/25 (8%)
First incidence (days)	—	—	—	658	733 (T)	733 (T)
Poly-3 test	P=0.196	—	—	P=0.283	P=0.142	P=0.252
Pancreatic Islets: Adenoma						
Overall rate	0/52 (0%)	2/52 (4%)	1/52 (2%)	3/52 (6%)	0/52 (0%)	0/47 (0%)
Adjusted rate	0.0%	5.6%	2.6%	7.0%	0.0%	0.0%
Terminal rate	0/21 (0%)	1/20 (5%)	0/25 (0%)	2/30 (7%)	0/28 (0%)	0/25 (0%)
First incidence (days)	—	658	504	602	—	—
Poly-3 test	P=0.220N	P=0.242	P=0.518	P=0.158	—	—
Pancreatic Islets: Adenoma or Carcinoma						
Overall rate	0/52 (0%)	2/52 (4%)	2/52 (4%)	3/52 (6%)	0/52 (0%)	0/47 (0%)
Adjusted rate	0.0%	5.6%	5.2%	7.0%	0.0%	0.0%
Terminal rate	0/21 (0%)	1/20 (5%)	1/25 (4%)	2/30 (7%)	0/28 (0%)	0/25 (0%)
First incidence (days)	—	658	504	602	—	—
Poly-3 test	P=0.177N	P=0.242	P=0.257	P=0.158	—	—

TABLE A2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
Pituitary Gland (Pars Distalis): Adenoma						
Overall rate	17/52 (33%)	24/52 (46%)	18/52 (35%)	24/52 (46%)	17/52 (33%)	4/52 (8%)
Adjusted rate	45.8%	63.7%	46.3%	53.8%	39.8%	10.0%
Terminal rate	11/21 (52%)	14/20 (70%)	15/25 (60%)	16/30 (53%)	10/28 (36%)	4/25 (16%)
First incidence (days)	440	608	617	582	407	733 (T)
Poly-3 test	P<0.001N	P=0.077	P=0.576	P=0.305	P=0.372N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma						
Overall rate	19/52 (37%)	25/52 (48%)	18/52 (35%)	24/52 (46%)	17/52 (33%)	4/52 (8%)
Adjusted rate	50.3%	65.9%	46.3%	53.8%	39.8%	10.0%
Terminal rate	12/21 (57%)	14/20 (70%)	15/25 (60%)	16/30 (53%)	10/28 (36%)	4/25 (16%)
First incidence (days)	440	608	617	582	407	733 (T)
Poly-3 test	P<0.001N	P=0.108	P=0.450N	P=0.462	P=0.227N	P<0.001N
Thyroid Gland (C-Cell): Adenoma						
Overall rate	9/51 (18%)	5/51 (10%)	5/51 (10%)	13/51 (25%)	5/52 (10%)	6/49 (12%)
Adjusted rate	24.1%	13.9%	13.1%	30.2%	12.3%	15.1%
Terminal rate	5/21 (24%)	2/20 (10%)	3/25 (12%)	8/30 (27%)	4/28 (14%)	4/25 (16%)
First incidence (days)	503	658	658	593	487	620
Poly-3 test	P=0.294N	P=0.206N	P=0.172N	P=0.358	P=0.143N	P=0.239N
Thyroid Gland (C-Cell): Adenoma or Carcinoma						
Overall rate	11/51 (22%)	6/51 (12%)	5/51 (10%)	14/51 (27%)	5/52 (10%)	6/49 (12%)
Adjusted rate	29.5%	16.5%	13.1%	32.6%	12.3%	15.1%
Terminal rate	7/21 (33%)	2/20 (10%)	3/25 (12%)	9/30 (30%)	4/28 (14%)	4/25 (16%)
First incidence (days)	503	620	658	593	487	620
Poly-3 test	P=0.183N	P=0.144N	P=0.068N	P=0.477	P=0.052N	P=0.104N
Uterus: Stromal Polyp						
Overall rate	3/52 (6%)	6/52 (12%)	6/52 (12%)	10/52 (19%)	8/52 (15%)	6/52 (12%)
Adjusted rate	8.4%	16.6%	15.6%	23.1%	19.7%	14.8%
Terminal rate	1/21 (5%)	4/20 (20%)	4/25 (16%)	6/30 (20%)	4/28 (14%)	4/25 (16%)
First incidence (days)	617	602	617	602	642	617
Poly-3 test	P=0.516N	P=0.240	P=0.273	P=0.071	P=0.138	P=0.303
Uterus: Squamous Cell Carcinoma						
Overall rate	0/52 (0%)	0/52 (0%)	3/52 (6%)	1/52 (2%)	1/52 (2%)	0/52 (0%)
Adjusted rate	0.0%	0.0%	7.9%	2.3%	2.5%	0.0%
Terminal rate	0/21 (0%)	0/20 (0%)	2/25 (8%)	0/30 (0%)	1/28 (4%)	0/25 (0%)
First incidence (days)	—	—	707	627	733 (T)	—
Poly-3 test	P=0.315N	—	P=0.132	P=0.539	P=0.525	—
Uterus: Carcinoma						
Overall rate	2/52 (4%)	2/52 (4%)	1/52 (2%)	3/52 (6%)	4/52 (8%)	3/52 (6%)
Adjusted rate	5.7%	5.5%	2.6%	7.1%	9.9%	7.5%
Terminal rate	2/21 (10%)	1/20 (5%)	0/25 (0%)	2/30 (7%)	2/28 (7%)	2/25 (8%)
First incidence (days)	733 (T)	602	595	730	669	672
Poly-3 test	P=0.427	P=0.685N	P=0.470N	P=0.585	P=0.402	P=0.561
All Organs: Malignant Lymphoma						
Overall rate	2/52 (4%)	1/52 (2%)	2/52 (4%)	2/52 (4%)	1/52 (2%)	3/52 (6%)
Adjusted rate	5.5%	2.8%	5.1%	4.6%	2.5%	7.5%
Terminal rate	1/21 (5%)	0/20 (0%)	0/25 (0%)	0/30 (0%)	0/28 (0%)	1/25 (4%)
First incidence (days)	119	637	163	582	487	673
Poly-3 test	P=0.323	P=0.501N	P=0.664N	P=0.628N	P=0.460N	P=0.550

TABLE A2a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
All Organs: Benign Neoplasms						
Overall rate	43/52 (83%)	39/52 (75%)	37/52 (71%)	45/52 (87%)	41/52 (79%)	36/52 (69%)
Adjusted rate	90.8%	91.2%	84.5%	91.1%	89.5%	82.4%
Terminal rate	19/21 (91%)	19/20 (95%)	22/25 (88%)	27/30 (90%)	26/28 (93%)	23/25 (92%)
First incidence (days)	320	386	412	238	407	433
Poly-3 test	P=0.109N	P=0.639	P=0.255N	P=0.632	P=0.565N	P=0.166N
All Organs: Malignant Neoplasms						
Overall rate	15/52 (29%)	18/52 (35%)	23/52 (44%)	14/52 (27%)	16/52 (31%)	39/52 (75%)
Adjusted rate	39.0%	44.8%	51.5%	30.9%	36.8%	86.9%
Terminal rate	9/21 (43%)	8/20 (40%)	9/25 (36%)	8/30 (27%)	8/28 (29%)	22/25 (88%)
First incidence (days)	119	298	163	300	298	433
Poly-3 test	P<0.001	P=0.379	P=0.170	P=0.291N	P=0.509N	P<0.001
All Organs: Benign or Malignant Neoplasms						
Overall rate	48/52 (92%)	43/52 (83%)	47/52 (90%)	47/52 (90%)	45/52 (87%)	45/52 (87%)
Adjusted rate	97.4%	94.6%	95.0%	92.4%	94.7%	97.5%
Terminal rate	21/21 (100%)	19/20 (95%)	23/25 (92%)	27/30 (90%)	27/28 (96%)	25/25 (100%)
First incidence (days)	119	298	163	238	298	433
Poly-3 test	P=0.343	P=0.403N	P=0.450N	P=0.219N	P=0.410N	P=0.809

(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, pancreas, pancreatic islets, 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 a 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 an animal that also had an hepatocellular adenoma.

TABLE A2b

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of PCB 118

	Vehicle Control	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Adrenal Cortex: Adenoma			
Overall rate ^a	2/52 (4%)	4/49 (8%)	1/49 (2%)
Adjusted rate ^b	5.6%	10.1%	2.5%
Terminal rate ^c	0/21 (0%)	2/25 (8%)	0/25 (0%)
First incidence (days)	658	673	671
Poly-3 test ^d		P=0.385	P=0.460N
Poly-3 test ^e			P=0.174N
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate	3/52 (6%)	0/49 (0%)	2/49 (4%)
Adjusted rate	8.3%	0.0%	5.0%
Terminal rate	1/21 (5%)	0/25 (0%)	2/25 (8%)
First incidence (days)	512	— ^f	733 (T)
Poly-3 test		P=0.104N	P=0.457N
Poly-3 test			P=0.240
Liver: Hepatocholangioma			
Overall rate	0/52 (0%)	4/49 (8%)	0/49 (0%)
Adjusted rate	0.0%	10.1%	0.0%
Terminal rate	0/21 (0%)	3/25 (12%)	0/25 (0%)
First incidence (days)	—	687	—
Poly-3 test		P=0.076	— ^g
Poly-3 test			P=0.058N
Liver: Cholangiocarcinoma			
Overall rate	0/52 (0%)	36/49 (73%)	29/49 (59%)
Adjusted rate	0.0%	84.6%	68.1%
Terminal rate	0/21 (0%)	22/25 (88%)	17/25 (68%)
First incidence (days)	—	433	567
Poly-3 test		P<0.001	P<0.001
Poly-3 test			P=0.048N
Liver: Hepatocellular Adenoma			
Overall rate	0/52 (0%)	24/49 (49%) ^h	1/49 (2%)
Adjusted rate	0.0%	59.3%	2.5%
Terminal rate	0/21 (0%)	17/25 (68%)	1/25 (4%)
First incidence (days)	—	617	733 (T)
Poly-3 test		P<0.001	P=0.525
Poly-3 test			P<0.001N
Lung: Cystic Keratinizing Epithelioma			
Overall rate	0/51 (0%)	20/50 (40%)	0/50 (0%)
Adjusted rate	0.0%	49.2%	0.0%
Terminal rate	0/21 (0%)	12/25 (48%)	0/25 (0%)
First incidence (days)	—	617	—
Poly-3 test		P<0.001	—
Poly-3 test			P<0.001N
Mammary Gland: Fibroadenoma			
Overall rate	25/52 (48%)	8/52 (15%)	17/50 (34%)
Adjusted rate	56.9%	19.4%	39.3%
Terminal rate	8/21 (38%)	5/25 (20%)	9/25 (36%)
First incidence (days)	320	433	302
Poly-3 test		P<0.001N	P=0.069N
Poly-3 test			P=0.036

TABLE A2b

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of PCB 118

	Vehicle Control	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Mammary Gland: Fibroadenoma or Carcinoma			
Overall rate	27/52 (52%)	10/52 (19%)	18/50 (36%)
Adjusted rate	60.5%	23.9%	41.0%
Terminal rate	9/21 (43%)	6/25 (24%)	9/25 (36%)
First incidence (days)	320	433	302
Poly-3 test		P<0.001N	P=0.046N
Poly-3 test			P=0.068
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	17/52 (33%)	4/52 (8%)	12/50 (24%)
Adjusted rate	45.8%	10.0%	30.0%
Terminal rate	11/21 (52%)	4/25 (16%)	10/25 (40%)
First incidence (days)	440	733 (T)	672
Poly-3 test		P<0.001N	P=0.107N
Poly-3 test			P=0.023
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rate	19/52 (37%)	4/52 (8%)	13/50 (26%)
Adjusted rate	50.3%	10.0%	32.5%
Terminal rate	12/21 (57%)	4/25 (16%)	11/25 (44%)
First incidence (days)	440	733 (T)	672
Poly-3 test		P<0.001N	P=0.077N
Poly-3 test			P=0.012
Thyroid Gland (C-Cell): Adenoma			
Overall rate	9/51 (18%)	6/49 (12%)	5/50 (10%)
Adjusted rate	24.1%	15.1%	12.4%
Terminal rate	5/21 (24%)	4/25 (16%)	4/25 (16%)
First incidence (days)	503	620	589
Poly-3 test		P=0.239N	P=0.147N
Poly-3 test			P=0.490N
Uterus: Stromal Polyp			
Overall rate	3/52 (6%)	6/52 (12%)	4/50 (8%)
Adjusted rate	8.4%	14.8%	10.1%
Terminal rate	1/21 (5%)	4/25 (16%)	4/25 (16%)
First incidence (days)	617	617	733 (T)
Poly-3 test		P=0.303	P=0.556
Poly-3 test			P=0.381N
Uterus: Carcinoma			
Overall rate	2/52 (4%)	3/52 (6%)	11/50 (22%)
Adjusted rate	5.7%	7.5%	26.9%
Terminal rate	2/21 (10%)	2/25 (8%)	6/25 (24%)
First incidence (days)	733 (T)	672	642
Poly-3 test		P=0.561	P=0.014
Poly-3 test			P=0.019
All Organs: Malignant Lymphoma			
Overall rate	2/52 (4%)	3/52 (6%)	0/50 (0%)
Adjusted rate	5.5%	7.5%	0.0%
Terminal rate	1/21 (5%)	1/25 (4%)	0/25 (0%)
First incidence (days)	119	673	—
Poly-3 test		P=0.550	P=0.216N
Poly-3 test			P=0.120N

TABLE A2b

Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of PCB 118

	Vehicle Control	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
All Organs: Benign Neoplasms			
Overall rate	43/52 (83%)	36/52 (69%)	30/50 (60%)
Adjusted rate	90.8%	82.4%	68.2%
Terminal rate	19/21 (91%)	23/25 (92%)	19/25 (76%)
First incidence (days)	320	433	302
Poly-3 test		P=0.166N	P=0.003N
Poly-3 test			P=0.082N
All Organs: Malignant Neoplasms			
Overall rate	15/52 (29%)	39/52 (75%)	37/50 (74%)
Adjusted rate	39.0%	86.9%	82.8%
Terminal rate	9/21 (43%)	22/25 (88%)	20/25 (80%)
First incidence (days)	119	433	545
Poly-3 test		P<0.001	P<0.001
Poly-3 test			P=0.397N
All Organs: Benign or Malignant Neoplasms			
Overall rate	48/52 (92%)	45/52 (87%)	44/50 (88%)
Adjusted rate	97.4%	97.5%	93.7%
Terminal rate	21/21 (100%)	25/25 (100%)	23/25 (92%)
First incidence (days)	119	433	302
Poly-3 test		P=0.809	P=0.323N
Poly-3 test			P=0.331N

(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 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 lower incidence in a dosed group is indicated by N.

^e Pairwise comparison between the 4,600 µg/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.

^h One hepatocellular carcinoma occurred in an animal that also had an hepatocellular adenoma.

TABLE A3a
Historical Incidence of Liver Neoplasms in Vehicle Control Female Sprague-Dawley Rats^a

Study (Study Start)	Incidence in Controls				
	Cholangiocarcinoma	Hepatocellular Adenoma	Hepatocellular Carcinoma	Cholangioma	Hepatocholangioma
PCB 126 (February, 1998)	0/53	1/53	0/53	0/53	0/53
TCDD (June, 1998)	0/53	0/53	0/53	0/53	0/53
PeCDF (April, 1999)	0/53	1/53	0/53	0/53	0/53
TEF Mixture (June, 1998)	0/53	0/53	0/53	0/53	0/53
PCB 153 (August, 1998)	0/53	0/53	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 153 (September, 1998)	0/53	0/53	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 118 (October, 1999)	0/53	2/53	0/53	0/53	0/53
PCB 118 (March, 2004)	0/52	0/52	0/52	1/52	0/52
3,3',4,4'-Tetrachloroazobenzene (January, 2003)	1/50	2/50	0/50	0/50	0/50
Overall Historical Incidence					
Total (%)	1/473 (0.2%)	6/473 (1.3%)	0/473	1/473 (0.2%)	0/473
Mean $\pm$ standard deviation	0.2% $\pm$ 0.7%	1.3% $\pm$ 1.7%		0.2% $\pm$ 0.6%	
Range	0%-2%	0%-4%		0%-2%	

^a Data as of November 13, 2008

TABLE A3b
Historical Incidence of Cystic Keratinizing Epithelioma in the Lung of Vehicle Control Female Sprague-Dawley Rats^a

Study (Study Start)	Incidence in Controls
PCB 126 (February, 1998)	0/53
TCDD (June, 1998)	0/53
PeCDF (April, 1999)	0/53
TEF Mixture (June, 1998)	0/53
PCB 153 (August, 1998)	0/52
Binary Mixture of PCB 126/PCB 153 (September, 1998)	0/53
Binary Mixture of PCB 126/PCB 118 (October, 1999)	0/53
PCB 118 (March, 2004)	0/51
3,3',4,4'-Tetrachloroazobenzene (January, 2003)	0/50
Overall Historical Incidence	
Total	0/471

^a Data as of November 13, 2008

TABLE A3c
Historical Incidence of Uterus Neoplasms in Vehicle Control Female Sprague-Dawley Rats^a

Study (Study Start)	Incidence in Controls	
	Carcinoma	Squamous Cell Carcinoma
PCB 126 (February, 1998)	0/53	0/53
TCDD (June, 1998)	0/53	0/53
PeCDF (April, 1999)	1/53	0/53
TEF Mixture (June, 1998)	1/53	0/53
PCB 153 (August, 1998)	0/53	0/53
Binary Mixture of PCB 126/PCB 153 (September, 1998)	0/53	1/53
Binary Mixture of PCB 126/PCB 118 (October, 1999)	1/53	0/53
PCB 118 (March, 2004)	2/52	0/52
3,3',4,4'-Tetrachloroazobenzene (January, 2003)	1/50	1/50
Overall Historical Incidence		
Total (%)	6/473 (1.3%)	2/473 (0.4%)
Mean $\pm$ standard deviation	1.3% $\pm$ 1.4%	0.4% $\pm$ 0.9%
Range	0%-4%	0%-2%

^a Data as of November 13, 2008

TABLE A3d
Historical Incidence of Pancreas Neoplasms in Vehicle Control Female Sprague-Dawley Rats^a

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
PCB 126 (February, 1998)	1/51	0/51	1/51
TCDD (June, 1998)	0/51	0/51	0/51
PeCDF (April, 1999)	0/53	0/53	0/53
TEF Mixture (June, 1998)	0/52	0/52	0/52
PCB 153 (August, 1998)	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 153 (September, 1998)	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 118 (October, 1999)	0/53	0/53	0/53
PCB 118 (March, 2004)	0/52	0/52	0/52
3,3',4,4'-Tetrachloroazobenzene (January, 2003)	0/50	1/50	1/50
Overall Historical Incidence			
Total	1/468 (0.2%)	1/468 (0.2%)	2/468 (0.4%)
Mean $\pm$ standard deviation	0.2% $\pm$ 0.7%	0.2% $\pm$ 0.7%	0.4% $\pm$ 0.9%
Range	0%-2%	0%-2%	0%-2%

^a Data as of November 13, 2008

TABLE A4a

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 PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
Disposition Summary				
Animals initially in study	28	30	30	28
Natural deaths		2 ^b		
14-Week interim evaluation	10	10	10	10
31-Week interim evaluation	10	10	10	10
53-Week interim evaluation	8	8	10	8
Animals examined microscopically	28	28	30	28
14-Week Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Clear cell focus				
Eosinophilic focus				
Fatty change, diffuse				
Hepatodiaphragmatic nodule				2 (20%)
Inflammation	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Necrosis				
Pigmentation				
Toxic hepatopathy				
Hepatocyte, hypertrophy			1 (10%)	1 (10%)
Hepatocyte, multinucleated				
Pancreas	(10)	(10)	(10)	(10)
Acinus, vacuolization cytoplasmic				
Stomach, forestomach	(10)			
Edema				
Inflammation				
Stomach, glandular	(10)			
Erosion	1 (10%)			
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy		2 (20%)		
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland	(10)			
Thyroid gland	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy	1 (10%)		1 (10%)	
Hematopoietic System				
Lymph node				
Spleen	(10)			
Hematopoietic cell proliferation	6 (60%)			
Pigmentation	10 (100%)			
Thymus	(10)	(10)	(10)	(10)
Atrophy				

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

^b Data not presented

TABLE A4a

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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
Disposition Summary				
Animals initially in study	28	28	28	28
Natural deaths				
14-Week interim evaluation	10	10	10	10
31-Week interim evaluation	10	10	10	10
53-Week interim evaluation	8	8	8	8
Animals examined microscopically	28	28	28	28
14-Week Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Clear cell focus		1 (10%)	1 (10%)	
Eosinophilic focus				1 (10%)
Fatty change, diffuse				10 (100%)
Hepatodiaphragmatic nodule				
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Necrosis			3 (30%)	9 (90%)
Pigmentation			5 (50%)	8 (80%)
Toxic hepatopathy				10 (100%)
Hepatocyte, hypertrophy	5 (50%)	6 (60%)	9 (90%)	10 (100%)
Hepatocyte, multinucleated				10 (100%)
Pancreas	(10)	(10)	(10)	(10)
Acinus, vacuolization cytoplasmic				1 (10%)
Stomach, forestomach				(10)
Edema				0
Inflammation				0
Stomach, glandular				(10)
Erosion				
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy	1 (10%)			
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland				(10)
Thyroid gland	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy	1 (10%)	2 (20%)	2 (20%)	4 (40%)
Hematopoietic System				
Lymph node			(1)	
Spleen				10
Hematopoietic cell proliferation				
Pigmentation				10 (100%)
Thymus	(10)	(10)	(10)	(10)
Atrophy				1 (10%)

TABLE A4a

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
14-Week Interim Evaluation (continued)				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage				1 (10%)
Inflammation		1 (10%)		
Alveolus, infiltration cellular, histiocyte		1 (10%)		1 (10%)
Serosa, inflammation, chronic active		1 (10%)		
Systems Examined at 14 Weeks with No Lesions Observed				
Cardiovascular System				
General Body System				
Genital System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
31-Week Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Cholangiofibrosis				
Clear cell focus	2 (20%)	3 (30%)	4 (40%)	
Clear cell focus, multiple				
Fatty change, diffuse				
Hematopoietic cell proliferation				
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus			1 (10%)	
Necrosis				
Pigmentation				
Toxic hepatopathy				
Bile duct, fibrosis	1 (10%)			
Bile duct, hyperplasia				
Hepatocyte, hypertrophy			1 (10%)	1 (10%)
Hepatocyte, multinucleated				
Oval cell, hyperplasia				
Pancreas	(10)	(10)	(10)	(10)
Inflammation, chronic active	1 (10%)			
Acinus, atrophy, focal				
Acinus, vacuolization cytoplasmic				
Stomach, forestomach	(10)			
Edema				
Inflammation				
Stomach, glandular	(10)			
Hyperplasia				

TABLE A4a

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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
14-Week Interim Evaluation (continued)				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage				
Inflammation				1 (10%)
Alveolus, infiltration cellular, histiocyte				1 (10%)
Serosa, inflammation, chronic active				
Systems Examined at 14 Weeks with No Lesions Observed				
Cardiovascular System				
General Body System				
Genital System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
31-Week Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Cholangiofibrosis				1 (10%)
Clear cell focus	2 (20%)	1 (10%)	3 (30%)	
Clear cell focus, multiple		1 (10%)		
Fatty change, diffuse				10 (100%)
Hematopoietic cell proliferation			1 (10%)	
Inflammation	10 (100%)	9 (90%)	10 (100%)	10 (100%)
Mixed cell focus		1 (10%)	1 (10%)	1 (10%)
Necrosis			1 (10%)	
Pigmentation		1 (10%)	6 (60%)	10 (100%)
Toxic hepatopathy				10 (100%)
Bile duct, fibrosis				
Bile duct, hyperplasia				2 (20%)
Hepatocyte, hypertrophy	3 (30%)	8 (80%)	10 (100%)	10 (100%)
Hepatocyte, multinucleated				10 (100%)
Oval cell, hyperplasia			1 (10%)	9 (90%)
Pancreas	(10)	(10)	(10)	(10)
Inflammation, chronic active			1 (10%)	
Acinus, atrophy, focal				1 (10%)
Acinus, vacuolization cytoplasmic				6 (60%)
Stomach, forestomach				(10)
Edema				1 (10%)
Inflammation				2 (20%)
Stomach, glandular	(1)			(10)
Hyperplasia	1 (100%)			

TABLE A4a

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
31-Week Interim Evaluation (continued)				
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Atrophy				
Degeneration, cystic				
Hyperplasia	1 (10%)			1 (10%)
Hypertrophy	2 (20%)		1 (10%)	1 (10%)
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland	(10)			
Pars distalis, cyst				
Thyroid gland	(10)	(10)	(10)	(10)
C-cell, hyperplasia				
Follicular cell, hypertrophy	1 (10%)			
Genital System				
Ovary	(10)			
Uterus	(10)	(10)	(10)	(10)
Dilatation		2 (20%)		
Inflammation	1 (10%)			
Inflammation, suppurative		1 (10%)		1 (10%)
Metaplasia, squamous	4 (40%)	3 (30%)	2 (20%)	3 (30%)
Endometrium, hyperplasia, cystic	1 (10%)		2 (20%)	
Vagina	(10)			
Hematopoietic System				
Spleen	(10)			
Pigmentation	10 (100%)			
Thymus	(10)	(10)	(10)	(10)
Atrophy		1 (10%)	1 (10%)	1 (10%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage				
Inflammation	1 (10%)		1 (10%)	2 (20%)
Alveolar epithelium, hyperplasia				
Alveolar epithelium, infiltration cellular, histiocyte			1 (10%)	
Alveolus, infiltration cellular, histiocyte	4 (40%)	1 (10%)		
Systems Examined at 31 Weeks with No Lesions Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				

TABLE A4a

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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
31-Week Interim Evaluation (continued)				
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Atrophy				1 (10%)
Degeneration, cystic			1 (10%)	1 (10%)
Hyperplasia				
Hypertrophy	3 (30%)		3 (30%)	2 (20%)
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland		(1)		(10)
Pars distalis, cyst		1 (100%)		
Thyroid gland	(10)	(10)	(10)	(10)
C-cell, hyperplasia		1 (10%)	1 (10%)	
Follicular cell, hypertrophy			2 (20%)	8 (80%)
Genital System				
Ovary				(10)
Uterus	(10)	(10)	(10)	(10)
Dilatation	1 (10%)	2 (20%)	3 (30%)	1 (10%)
Inflammation				
Inflammation, suppurative	1 (10%)	2 (20%)	1 (10%)	
Metaplasia, squamous	1 (10%)	2 (20%)	4 (40%)	
Endometrium, hyperplasia, cystic	1 (10%)			
Vagina				(10)
Hematopoietic System				
Spleen				(10)
Pigmentation				10 (100%)
Thymus	(10)	(10)	(10)	(10)
Atrophy	3 (30%)	2 (20%)	1 (10%)	2 (20%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage				2 (20%)
Inflammation		2 (20%)		
Alveolar epithelium, hyperplasia			1 (10%)	
Alveolar epithelium, infiltration cellular, histiocyte				
Alveolus, infiltration cellular, histiocyte			1 (10%)	3 (30%)
Systems Examined at 31 Weeks with No Lesions Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				

TABLE A4a

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
53-Week Interim Evaluation				
Alimentary System				
Liver	(8)	(8)	(10)	(8)
Cholangiofibrosis		1 (13%)		
Clear cell focus	1 (13%)	3 (38%)	2 (20%)	4 (50%)
Clear cell focus, multiple	3 (38%)	1 (13%)	1 (10%)	1 (13%)
Eosinophilic focus				
Eosinophilic focus, multiple				
Fatty change, diffuse				
Hepatodiaphragmatic nodule				
Hyperplasia, nodular				
Inflammation	8 (100%)	7 (88%)	8 (80%)	5 (63%)
Mixed cell focus	1 (13%)			
Mixed cell focus, multiple				
Necrosis				
Pigmentation				
Toxic hepatopathy				
Bile duct, cyst			1 (10%)	1 (13%)
Bile duct, hyperplasia	1 (13%)			
Centrilobular, degeneration				
Hepatocyte, hypertrophy	2 (25%)		4 (40%)	5 (63%)
Hepatocyte, multinucleated				
Oval cell, hyperplasia				
Pancreas	(8)	(8)	(10)	(8)
Basophilic focus	1 (13%)			
Acinus, vacuolization cytoplasmic				
Stomach, forestomach	(8)			
Stomach, glandular	(8)			
Endocrine System				
Adrenal cortex	(8)	(8)	(10)	(8)
Atrophy				
Degeneration, cystic				1 (13%)
Hyperplasia	1 (13%)			
Hypertrophy	5 (63%)	7 (88%)	6 (60%)	4 (50%)
Vacuolization cytoplasmic	1 (13%)			
Adrenal medulla	(8)	(8)	(10)	(8)
Pituitary gland	(8)			
Angiectasis	1 (13%)			
Pars distalis, hyperplasia	1 (13%)			
Thyroid gland	(8)	(8)	(10)	(8)
C-cell, hyperplasia	1 (13%)			1 (13%)
Follicular cell, hypertrophy	1 (13%)		1 (10%)	1 (13%)
Genital System				
Ovary	(8)			
Uterus	(8)	(8)	(10)	(8)
Dilatation				
Inflammation		1 (13%)		
Inflammation, suppurative	3 (38%)	1 (13%)	3 (30%)	4 (50%)
Metaplasia, squamous	6 (75%)	5 (63%)	7 (70%)	7 (88%)
Endometrium, hyperplasia, cystic	4 (50%)	3 (38%)	6 (60%)	5 (63%)

TABLE A4a

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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
53-Week Interim Evaluation				
Alimentary System				
Liver	(8)	(8)	(8)	(8)
Cholangiofibrosis				4 (50%)
Clear cell focus	2 (25%)	5 (63%)	2 (25%)	
Clear cell focus, multiple	3 (38%)		6 (75%)	
Eosinophilic focus				3 (38%)
Eosinophilic focus, multiple				1 (13%)
Fatty change, diffuse				8 (100%)
Hepatodiaphragmatic nodule			1 (13%)	
Hyperplasia, nodular				3 (38%)
Inflammation	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Mixed cell focus		1 (13%)		
Mixed cell focus, multiple			1 (13%)	7 (88%)
Necrosis		1 (13%)	2 (25%)	1 (13%)
Pigmentation		4 (50%)	7 (88%)	8 (100%)
Toxic hepatopathy				8 (100%)
Bile duct, cyst				
Bile duct, hyperplasia				6 (75%)
Centrilobular, degeneration			1 (13%)	
Hepatocyte, hypertrophy	6 (75%)	7 (88%)	8 (100%)	8 (100%)
Hepatocyte, multinucleated			1 (13%)	7 (88%)
Oval cell, hyperplasia				8 (100%)
Pancreas	(8)	(8)	(8)	(8)
Basophilic focus				
Acinus, vacuolization cytoplasmic				8 (100%)
Stomach, forestomach				(8)
Stomach, glandular				(8)
Endocrine System				
Adrenal cortex	(8)	(8)	(8)	(8)
Atrophy				2 (25%)
Degeneration, cystic			1 (13%)	3 (38%)
Hyperplasia				4 (50%)
Hypertrophy	5 (63%)	5 (63%)	5 (63%)	4 (50%)
Vacuolization cytoplasmic			1 (13%)	
Adrenal medulla	(8)	(8)	(8)	(8)
Pituitary gland				(8)
Angiectasis				
Pars distalis, hyperplasia				1 (13%)
Thyroid gland	(8)	(8)	(8)	(8)
C-cell, hyperplasia		1 (13%)		
Follicular cell, hypertrophy	1 (13%)	5 (63%)	5 (63%)	7 (88%)
Genital System				
Ovary				(8)
Uterus	(8)	(8)	(8)	(8)
Dilatation		1 (13%)	4 (50%)	
Inflammation				
Inflammation, suppurative	3 (38%)	3 (38%)	5 (63%)	2 (25%)
Metaplasia, squamous	4 (50%)	4 (50%)	3 (38%)	4 (50%)
Endometrium, hyperplasia, cystic	4 (50%)	3 (38%)	4 (50%)	3 (38%)

TABLE A4a

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg
53-Week Interim Evaluation (continued)				
Genital System (continued)				
Vagina	(8)			
Inflammation, chronic active	1 (13%)			
Necrosis	1 (13%)			
Hematopoietic System				
Spleen	(8)			
Pigmentation	8 (100%)			
Thymus	(8)	(8)	(10)	(8)
Atrophy	8 (100%)	8 (100%)	10 (100%)	8 (100%)
Cyst	1 (13%)			
Integumentary System				
Mammary gland	(8)	(2)	(2)	(1)
Cyst		2 (100%)		
Respiratory System				
Lung	(8)	(8)	(10)	(8)
Inflammation	1 (13%)		1 (10%)	
Alveolar epithelium, hyperplasia				
Alveolar epithelium, metaplasia, bronchiolar				
Alveolus, infiltration cellular, histiocyte	2 (25%)	2 (25%)	4 (40%)	2 (25%)
Urinary System				
Kidney		(1)		
Infarct		1 (100%)		
Nephropathy		1 (100%)		
Pelvis, dilatation		1 (100%)		
Pelvis, inflammation		1 (100%)		
Transitional epithelium, hyperplasia		1 (100%)		
Systems Examined at 53 Weeks with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				

TABLE A4a

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 PCB 118

	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
53-Week Interim Evaluation (continued)				
Genital System (continued)				
Vagina				(8)
Inflammation, chronic active				
Necrosis				
Hematopoietic System				
Spleen				(8)
Pigmentation				8 (100%)
Thymus	(8)	(8)	(8)	(8)
Atrophy	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Cyst				
Integumentary System				
Mammary gland	(2)		(1)	(8)
Cyst				
Respiratory System				
Lung	(8)	(8)	(8)	(8)
Inflammation				1 (13%)
Alveolar epithelium, hyperplasia				1 (13%)
Alveolar epithelium, metaplasia, bronchiolar				3 (38%)
Alveolus, infiltration cellular, histiocyte	3 (38%)	4 (50%)	3 (38%)	3 (38%)
Urinary System				
Kidney				
Infarct				
Nephropathy				
Pelvis, dilatation				
Pelvis, inflammation				
Transitional epithelium, hyperplasia				
Systems Examined at 53 Weeks with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118^a

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Disposition Summary							
Animals initially in study	52	52	52	52	52	52	50
Early deaths							
Accidental deaths		1					1
Moribund	27	22	22	17	16	16	18
Natural deaths	4	9	5	5	8	11	6
Survivors							
MSAC last week of study							1
Terminal sacrifice	21	20	25	30	28	25	24
Animals examined microscopically	52	52	52	52	52	52	50
Alimentary System							
Esophagus	(51)	(52)	(52)	(52)	(52)	(52)	(50)
Ulcer				1 (2%)			
Muscularis, degeneration					1 (2%)		
Muscularis, inflammation		3 (6%)		1 (2%)			1 (2%)
Intestine large, cecum	(52)	(51)	(51)	(52)	(52)	(48)	(49)
Degeneration, fatty					1 (2%)		
Inflammation			1 (2%)	1 (2%)			
Ulcer			1 (2%)				
Artery, inflammation, chronic active				1 (2%)	3 (6%)	1 (2%)	2 (4%)
Intestine large, colon	(52)	(52)	(52)	(52)	(52)	(48)	(49)
Parasite metazoan	1 (2%)				1 (2%)	1 (2%)	1 (2%)
Artery, inflammation, chronic active				1 (2%)	3 (6%)	1 (2%)	2 (4%)
Intestine large, rectum	(52)	(52)	(52)	(52)	(52)	(50)	(49)
Inflammation		1 (2%)					
Parasite metazoan	2 (4%)	2 (4%)	3 (6%)	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Artery, inflammation, chronic active	1 (2%)			2 (4%)	5 (10%)	1 (2%)	5 (10%)
Intestine small, duodenum	(52)	(52)	(52)	(52)	(52)	(48)	(49)
Inflammation					1 (2%)		
Ulcer					1 (2%)		
Intestine small, ileum	(52)	(51)	(50)	(52)	(52)	(47)	(49)
Artery, inflammation, chronic active					1 (2%)		
Intestine small, jejunum	(52)	(52)	(50)	(52)	(52)	(48)	(49)
Inflammation, chronic active					1 (2%)		
Artery, inflammation, chronic active					1 (2%)		
Liver	(52)	(51)	(52)	(52)	(52)	(49)	(49)
Angiectasis		1 (2%)	1 (2%)	2 (4%)		2 (4%)	1 (2%)
Basophilic focus	11 (21%)	5 (10%)	8 (15%)	4 (8%)	8 (15%)	1 (2%)	5 (10%)
Basophilic focus, multiple	4 (8%)	2 (4%)	3 (6%)	2 (4%)	1 (2%)		4 (8%)
Cholangiofibrosis		2 (4%)	2 (4%)	3 (6%)	2 (4%)	22 (45%)	10 (20%)
Clear cell focus	6 (12%)	3 (6%)	4 (8%)	5 (10%)	2 (4%)		3 (6%)
Clear cell focus, multiple	9 (17%)	7 (14%)	3 (6%)	9 (17%)	3 (6%)		10 (20%)
Degeneration, cystic	1 (2%)		1 (2%)		1 (2%)	2 (4%)	4 (8%)

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

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Alimentary System (continued)							
Liver (continued)	(52)	(51)	(52)	(52)	(52)	(49)	(49)
Eosinophilic focus	5 (10%)	5 (10%)	4 (8%)	4 (8%)	5 (10%)		7 (14%)
Eosinophilic focus, multiple		3 (6%)	5 (10%)	11 (21%)	20 (38%)	41 (84%)	13 (27%)
Fatty change, focal	2 (4%)	1 (2%)	6 (12%)	4 (8%)	3 (6%)		9 (18%)
Fatty change, diffuse	1 (2%)	2 (4%)	1 (2%)	9 (17%)	39 (75%)	48 (98%)	8 (16%)
Hematopoietic cell proliferation	19 (37%)	20 (39%)	21 (40%)	28 (54%)	19 (37%)	21 (43%)	31 (63%)
Hepatodiaphragmatic nodule		1 (2%)	2 (4%)	1 (2%)			1 (2%)
Hyperplasia, nodular					12 (23%)	43 (88%)	4 (8%)
Inflammation	21 (40%)	30 (59%)	35 (67%)	36 (69%)	43 (83%)	44 (90%)	47 (96%)
Mixed cell focus	6 (12%)	5 (10%)	7 (13%)	6 (12%)	1 (2%)	1 (2%)	2 (4%)
Mixed cell focus, multiple	15 (29%)	14 (27%)	22 (42%)	30 (58%)	30 (58%)	6 (12%)	34 (69%)
Necrosis	1 (2%)	2 (4%)	1 (2%)	2 (4%)	20 (38%)	22 (45%)	14 (29%)
Pigmentation	1 (2%)	5 (10%)	12 (23%)	41 (79%)	50 (96%)	48 (98%)	43 (88%)
Toxic hepatopathy			3 (6%)	14 (27%)	33 (63%)	46 (94%)	36 (73%)
Bile duct, cyst	2 (4%)	3 (6%)	5 (10%)	6 (12%)	6 (12%)	21 (43%)	14 (29%)
Bile duct, fibrosis	2 (4%)	1 (2%)		3 (6%)	2 (4%)		7 (14%)
Bile duct, hyperplasia	5 (10%)	6 (12%)	7 (13%)	8 (15%)	21 (40%)	40 (82%)	25 (51%)
Capsule, inflammation	1 (2%)						
Centrilobular, degeneration	1 (2%)	2 (4%)	4 (8%)	3 (6%)	6 (12%)	1 (2%)	2 (4%)
Hepatocyte, hypertrophy		12 (24%)	15 (29%)	20 (38%)	44 (85%)	48 (98%)	30 (61%)
Hepatocyte, multinucleated		1 (2%)	3 (6%)	21 (40%)	40 (77%)	43 (88%)	32 (65%)
Oval cell, hyperplasia		12 (24%)	9 (17%)	29 (56%)	40 (77%)	46 (94%)	29 (59%)
Mesentery	(2)	(1)	(3)	(3)	(9)	(9)	(9)
Hemorrhage				1 (33%)			
Artery, inflammation, chronic active	1 (50%)			2 (67%)	5 (56%)	8 (89%)	5 (56%)
Artery, thrombosis						1 (11%)	
Fat, necrosis					1 (11%)	1 (11%)	
Oral mucosa	(1)		(1)	(1)	(1)	(3)	
Gingival, cyst						1 (33%)	
Gingival, hyperplasia, squamous				1 (100%)	1 (100%)		
Pancreas	(52)	(52)	(52)	(52)	(52)	(47)	(49)
Amyloid deposition							1 (2%)
Degeneration	1 (2%)						
Inflammation, chronic active		1 (2%)	2 (4%)	2 (4%)	3 (6%)	2 (4%)	4 (8%)
Acinus, atrophy, focal	4 (8%)	2 (4%)	3 (6%)	4 (8%)	3 (6%)	1 (2%)	4 (8%)
Acinus, atrophy, diffuse						1 (2%)	
Acinus, hyperplasia		2 (4%)			1 (2%)		
Acinus, vacuolization cytoplasmic					4 (8%)	42 (89%)	10 (20%)
Artery, inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	7 (13%)	7 (13%)	12 (26%)	5 (10%)
Duct, dilatation						3 (6%)	
Duct, inflammation						2 (4%)	
Duct, necrosis						1 (2%)	
Salivary glands	(51)	(51)	(52)	(51)	(52)	(51)	(50)
Degeneration					1 (2%)		
Stomach, forestomach	(52)	(52)	(52)	(52)	(52)	(51)	(49)
Hyperplasia, squamous		3 (6%)			2 (4%)	3 (6%)	5 (10%)
Inflammation	2 (4%)	1 (2%)				1 (2%)	4 (8%)
Ulcer	2 (4%)						3 (6%)
Artery, inflammation, chronic active					1 (2%)	1 (2%)	1 (2%)

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Alimentary System (continued)							
Stomach, glandular	(52)	(52)	(52)	(52)	(52)	(51)	(49)
Cyst	1 (2%)						
Erosion		1 (2%)		1 (2%)			2 (4%)
Mineralization					1 (2%)		1 (2%)
Artery, inflammation, chronic active						1 (2%)	
Artery, mineralization		1 (2%)					
Glands, cyst			1 (2%)				
Tongue							(1)
Degeneration							1 (100%)
Tooth	(10)	(5)	(5)	(5)	(4)	(7)	(7)
Peridental tissue, inflammation	7 (70%)	5 (100%)	5 (100%)	5 (100%)	4 (100%)	6 (86%)	7 (100%)
Cardiovascular System							
Blood vessel	(52)	(52)	(52)	(52)	(52)	(51)	(50)
Aorta, mineralization		1 (2%)					
Heart	(52)	(52)	(52)	(52)	(52)	(50)	(50)
Cardiomyopathy	13 (25%)	19 (37%)	14 (27%)	16 (31%)	19 (37%)	16 (32%)	19 (38%)
Inflammation					1 (2%)	1 (2%)	
Artery, inflammation, chronic active					1 (2%)	1 (2%)	
Artery, mineralization		1 (2%)	1 (2%)	1 (2%)			
Coronary artery, thrombosis		1 (2%)				1 (2%)	
Endocardium, hyperplasia		1 (2%)			1 (2%)	2 (4%)	
Endocardium, infiltration cellular		1 (2%)					
Epicardium, fibrosis						1 (2%)	
Epicardium, inflammation		1 (2%)					
Myocardium, mineralization		1 (2%)					
Endocrine System							
Adrenal cortex	(52)	(52)	(52)	(51)	(52)	(49)	(49)
Angiectasis				1 (2%)			
Atrophy	1 (2%)			2 (4%)	9 (17%)	35 (71%)	4 (8%)
Degeneration, cystic	9 (17%)	8 (15%)	9 (17%)	12 (24%)	6 (12%)	8 (16%)	12 (24%)
Fibrosis							1 (2%)
Hematopoietic cell proliferation	1 (2%)				1 (2%)		
Hyperplasia	14 (27%)	18 (35%)	13 (25%)	16 (31%)	13 (25%)	13 (27%)	21 (43%)
Hypertrophy	37 (71%)	37 (71%)	39 (75%)	43 (84%)	44 (85%)	34 (69%)	38 (78%)
Inflammation			1 (2%)				
Necrosis		1 (2%)	2 (4%)		3 (6%)		4 (8%)
Vacuolization cytoplasmic	10 (19%)	12 (23%)	13 (25%)	12 (24%)	12 (23%)	18 (37%)	21 (43%)
Adrenal medulla	(52)	(52)	(52)	(52)	(52)	(49)	(49)
Hyperplasia	11 (21%)	12 (23%)	14 (27%)	16 (31%)	10 (19%)	1 (2%)	16 (33%)
Necrosis					1 (2%)		
Islets, pancreatic	(52)	(52)	(52)	(52)	(52)	(47)	(49)
Hyperplasia		1 (2%)					
Parathyroid gland	(47)	(46)	(47)	(50)	(50)	(47)	(49)
Hyperplasia		1 (2%)					

TABLE A4b

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Endocrine System (continued)							
Pituitary gland	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Angiectasis	1 (2%)	1 (2%)			1 (2%)		
Cyst		1 (2%)					
Vacuolization cytoplasmic							1 (2%)
Pars distalis, cyst					1 (2%)		
Pars distalis, hyperplasia	10 (19%)	6 (12%)	13 (25%)	13 (25%)	16 (31%)	10 (19%)	10 (20%)
Thyroid gland	(51)	(51)	(51)	(51)	(52)	(49)	(50)
Infiltration cellular, lymphocyte					1 (2%)	1 (2%)	
Inflammation	1 (2%)						
C-cell, hyperplasia	10 (20%)	14 (27%)	10 (20%)	6 (12%)	12 (23%)	11 (22%)	9 (18%)
Follicular cell, hyperplasia					1 (2%)		
Follicular cell, hypertrophy	6 (12%)	7 (14%)	13 (25%)	18 (35%)	21 (40%)	23 (47%)	12 (24%)
General Body System							
None							
Genital System							
Clitoral gland	(52)	(52)	(51)	(52)	(51)	(49)	(48)
Hyperplasia, squamous			1 (2%)				1 (2%)
Inflammation	41 (79%)	38 (73%)	39 (76%)	40 (77%)	35 (69%)	13 (27%)	29 (60%)
Duct, cyst	26 (50%)	39 (75%)	31 (61%)	35 (67%)	37 (73%)	30 (61%)	28 (58%)
Ovary	(52)	(52)	(52)	(52)	(52)	(48)	(49)
Cyst	8 (15%)	10 (19%)	13 (25%)	14 (27%)	14 (27%)	7 (15%)	10 (20%)
Fibrosis				1 (2%)			
Inflammation				2 (4%)	1 (2%)	2 (4%)	1 (2%)
Pigmentation				1 (2%)			
Bilateral, cyst			1 (2%)				
Uterus	(52)	(52)	(52)	(52)	(52)	(49)	(49)
Adenomyosis				1 (2%)		1 (2%)	
Cyst			1 (2%)	1 (2%)		1 (2%)	
Hemorrhage					1 (2%)	1 (2%)	2 (4%)
Inflammation	4 (8%)	6 (12%)	6 (12%)	8 (15%)	8 (15%)	4 (8%)	10 (20%)
Metaplasia, squamous	29 (56%)	26 (50%)	27 (52%)	34 (65%)	35 (67%)	5 (10%)	23 (47%)
Thrombosis	1 (2%)			2 (4%)	1 (2%)		
Ulcer					2 (4%)		
Artery, inflammation, chronic active				1 (2%)			
Cervix, cyst					1 (2%)		
Endometrium, hyperplasia, cystic	28 (54%)	27 (52%)	22 (42%)	23 (44%)	13 (25%)	9 (18%)	21 (43%)
Epithelium, hyperplasia					1 (2%)		
Vagina	(7)			(1)	(1)		
Hematopoietic System							
Bone marrow	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Atrophy	4 (8%)	1 (2%)	1 (2%)		1 (2%)		
Hyperplasia	31 (60%)	30 (58%)	30 (58%)	32 (62%)	34 (65%)	47 (90%)	43 (86%)
Myelofibrosis			1 (2%)				
Necrosis						1 (2%)	

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Hematopoietic System (continued)							
Lymph node		(2)	(1)	(1)		(2)	(1)
Bronchial, ectasia						1 (50%)	
Bronchial, hemorrhage						1 (50%)	
Mediastinal, hemorrhage		1 (50%)					
Lymph node, mandibular	(51)	(51)	(52)	(51)	(52)	(51)	(50)
Atrophy					1 (2%)	1 (2%)	
Hyperplasia, lymphoid			1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia, plasma cell	24 (47%)	34 (67%)	36 (69%)	33 (65%)	30 (58%)	19 (37%)	22 (44%)
Lymph node, mesenteric	(52)	(51)	(52)	(52)	(52)	(47)	(49)
Atrophy	1 (2%)	1 (2%)			1 (2%)		1 (2%)
Ectasia						1 (2%)	
Hemorrhage					1 (2%)		2 (4%)
Hyperplasia, plasma cell	1 (2%)						
Spleen	(52)	(52)	(52)	(52)	(52)	(47)	(49)
Hematopoietic cell proliferation	42 (81%)	39 (75%)	39 (75%)	39 (75%)	32 (62%)	34 (72%)	43 (88%)
Hemorrhage	1 (2%)						
Necrosis	1 (2%)			1 (2%)			
Pigmentation	39 (75%)	35 (67%)	31 (60%)	36 (69%)	40 (77%)	28 (60%)	31 (63%)
Capsule, hemorrhage				1 (2%)			
Lymphoid follicle, atrophy	3 (6%)	4 (8%)	2 (4%)	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Red pulp, atrophy		2 (4%)	2 (4%)	1 (2%)	1 (2%)	3 (6%)	
Thymus	(51)	(51)	(51)	(50)	(50)	(49)	(50)
Atrophy	41 (80%)	38 (75%)	44 (86%)	44 (88%)	46 (92%)	44 (90%)	46 (92%)
Cyst		2 (4%)	1 (2%)				
Hemorrhage		1 (2%)	1 (2%)		1 (2%)	3 (6%)	
Inflammation		1 (2%)					
Artery, inflammation, chronic active						2 (4%)	
Integumentary System							
Mammary gland	(52)	(51)	(52)	(52)	(52)	(50)	(50)
Cyst	1 (2%)	2 (4%)		2 (4%)			5 (10%)
Hyperplasia	4 (8%)	5 (10%)	4 (8%)	5 (10%)		1 (2%)	4 (8%)
Inflammation, granulomatous			2 (4%)	1 (2%)			4 (8%)
Inflammation, chronic active		1 (2%)					
Skin	(52)	(51)	(52)	(52)	(52)	(51)	(50)
Cyst epithelial inclusion			1 (2%)	1 (2%)			
Hyperkeratosis		1 (2%)					
Hyperplasia, squamous		2 (4%)					
Inflammation		3 (6%)					
Musculoskeletal System							
Bone	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Fracture							1 (2%)
Skeletal muscle		(1)			(1)		(2)

TABLE A4b

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Nervous System							
Brain	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Angiectasis			1 (2%)				
Gliosis	1 (2%)			1 (2%)			
Hemorrhage	3 (6%)			1 (2%)			1 (2%)
Hydrocephalus	1 (2%)	2 (4%)	1 (2%)				
Necrosis	2 (4%)						
Vacuolization cytoplasmic		1 (2%)					
Meninges, inflammation				1 (2%)			
Spinal cord			(1)			(1)	
Nerve, degeneration						1 (100%)	
Respiratory System							
Lung	(51)	(52)	(52)	(52)	(52)	(50)	(50)
Congestion					1 (2%)		
Hemorrhage				1 (2%)		1 (2%)	
Inflammation	5 (10%)	3 (6%)	5 (10%)	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Metaplasia, squamous	1 (2%)			1 (2%)	1 (2%)	13 (26%)	
Pigmentation			1 (2%)			1 (2%)	
Proteinosis	1 (2%)						
Alveolar epithelium, hyperplasia	4 (8%)	2 (4%)					3 (6%)
Alveolar epithelium, metaplasia, bronchiolar	6 (12%)	7 (13%)	14 (27%)	18 (35%)	24 (46%)	40 (80%)	32 (64%)
Alveolus, infiltration cellular, histiocyte	36 (71%)	35 (67%)	37 (71%)	39 (75%)	34 (65%)	40 (80%)	40 (80%)
Artery, mediastinum, inflammation, chronic active						1 (2%)	
Serosa, inflammation		1 (2%)					
Nose	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Cyst	1 (2%)						
Inflammation	1 (2%)	5 (10%)	5 (10%)	3 (6%)	5 (10%)	23 (44%)	8 (16%)
Glands, cyst							1 (2%)
Nasolacrimal duct, inflammation, suppurative						1 (2%)	
Olfactory epithelium, degeneration	1 (2%)	1 (2%)				1 (2%)	
Olfactory epithelium, metaplasia						1 (2%)	1 (2%)
Respiratory epithelium, degeneration, focal	1 (2%)						
Respiratory epithelium, hyperplasia	5 (10%)	5 (10%)	7 (13%)	7 (13%)	14 (27%)	27 (52%)	11 (22%)
Respiratory epithelium, metaplasia, squamous							1 (2%)
Respiratory epithelium, necrosis				1 (2%)			
Trachea	(51)	(52)	(52)	(52)	(52)	(52)	(50)
Inflammation						1 (2%)	

TABLE A4b

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PCB 118

	Vehicle Control	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg	4,600 µg/kg (Stop-Exposure)
Special Senses System							
Eye	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Cornea, inflammation	1 (2%)	1 (2%)	1 (2%)	1 (2%)			
Retina, atrophy	1 (2%)				1 (2%)	6 (12%)	
Harderian gland	(52)	(52)	(52)	(52)	(52)	(52)	(50)
Hyperplasia		1 (2%)	2 (4%)				
Infiltration cellular, mononuclear cell	7 (13%)	10 (19%)	3 (6%)	10 (19%)	4 (8%)	13 (25%)	11 (22%)
Vacuolization cytoplasmic					1 (2%)		
Urinary System							
Kidney	(52)	(52)	(52)	(52)	(52)	(50)	(49)
Accumulation, hyaline droplet	1 (2%)		1 (2%)				2 (4%)
Amyloid deposition							1 (2%)
Calculus microscopic observation only	3 (6%)			2 (4%)	1 (2%)		
Cyst		2 (4%)				1 (2%)	1 (2%)
Dilatation				1 (2%)			
Inflammation					1 (2%)		
Mineralization	25 (48%)	28 (54%)	30 (58%)	18 (35%)	22 (42%)	25 (50%)	28 (57%)
Necrosis			1 (2%)				
Nephropathy	42 (81%)	40 (77%)	46 (88%)	44 (85%)	44 (85%)	46 (92%)	48 (98%)
Pigmentation	2 (4%)	3 (6%)	3 (6%)	4 (8%)	6 (12%)	42 (84%)	6 (12%)
Artery, inflammation, chronic active	1 (2%)				1 (2%)		1 (2%)
Capsule, inflammation, chronic active					1 (2%)		
Pelvis, dilatation		1 (2%)		1 (2%)	1 (2%)		3 (6%)
Pelvis, inflammation		1 (2%)			2 (4%)	2 (4%)	2 (4%)
Renal tubule, hyperplasia				1 (2%)			
Transitional epithelium, hyperplasia					3 (6%)	3 (6%)	
Ureter					(1)		(2)
Cyst							2 (100%)
Urinary bladder	(52)	(52)	(52)	(52)	(52)	(50)	(49)
Hyperplasia		1 (2%)					
Inflammation		1 (2%)			1 (2%)		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 PCB 118	130

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 PCB 118^a

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Necropsy body wt								
Week 14	251 ± 5	262 ± 4	269 ± 6	273 ± 5*	266 ± 6	272 ± 7*	263 ± 4	242 ± 7
Week 31	284 ± 5	295 ± 8	306 ± 5	301 ± 8	287 ± 7	297 ± 7	286 ± 12	249 ± 6**
Week 53	332 ± 12	332 ± 12	325 ± 11	339 ± 12	367 ± 21	317 ± 12	295 ± 5*	268 ± 5**
L. Kidney								
Week 14								
Absolute	0.724 ± 0.014	0.747 ± 0.023	0.750 ± 0.023	0.750 ± 0.023	0.737 ± 0.032	0.756 ± 0.020	0.760 ± 0.022	0.729 ± 0.025
Relative	2.887 ± 0.055	2.844 ± 0.065	2.792 ± 0.072	2.751 ± 0.091	2.767 ± 0.084	2.790 ± 0.089	2.891 ± 0.071	3.015 ± 0.080
Week 31								
Absolute	0.773 ± 0.027	0.823 ± 0.033	0.823 ± 0.030	0.823 ± 0.018	0.822 ± 0.029	0.823 ± 0.031	0.805 ± 0.025	0.806 ± 0.020
Relative	2.721 ± 0.072	2.792 ± 0.096	2.689 ± 0.093	2.745 ± 0.082	2.872 ± 0.086	2.777 ± 0.113	2.831 ± 0.081	3.247 ± 0.100**
Week 53								
Absolute	0.949 ± 0.016	0.989 ± 0.044	0.907 ± 0.020	0.981 ± 0.034	0.988 ± 0.038	0.992 ± 0.019	0.995 ± 0.030	0.934 ± 0.023
Relative	2.888 ± 0.118	2.982 ± 0.100	2.808 ± 0.075	2.904 ± 0.101	2.726 ± 0.108	3.150 ± 0.066	3.375 ± 0.068**	3.480 ± 0.064**
Liver								
Week 14 ^b								
Absolute	7.50 ± 0.37	9.12 ± 0.30*	9.40 ± 0.48*	8.88 ± 0.37*	8.95 ± 0.59*	9.20 ± 0.40*	10.18 ± 0.34**	13.09 ± 0.62**
Relative	30.277 ± 1.154	34.041 ± 0.936*	34.396 ± 0.609*	32.356 ± 0.655*	32.968 ± 1.282	34.715 ± 1.158**	38.416 ± 0.782**	54.678 ± 1.508**
Week 31 ^b								
Absolute	9.01 ± 0.38	9.28 ± 0.25	10.34 ± 0.70	9.52 ± 0.40	9.58 ± 0.15	10.08 ± 0.41	11.07 ± 0.81**	14.65 ± 0.50**
Relative	30.798 ± 0.878	31.338 ± 0.640	33.698 ± 1.633	31.394 ± 0.642	32.856 ± 0.581	33.998 ± 0.299	37.133 ± 0.352**	61.185 ± 2.103**
Week 53								
Absolute	10.03 ± 0.43	10.73 ± 0.80	10.23 ± 0.48	10.41 ± 0.40	11.82 ± 0.80	9.94 ± 0.33	10.80 ± 0.26	17.59 ± 0.75**
Relative	30.262 ± 0.930	32.066 ± 1.346	31.435 ± 0.797	30.708 ± 0.662	32.369 ± 1.681	31.557 ± 1.050	36.626 ± 0.460**	65.770 ± 3.238**

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Necropsy body wt								
Week 14	251 ± 5	262 ± 4	269 ± 6	273 ± 5*	266 ± 6	272 ± 7*	263 ± 4	242 ± 7
Week 31	284 ± 5	295 ± 8	306 ± 5	301 ± 8	287 ± 7	297 ± 7	286 ± 12	249 ± 6**
Week 53	332 ± 12	332 ± 12	325 ± 11	339 ± 12	367 ± 21	317 ± 12	295 ± 5*	268 ± 5**
Lung								
Week 14								
Absolute	1.56 ± 0.07	1.63 ± 0.08	1.66 ± 0.06	1.63 ± 0.07	1.71 ± 0.05	1.63 ± 0.06	1.65 ± 0.05	1.53 ± 0.05
Relative	6.198 ± 0.249	6.217 ± 0.274	6.171 ± 0.231	5.966 ± 0.227	6.445 ± 0.142	6.026 ± 0.282	6.266 ± 0.156	6.344 ± 0.136
Week 31								
Absolute	1.74 ± 0.07	1.76 ± 0.05	1.93 ± 0.04	1.83 ± 0.09	1.68 ± 0.06	1.78 ± 0.06	1.77 ± 0.08	1.76 ± 0.08
Relative	6.131 ± 0.214	5.997 ± 0.192	6.302 ± 0.094	6.107 ± 0.334	5.854 ± 0.179	6.017 ± 0.270	6.249 ± 0.323	7.027 ± 0.220
Week 53								
Absolute	2.14 ± 0.12	2.28 ± 0.09	2.25 ± 0.07	2.24 ± 0.09	1.98 ± 0.05	2.10 ± 0.10	2.02 ± 0.09	1.89 ± 0.06
Relative	6.497 ± 0.394	6.922 ± 0.351	6.975 ± 0.224	6.607 ± 0.208	5.520 ± 0.319	6.714 ± 0.407	6.850 ± 0.245	7.047 ± 0.228
L. Ovary								
Week 14								
Absolute	0.053 ± 0.003	0.065 ± 0.003	0.064 ± 0.004	0.065 ± 0.002	0.067 ± 0.004*	0.061 ± 0.003	0.063 ± 0.004	0.051 ± 0.002
Relative	0.212 ± 0.013	0.247 ± 0.010	0.239 ± 0.012	0.237 ± 0.009	0.250 ± 0.010	0.224 ± 0.011	0.239 ± 0.012	0.212 ± 0.006
Week 31								
Absolute	0.051 ± 0.003	0.055 ± 0.005	0.060 ± 0.004	0.061 ± 0.004	0.057 ± 0.004	0.056 ± 0.005	0.053 ± 0.004	0.053 ± 0.003
Relative	0.179 ± 0.009	0.186 ± 0.016	0.196 ± 0.013	0.201 ± 0.010	0.196 ± 0.012	0.186 ± 0.013	0.184 ± 0.010	0.212 ± 0.016
Week 53								
Absolute	0.059 ± 0.006	0.060 ± 0.007	0.064 ± 0.006	0.058 ± 0.006	0.068 ± 0.006	0.055 ± 0.005	0.054 ± 0.002	0.050 ± 0.006
Relative	0.176 ± 0.014	0.180 ± 0.019	0.195 ± 0.015	0.168 ± 0.013	0.183 ± 0.009	0.175 ± 0.016	0.182 ± 0.004	0.186 ± 0.020

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Necropsy body wt								
Week 14	251 ± 5	262 ± 4	269 ± 6	273 ± 5*	266 ± 6	272 ± 7*	263 ± 4	242 ± 7
Week 31	284 ± 5	295 ± 8	306 ± 5	301 ± 8	287 ± 7	297 ± 7	286 ± 12	249 ± 6**
Week 53	332 ± 12	332 ± 12	325 ± 11	339 ± 12	367 ± 21	317 ± 12	295 ± 5*	268 ± 5**
Spleen								
Week 14								
Absolute	0.549 ± 0.024	0.607 ± 0.015	0.624 ± 0.022	0.636 ± 0.031	0.630 ± 0.031	0.598 ± 0.026	0.577 ± 0.010	0.476 ± 0.022
Relative	2.180 ± 0.072	2.314 ± 0.040	2.321 ± 0.070	2.321 ± 0.081	2.365 ± 0.092	2.203 ± 0.084	2.197 ± 0.047	1.963 ± 0.062
Week 31								
Absolute	0.580 ± 0.021	0.609 ± 0.018	0.612 ± 0.025	0.592 ± 0.023	0.547 ± 0.022	0.595 ± 0.027	0.531 ± 0.025	0.430 ± 0.019**
Relative	2.049 ± 0.074	2.075 ± 0.079	1.999 ± 0.073	1.964 ± 0.047	1.905 ± 0.049	1.998 ± 0.069	1.856 ± 0.046*	1.723 ± 0.057**
Week 53								
Absolute	0.609 ± 0.044	0.592 ± 0.024	0.560 ± 0.029	0.567 ± 0.025	0.613 ± 0.036	0.544 ± 0.036	0.496 ± 0.014*	0.462 ± 0.018**
Relative	1.865 ± 0.179	1.783 ± 0.047	1.725 ± 0.073	1.676 ± 0.057	1.688 ± 0.100	1.724 ± 0.107	1.687 ± 0.055	1.724 ± 0.073
Thymus								
Week 14								
Absolute	0.309 ± 0.016	0.321 ± 0.013	0.349 ± 0.013	0.316 ± 0.024	0.278 ± 0.018	0.345 ± 0.014	0.332 ± 0.012	0.263 ± 0.020
Relative	1.230 ± 0.053	1.226 ± 0.056	1.306 ± 0.066	1.158 ± 0.085	1.045 ± 0.066	1.272 ± 0.053	1.264 ± 0.050	1.088 ± 0.077

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 PCB 118

	Vehicle Control	10 µg/kg	30 µg/kg	100 µg/kg	220 µg/kg	460 µg/kg	1,000 µg/kg	4,600 µg/kg
n								
Week 14	10	10	10	10	10	10	10	10
Week 31	10	10	10	10	10	10	10	10
Week 53	8	8	10	8	8	8	8	8
Necropsy body wt								
Week 14	251 ± 5	262 ± 4	269 ± 6	273 ± 5*	266 ± 6	272 ± 7*	263 ± 4	242 ± 7
Week 31	284 ± 5	295 ± 8	306 ± 5	301 ± 8	287 ± 7	297 ± 7	286 ± 12	249 ± 6**
Week 53	332 ± 12	332 ± 12	325 ± 11	339 ± 12	367 ± 21	317 ± 12	295 ± 5*	268 ± 5**
Thyroid Gland								
Week 14								
Absolute	0.020 ± 0.001	0.023 ± 0.001	0.022 ± 0.001	0.022 ± 0.001	0.022 ± 0.001	0.022 ± 0.001	0.022 ± 0.001	0.021 ± 0.001
Relative	0.078 ± 0.003	0.089 ± 0.005	0.082 ± 0.006	0.081 ± 0.005	0.082 ± 0.004	0.080 ± 0.004	0.082 ± 0.005	0.088 ± 0.006
Week 31								
Absolute	0.020 ± 0.001	0.019 ± 0.002	0.023 ± 0.001	0.019 ± 0.001	0.020 ± 0.002	0.020 ± 0.001	0.020 ± 0.002	0.016 ± 0.001
Relative	0.069 ± 0.004	0.062 ± 0.005	0.074 ± 0.003	0.064 ± 0.004	0.069 ± 0.006	0.066 ± 0.003	0.070 ± 0.006	0.063 ± 0.003
Week 53								
Absolute	0.029 ± 0.003	0.029 ± 0.002	0.031 ± 0.002	0.030 ± 0.002	0.038 ± 0.002*	0.029 ± 0.002	0.024 ± 0.001	0.024 ± 0.002
Relative	0.087 ± 0.006	0.086 ± 0.005	0.094 ± 0.006	0.089 ± 0.007	0.105 ± 0.006	0.090 ± 0.004	0.082 ± 0.004	0.091 ± 0.007

* 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=5

APPENDIX C

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

PCB 118

PCB 118 was obtained from Cerilliant Corp. (Round Rock, TX), in one lot (35108-72) that was used in the 2-year study. Identity and purity analyses were conducted by the analytical chemistry laboratory (Research Triangle Institute, Research Triangle Park, NC) and the study laboratory (Battelle Columbus Operations, Columbus, OH), and stability analyses were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the study of PCB 118 are on file at the National Institute of Environmental Health Sciences.

Lot 35108-72 of the chemical, a white powder, was identified as PCB 118 by the analytical chemistry laboratory using 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. All spectra were consistent with the structure of PCB 118, and the IR spectrum obtained by the study laboratory matched a reference spectrum provided by the test article vendor. Infrared, proton NMR, and low resolution MS spectra are presented in Figures C1, C2, and C3.

The purity of lot 35108-72 was determined by the analytical chemistry laboratory (systems A and B) and the study laboratory (system C) using gas chromatography with electron capture detection (GC/ECD) (Table C1). The moisture content of lot 35108-72 was determined by the analytical chemistry laboratory using a gas chromatography moisture analysis (GCMA) purge and trap method by system D.

No moisture was detected by GCMA. GC/ECD by system A detected one major peak and three impurity peaks with areas of 0.023%, 0.15%, and 0.077% relative to the total integrated peak area. GC/ECD by system B detected one major peak and four impurity peaks with areas of 0.15%, 0.047%, 0.25%, and 0.13% relative to the total integrated peak area. The overall purity of lot 35108-72 was determined to be 99% or greater with trace quantities of other dioxins (Table C2).

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC/MS by system E (Table C1). Aliquots of the test chemical were stored at -20°C , 5°C , and 25°C in amber glass vials for 14 days. Samples stored protected from light were stable for at least 2 weeks at temperatures up to 25°C . Stability of the bulk chemical was monitored by the study laboratory using GC/ECD by system C. No degradation of the bulk chemical was detected during the study. The bulk chemical was stored at room temperature (approximately 25°C) in an amber glass bottle.

Formulation Materials

NF-grade acetone was obtained from EM Sciences/EMD (Gibbstown, NJ) in two lots and was used with corn oil (Spectrum Quality Products, Gardena, CA) as the vehicle in the 2-year gavage study. The identity of each lot of acetone 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 with flame ionization detection by system F prior to initial use and at intervals of no more than 6 months thereafter. All acetone lots showed a purity of at least 99%. 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 acetone and the corn oil vehicle (Table C3). 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 (approximately 25° C) in amber glass bottles sealed with Teflon[®]-lined lids for up to 42 days.

Homogeneity and stability studies of a 4 µg/mL PCB 118 formulation were performed by the study laboratory using GC/ECD by system C (Table C1). Homogeneity was confirmed, and 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 under simulated animal room conditions.

Analyses of the dose formulations of PCB 118 were conducted by the study laboratory using GC/ECD by system C every 2 to 3 months (Table C4). Of the dose formulations analyzed and used in the study, all 63 were within 10% of the target concentrations; all 25 animal room samples analyzed were within 10% of the target concentrations. Periodic analyses of the corn oil vehicle by the study laboratory demonstrated peroxide concentrations less than 3 mEq/kg.

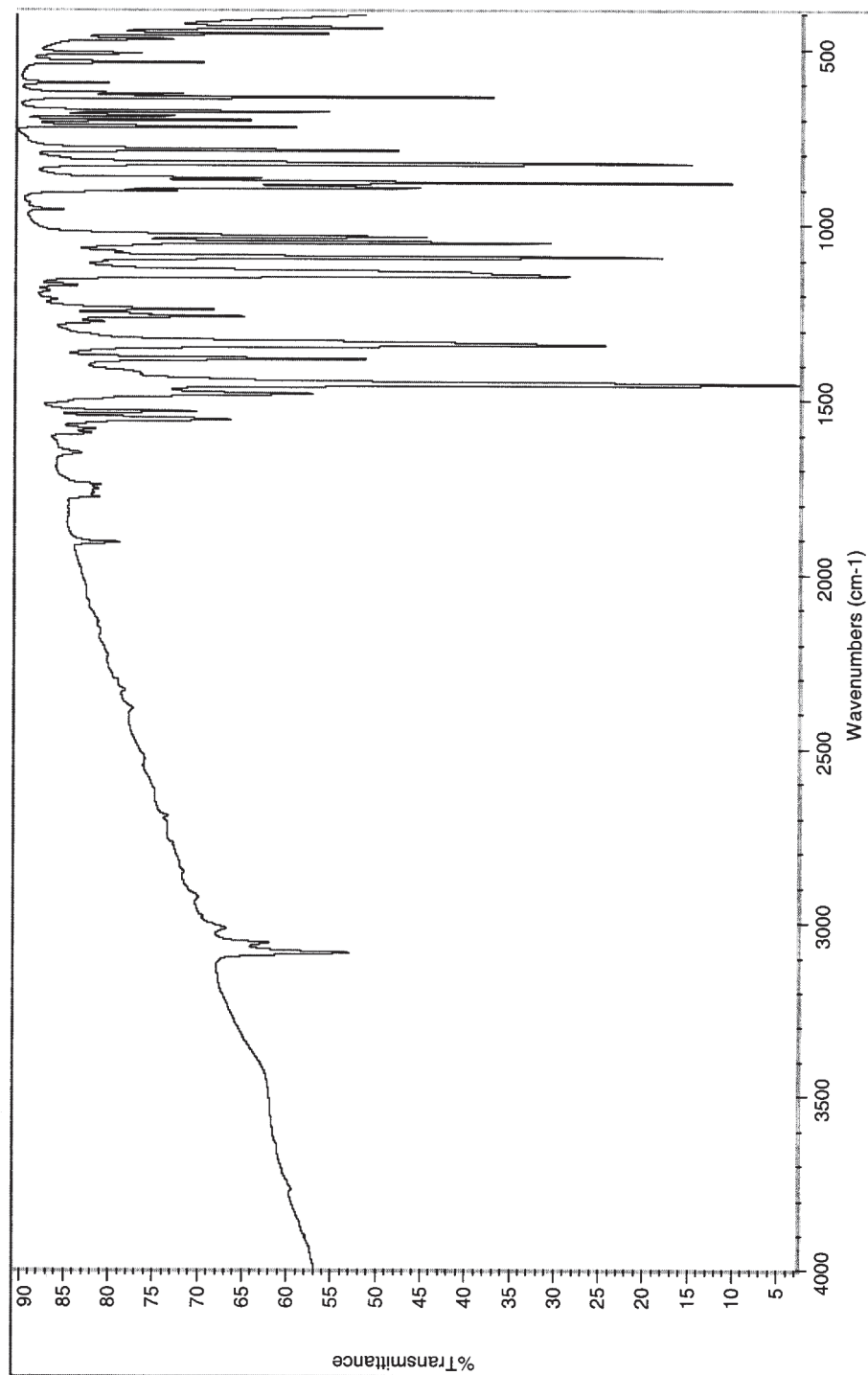


FIGURE C1
Infrared Absorption Spectrum of PCB 118

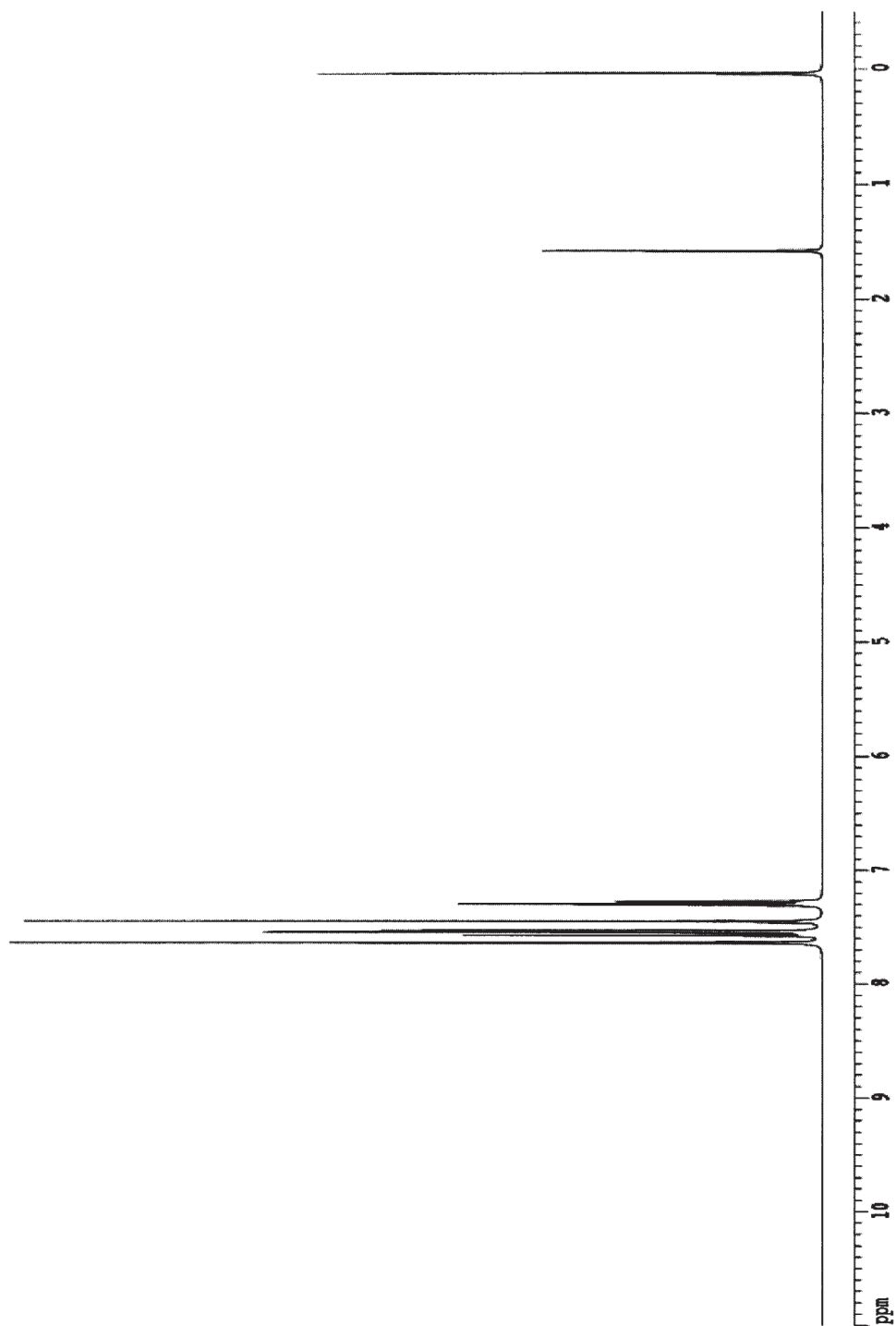


FIGURE C2
Proton Nuclear Magnetic Resonance Spectrum of PCB 118

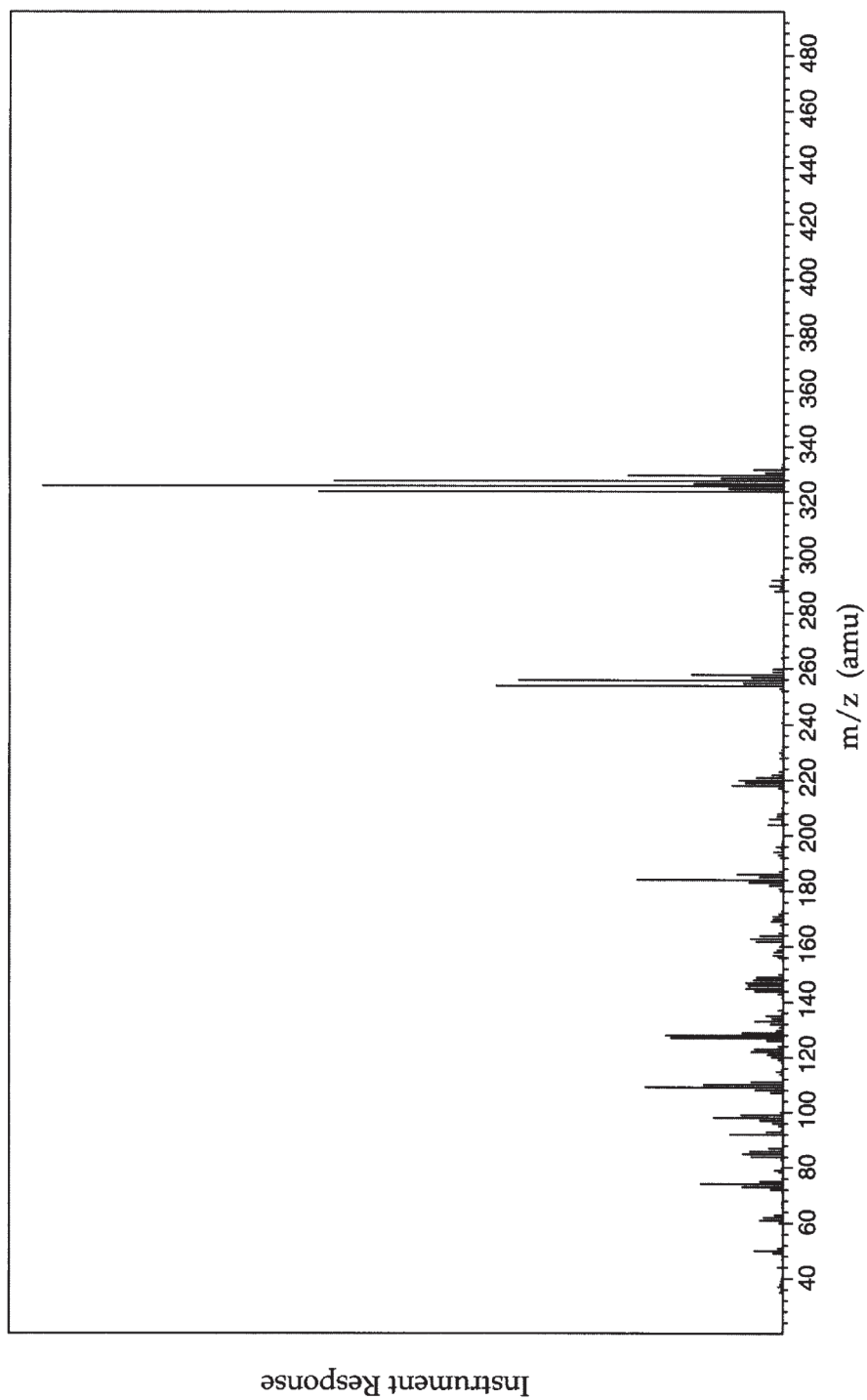


FIGURE C3
Mass Spectrum of PCB 118

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

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Electron capture	J&W DB-17, 30 m × 0.25 mm, 0.25-μm film thickness (J&W Scientific, Folsom, CA)	Nitrogen at 1.06 mL/minute	100° C, then 5° C/minute to 300° C, then held for 5 minutes
System B			
Electron capture	J&W DB-5 MS, 30 m × 0.32 mm, 0.5-μm film thickness (J&W Scientific)	Nitrogen at 1.8 mL/minute	50° C, then 10° C/minute to 300° C, then held for 5 minutes
System C			
Electron capture	RTX-5, 30 m × 0.32 mm, 1-μm film thickness (Restek, 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 D			
Thermal conductivity	Porapak QS 80/100 mesh 6 ft × 4 mm (Sigma-Aldrich, St. Louis, MO)	Helium at 100 mL/minute	40° C for 1 minute, then 15° C/minute to 120° C
System E			
Mass spectrometry	Agilent HP-5, 30 m × 0.32 mm, 0.25-μm film thickness (Agilent Technologies, Santa Clara, CA)	Nitrogen at 1.5 mL/minute	50° C for 1 minute, then 10° C/minute to 300° C, held for 10 minutes
System F			
Flame ionization	Supelcowax-10, 30 m × 0.53 mm, 0.5-μm film thickness (Sigma-Aldrich)	Helium at 10 mL/minute	40° C for 5 minutes, then 10° C/minute to 220° C

^a The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA) (systems A, B, and E), Agilent Technologies (Santa Clara, CA) (systems C and F), and Varian, Inc., (Palo Alto, CA) (system D).

TABLE C2
Dioxin-like Chemicals Measured in the Bulk Analysis of PCB 118^a

Compound	WHO ₉₈ TEF	% in Bulk PCB 118
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	0.0001	>99
2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin (TCDD)	1	0.000005
1,2,3,7,8-Pentachlorodibenzo- <i>p</i> -dioxin (PeCDD)	1	ND
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	0.5	ND
1,2,3,4,7,8-Hexachlorodibenzo- <i>p</i> -dioxin (HxCDD)	0.1	ND
1,2,3,6,7,8-Hexachlorodibenzo- <i>p</i> -dioxin (HxCDD)	0.1	ND
1,2,3,7,8,9-Hexachlorodibenzo- <i>p</i> -dioxin (HxCDD)	0.1	ND
2,3,7,8-Tetrafurane (TCDF)	0.1	0.000005
1,2,3,4,7,8-Hexafuran (HxCDF)	0.1	ND
1,2,3,7,8,9-Hexafuran (HxCDF)	0.1	ND
2,3,4,6,7,8-Hexafuran (HxCDF)	0.1	ND
1,2,3,6,7,8-Hexafuran (HxCDF)	0.1	ND
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	0.05	ND
1,2,3,4,6,7,8-Heptachlorodibenzo- <i>p</i> -dioxin (HpCDD)	0.01	ND
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	0.01	ND
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	0.01	ND ^b
Octachlorodibenzodioxin (OCDD)	0.0001	ND ^c
Octafuran (OCDF)	0.0001	ND ^b
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	0.1	0.0000170
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	0.01	0.0000003
2,3,4,4',5-Pentachlorobiphenyl (PCB 114)	0.0005	co-elutes with PCB 118
2,3,4,4',5-Pentachlorobiphenyl (PCB 156)	0.0005	0.06
2,3',4,4',5-Pentachlorobiphenyl (PCB 157)	0.0005	0.001
3,3',4,4'-Tetrachlorobiphenyl (PCB 77)	0.0001	0.0003
3,4,4',5-Tetrachlorobiphenyl (PCB 81)	0.0001	0.001
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	0.0001	co-elutes with PCB 118
2,3,3',4,4'-Pentachlorobiphenyl (PCB 123)	0.0001	co-elutes with PCB 118
2,3',4,4',5-Pentachlorobiphenyl (PCB 189)	0.0001	did not elute
2,3,4,4',5-Pentachlorobiphenyl (PCB 167)	0.00001	0.1000
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	0	0.001
2,2',3,4,4',5'-Hexachlorobiphenyl (PCB 138)	0	0.001
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	0	co-elutes with PCB 118
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	0	0.001
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	0	0.001

^a % is the detected level or limit of quantitation. Total non-PCB 118 TEQ contribution is 0.39 ng TEQ/1,000 µg PCB 118 bulk test article; at the highest dose of 4,600 µg/kg PCB 118 this corresponds to a maximum TEQ contribution from other dioxin-like chemicals of 1.79 ng TEQ/kg. Estimated limit of detection (ELOD)=100 ppb; ND=none detected

^b ELOD=50 ppb based on TCDF

^c ELOD=50 ppb based on TCDD

TABLE C3**Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of PCB 118**

Preparation

Dose formulations were prepared approximately every 5 weeks. A working stock of 8 mg/mL PCB 118 was prepared by adding the appropriate amount of test article to a 10-mL volumetric flask; approximately 5 mL of acetone were added, and the flask was capped and shaken well. The contents were diluted to volume with acetone, vortexed for approximately 2 minutes, sonicated for approximately 30 minutes, and inverted at least 10 times.

To prepare the 4 µg/mL dose formulation, approximately one-half of the required total volume of corn oil was placed in a calibrated glass mixing container. A specified volume of the 8 µg/mL stock solution was pipetted into the mixing container. A volume of acetone was measured in a graduated cylinder and transferred to the mixing container. The mixing container was capped and shaken to mix. After the corn oil settled, the contents were diluted to final volume with corn oil, mixed by shaking, and stirred overnight on a stir plate.

To prepare the 12, 40, 88, 184, 400, and 1,840 µg/mL dose formulations, approximately one-half of the required total volume of corn oil was placed in a calibrated glass mixing container. The test article was weighed and then added to the mixing container. Acetone equivalent to 1.0% of the volume of the formulation was measured. The container in which the test article was weighed was rinsed with the acetone into the mixing container. The remainder of the acetone was added to the mixing container. The mixing container was capped and shaken to mix, and the contents were then diluted to final volume with corn oil. The container was capped, mixed by shaking, and stirred overnight on a stir plate.

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

Chemical Lot Number

35108-72

Maximum Storage Time

42 days

Storage Conditions

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

Study Laboratory

Battelle Columbus Operations (Columbus, OH)

TABLE C4
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of PCB 118

Date Prepared	Date Analyzed	Target Concentration (µg/mL)	Determined Concentration ^a (µg/mL)	Difference from Target (%)
March 11, 2004	March 15-16, 2004	4	3.825 ± 0.006	-4
		12	11.36 ± 0.03	-5
		40	38.13 ± 0.29	-5
		88	84.87 ± 0.28	-4
		184	179.1 ± 2.2	-3
		1,840	1,831 ± 9	0
		1,840	1,772 ± 6	-4
	April 20-21, 2004 ^b	4	3.807 ± 0.019	-5
		12	11.29 ± 0.05	-6
		40	38.02 ± 0.10	-5
		88	84.85 ± 0.47	-4
		184	179.3 ± 0.5	-3
		1,840	1,834 ± 11	0
		1,840	1,760 ± 6	-4
March 17, 2004	March 22, 2004	400	391.3 ± 2.0	-2
	April 20-21, 2004 ^b	400	385.4 ± 1.6	-4
June 2, 2004	June 4-5, 2004	4	3.529 ± 0.015 ^c	-12
		12	11.59 ± 0.05	-3
		40	39.45 ± 0.79	-1
		88	86.31 ± 0.45	-2
		184	182.9 ± 1.5	-1
		400	392.1 ± 2.8	-2
		1,840	1,861 ± 8	+1
		1,840	1,781 ± 20	-3
June 8, 2004	June 8-9, 2004	4	3.951 ± 0.014 ^d	-1
August 23, 2004	August 25-26, 2004	4	4.120 ± 0.109	+3
		12	11.07 ± 0.03	-8
		40	37.93 ± 0.23	-5
		88	81.31 ± 1.04	-8
		184	178.2 ± 1.4	-3
		400	383.1 ± 4.2	-4
		1,840	1,836 ± 34	0
		1,840	1,739 ± 15	-5

TABLE C4
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of PCB 118

Date Prepared	Date Analyzed	Target Concentration (µg/mL)	Determined Concentration (µg/mL)	Difference from Target (%)
November 11, 2004	November 16-17, 2004	4	4.312 ± 0.097	+8
		12	12.55 ± 0.19	+5
		40	42.49 ± 0.71	+6
		88	92.80 ± 0.95	+5
		184	191.8 ± 2.2	+4
		400	411.0 ± 4.3	+3
		1,840	1,947 ± 13	+6
	December 21-22, 2004 ^b	4	4.175 ± 0.169	+4
		12	12.14 ± 0.28	+1
		40	40.97 ± 0.41	+2
		88	91.78 ± 1.18	+4
		184	196.3 ± 7.4	+7
		400	427.5 ± 14.4	+7
		1,840	2,025 ± 77	+10
February 1, 2005	February 8-9, 2005	4	4.070 ± 0.013	+2
		12	11.96 ± 0.10	0
		40	38.40 ± 0.13	-4
		88	84.46 ± 1.02	-4
		184	185.6 ± 0.8	+1
		400	398.9 ± 1.2	0
		1,840	1,895 ± 8	+3
April 20, 2005	April 26-27, 2005	40	37.65 ± 0.20	-6
		88	71.69 ± 0.55 ^c	-19
		184	174.0 ± 1.4	-5
		400	370.7 ± 4.6	-7
		1,840	1,720 ± 4	-7
April 27, 2005	April 29-30, 2005	88	83.78 ± 0.40 ^d	-5
July 12, 2005	July 14-18, 2005	40	38.04 ± 1.28	-5
		88	82.65 ± 1.68	-6
		184	170.7 ± 6.3 ^e	-7
		400	378.1 ± 3.2	-5
		1,840	1,811 ± 14	-2
	August 18-19, 2005 ^b	40	36.35 ± 0.28	-9
		88	79.90 ± 1.02	-9
		184	170.2 ± 0.7	-8
		400	364.6 ± 2.5	-9
		1,840	1,800 ± 35	-2

TABLE C4
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of PCB 118

Date Prepared	Date Analyzed	Target Concentration (µg/mL)	Determined Concentration (µg/mL)	Difference from Target (%)
October 3, 2005	October 5-6, 2005	40	38.37 ± 0.43	-4
		88	86.90 ± 1.08	-1
		184	177.4 ± 2.8	-4
		400	391.4 ± 4.9	-2
		1,840	1,789 ± 8	-3
December 19, 2005	December 21-22, 2005	40	38.36 ± 0.59	-4
		88	84.89 ± 1.20	-4
		184	185.9 ± 4.2	+1
		400	372.1 ± 4.5	-7
		1,840	1,798 ± 47	-2
March 6, 2006	March 13-14, 2006	40	38.97 ± 1.04	-3
		88	85.49 ± 0.51	-3
		184	180.5 ± 3.6	-2
		400	388.7 ± 7.6	-3
		1,840	1,907 ± 22	+4
	April 6-7, 2006 ^b	40	42.08 ± 0.95	+5
		88	92.61 ± 1.34	+5
		184	200.2 ± 12.5	+9
		400	422.0 ± 10.6	+6
		1,840	1,950 ± 6.7	+6

^a Determined concentration is the average of quadruplicate analyses ± standard deviation.

Dosing volume=2.5 mL/kg; 4 µg/mL=10 µg/kg, 12 µg/mL=30 µg/kg, 40 µg/mL=100 µg/kg, 88 µg/mL=220 µg/kg, 184 µg/mL=460 µg/kg, 400 µg/mL=1,000 µg/kg, 1,840 µg/mL=4,600 µg/kg

^b Animal room samples

^c Remixed; not used in study

^d Result of remix

^e Average of eight analyses

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	148
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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 $\pm$ Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 $\pm$ 0.72	13.5 – 16.3	25
Crude fat (% by weight)	8.2 $\pm$ 0.37	7.6 – 9.3	25
Crude fiber (% by weight)	9.3 $\pm$ 0.48	8.2 – 10.0	25
Ash (% by weight)	5.0 $\pm$ 0.23	4.6 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.770 $\pm$ 0.070	0.670 – 0.970	18
Cystine	0.225 $\pm$ 0.023	0.150 – 0.250	18
Glycine	0.706 $\pm$ 0.043	0.620 – 0.800	18
Histidine	0.362 $\pm$ 0.082	0.310 – 0.680	18
Isoleucine	0.542 $\pm$ 0.046	0.430 – 0.660	18
Leucine	1.087 $\pm$ 0.066	0.960 – 1.240	18
Lysine	0.712 $\pm$ 0.118	0.310 – 0.840	18
Methionine	0.407 $\pm$ 0.051	0.260 – 0.490	18
Phenylalanine	0.626 $\pm$ 0.043	0.540 – 0.720	18
Threonine	0.500 $\pm$ 0.046	0.430 – 0.610	18
Tryptophan	0.142 $\pm$ 0.024	0.110 – 0.200	18
Tyrosine	0.388 $\pm$ 0.058	0.280 – 0.540	18
Valine	0.667 $\pm$ 0.045	0.550 – 0.730	18
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 $\pm$ 0.243	3.49 – 4.54	18
Linolenic	0.30 $\pm$ 0.035	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	4,207 $\pm$ 9,048	2,340 – 6,160	25
Vitamin D (IU/kg)	1,000 ^a		
α -Tocopherol (ppm)	84.2 $\pm$ 16.60	52.0 – 110.0	15
Thiamine (ppm) ^b	7.9 $\pm$ 1.20	6.3 – 10.5	25
Riboflavin (ppm)	6.8 $\pm$ 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 $\pm$ 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 $\pm$ 3.73	17.4 – 29.8	15
Pyridoxine (ppm) ^b	9.21 $\pm$ 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 $\pm$ 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 $\pm$ 0.12	0.225 – 0.704	15
Vitamin B ₁₂ (ppb)	60.5 $\pm$ 46.5	18.3 – 174.0	15
Choline (ppm) ^c	3,064 $\pm$ 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.972 $\pm$ 0.051	0.884 – 1.080	25
Phosphorus (%)	0.570 $\pm$ 0.030	0.515 – 0.623	25
Potassium (%)	0.665 $\pm$ 0.023	0.626 – 0.694	15
Chloride (%)	0.376 $\pm$ 0.041	0.300 – 0.474	15
Sodium (%)	0.191 $\pm$ 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 $\pm$ 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 $\pm$ 0.029	0.116 – 0.209	15
Iron (ppm)	182 $\pm$ 46.7	135 – 311	15
Manganese (ppm)	54.1 $\pm$ 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 $\pm$ 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 $\pm$ 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 $\pm$ 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 $\pm$ 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 $\pm$ 0.074	0.20 – 0.47	14

^a From formulation

^b As hydrochloride

^c As chloride

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

	Mean $\pm$ Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 $\pm$ 0.061	0.15 – 0.39	25
Cadmium (ppm)	0.51 $\pm$ 0.010	0.04 – 0.09	25
Lead (ppm)	0.09 $\pm$ 0.018	0.06 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.28 $\pm$ 0.100	0.18 – 0.49	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm) ^c	13.8 $\pm$ 5.77	4.76 – 24.4	25
Nitrite nitrogen (ppm) ^c	<2.1		25
BHA (ppm) ^d	<1.0		25
BHT (ppm) ^d	<1.0		25
Aerobic plate count (CFU/g)	10	10 – 10	25
Coliform (MPN/g)	3.0	3.0 – 3.0	25
<i>Escherichia coli</i> (MPN/g)	<10		25
<i>Salmonella</i> (MPN/g)	Negative		25
Total nitrosoamines (ppb) ^e	5.3 $\pm$ 1.64	3.1 – 9.9	25
<i>N</i> -Nitrosodimethylamine (ppb) ^e	3.0 $\pm$ 1.31	1.3 – 6.3	25
<i>N</i> -Nitrosopyrrolidine (ppb) ^e	2.3 $\pm$ 0.76	1.1 – 4.1	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	0.098 $\pm$ 0.111	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.247 $\pm$ 0.254	0.020 – 0.997	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	8.86	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 of six samples with measurable concentrations; blanks indicate concentrations below the limit of detection in all samples

APPENDIX E

SENTINEL ANIMAL PROGRAM

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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, 12, and 18 months; and five 4,600 µg/kg female rats 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/sialodacryodentitis virus)	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
<i>Mycoplasma arthritidis</i>	Study termination
Parvovirus	1, 6, 12, and 18 months, study termination

RESULTS

All serology tests were negative.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model for PCB 118 was developed in support of the dioxin toxic equivalency factor (TEF) studies. The model is based on a PBPK model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). 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 chemicals (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 in 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, 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 models 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, and tissue volumes). 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 the 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. These processes for TCDD and related DLCs such as PCB 118 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). A model for PCB 118 was built from a model initially designed for TCDD and modified to handle other DLCs that are ligands of the AhR. The current PBPK model describes the pharmacokinetics of PCB 118 with a series of mass-balance differential equations in which the state variables represent the concentration of PCB 118 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.

In the current model, PCB 118 can bind to the AhR and cytochrome P450 1A2 (CYP1A2). Data for tissue concentrations, 7-ethoxyresorufin-*O*-deethylase (EROD) activity, and acetanilide-4-hydroxylase (A4H) activity in female Sprague-Dawley rats chronically dosed with PCB 118 were available to aid the model development (NTP, 2006d). These data were used to estimate model parameters that should be different for TCDD and PCB 118 (Tables F1 and F2). With the estimated parameters, the model fits the data across the dose range. The key result is that the model fits the fat and liver data when there is almost no binding of PCB 118 to the AhR or CYP1A2.

MODEL DEVELOPMENT

The same basic model structure was used for all compounds studied in the dioxin TEF evaluation, with some of the model parameters unique to each compound, such as those parameters involved in metabolism or binding to the AhR. 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); these models have gone through rounds of refinement and include increasing levels of biological complexity. A thorough summary of PBPK modeling for TCDD, including the basic model used in the current 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 amounts of the 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 the AhR. A key to the model is that the induction rates for 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. Since 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. 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 the PBPK model used for chemicals studied under the dioxin TEF evaluation including addition of a lung compartment, converting the body weight function, functional links between 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 includes lung tissue concentrations. The lung compartment is diffusion limited and includes the same equations used in the liver for the 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 (fat, liver, lung, and blood) data.

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

The TCDD model of Kohn *et al.* (2001) was used to develop the functional relationships linking CYP1A1 to EROD activity and CYP1A2 to A4H activity. The model was run for the TEF study TCDD doses (0, 3, 10, 22, 46, or 100 ng/kg per day) to obtain model-predicted liver content of CYP1A1 and CYP1A2 at 14, 31, and 53 weeks for each dose. EROD data was fit as a combination of two Hill functions. The first Hill function was for the sharp

change from baseline levels of EROD to induced levels of EROD while the second Hill function was for the increase in EROD activity once induction from baseline had happened. Model-predicted A4H activity was fit as a linear function of CYP1A2.

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

$$PC_{PCB\ 118} = PC_{TCDD} \bullet \frac{\log P_{PCB\ 118}}{\log P_{TCDD}}$$

While many model parameters might be different for each DLC, the procedure was to start with a small set of the most likely parameters and estimate the small set of parameters by fitting the model predictions to the data. The parameters for binding to the AhR, CYP1A2, and blood protein were the first group. The relative binding affinity to the AhR in rats (Safe, 1990) was used to scale the AhR binding of PCB 118 from the TCDD value. The relative affinity for PCB 118 was 0.0011 resulting in an AhR dissociation constant in the model of 245 as compared to 0.27 for TCDD (Table F1). There was no information on the relative binding to CYP1A2, so this parameter was the first parameter assumed to be different for PCB 118 in the model. 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 (fat, liver, lung, blood). Two parameters describing hepatotoxicity, k_{lysis} and $k_{recovery}$, were included in the optimizations because they were multipliers of 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 DLCs.

The presence of a rat liver cytosolic protein different from the AhR and CYP1A2 that binds PCBs but not dioxin (Buff and Bründl, 1992; Bründl and Buff, 1993) is one potentially important difference for the modeling of PCBs and TCDD that is not included in Kohn's TCDD model. While little is known about this PCB-specific 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

The Kohn model could not be fit to the PCB 118 data without adding a PCB-specific binding protein to the liver (results not shown). As many as 11 parameters were allowed to differ from the TCDD values but no combination could be identified that matched both the tissue disposition data and the EROD and A4H activity data. The addition of the PCB binding protein added four parameters to the model to describe the synthesis, degradation, and dissociation of the PCB binding protein. Seven parameters from Kohn's model were fit for the PCB 118 model. All other model parameters were the same as those in the TCDD model. The estimate of the dissociation constant for CYP1A2 is much higher for PCB 118 than for TCDD (Table F1). Simulations show that nearly all of the PCB 118 is bound to the PCB protein. However, even with the PCB binding protein accounting for nearly all of the PCB 118 in the liver, there is still enough PCB 118 binding to the AhR or CYP1A2 to detect the induction of the P450s as measured by EROD and A4H activities. The model captures the complex dynamics in the liver, accurately predicting liver enzyme activities and liver tissue concentrations across all doses (Figures F1 to F9). A model without the PCB binding protein always failed in one of two ways, either fitting the tissue concentration and

over-predicting the P450 activity data or drastically under-predicting the tissue concentration and fitting the activity data. While the model predictions for fat are slightly low, the model captures the important dynamics in the liver for DLCs. There is a general trend in the data for fat and liver concentrations to be higher at week 104 than at week 53. The changing body weights in the model do not account for this change and the model does not match the data. It is possible that a time-dependent partition coefficient could be used to represent changes in the liver over time. Another option is that synthesis of the PCB binding protein could increase during the second year. Either of these options could produce improved model predictions and both will be considered at a later time.

The PBPK model was used to calculate the liver AUC for each dose group (Table F3). PCB 118 in the model liver may be free, or bound to CYP1A2, the AhR, or the PCB binding protein. In all runs, PCB 118 is predicted to be bound to the PCB binding protein.

TABLE F1
Model Parameters for TCDD and PCB 118^a

Parameter	TCDD	PCB 118	Unit
<i>Background^b</i>	0.082	15.15	ng/kg per day
<i>K_{AhR}^c</i>	0.27	245	nM
<i>K_{CYP1A2}</i>	30	2,640	nM
<i>V_{metabolism}</i>	9.12	1×10^{-8}	nmole/L per day
<i>k_{absorption}</i>	4.8	56.4	kg ^{0.75} /day
<i>k_{lysis}</i>	200	1.5×10^{-5}	/day
<i>critical_{accumulation}</i>	0.6	0.3	nmole
<i>k_{recovery}</i>	0.01	1×10^{-7}	/day
<i>critical_{concentration}</i>	2	9.0×10^{-8}	nM
<i>k_{PCBprotChem_{degradation}}</i>	—	1×10^{-10}	/day
<i>liverInitPCBprot_{Chem}</i>	—	1.3	nmole
<i>K_{PCBprotein}</i>	—	806	nM
<i>PCBbinding_{synthesis}</i>	—	43.3	nmole/day

^a From optimization unless otherwise indicated. Values for TCDD are taken from Kohn et al. (2001).

^b Measured in NTP rodent diet

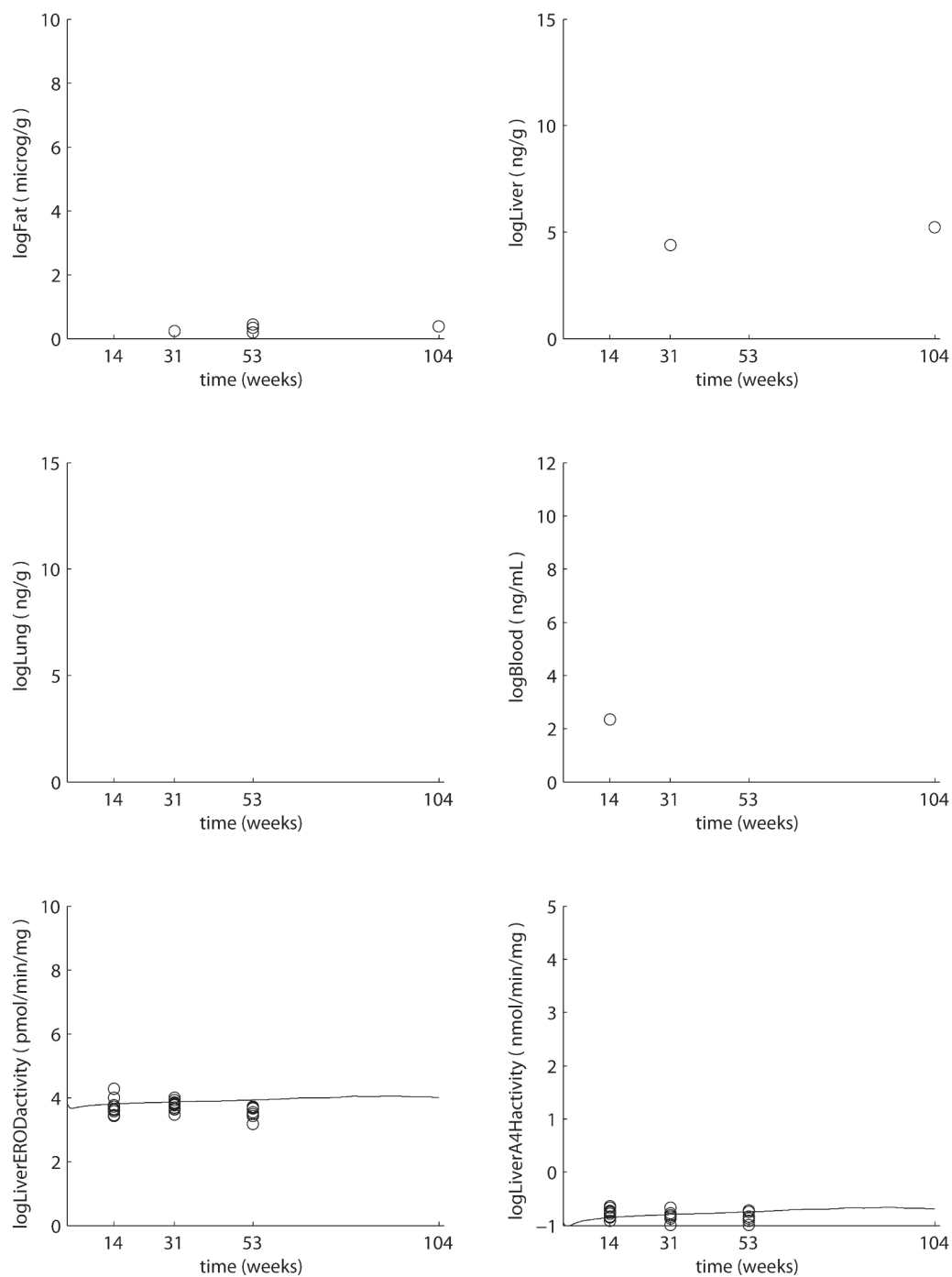
^c Calculated using relative binding affinity.

TABLE F2
Partition Coefficients for TCDD and PCB 118^a

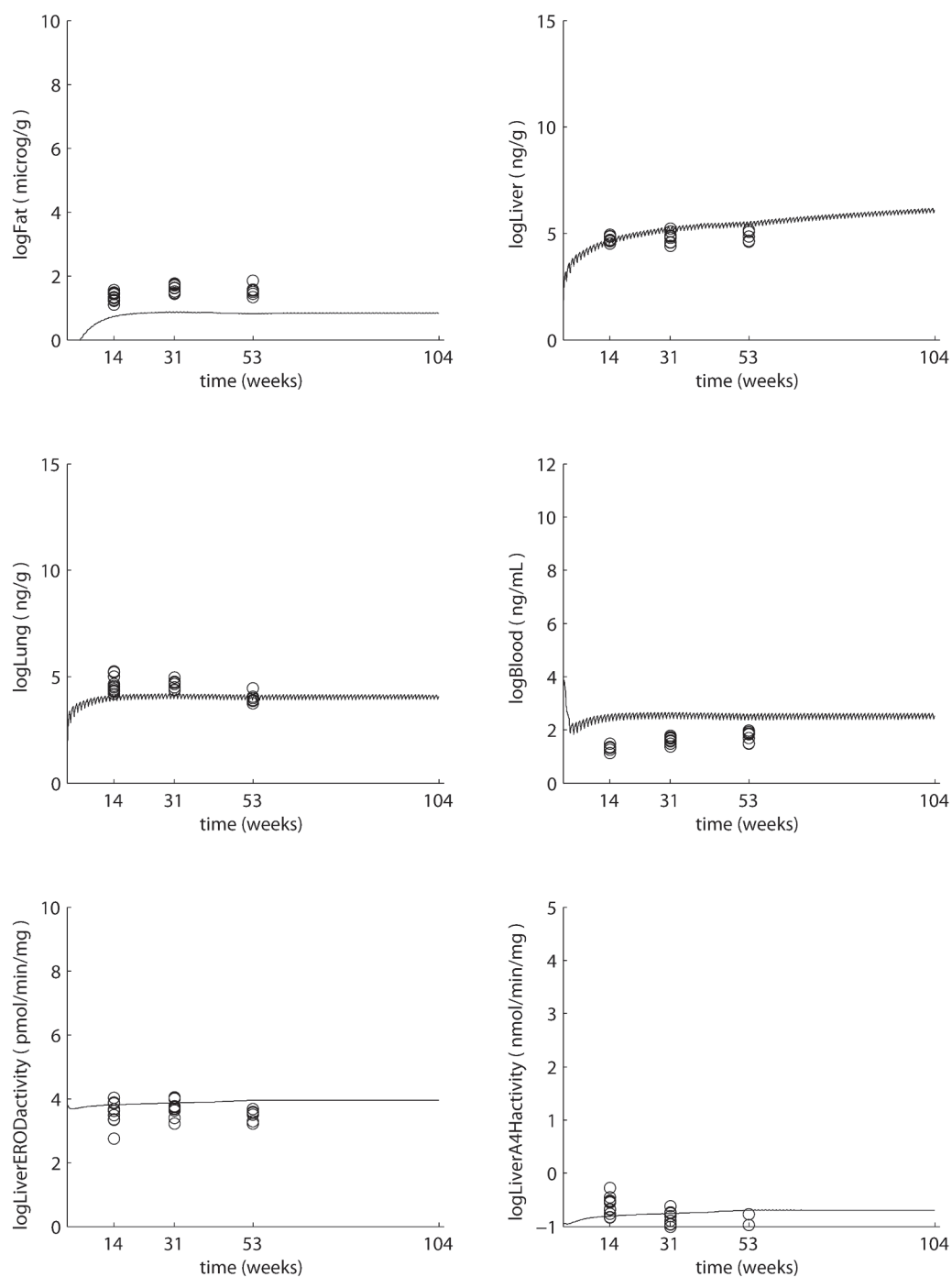
	TCDD	PCB 118
Fat	187.0	188.62
Muscle	4.48	4.52
Viscera	3.35	3.38
Liver	4.60	4.64
Kidney	3.35	3.38
Gastrointestinal tract	3.35	3.38
Lung	4.57 ^b	4.64 ^b

^a Values for TCDD are taken from Kohn et al. (2001).

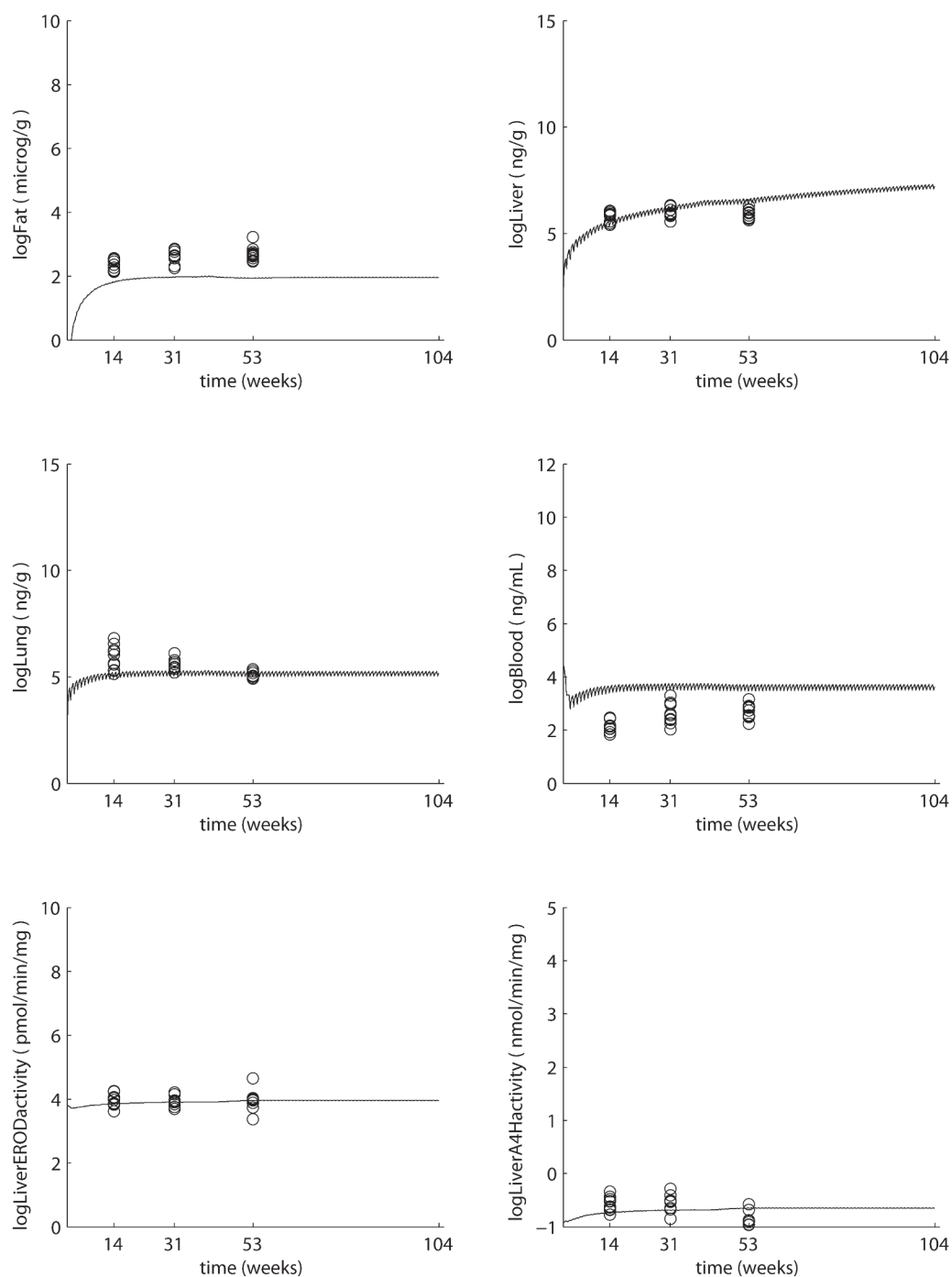
^b Estimated by optimization

**FIGURE F1**

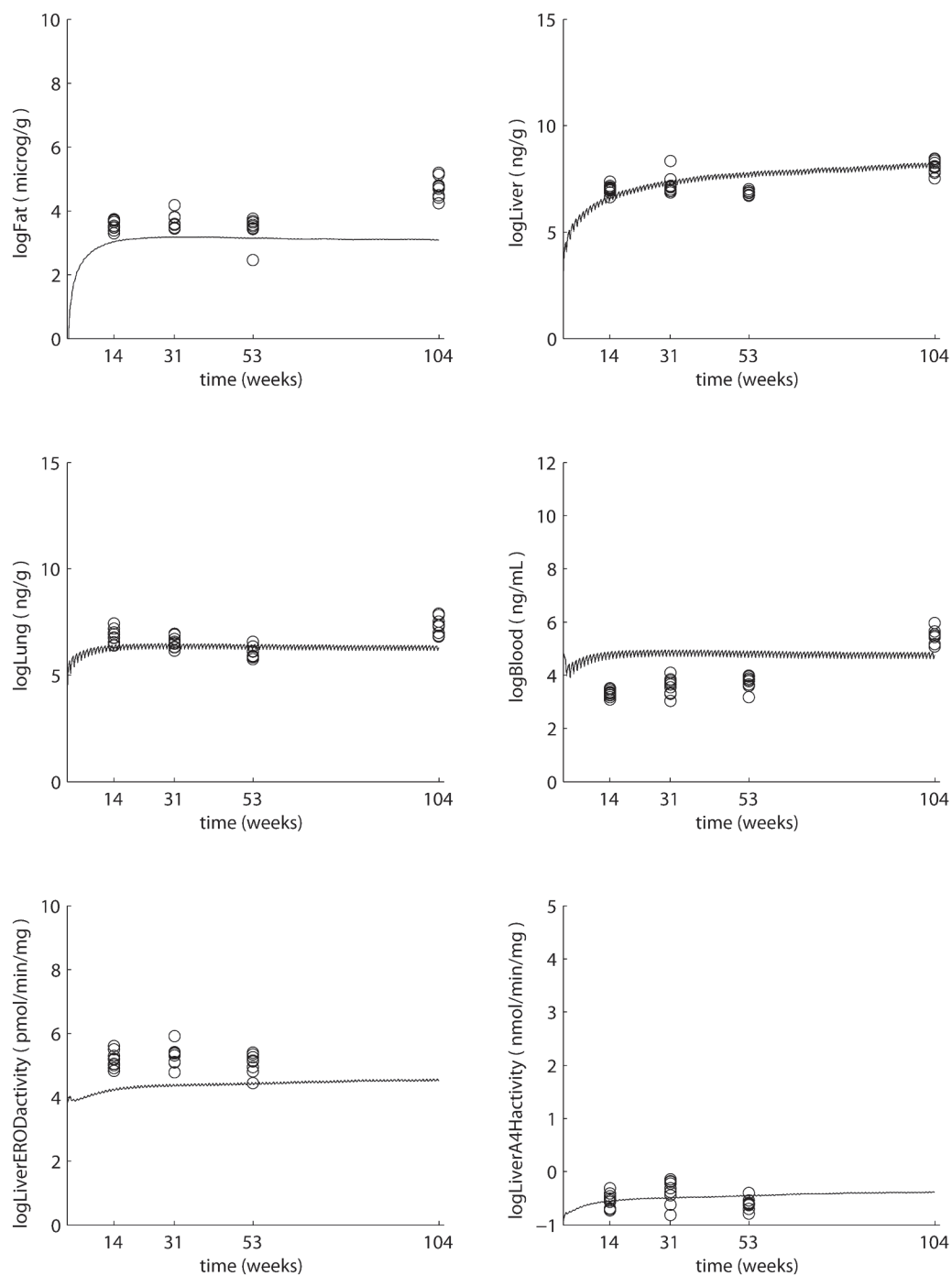
Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the Vehicle Control Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F2**

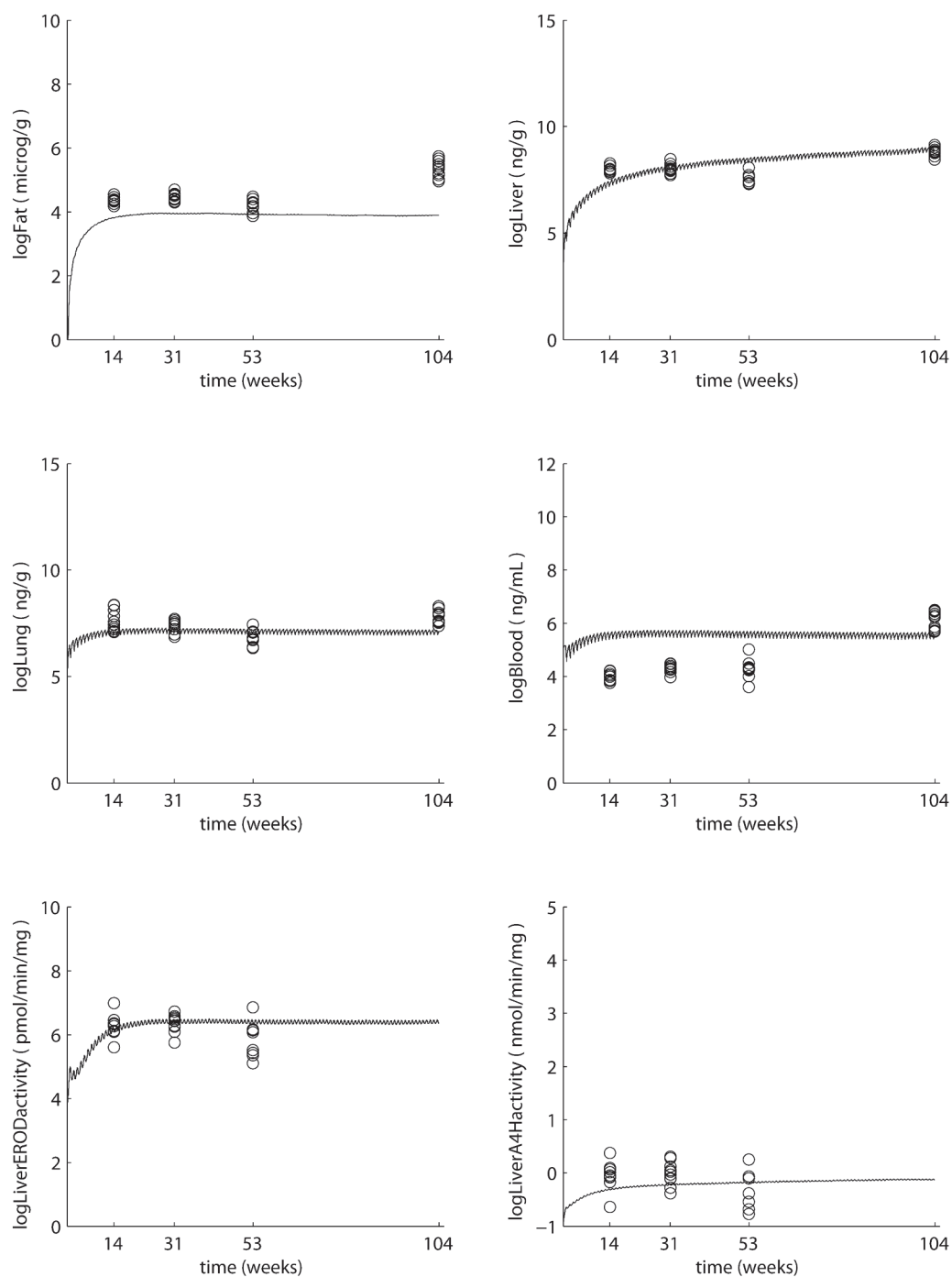
Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 10 µg/kg Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F3**

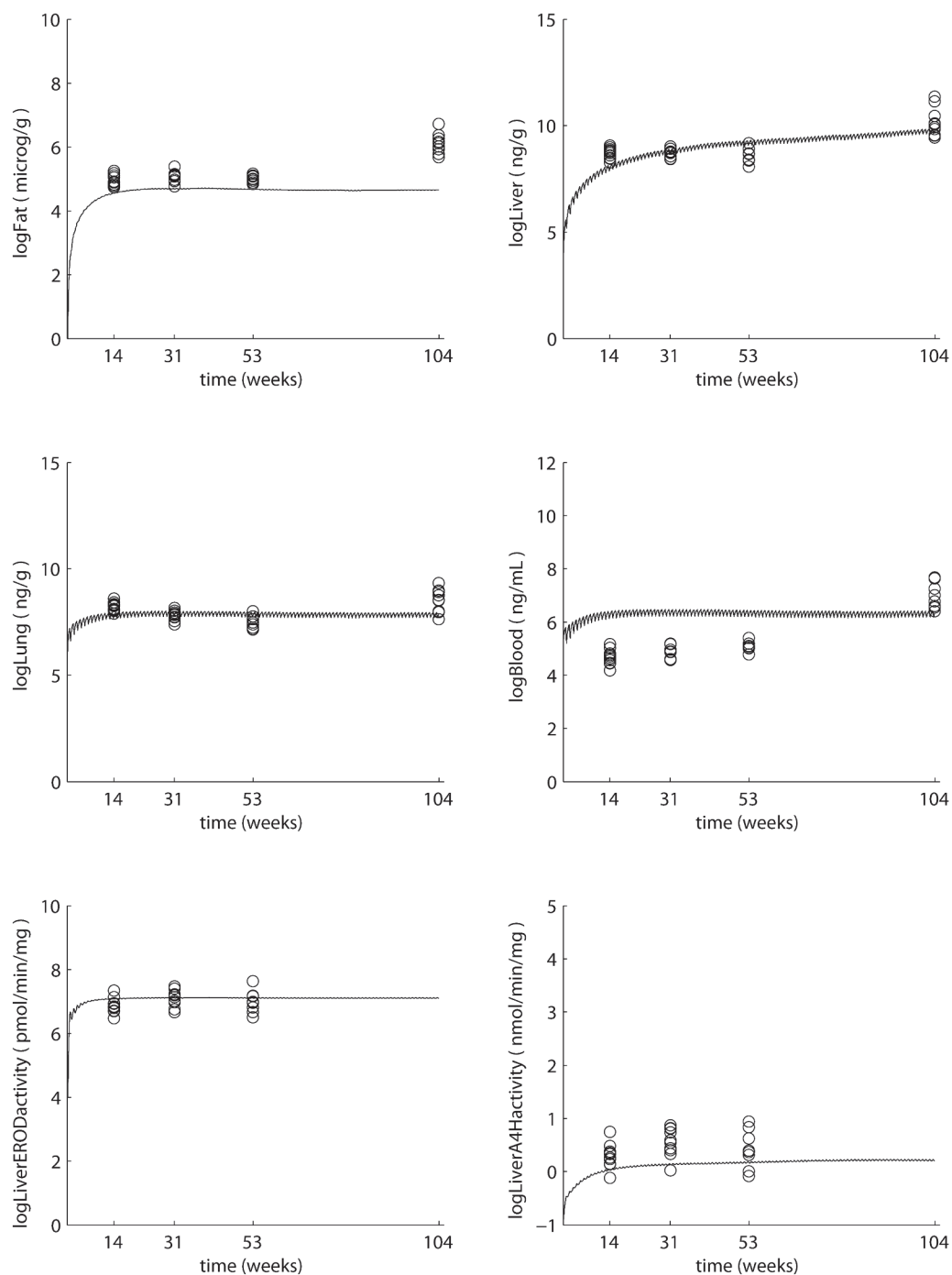
Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 30 µg/kg Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F4**

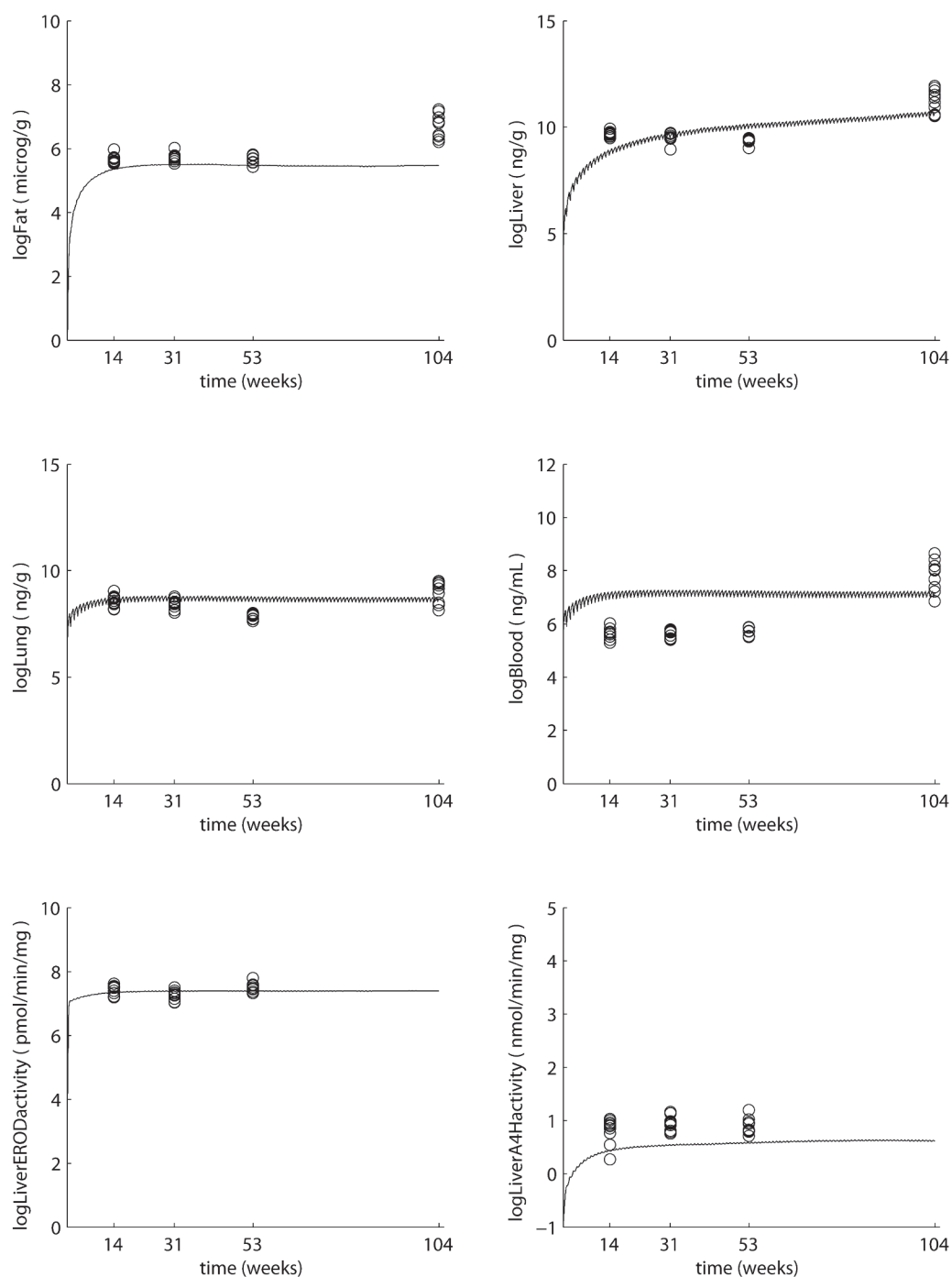
Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 100 µg/kg Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-O-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F5**

Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 220 µg/kg Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F6**

Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 460 µg/kg Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F7**

Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 1,000 µg/kg Group in the 2-Year Gavage Study of PCB 118
EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

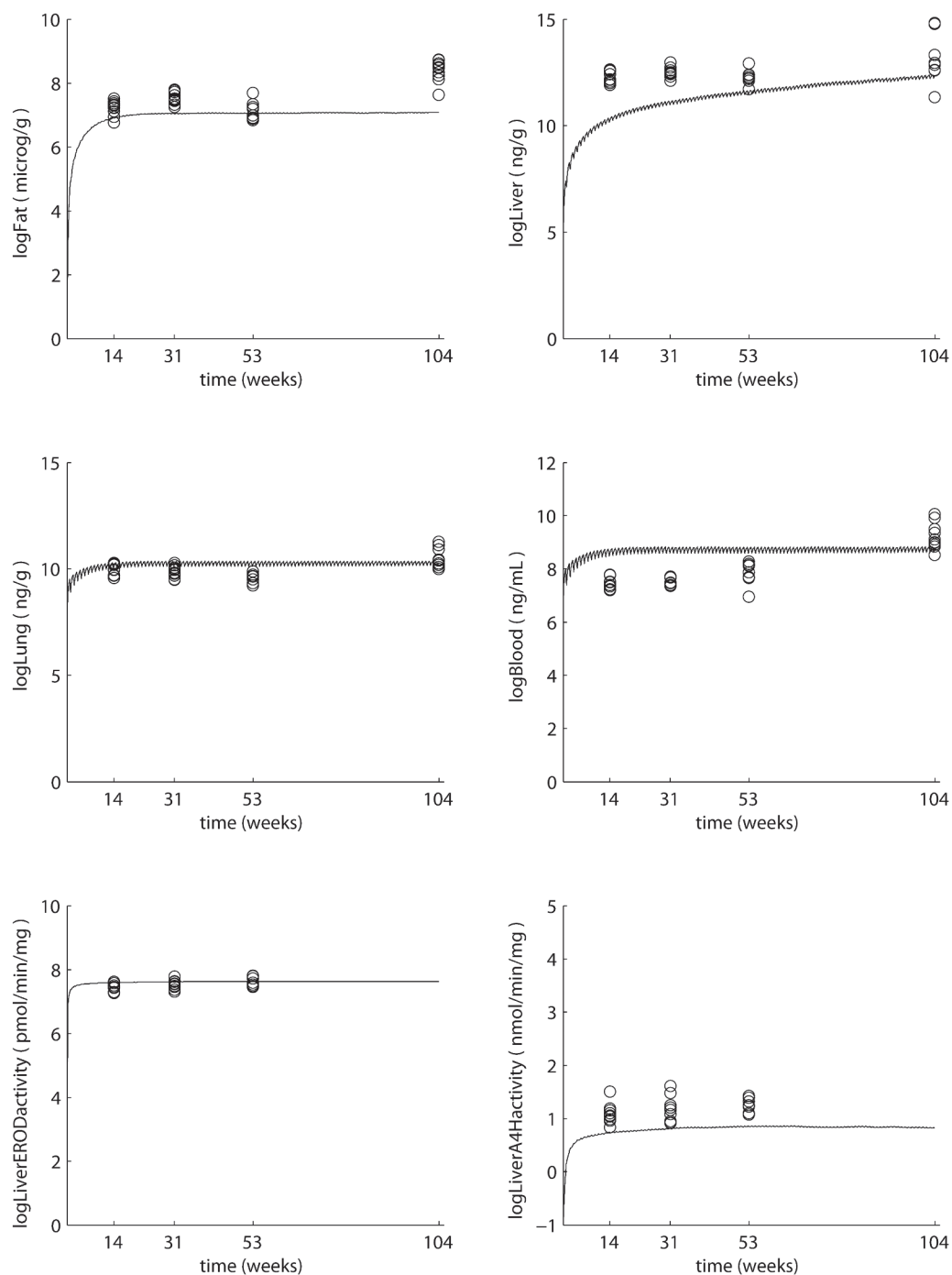
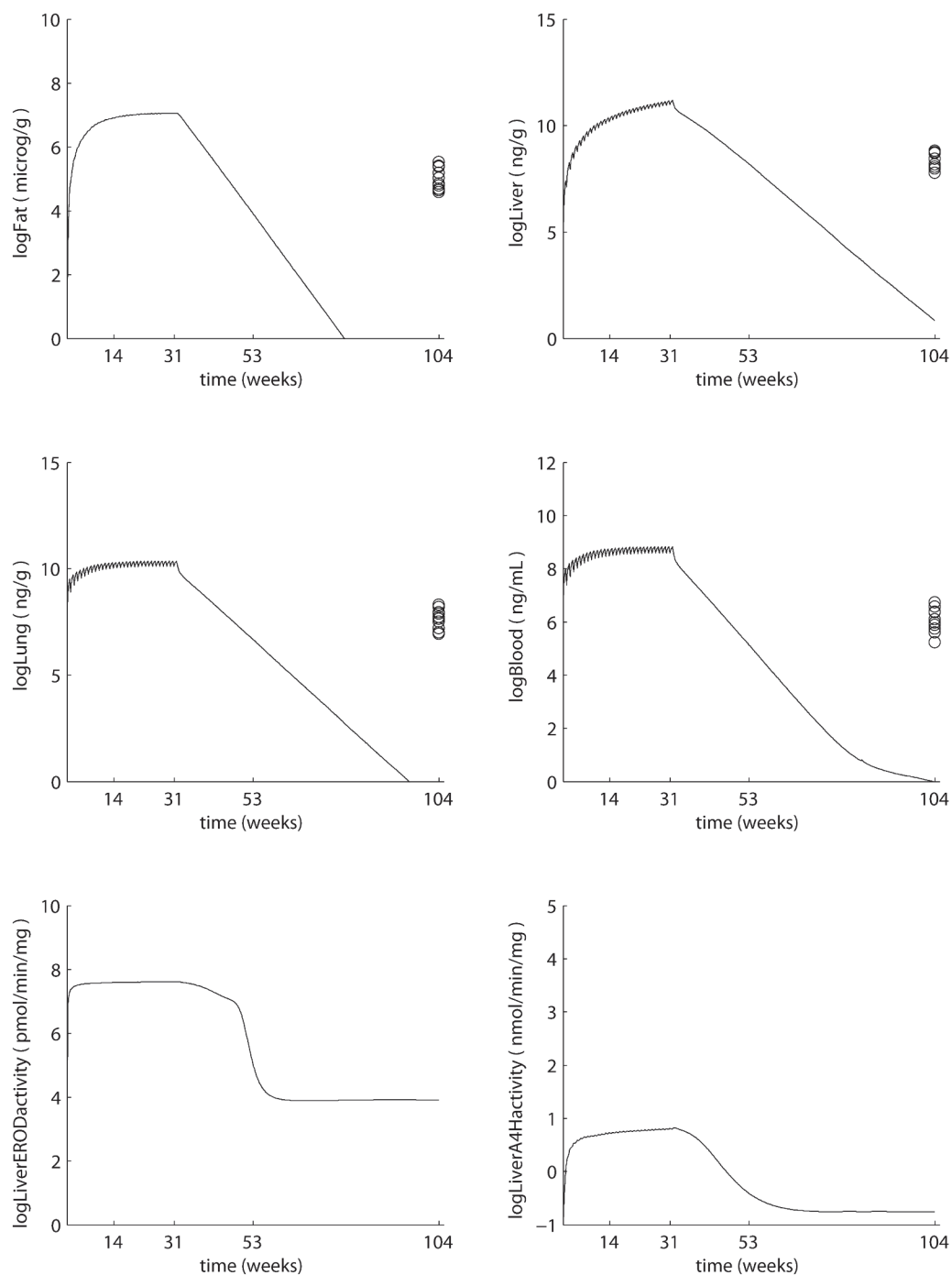


FIGURE F8

Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 4,600 µg/kg Group in the 2-Year Gavage Study of PCB 118
 EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

**FIGURE F9**

Model Predictions (—) and Observed Data (○) for Tissue PCB 118 Concentrations and Liver Enzyme Activities for the 4,600 µg/kg Stop-Exposure Group in the 2-Year Gavage Study of PCB 118
 EROD=7-ethoxyresorufin-*O*-deethylase; A4H=acetanilide-4-hydroxylase

TABLE F3
Liver Area Under the Curve (AUC) for PCB 118 in the 2-Year Gavage Study of PCB 118

Dose	AUC (days • ng/g)
Vehicle control	0.0
10 µg/kg	1.8×10^5
30 µg/kg	5.3×10^5
100 µg/kg	1.5×10^6
220 µg/kg	3.2×10^6
460 µg/kg	6.9×10^6
1,000 µg/kg	1.6×10^7
4,600 µg/kg	8.0×10^7
4,600 µg/kg (stop-exposure)	1.5×10^7

APPENDIX G

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.

Publications based on analyses of data from the dioxin TEF initiative

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 (PCB 126). *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.

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.

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.D., 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.

Sells, D.M., Brix, A.E., Nyska, A., Jokinen, M.P., Orzech, D.P., and Walker, N.J. (2007). Respiratory tract lesions in noninhalation studies. *Toxicol. Pathol.* **35**, 170-177.

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.

Toyoshiba, H., Yamanaka, T., Sone, H., Parham, F.M., Walker, N.J., Martinez, J., and Portier, C.J. (2004). Gene interaction network suggests dioxin induces a significant linkage between aryl hydrocarbon receptor and retinoic acid receptor beta. *Environ. Health Perspect.* **112**, 1217-1224.

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.

Walker, N.J., Wyde, M.E., Fischer, L.J., Nyska, A., and Bucher, J.R. (2006). Comparison of chronic toxicity and carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in two-year bioassays in female Sprague-Dawley rats. *Mol. Nutr. Food Res.* **50**, 934-944.

Walker, N.J., Yoshizawa, K., Miller, R.A., Brix, A.E., Sells, D.M., Jokinen, M.P., Wyde, M.E., Easterling, M., and Nyska, A. (2007). Pulmonary lesions in female Harlan Sprague-Dawley rats following two-year oral treatment with dioxin-like compounds. *Toxicol. Pathol.* **35**, 880-889.

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.

Yoshizawa, K., Heatherly, A., Malarkey, D.E., Walker, N.J., and Nyska, A. (2007). A critical comparison of murine pathology and epidemiological data of TCDD, PCB126, and PeCDF. *Toxicol. Pathol.* **35**, 865-879.

Publications based on analyses using tissues from "special study" animals from the dioxin TEF initiative studies.

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.

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.

Ovando, B.J., Vezina, C.M., McGarrigle, B.P., and Olson, J.R. (2006). Hepatic gene downregulation following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Sci.* **94**, 428-438.

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.



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