NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF A BINARY MIXTURE OF

3,3′,4,4′,5-PENTACHLOROBIPHENYL (PCB 126)

(CAS NO. 57465-28-8)

AND

2,2′,4,4′,5,5′-HEXACHLOROBIPHENYL (PCB 153)

(CAS NO. 35065-27-1)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)

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

August 2006

NTP TR 530

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

FOREWORD

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

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

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SUMMARY

Background

3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) are members 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. Contaminated sites usually contain many different varieties of these dioxin-like compounds. The National Toxicology Program conducted a series of studies to try to gauge the relative toxicity of the more common of these compounds, both alone and in mixtures. This study evaluated the effects of mixtures of PCB 126 and PCB 153 on female rats.

Methods

We exposed groups of 53 female rats by depositing mixtures of PCB 126 and PCB 153 dissolved in corn oil through a tube directly into their stomachs five days a week for two years. Daily doses were 10, 100, 300, or 1,000 nanograms of PCB 126, each with 1,000 times as much PCB 153, per kilogram body weight. Animals receiving the corn oil alone served as the control group. Tissues from more than 40 sites were examined for every animal.

Results

Female rats exposed to the mixtures of PCB 126 and PCB 153 developed a variety of diseases in several organs, including cancers of the liver, lung, mouth, and pancreas. A variety of other toxic lesions observed in exposed animals included hypertrophy, hyperplasia, fibrosis, and necrosis of the liver, hyperplasia of the oral mucosa, metaplasia of the lung, vacuolization and atrophy of the pancreas, kidney nephropathy, atrophy and hyperplasia of the adrenal cortex, atrophy of the thymus, hyperplasia of the nose, and hyperplasia of the forestomach.

Conclusions

We conclude that the mixtures of PCB 126 and PCB 153 caused cancer and other toxic effects at several sites in female rats.

ABSTRACT

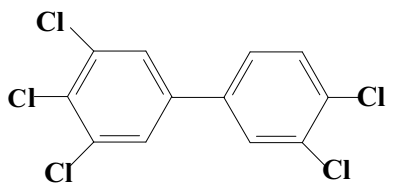
Dioxin Toxic Equivalency Factor Evaluation Overview

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

Since human exposure to DLCs always occurs as a complex mixture, the toxic equivalency factor (TEF) methodology has been developed as a mathematical tool to assess the health risk posed by complex mixtures of these compounds. The TEF methodology is a relative potency scheme that ranks the dioxin-like activity of a compound relative to TCDD, 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.

PCBs, including 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), 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). PCBs were selected for study by the National Toxicology Program as a part of the dioxin TEF evaluation to assess the cancer risk posed by complex mixtures of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). The dioxin TEF evaluation includes conducting multiple 2-year rat bioassays to evaluate the relative chronic toxicity and carcinogenicity of dioxin-like compounds, structurally related PCBs, and mixtures of these compounds. Female Harlan Sprague-Dawley rats were administered a binary mixture of PCB 126 and PCB 153 (at least 99% pure) in corn oil:acetone (99:1) by gavage for 14, 31, or 53 weeks or 2 years. While one of the aims of this study was a comparative analysis of effects seen with PCB 126 and the mixture of PCB 126 and PCB 153, in this Technical Report only the results of the present study of PCB 126 and PCB 153 are presented and discussed.

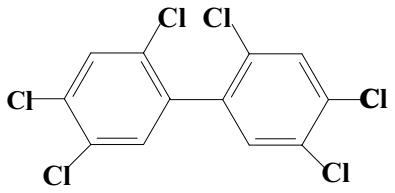


3,3′,4,4′,5-Pentachlorobiphenyl
PCB 126

CAS No. 57465-28-8

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

Synonyms: 1,1′-Biphenyl, 3,3′,4,4′,5-pentachloro-(9CI)



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

Synonyms: 1,1′-Biphenyl, 2,2′,4,4′,5,5′-hexachloro-(9CI)

2-Year Study

The 2-year study of a binary mixture of PCB 126 and PCB 153 was designed to assess the carcinogenicity of a constant ratio mixture of PCB 126 and PCB 153. In addition, varying ratio mixture groups were used to assess the impact of increasing PCB 153 on the carcinogenicity of PCB 126. Dose groups were divided into two study arms (Figure 1). TCDD equivalent (TEQ) doses are based on the PCB 126 doses after adjustment for the PCB 126 TEF of 0.1.

Groups of 81 (Groups 2, 3, 5, and 7) or 80 (Groups 4 and 6) female rats received a mixture of PCB 126 and PCB 153 in corn oil:acetone (99:1) by gavage 5 days per week for up to 105 weeks; a group of 81 female rats received the corn oil:acetone (99:1) vehicle only and served as the vehicle control (Group 1). Up to 10 rats per group were evaluated at 14, 31, and 53 weeks.

Survival of all dosed groups was similar to that of the vehicle controls. The mean body weights of Groups 4 and 5 were generally less than those of the vehicle controls after week 25. The mean body weights of Group 6 were less after week 12, and those of Group 7 were less after week 8.

Constant ratio mixture groups:

Group 1: Vehicle Control
Group 2: 10 ng/kg PCB 126 plus 10 µg/kg PCB 153 (1 ng TEQ/kg)
Group 3: 100 ng/kg PCB 126 plus 100 µg/kg PCB 153 (10 ng TEQ/kg)
Group 5: 300 ng/kg PCB 126 plus 300 µg/kg PCB 153 (30 ng TEQ/kg)
Group 7: 1,000 ng/kg PCB 126 plus 1,000 µg/kg PCB 153 (100 ng TEQ/kg)

Varying ratio mixture groups:

Group 4: 300 ng/kg PCB 126 plus 100 µg/kg PCB 153 (30 ng TEQ/kg)
Group 5: 300 ng/kg PCB 126 plus 300 µg/kg PCB 153 (30 ng TEQ/kg)
Group 6: 300 ng/kg PCB 126 plus 3,000 µg/kg PCB 153 (30 ng TEQ/kg)

Figure 1
Study Arms and Dose Groups in the 2-Year Gavage Study of the Binary Mixture of PCB 153 and PCB 126 [TCDD equivalent (TEQ) doses are shown in parentheses]

Thyroid Hormone Concentrations

Alterations in serum thyroid hormone levels were evaluated at the 14-, 31-, and 53-week interim evaluations. In the constant ratio groups, serum total thyroxine (T_4) and free T_4 generally showed a treatment-related decrease relative to controls. Serum total triiodothyronine (T_3) exhibited a treatment-related increase at the 14-, 31-, and 53-week interim evaluations, but serum thyroid stimulating hormone (TSH) levels were increased at the 14-week time point only. In the varying ratio groups, the decrease in total and free T_4 was more pronounced in those groups dosed with the increasing proportion of PCB 153 at the 31- and 53-week time points.

Hepatic Cell Proliferation Data

To evaluate hepatocyte replication, analysis of labeling of replicating hepatocytes with 5-bromo-2′-deoxyuridine was conducted at the 14-, 31-, and 53-week interim evaluations. At 31 and 53 weeks, a significant increase in the hepatocellular labeling index occurred in Group 7. In the varying ratio groups, the labeling index at the 53-week interim time point was significantly higher in Group 6, which had the highest proportion of PCB 153 compared to the other varying ratio groups.

Cytochrome P450 Enzyme Activities

To evaluate the expression of known PCB 126-responsive genes, CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) and CYP1A2-associated acetanilide-4-hydroxylase (A4H) activities were evaluated at the 14-, 31-, and 53-week interim evaluations. In addition, PCB 153-inducible CYP2B-associated 7-pentoxyresorufin-*O*-dealkylase (PROD) activity was analyzed. In the constant ratio Groups 2, 3, 5, and 7, hepatic and pulmonary EROD (CYP1A1) activities, hepatic A4H (CYP1A2) activities, and hepatic PROD (CYP2B) activities were significantly greater in all dosed groups compared to the vehicle controls at weeks 14, 31, and 53. In the varying ratio groups, hepatic EROD, A4H, and PROD activities at 14 weeks were higher in groups receiving a greater proportion of PCB 153 in the PCB mixture. At 31 and 53 weeks, hepatic CYP1A1 and CYP1A2 enzyme activities in Group 6 were generally lower than in Groups 4 and 5.

Determinations of PCB 126 and PCB 153 Concentrations in Tissues

Concentrations of PCB 126 and PCB 153 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). PCB 126 was not detectable in vehicle control animals, but increased with increasing dose of PCB 126 and duration of exposure; the highest concentrations were found in liver and fat, and lower levels were seen in lung and blood. Increasing the proportion of PCB 153 in the mixture relative to PCB 126 led to a general decrease in the amount of PCB 126 in liver and lung at the later time points, whereas in fat and blood, there was generally either no effect of PCB 153 on the disposition of PCB 126, or there was an increase in the amount of PCB 126 in the tissue. In vehicle control animals, PCB 153 was detectable in the fat at all time points, in the lung at all time points except 53 weeks, and in the liver and blood at 2 years. PCB 153 was measurable in all examined tissues of treated animals, with the highest concentrations found in fat at the end of the 2-year study in groups administered the highest doses of PCB 153.

Pathology and Statistical Analyses

Constant Ratio Mixture of PCB 126 and PCB 153

At 14, 31, and 53 weeks, the absolute and relative liver weights of all dosed groups were generally greater than those of the vehicle controls.

Exposure to the PCB mixture led to significant toxicity in the liver. At 14 weeks, the incidences of several nonneoplastic liver lesions were increased compared to the vehicle controls including hepatocyte hypertrophy, pigmentation, multinucleated hepatocytes, and diffuse fatty change. The spectrum and severity of effects increased with dose and duration of exposure. At the end of the 2-year study, there were significantly increased incidences and severities of toxic hepatopathy characterized by hepatocyte hypertrophy, multinucleated hepatocytes, pigmentation, diffuse and focal fatty change, eosinophilic focus, nodular hyperplasia, cholangiofibrosis, oval cell hyperplasia, bile duct cysts, bile duct hyperplasia, necrosis, and portal fibrosis.

Significantly increased incidences of hepatocellular adenoma, cholangiocarcinoma, and hepatocholangioma were observed in the study. In addition, two animals in the highest dose group had hepatocellular carcinoma. The incidences of these lesions generally exceeded the historical vehicle control ranges.

At 2 years, a significantly increased incidence of cystic keratinizing epithelioma of the lung was observed in

Group 7. In addition, single occurrences of squamous cell carcinoma were seen in the top two dose groups. Nonneoplastic effects whose incidences were increased in the lung included bronchiolar metaplasia of the alveolar epithelium and squamous metaplasia.

Significantly increased incidences of squamous cell carcinoma (gingival) of the oral mucosa were seen at the end of the 2-year study and were accompanied by increased incidences of gingival squamous hyperplasia.

In the pancreas at 53 weeks, the incidence of acinar cytoplasmic vacuolization was significantly increased in the highest dose group. At 2 years, increased incidences of acinar atrophy and acinar cytoplasmic vacuolization were seen in addition to pancreatic acinar neoplasms in dosed groups. In Groups 5 and 7, these incidences exceeded the historical vehicle control ranges.

In the uterus at 2 years, there was a marginal increase in the incidence of squamous cell carcinoma in Group 5.

Numerous nonneoplastic effects were seen in other organs at the interim time points including atrophy of the thymus and follicular cell hypertrophy of the thyroid gland. These responses were also affected by administration of the mixture of PCB 126 and PCB 153 at the end of the 2-year study and were accompanied by additional nonneoplastic responses in numerous organs including atrophy of the adrenal cortex and cortical hyperplasia, severity of nephropathy, and incidences of pigmentation of the kidney. Other nonneoplastic lesions that were treatment related were forestomach hyperplasia, hyperplasia of the nasal respiratory epithelium, metaplasia of the olfactory epithelium, and ectasia of the mandibular lymph node.

Varying Ratio Mixture of PCB 126 and PCB 153

An effect of increasing the proportion of PCB 153 in the PCB mixture was seen in several tissues, most notably in the liver. Treatment-related nonneoplastic effects seen across the varying ratio groups were generally the same as those seen in the constant ratio groups. In general there was a positive effect of PCB 153 in the mixture on the incidences and severities of these lesions with higher incidences and higher severities being seen in Group 6, which had the highest proportion of PCB 153. A significant positive effect of increasing the proportion of PCB 153 in the PCB mixture was seen for hepatocyte hypertrophy, cholangiofibrosis, eosinophilic focus, clear cell focus, basophilic focus, diffuse and focal fatty change, bile duct hyperplasia, and hematopoietic cell proliferation. In contrast, the incidences of pigmentation decreased with increasing proportions of PCB 153.

At 2 years, there was a significant positive effect of increasing PCB 153 in the mixture on the incidences of hepatocellular adenoma and cholangiocarcinoma. In addition, hepatocholangiomas were observed only in Groups 5 and 6.

A significant effect of increasing the proportion of PCB 153 in the PCB mixture was also seen for nonneoplastic lesions in the lung (bronchiolar metaplasia of alveolar epithelium), pancreas (acinar cytoplasmic vacuolization), thyroid gland (follicular cell hypertrophy) and kidney (pigmentation and pelvic inflammation of the kidney).

Conclusions

Under the conditions of this 2-year gavage study 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. Increased incidences of pancreatic acinar neoplasms were also considered to be related to administration of the binary mixture of PCB 126 and PCB 153. The 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 caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, pancreas, adrenal cortex, thyroid gland, thymus, kidney, nose, and forestomach.

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

Summary of the 2-Year Carcinogenesis Studies of a Binary Mixture of PCB 126 and PCB 153 in Female Sprague-Dawley Rats

Constant Ratio Mixture (Groups 1, 2, 3, 5, and 7)	Varying Ratio Mixture[a] (Groups 4, 5, and 6)
Doses in corn oil/acetone by gavage	
Group 1: Vehicle control; Group 2: 10 ng/kg PCB 126 plus 10 µg/kg PCB 153; Group 3: 100 ng/kg PCB 126 plus 100 µg/kg PCB 153; Group 5: 300 ng/kg PCB 126 plus 300 µg/kg PCB 153; Group 7: 1,000 ng/kg PCB 126 plus 1,000 µg/kg PCB 153	Group 4: 300 ng/kg PCB 126 plus 100 µg/kg PCB 153; Group 5: 300 ng/kg PCB 126 plus 300 µg/kg PCB 153; Group 6: 300 ng/kg PCB 126 plus 3,000 µg/kg PCB 153
Body weights	
Groups 5 and 7 were less than Group 1 (vehicle controls)	Groups 4, 5, and 6 were less than Group 1 (vehicle controls)
Survival rates	
22/53, 21/53, 22/53, 24/53, 24/53	28/50, 24/53, 27/51
Nonneoplastic effects	
Liver: hepatocyte, hypertrophy (1/53, 7/53, 17/52, 33/52, 50/51) hepatocytes, multinucleated (0/53, 0/53, 14/52, 46/52, 48/51) pigmentation (2/53, 5/53, 38/52, 50/52, 50/51) fatty change, diffuse (3/53, 1/53, 9/52, 31/52, 38/51) fatty change, focal (3/53, 4/53, 7/52, 1/52, 12/51) eosinophilic focus (includes multiple) (14/53, 16/53, 30/52, 40/52, 18/51) toxic hepatopathy (0/53, 2/53, 34/52, 48/52, 49/51) bile duct, cyst (4/53, 3/53, 1/52, 5/52, 23/51) bile duct, hyperplasia (8/53, 2/53, 9/52, 29/52, 46/51) necrosis (4/53, 8/53, 5/52, 4/52, 20/51) oval cell, hyperplasia (2/53, 2/53, 15/52, 39/52, 46/51) portal fibrosis (0/53, 0/53, 0/52, 7/52, 34/51) hyperplasia, nodular (0/53, 0/53, 2/52, 24/52, 42/51) cholangiofibrosis (0/53, 1/53, 0/52, 7/52, 39/51)	Liver: hepatocyte, hypertrophy (22/50, 33/52, 47/51) pigmentation (50/50, 50/52, 44/51) fatty change, diffuse (28/50, 31/52, 47/51) fatty change, focal (4/50, 1/52, 11/51) basophilic focus (5/50, 3/52, 18/51) eosinophilic focus (includes multiple) (27/50, 40/52, 45/51) clear cell focus (5/50, 3/52, 11/51) bile duct, hyperplasia (20/50, 29/52, 40/51) hematopoietic cell proliferation (18/50, 19/52, 29/51) cholangiofibrosis (5/50, 7/52, 13/51)
Lung: metaplasia, squamous (0/53, 0/53, 1/52, 2/53, 11/52) alveolar epithelium, metaplasia, bronchiolar (0/53, 6/53, 23/52, 34/53, 32/52)	Lung: alveolar epithelium, metaplasia, bronchiolar (39/50, 34/53, 30/50)
Oral Mucosa: gingival, hyperplasia, squamous (8/12, 8/11, 18/25, 22/30, 24/36)	
Pancreas: acinus, vacuolization cytoplasmic (0/53, 0/53, 0/52, 7/52, 40/50) acinus, atrophy (0/53, 2/53, 1/52, 1/52, 8/50)	Pancreas: acinus, vacuolization cytoplasmic (3/49, 7/52, 44/49)
Adrenal Cortex: atrophy (0/53, 0/53, 0/52, 3/52, 35/51) hyperplasia (11/53, 18/53, 23/52, 25/52, 18/51)	
Thyroid Gland: follicular cell, hypertrophy (14/53, 17/53, 34/51, 35/52, 42/52)	Thyroid Gland: follicular cell, hypertrophy (28/49, 35/52, 44/50)

[a] Effects shown for the varying ratio mixture groups are those data where there was a significant effect of varying ratio on the incidence. Not all effects in Groups 4, 5, and 6 that were related to treatment are shown.

Summary of the 2-Year Carcinogenesis Studies of a Binary Mixture of PCB 126 and PCB 153 in Female Sprague-Dawley Rats

Constant Ratio Mixture (Groups 1, 2, 3, 5, and 7)	Varying Ratio Mixture (Groups 4, 5, and 6)
Nonneoplastic effects (continued)	
Thymus:	
atrophy (33/53, 33/50, 43/48, 42/50, 49/51)	
Kidney:	Kidney:
severity of nephropathy (1.2, 1.0, 1.1, 1.3, 2.2)	pigmentation (2/48, 7/52, 17/51)
pigmentation (0/53, 1/53, 3/52, 7/52, 35/51)	pelvis, inflammation (1/48, 3/52, 8/51)
Nose:	
respiratory epithelium, hyperplasia (10/53, 5/53, 7/53, 11/53, 20/53)	
olfactory epithelium, metaplasia (4/53, 3/53, 5/53, 6/53, 15/53)	
Forestomach:	
hyperplasia, squamous (1/53, 1/53, 2/52, 7/52, 8/51)	
Neoplastic effects	
Liver:	Liver:
cholangiocarcinoma (0/53, 0/53, 1/52, 9/52, 30/51)	cholangiocarcinoma (7/50, 9/52, 25/51)
hepatocholangioma (includes multiple) (0/53, 0/53, 0/52, 2/52, 6/51)	hepatocellular adenoma (2/50, 5/52, 21/51)
hepatocellular adenoma (0/53, 0/53, 3/52, 5/52, 27/51)	
hepatocellular carcinoma (0/53, 0/53, 0/52, 0/52, 2/51)	
Lung:	
cystic keratinizing epithelioma (0/53, 0/53, 0/52, 1/53, 11/52)	
squamous cell carcinoma (0/53, 0/53, 0/52, 1/53, 1/52)	
Oral Mucosa:	
squamous cell carcinoma (gingival) (0/53, 0/53, 2/53, 5/53, 9/53)	
Pancreas:	
adenoma (0/53, 1/53, 1/52, 3/52, 1/50)	
adenoma or carcinoma (0/53, 1/53, 1/52, 4/52, 2/50)	
Equivocal findings	
Uterus:	
squamous cell carcinoma (1/53, 1/53, 1/53, 4/53, 0/53)	
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 a binary mixture of PCB 126 and PCB 153 on December 9, 2004, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 9, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) and 2,2′,4,4′,5-hexachlorobiphenyl (PCB 153) received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. N.J. Walker, NIEHS, presented the background, design, and goals of the NTP study series on the toxic equivalency factor (TEF) evaluations of mixtures of dioxin-like compounds (dioxins, PCBs, furans) and the background, design, and goals of a series of NTP studies of some representative dioxin-like chemicals and mixtures of chemicals. Four reports in that series were presented at the previous peer review meeting and Dr. Walker summarized the design and results of those studies on 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and a mixture of those three compounds. He described development of dose-response models for various endpoints using these results.

Dr. N.J. Walker introduced the study on a binary mixture of PCB 126 and PCB 153 by noting that PCB 126 is the most abundant of the coplanar dioxin-like compounds and PCB 153 is the most abundant of the non-dioxin-like PCBs. Part of the study rationale was to examine for any interactive effect for the different types of PCBs. He described the study design, incorporating one set of animal groups with increasing doses of both compounds in a fixed ratio, and another set of groups all receiving the same amount of PCB 126 plus varying amounts of PCB 153. The proposed conclusion, based on the first set of animal groups, was *clear evidence of carcinogenic activity* of a constant ratio binary mixture of PCB 126 and PCB 153 in female Harlan Sprague-Dawley rats.

Dr. Elwell, the first principal reviewer, said the study was well designed and well presented and included a number of supplemental mechanistic studies.

Dr. Storer, the second principal reviewer, commented on the wealth of detail contained in the report. He inquired if a second set of conclusions would be given for the varying ratio set of mixtures and suggested that cholangiocarcinoma be added to the list of effects of interactions between PCB 126 and PCB 153.

Dr. Gasiewicz, the third principal reviewer, suggested that results from the other TEF studies be included in the report for comparison.

Dr. Walker explained that making a conclusion for the PCB 126/PCB 153 varying ratio combination would require examining the result for both chemicals individually, and drawing conclusions about potential interactions could entail policy considerations beyond the purview of these reports. He added that summarizing all the findings for the several TEF studies as cross-references in each report could make the reports unwieldy. A separate report analyzing and comparing the effects from the whole TEF series will be produced once all the studies are completed.

Dr. Boekelheide noted that a number of endocrine-related tumors seemed to be affected by high doses or by varying dose ratios. He also suggested that the nomenclature of binary mixture be footnoted to clarify the distinction between the constant-ratio and varying-ratio dose groups.

Dr. C.L. Walker moved, and Dr. Vore seconded, that the conclusions be edited by the panel. Dr. Boekelheide suggested that the parenthetical description of hepatocellular neoplasms be changed to "predominantly adenomas." For the lung neoplasms, he proposed removing squamous cell carcinoma and adding "predominantly" before the cystic keratinizing epithelioma. Dr. Klaunig suggested including the term "contant ratio" before the term binary mixture to specify the groups on which conclusions were being drawn.

Dr. Gasiewicz moved that the conclusions be accepted as edited. Dr. Elwell seconded the motion. The motion was passed unanimously with nine votes.

OVERVIEW

Dioxin Toxic Equivalency Factor Evaluation

Polyhalogenated Aromatic Hydrocarbons and Human Exposure

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

PCDDs and PCDFs were 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 persistent in the environment, they have the ability to bioaccumulate and become more concentrated. Ambient human exposure to PHAHs occurs through the ingestion of foods containing PHAH residues. Due to their persistence and lipophilicity, once internalized, they accumulate in body tissue, mainly adipose, resulting in chronic lifetime human exposure (Schecter *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 the United States Environmental Protection Agency (2000a,b,c); therefore, it will not be re-reviewed in depth in this Technical Report.

Mechanism of Action via the Aryl Hydrocarbon Receptor

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

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

Toxicity of Dioxin-like Compounds

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

The broad spectrum of DLC effects on hormone and growth factor systems, cytokines, and signal transduction pathways 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 differentiation; disruption of normal cellular processes could be a risk factor for carcinogenicity.

The initial involvement of the AhR in initiating this cascade of events is supported by studies showing the lower potency of structurally related compounds with lower affinity for the AhR, reduction of effects in rodents with lower AhR affinities (Pohjanvirta *et al.,* 1993; Birnbaum, 1994), 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 risk posed by complex mixtures of PCDDs, PCDFs, and PCBs (Ahlborg *et al.,* 1992; Van den Berg *et al.,* 1998; USEPA, 2000c). The TEF methodology is a relative potency scheme to estimate the total exposure and dioxin-like effects of a mixture of chemicals based on a common mechanism of action involving an initial binding of the compound to the AhR. The TEF methodology is currently the most feasible interim approach for assessing and managing the risk posed by these mixtures and has been formally adopted by a number of countries including Canada, Germany, Italy, the Netherlands, Sweden, the United Kingdom, and the United States. The method is also used by the International Programme on Chemical Safety and the World Health Organization (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. 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 ED_{50} or EC_{50}) 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.

$$TEQ = \Sigma_{ni} (PCDD_i \times TEF_i)_n + \Sigma_{ni} (PCDF_i \times TEF_i) + \Sigma_{ni}(PCB_i \times TEF_i)_n$$

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

Uncertainties in the Use of Toxic Equivalency Factors

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

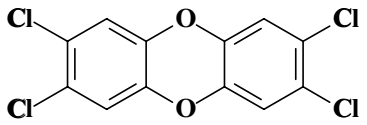
One of the implicit assumptions in the use of TEFs is that the TEQ for different compounds is dose additive. While dose additivity is supported for certain mixtures, for some biological endpoints in some models, this may not be true. As outlined by Van den Berg *et al.* (1998), the TEF methodology is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

The Dioxin Toxic Equivalency Factor Evaluation Studies

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

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

Individual Compounds, Mixtures, and Rationale for Choice

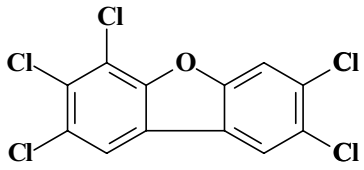


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD

CAS No. 1746-01-6

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

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

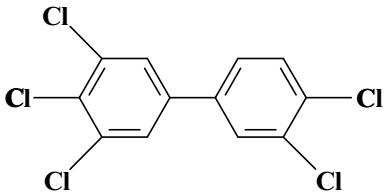


2,3,4,7,8-Pentachlorodibenzofuran
PeCDF

CAS No. 57117-31-4

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

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

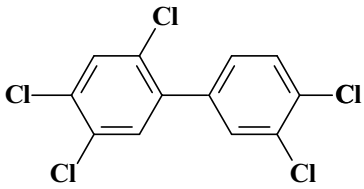


3,3′,4,4′,5-Pentachlorobiphenyl
PCB 126

CAS No. 57465-28-8

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

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

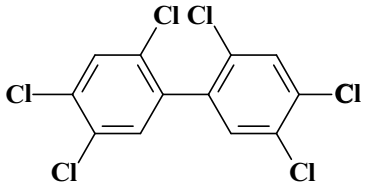


2,3′,4,4′,5-Pentachlorobiphenyl
PCB 118

CAS No. 31508-00-6

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

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



2,2′,4,4′,5,5′-Hexachlorobiphenyl

PCB 153

CAS No. 35065-27-1

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

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

Mixture Studies

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

Mixture of TCDD, PCB 126, and PeCDF

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

Binary mixture study of PCB 126 and PCB 153

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

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

Binary mixture study of PCB 118 and PCB 126

This binary mixture was not designed *a priori* as part of the dioxin TEF evaluation. While the individual PCB 118 study was at the in-life phase, it was found that the PCB 118 compound being used contained not only PCB 118 but also 0.622% PCB 126 (PCB 118:PCB 126 of 161:1). Given the large TEF difference between PCB 118 (0.0001) and PCB 126 (0.1), this resulted in a TEQ ratio for PCB 126:PCB 118 of 6:1. As such, the effects of the mixture 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, checked for the absence of high TEQ-contributing compounds, and a new study was started.

Study Design, Species, and Dose Selection Rationale

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

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

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

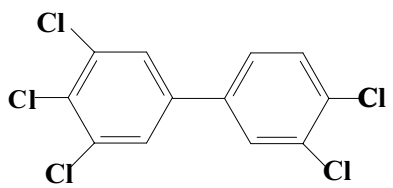
Additional "special study" animals were included at each interim necropsy. Tissues from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies. These animals were not evaluated as part of the core study.

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

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

[a] Van den Berg *et al.* (1998)
[b] 14-, 31-, and 53-week scheduled sacrifices only
[c] 10, 22, 46, 100 ng TEQ/kg (TCDD:PeCDF:PCB 126, 1:2:10)
[d] PCB 126 dose units are ng/kg, PCB 153 units are µg/kg.
[e] PCB 126 dose units are ng/kg, PCB 118 units are µg/kg. Doses are based on PCB 126 levels that are 0.622% of the administered PCB 118 bulk.

INTRODUCTION

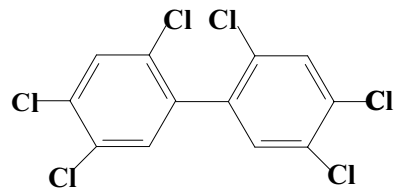


3,3′,4,4′,5-Pentachlorobiphenyl
PCB 126

CAS No. 57465-28-8

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

Synonyms: 1,1′-Biphenyl, 3,3′,4,4′,5-pentachloro-(9CI)



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

Synonyms: 1,1′-Biphenyl, 2,2′,4,4′,5,5′-hexachloro-(9CI)

Chemical and Physical Properties

PCB 126 is a coplanar polychlorinated biphenyl (PCB) produced commercially before 1977 as a component of technical grade PCB mixtures, including Aroclors 1016, 1242, 1248, and 1254 (Mayes *et al*., 1998). PCB 153 is a di-*ortho*-substituted nonplanar PCB that was commercially produced as a component of Aroclors 1242, 1248, 1254, 1260, and 1262 (Frame *et al*., 1996; ATSDR, 2000). Lower chlorinated Aroclors (1016, 1242, and 1248) are colorless mobile oils. Increasing the chlorine content results in the mixture taking on the consistency of a viscous liquid (Aroclor 1254) or sticky resin (Aroclors 1260 and 1262) (ATSDR, 2000). PCB 126 has a melting point of 160° to 161° C, a water solubility of 1.03×10^{-3} at 25° C, a vapor pressure of 2.96×10^{-7} at 25° C, and a log octanol:water partition coefficient of 6.89. PCB 153 has a melting point of 102° C, a vapor pressure of 1.2×10^{-4} (solid) and 7.0×10^{-4} (liquid) at 25° C, and a log octanol:water partition coefficient of 6.9 (Hansen, 1999).

Production, Use, and Human Exposure

PCB mixtures, including PCBs 126 and 153, 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 (log octanol:water partition coefficient of 6.5 to 7.71) and resistance to biodegradation, specific PCBs have the ability to bioconcentrate

and bioaccumulate. PCBs are widespread in their distribution and are found in virtually all media, including air, soil, water, sediment, and biota (USEPA, 2000b).

The majority of ambient human exposure to PCBs occurs through the ingestion of food containing PCB residues. PCB-contaminated fish, milk and dairy products, vegetables, and meat and animal fat are estimated to account for a majority of the exposure (Duarte-Davidson and Jones, 1994). Levels of PCB 126 in food range from 0.05 to 0.83 pg/g. Human exposure to PCB 126, which is a dioxin-like compound (DLC), is usually calculated in terms of toxic equivalencies (TEQs). On a TEQ basis, it is estimated that humans are exposed via food to 22 pg TEQ/day (for a 70 kg person) from dioxin-like PCBs of which PCB 126 (13 pg/day) accounts for 60% of the TEQ intake. The environmental occurrence of PCB 153 is widespread, and it is relatively abundant in food (Jones, 1988; McFarland and Clarke, 1989). Bioaccumulation of PCBs 126 and 153 results in persistent levels of these PCBs in human tissues. PCB 126 (12 pg TEQ/g lipid) accounts for 52% of the PCB TEQ in human tissues (USEPA, 2000b). PCB 153 is present at the highest concentrations in human tissue samples on a molar basis (McFarland and Clarke, 1989; Schecter *et al*., 1994; Heudorf *et al*., 2002; Ayotte *et al*., 2003; Chu *et al*., 2003).

TOXICOKINETICS

There is an extensive body of literature examining the toxicokinetics of mixtures and some individual congeners of PCBs (ATSDR, 2000) and DLCs such as PCB 126 (USEPA, 2000c). Since PCB 126 is a DLC with properties similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the toxicokinetics of PCB 126 are expected to be similar to TCDD. In the gastrointestinal tract, PCBs are well absorbed by passive diffusion. Several studies have examined absorption of TCDD and demonstrate that gastrointestinal absorption of a single dose of 1 µg TCDD/kg body weight in acetone:corn oil (1:25) in Sprague-Dawley rats is 84% (range 66% to 93%) (Piper *et al*., 1973; Rose *et al*., 1976). Similar results have been observed after repeated exposure (0.1 to 1 µg/kg per day) and higher doses. 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). Cytochrome P450 1A2 is a known binding protein for DLCs and is also inducible by exposure to aryl hydrocarbon receptor (AhR) ligands. Since CYP1A2 is inducible only in the liver and nasal passages, DLCs tend to sequester in the liver at levels that would not be predicted based on their lipophilicity alone. The hepatic sequestration by TCDD is not seen in CYP1A2 knockout mice, demonstrating the critical involvement of CYP1A2 in this process (Diliberto *et al*., 1999).

Unlike TCDD and dioxin-like PCBs, hepatic retention of PCB 153 in rats is low (Diliberto *et al*., 1997; van der Plas *et al*., 1998). Studies in CYP1A2 knockout mice demonstrate that PCB 153 is not sequestered in the liver by CYP1A2 like TCDD and DLCs (Diliberto *et al*., 1997, 1999). However, coadministration of PCB 153 and TCDD results in an interactive effect resulting in higher concentrations of both compounds in the liver and lower concentrations of TCDD in other tissues (Van Birgelen *et al*., 1996). Interactions between DLCs and non-dioxin-like PCBs may influence the toxicity of mixtures of these compounds.

Following absorption, PCB 153 is initially distributed to the liver and muscle, and then redistributed into the adipose tissue and skin (Matthews and Tuey, 1980; Birnbaum, 1983). PCB 153 is not well metabolized, and it is excreted primarily as the parent compound in the feces (Kato *et al*., 1980; Muhlebach and Bickel, 1981). Muhlebach and Bickel (1981) demonstrated that 40 weeks after a single dose of PCB 153 in rats, 16% of the dose was excreted in feces and less than 1% in urine. Approximately 75% of the dose was sequestered in adipose tissue and the excretion half-life for terminal elimination was 100 days. The distribution and elimination of PCB 153 in nonhuman primates is similar to rats, but PCB 153 is readily eliminated by dogs (Sipes *et al*., 1982).

There are limited data available on the distribution and excretion of PCBs in humans. However, in humans, PCBs are found in the highest concentrations in adipose tissue, and they tend to accumulate to a lesser extent in

other lipid-rich tissues such as liver, skin, and breast milk (ATSDR, 2000). PCB concentrations of 0.5 to 10 ppm have been reported for human adipose tissue and 0.5 to 4 ppm for human milk fat (Jensen, 1987). Human metabolism of PCB 153 to 3-hydroxy-2,2′,4,4′,5,5′-hexachlorobiphenyl is mediated by CYP2B6 (Ariyoshi *et al*., 1995). However, CYP2B6 is expressed only at low levels, accounting for only 1% to 2% of the total CYP enzymes in the human liver. Calculated estimates for the apparent half-life of PCB 153 in humans range from 3.8 to 47 years (Chen *et al*., 1982; Brown *et al*., 1989; Ryan *et al*., 1993).

PCB 126 and PCB 153 Toxic Equivalency Factors

The World Health Organization WHO_{98} toxic equivalency factor (TEF) for PCB 126 is 0.1 (Van den Berg *et al*., 1998). PCB 153 is a di-*ortho*-substituted nonplanar PCB. Nonplanar PCBs do not have dioxin-like activity and are not included in the TEF methodology; therefore, PCB 153 has no TEF value.

TOXICITY

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

PCB 153 is a di-*ortho*-substituted nonplanar PCB. Nonplanar PCB congeners with two or more chlorines in the *ortho* position do not have dioxin-like activity and exhibit toxicity profiles that are different than the dioxin-like coplanar PCB congeners (Fischer *et al*., 1998). PCB 153 is a phenobarbital-like inducer of hepatic cytochrome P450 (Denomme *et al*., 1983). Exposure to PCB 153 induces hepatic lipid peroxidation (Fadhel *et al*., 2002) and increases glutathione-S-transferase activity (Lamartiniere *et al*., 1979). In Sprague-Dawley rats, subchronic exposure to PCB 153 reduces hepatic and pulmonary vitamin A, induces histological changes in the thyroid and liver, and decreases dopamine and its metabolites in the brain (Chu *et al*., 1996). In tumor promotion studies, PCB 153 induces preneoplastic altered hepatocellular foci (Bager *et al*., 1995; van der Plas *et al*., 2000; Dean *et al*., 2002). In male Sprague-Dawley rats, PCB 153 induces hepatocyte proliferation and activation of the NF-κB transcription factor (Lu *et al*., 2003). NF-κB regulates the expression of cell proliferation and apoptosis-related genes; therefore it may play a critical role in tumor promotion and carcinogenesis.

Several studies have investigated the interaction between PCB 153 and dioxin-like congeners on tumor promotion. These studies demonstrate that coexposure of female Sprague-Dawley rats to PCB 153 and PCB 126 antagonizes the formation and development of altered hepatocellular foci expressing the placental form of glutathione-S-transferase (Haag-Grönlund *et al*., 1998; Dean *et al*., 2002). PCB 153-mediated antagonism of PCB 77-induced altered hepatocellular foci has also been reported (Berberian *et al*., 1995). PCB 153 also antagonizes the promoting effect of TCDD on malignant transformation (Wolfle, 1998). The antagonistic interaction of PCB 153 with TCDD and other dioxin-like PCB congeners may occur via interference with the AhR. Although it does not elicit dioxin-like biological effects, PCB 153 binds the AhR with a binding affinity relative to TCDD of 3×10^{-5} (Schneider *et al*., 1995). PCB 153 has also been demonstrated to antagonize TCDD-mediated cleft palate, hydronephrosis, and immunotoxicity in mice (Biegel *et al*., 1989; Morrissey *et al*., 1992). Although antagonism has been well established, Bager *et al*. (1995) demonstrated that coexposure of female Sprague-Dawley rats to PCB 153 and PCB 126 induced a synergistic effect on the development of altered hepatocellular foci expressing γ-glutamyltranspeptidase.

Nonplanar *ortho*-substituted PCBs have been shown to induce neurobehavioral toxicity, neurotoxicity, and endocrine alterations (Fischer *et al*., 1998; 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 PCB congeners 28, 47, and 52 in 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). PCB 153 and Aroclors 1242 and 1254, which contain relatively low concentrations of dioxin-like PCB congeners, also induce death in cultured cerebellar granule cells and formation of reactive oxygen species (Mariussen *et al*., 2002).

CARCINOGENICITY

Experimental Animals

There is an extensive body of literature examining the carcinogenicity of mixtures of PCBs in rodents (Silberhorn *et al*., 1990). In general, these studies indicate that PCB mixtures have the potential to be carcinogenic, primarily within the liver (hepatocellular neoplasms). Mixtures of PCBs contain both dioxin-like coplanar PCBs as well as non-dioxin-like PCBs, which may elicit responses via different mechanisms. While these mixtures of PCBs have been shown to be carcinogenic in rats and mice (Nagasaki *et al*., 1972; Ito *et al*., 1973; Kimbrough *et al*., 1975; Mayes *et al*., 1998), there have been no studies on the carcinogenicity of PCB 153 alone. Until the recent study of PCB 126 as part of the NTP dioxin toxic equivalency factor (TEF) evaluation (NTP, 2006a), there have been no individual studies on the carcinogenicity of PCB 126. No epidemiology studies of either PCB 126 or PCB 153 were found in a review of the literature. With the exception of PCB 126, there have been no published studies examining the carcinogenicity of an individual PCB congener. In the NTP (NTP, 2006a) carcinogenicity study of PCB 126 in female Harlan Sprague-Dawley rats, 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. In addition there were increased incidences of nonneoplastic lesions in the liver, lung, adrenal coretex, pancreas, kidney, heart, thyroid gland, thymus, spleen, clitoral gland, and mesenteric artery that were due to treatment with PCB 126.

The most recent study of PCB mixtures, conducted by Mayes *et al*. (1998), examined the comparative carcinogenicity of Aroclors 1016, 1242, 1254, and 1260 in male and female Sprague-Dawley rats. Increased incidences of hepatocellular adenoma, hepatocellular carcinoma, hepatocholangioma, hepatocholangiocarcinoma, and follicular cell adenoma of the thyroid gland were seen in this study. 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 tumors were induced by exposure to Aroclors 1242, 1254, and 1260, and liver tumors by Aroclor 1260. Within this context, compared to the other PCB mixtures, Aroclor 1254 has the highest dioxin-like activity measured on a TEQ basis due to the presence of specific coplanar PCBs, PCDDs and PCDFs in the mixture. Aroclors 1254 and 1260 are composed of 5.6% and 12.2% PCB 153 by weight, respectively (Frame *et al*., 1996). The incidences of liver tumors in rats were greater in females than in males. Female tumor incidences were 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 similar mechanisms for dioxin-like PCBs and 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 incidences of thyroid gland follicular cell adenoma in male and female Osborne-Mendel rats and female $B6C3F_1$ 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 Harlan Sprague-Dawley rats, there was clear evidence of

carcinogenicity of TCDD at doses 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, 2006b). Increased incidences of squamous cell carcinoma of the uterus were also considered to be related to treatment with TCDD, and marginal increases in the incidences of pancreatic neoplasms and hepatocholangioma and cholangioma of the liver may have been related to administration of TCDD. 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 splenic and mesenteric arteries that were due to treatment.

Humans

Humans have not been exposed to significant amounts of PCB 153 alone. Exposures to PCB 153 occur through mixtures, containing other structurally related PCBs and compounds such as PCDDs and PCDFs.

Two accidental poisoning incidents in Japan and Taiwan resulted from exposures to cooking oil that was highly contaminated with PCDFs and PCBs (Masuda, 1985). In addition to extensive reproductive and developmental effects in these populations, early follow-up studies indicated increased mortality from liver disease and cancer, particularly liver cancer (IARC, 1997). Although recent 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.

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

Study Design Overview

The design of this study of a mixture of PCB 126 and PCB 153 should be considered within the context of the dioxin TEF evaluation. The aim of these 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-sex, two-species carcinogenicity testing paradigm. Consequently, many of the design rationales are based on the prior observations of the carcinogenicity of TCDD.

Study Design, Species, and Dose Selection Rationale

PCB 126 is a dioxin-like PCB with a relative potency of 0.1. PCB 153, the most abundant PCB in human tissues on a mass basis, is a di-*ortho*-substituted nonplanar PCB that does not demonstrate dioxin-like activity, and therefore, has no TEF value. The dosages for the mixture were selected to: 1) evaluate the dose response of a constant ratio of each of the PCB congeners, and 2) evaluate the effect of increasing concentrations of PCB 153 on the PCB 126-induced responses. The dosages of PCB 126 for the current study were designed to match the range of known carcinogenicity of TCDD with an adjustment for the TEF of 0.1. The TEQ doses used in the constant ratio groups were 0, 1, 10, 30, and 100 ng TEQ/kg, which are comparable to the doses in the study of TCDD (0, 3, 10, 22, 46, and 100 ng TCDD/kg; NTP, 2006b). The doses of PCB 153 are similar to those previously used in tumor promotion studies. The Sprague-Dawley female rat was used for the dioxin TEF evaluation studies based upon the prior observation of high hepatocarcinogenic potency of TCDD within this strain and the extensive literature on the effects of TCDD and related compounds in this model.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Reports on analyses performed in support of the study of a binary mixture of PCB 126 and PCB 153 are on file at the National Institute of Environmental Health Sciences.

PCB 126

PCB 126 was obtained from AccuStandard, Inc. (New Haven, CT), in one lot (130494) that was used in the 2-year study. One additional lot (DK-130) was procured by Midwest Research Institute (Kansas City, MO) from Cambridge Isotope Laboratories, Inc. (Andover, MA), solely for dose formulation stability studies and was not used in the 2-year animal study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Chemistry Support Services) (Columbus, OH), and the study laboratory (Battelle Columbus Operations, Columbus, OH).

Lot 130494 of the chemical, a white powder, was identified as PCB 126 by proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and melting point determination. All spectra were consistent with the structure of a pentachlorobiphenyl, and determination of the melting point (156.9° C) by differential scanning calorimetry agreed with the literature (Bolgar *et al*., 1995).

The purity of lot 130494 was determined by the analytical chemistry laboratory using gas chromatography (GC) coupled to a high resolution mass spectrometer (MS) and by the study laboratory using GC. The purity profile obtained detected four impurities with a combined relative area of 0.49%. Two impurities were tetrachlorinated biphenyls and one was a pentachlorinated biphenyl. One impurity was not identified, but was determined not to be a dioxin, dibenzofuran, or PCB. GC indicated a purity of 100.3% ± 0.7% for lot 130494 relative to the reference sample. The overall purity of lot 130494 was determined to be greater than 99%.

PCB 153

PCB 153 was obtained from Radian International LLC (Austin, TX) by Midwest Research Institute and provided to the study laboratory in one lot (31532-78) that was used in the 2-year study. Additional lots (HE-553, HF-440, and HD-175) were procured by Midwest Research Institute from Cambridge Isotope Laboratories, Inc., solely for dose formulation stability studies and were not used in the 2-year animal study. Identity and purity analyses were conducted by the analytical chemistry laboratory and the study laboratory.

Lot 31532-78 of the chemical, a white powder, was identified as PCB 153 by the analytical chemistry laboratory using proton and carbon-13 NMR spectroscopy. In addition, identity analysis was conducted by the study laboratory using proton NMR; spectra of a purity analysis sample and a frozen reference sample were compared to each other and to the spectrum of the same lot previously reported by the analytical chemistry laboratory. All spectra were consistent with the structure of PCB 153.

The purity of lot 31532-78 was determined by the analytical chemistry laboratory to be approximately 99.8% using GC/MS. The purity profile detected two significant impurities: 0.21% of the test article was identified as a pentachlorobiphenyl and 0.002% of the test article was identified as a heptachlorobiphenyl. Standards of the possible impurities were obtained by the analytical chemistry laboratory from Cambridge Isotope Laboratories, Inc., and analyzed using GC/MS; the pentachlorobiphenyl impurity was identified as 2,2′,4,5,5′-pentachlorobiphenyl (PCB 101), and the heptachlorobiphenyl impurity was identified as 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180). Since PCB 101 and PCB 180 are di-*ortho* PCBs, it was predicted that they do not have dioxin-like activity. Di-*ortho* PCBs were not included in the dioxin TEF scheme.

Additional evaluations of the purity of lot 31532-78 were performed by the study laboratory. Initial evaluation using flame ionization indicated an average purity

of 96.1% for the test article relative to that of a frozen reference sample supplied by the analytical chemistry laboratory. To resolve the discrepancy in the purity estimates for the test article by the analytical chemistry and study laboratories, additional purity studies were conducted by the study laboratory. A new frozen reference sample of the same lot was obtained from the analytical chemistry laboratory, and comparative purity analysis by flame ionization indicated that the relative purity of the test article was 101.1%. Subsequent analyses of these samples using GC/MS detected single impurities in each sample with peak areas of 0.5% relative to the major peak areas. The overall purity of lot 31532-78 was determined to be greater than 99%.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving the PCB 126 working stocks in acetone and diluting in the corn oil vehicle that contained either an aliquot of a PCB 153 working stock (for the 4 ng/mL PCB 126: 4 µg/mL PCB 153 dose formulation only) or neat PCB 153. The final dose formulations contained 1% acetone and were stored at room temperature in amber glass bottles with minimal headspeace, sealed with Teflon®-lined lids for up to 35 days with four exceptions. Formulations prepared on December 17, 1999, March 10, 2000, and June 2, 2000, were used for 41, 38, and 40 days after formulating, respectively, pending completion of analysis of subsequent sets of formulations. Formulations prepared on September 1, 1998, were used 2 days after expiration due to an oversight.

Homogeneity of 4 ng/mL PCB 126:4 µg/mL PCB 153 and 120 ng/mL:1,200 µg/mL dose formulations and gavageability of a 120 ng/mL:1,200 µg/mL dose formulation were confirmed by the study laboratory using GC/MS for PCB 126 and GC for PCB 153. Stability studies of a 4 ng/mL:4 µg/mL formulation of lots DK-130 (PCB 126) and HE-553, HF-440, or HD-175 (PCB 153) with 0.04% hexane and 0.08% isooctane were conducted by Midwest Research Institute using GC/MS for PCB 126 and GC for PCB 153. Stability was confirmed for at least 35 days for the formulations stored in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids at 5° C and room temperature, and for 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of the binary mixture of PCB 126 and PCB 153 were conducted by the study laboratory using GC/MS for PCB 126 concentrations and GC for PCB 153 concentrations. During the 2-year study, the dose formulations were analyzed at least every 3 months to determine the concentrations of PCB 126 and PCB 153 in the binary mixture. For the dose formulations analyzed and used in the study, 80% (44/55) and 98% (54/55) were within 10% of the target concentrations for PCB 126 and PCB 153, respectively; all were within 15% of target. Of the animal room samples, 64% (16/25) for PCB 126 and all 25 for PCB 153 were within 10% of the target concentrations; all PCB 126 concentrations were within 14% of target.

2-Year Study

Study Design

The 2-year study of a binary mixture of PCB 126 and PCB 153 was designed to assess the carcinogenicity of a constant ratio binary mixture of PCB 126 and PCB 153. In addition, varying ratio mixture groups were used to assess the impact of increasing PCB 153 on the carcinogenicity of PCB 126. Dose groups were divided into two study arms. TCDD equivalent (TEQ) doses for each group based on the PCB 126 doses after adjustment for the PCB 126 TEF of 0.1 (Figure 1 and Table 2).

Groups of 81 (Groups 2, 3, 5, and 7) or 80 (Groups 4 and 6) female rats received a mixture of PCB 126 and PCB 153 in corn oil:acetone (99:1) by gavage 5 days per week for up to 105 weeks; a group of 81 female rats received the corn oil:acetone (99:1) vehicle only and served as the vehicle control (Group 1). Up to 10 female rats per group were evaluated at 14, 31, and 53 weeks.

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

Source and Specification of Animals

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

for 13 days before the study and were approximately 8 weeks old at the beginning of the study. Rats were evaluated for parasites and gross observation of disease, and the health of the rats was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix F). Sentinel rats included five males and five females at 1 month, six males at 6 months, five males at 12 and 18 months, and five Group 7 females at the end of the study.

Animal Maintenance

Male rats were housed three per cage and female rats were housed three or five per cage. Feed and water were available *ad libitum*. Cages were changed twice weekly, and cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix E.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded on day 29, monthly thereafter, and at the end of the study. Body weights were recorded on the first day prior to dose initiation, weekly for 13 weeks, monthly 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 and processed into serum for thyroid hormone determinations. Radioimmunoassays were performed for thyroid stimulating hormone (TSH), triiodothyronine, 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, NH).

For cell proliferation analysis at 14, 31, and 53 weeks, up to 10 female rats per group received drinking water containing 40 mg BrdU in 100 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. A sample of duodenum (positive control) and liver was fixed in 10% neutral buffered formalin for 18 to 24 hours then transferred to 70% ethanol. Representative sections of the duodenum and liver were trimmed and embedded, and two sections were cut. One of these sections was stained with hematoxylin and eosin and the other with anti-BrdU antibody complexed with avidin and biotin. At the 14-week interim evaluation, potential interlobular variation was determined in vehicle control and Group 7 rats by counting stained cells in the left lobe and right median lobe. Interlobular variation greater than 25% was considered significant. For the remaining rats, stained cells were counted only in the left lobe. At least 2,000 labeled or unlabeled hepatocyte nuclei were counted using a 20× objective and ocular grid. The labeling index was calculated 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 at 14, 31, and 53 weeks and stored frozen at –70° C. Microsomal suspensions were prepared using the Pearce Method (Pearce *et al*., 1996). The concentration of protein in each suspension was determined using the microtiter plate method of the Coomassie Plus Protein Assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. Enzyme activities were determined by fluorometric analysis of *O*-deethylation of 7-ethoxyresorufin, 7-pentoxyresorufin, and by the acetanilide-4-hydroxylase activity assay. Cytochrome P450 1A1 (CYP1A1) associated 7-ethoxyresorufin-*O*-deethylase (EROD), CYP2B associated 7-pentoxyresorufin-*O*-deethylase (PROD), and CYP1A2 associated acetanilide-4-hydroxylase (A4H) activities were determined in microsomal protein isolated from frozen liver or lung tissue according to established procedures. Data are shown as pmol/min per mg (EROD, PROD) or nmol/min per mg (A4H) microsomal protein.

For analysis of tissue concentrations of PCB 126 or PCB 153, samples of fat, liver, lung, and blood were taken from up to 10 female rats per dose group at 14, 31, and 53 weeks and at 2 years. Tissue sample preparation included overnight saponification with ethanolic potassium hydroxide, extraction of the saponificate with hexanes, and two-stage sample extract clean up on columns

using silica gel with hexanes elution and magnesium silicate with hexanes:ethyl ether (80:20) elution by automated solid phase extraction. The concentrations of PCB 126 or PCB 153 in the tissue extracts were measured by capillary gas chromatograpy with high resolution mass spectrometry detection.

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 potential target organs, which included the adrenal cortex, bone marrow, heart, kidney, liver, lung, mandibular lymph node, mesenteric artery, nose, oral mucosa, ovary, pancreas, spleen, stomach, thymus, thyroid gland, tooth, and uterus.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the 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 pathologists were involved in all phases of the pathology evaluation including the initial examination and the pathology peer review. Because of the need for a consistent diagnostic approach across all studies and the unusual nature of some of the lesions, five other studies (PCB 126, TCDD, the TEF Dioxin Mixture, PeCDF, and PCB 153; NTP, 2006a,b,c,d,e) were subjected to additional PWG reviews. Within many of these studies, there were hepatocellular proliferative lesions for which the criteria used for common diagnoses did not appear to fit. Furthermore, classification was sometimes confounded by significant liver damage (toxic hepatopathy) that was present in many animals from these studies. With the consecutive pathology peer review of each of these studies, the morphological spectrum of proliferative lesions became more apparent to those involved, and the diagnostic criteria for the proliferative lesions further refined. Therefore, a PWG was held to ensure that these important proliferative lesions were sufficiently and consistently categorized across all seven studies for which data are to be compared. PWG participants for this review were primarily those involved in previous PWGs. Additionally, a 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. Participants included Drs. Jerrold Ward, Ernest McConnell, James Swenberg, Michael Elwell, Peter Bannasch, Douglas Wolf, John Cullen, and Rick Hailey. Final diagnoses for the hepatocellular proliferative lesions reflect the consensus of this complete review process.

TABLE 2
Experimental Design and Materials and Methods in the 2-Year Gavage Study of the Binary Mixture of PCB 153 and PCB 126

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

Strain and Species
Harlan Sprague-Dawley rats; Hsd; Sprague-Dawley™

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

Time Held Before Studies
13 days

Average Age When Studies Began
8 weeks

Date of First Dose (female rats only)
September 16, 1998

Duration of Dosing
5 days/week for 14, 31, 53 (interim evaluations), or 104 to 105 (core study) weeks

Date of Last Dose
September 12-14, 2000

Necropsy Dates
September 13-15, 2000

Average Age at Necropsy
112 weeks

Size of Study Groups
80 (Groups 4 and 6) or 81 (Groups 1, 2, 3, 5, and 7)

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

Animals per Cage
Male Rats: 3
Female Rats: 3 or 5

Method of Animal Identification
Tail tattoo

Diet
Irradiated NTP-2000 wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, checked daily, changed weekly

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

Cages
Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed twice weekly, rotated every 2 weeks

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

Cage Filters
DuPont 2024 spun-bonded polyester filter (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks

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

TABLE 2
Experimental Design and Materials and Methods in the 2-Year Gavage Study of the Binary Mixture of PCB 153 and PCB 126

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

Doses
Constant ratio mixture groups:
Group 1: Vehicle control
Group 2: 10 ng/kg PCB 126 plus 10 µg/kg PCB 153 (1 ng TEQ/kg)
Group 3: 100 ng/kg PCB 126 plus 100 µg/kg PCB 153 (10 ng TEQ/kg)
Group 5: 300 ng/kg PCB 126 plus 300 µg/kg PCB 153 (30 ng TEQ/kg)
Group 7: 1,000 ng/kg PCB 126 plus 1,000 µg/kg PCB 153 (100 ng TEQ/kg)
Varying ratio mixture groups:
Group 4: 300 ng/kg PCB 126 plus 100 µg/kg PCB 153 (30 ng TEQ/kg)
Group 5: 300 ng/kg PCB 126 plus 300 µg/kg PCB 153 (30 ng TEQ/kg)
Group 6: 300 ng/kg PCB 126 plus 3,000 µg/kg PCB 153 (30 ng TEQ/kg)

Type and Frequency of Observation
Animals were observed twice daily and weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the study. Clinical findings were recorded on day 29, monthly thereafter, and at the end of the study.

Method of Sacrifice
Carbon dioxide asphyxiation

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

Thyroid Hormone Analyses
At 14, 31, and 53 weeks, blood was collected from the retroorbital sinus of up to 10 rats per group for thyroid stimulating hormone, triiodothyronine, and total and free thyroxine determinations.

Cell Proliferation
At 14, 31, and 53 weeks, up to 10 rats per group received BrdU in drinking water for 5 days. Samples from the liver and duodenum were taken for BrdU labeled and unlabeled hepatocyte determinations.

Cytochrome P450 Activities
At 14, 31, and 53 weeks, tissue samples from the liver were taken from up to 10 rats per group for 7-ethoxyresorufin-*O*-deethylase, 7-pentoxyresorufin-*O*-deethylase, and acetanilide-4-hydroxylase activities. Lung samples from these rats were analyzed for 7-ethoxyresorufin-*O*-deethylase activity.

Tissue Concentration Analysis
At 14, 31, 53, and 104 weeks, samples of blood, fat, liver, and lung were taken from up to 10 rats per group for analysis of PCB 126 and PCB 153 concentrations.

Histopathology
Complete histopathology was performed on all core study 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 with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, 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. At 14, 31, and 53 weeks, the adrenal gland, liver, lung, mammary gland, ovary, pancreas, pituitary gland, spleen, stomach, thymus, thyroid gland, uterus, and vagina were examined in the vehicle control and 300 ng/kg plus 3,000 µg/kg groups. In all remaining dose groups, the following tissues were examined: liver at 14, 31, and 53 weeks, and thymus at 31 and 53 weeks. In addition, the following tissues were examined: at 14 weeks, the thymus in the 300 ng/kg plus 300 µg/kg, 300 ng/kg plus 3,000 µg/kg, and 1,000 ng/kg plus 1,000 µg/kg groups, and the pancreas in the 1,000 ng/kg plus 1,000 µg/kg group; at 31 weeks, the pancreas in the 1,000 ng/kg plus 1,000 µg/kg group; and at 53 weeks, the pancreas in the 300 ng/kg plus 100 µg/kg, 300 ng/kg plus 300 µg/kg, and 1,000 ng/kg plus 1,000 µg/kg groups.

Statistical Methods

Analyses were conducted separately on the constant ratio dose groups and the varying ratio dose groups. In the analyses of the varying ratio dose groups, the vehicle control group was excluded from trend tests and pairwise comparisons.

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1a, A1b, A5a, A5b, B1a, B1b, B4a, and B4b 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 A3 and B3) 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 A3 and B3 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 $B6C3F_1$ 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 trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N). For neoplasms and nonneoplastic lesions detected at the interim evaluation, the Fisher exact test (Gart *et al.,* 1979), a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

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

which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). PCB tissue concentrations were analyzed using Scheffé's test (Scheffé, 1953) for pairwise comparisons of multiple dosed groups.

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 limited to the seven gavage studies conducted as part of the dioxin TEF evaluation (the current binary mixture of PCB 126 plus PCB 153 and PCB 126, TCDD, the TEF mixture, PeCDF, PCB 153, and the PCB Mixture of PCB 126 and PCB 118; NTP, 2006a,b,c,d,e,f).

Quality Assurance Methods

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

RESULTS

2-Year Study

Survival

Constant Ratio Mixture of PCB 126 and PCB 153

Estimates of 2-year survival probabilities for female rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 2). Survival of all dosed groups was similar to that of the vehicle controls.

Varying Ratio Mixture of PCB 126 and PCB 153

Estimates of 2-year survival probabilities for female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 3). Survival of all dosed groups was similar to that of the vehicle controls.

Table 3
Survival of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Animals initially in study	81	81	81	81	81
14-Week interim evaluation[b]	10	10	10	10	10
31-Week interim evaluation[b]	10	10	10	10	10
53-Week interim evaluation[b]	8	8	8	8	8
Accidental deaths[b]	1	1	1	0	2
Moribund	22	19	24	19	20
Natural deaths	8	12	6	10	7
Animals surviving to study termination	22	21	22	24	24
Percent probability of survival at end of study[c]	42	40	42	45	47
Mean survival (days)[d]	629	618	635	616	602
Survival analysis[e]	P=0.798N	P=0.942	P=1.000N	P=1.000N	P=0.942N

[a] Dosed groups are presented as a ratio of PCB 126:PCB 153
[b] Censored from survival analyses
[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**.

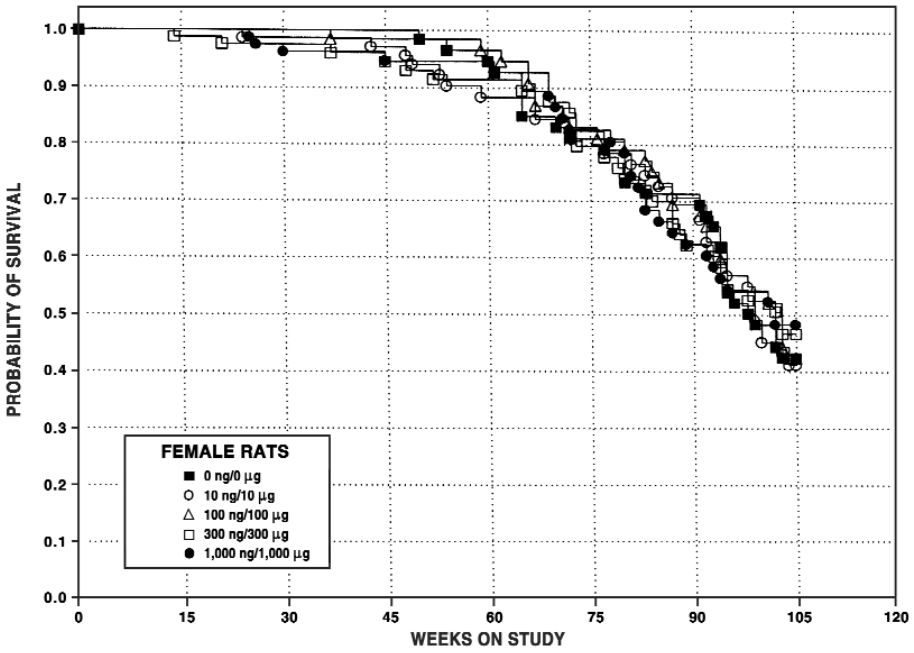


FIGURE 2
Kaplan-Meier Survival Curves for Female Rats Administered a Binary Mixture of PCB 126 and PCB 153 by Gavage for 2 Years (Constant Ratio Mixtures)

TABLE 4
Survival of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Animals initially in study	81	80	81	80
14-Week interim evaluation[b]	10	10	10	10
31-Week interim evaluation[b]	10	10	10	10
53-Week interim evaluation[b]	8	10	8	9
Accidental deaths[b]	1	0	0	1
Moribund	22	10	19	13
Natural deaths	8	12	10	10
Animals surviving to study termination	22	28	24	27
Percent probability of survival at end of study[c]	42	56	45	54
Mean survival (days)[d]	629	663	616	656
Survival analysis[e]	P=0.463N	P=0.280N	P=1.000N	P=0.301N

[a] Dosed groups are presented as a ratio of PCB 126:PCB 153
[b] Censored from survival analyses
[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**.

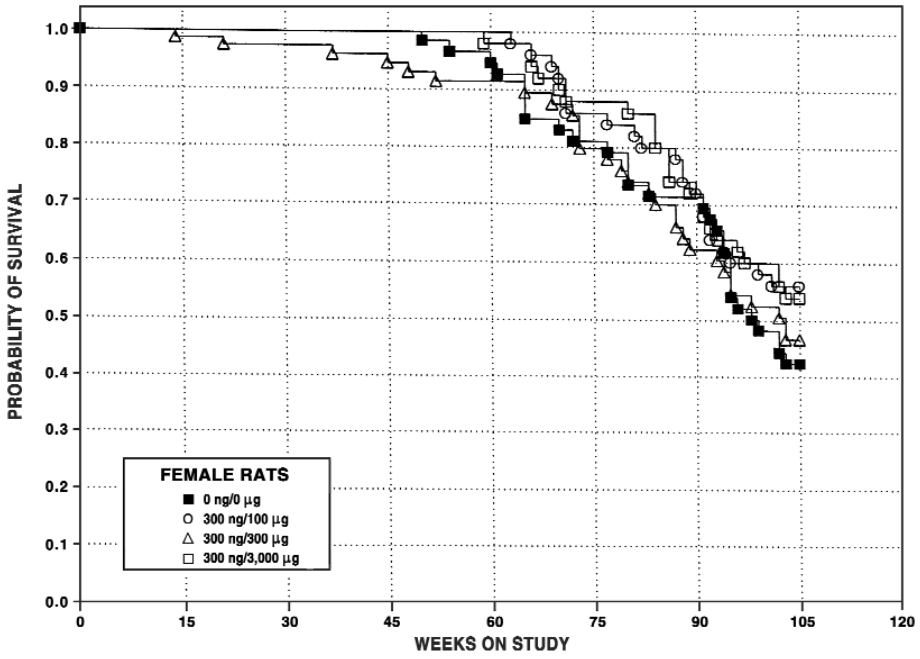


FIGURE 3
Kaplan-Meier Survival Curves for Female Rats Administered a Binary Mixture of PCB 126 and PCB 153 by Gavage for 2 Years (Varying Ratio Mixtures)

Body Weights and Clinical Findings

Constant Ratio Mixture of PCB 126 and PCB 153

The mean body weights of Group 7 were less than those of the vehicle controls after week 8, and those of Group 5 were generally less after week 25 (Figure 4 and Table 5). Mean body weights of Group 2 and Group 3 were generally similar to those of the vehicle controls throughout the study. No clinical findings related to the administration of the binary mixture of PCB 126 and PCB 153 were observed.

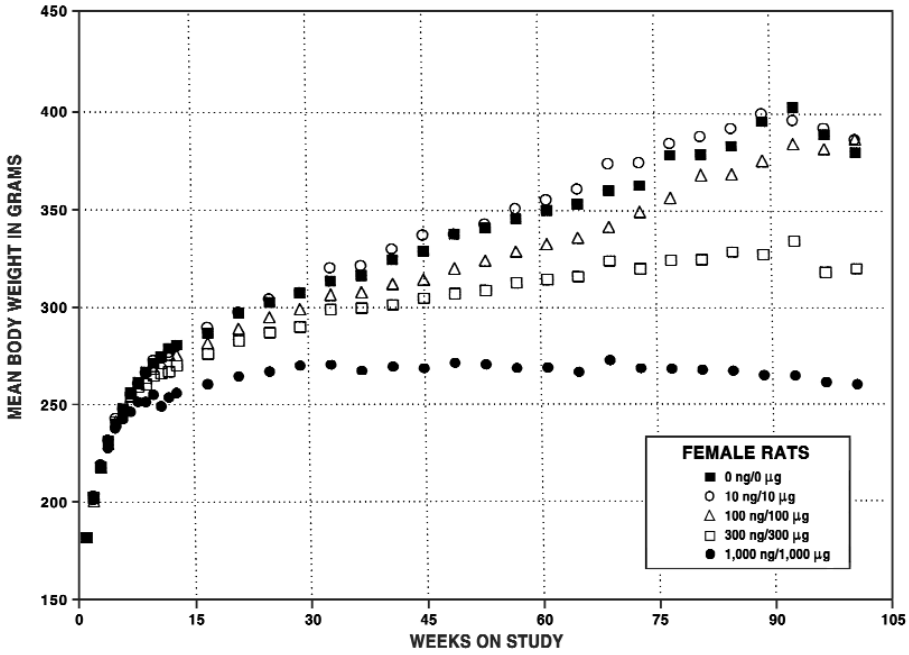


FIGURE 4
Growth Curves for Female Rats Administered a Binary Mixture of PCB 126 and PCB 153 by Gavage for 2 Years (Constant Ratio Mixtures)

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control		Group 2 10 ng/kg:10 µg/kg			Group 3 100 ng/kg:100 µg/kg			Group 5 300 ng/kg:300 µg/kg			Group 7 1,000 ng/kg:1,000 µg/kg		
Weeks on Study	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	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	181	98	182	100	86	182	100	98	182	100	98	182	100	98
2	202	98	201	100	86	201	100	98	202	100	98	203	101	98
3	218	98	219	101	86	218	100	98	218	100	98	218	100	98
4	232	98	232	100	86	230	99	98	231	100	98	228	98	98
5	240	98	243	101	86	241	101	98	241	100	98	238	99	98
6	248	98	248	100	86	247	100	98	248	100	98	243	98	98
7	256	98	255	100	86	254	99	98	254	99	98	246	96	98
8	262	98	261	100	86	261	100	98	259	99	98	252	96	98
9	267	98	266	100	86	264	99	98	260	98	98	251	94	98
10	272	98	273	101	86	268	99	98	265	97	98	255	94	98
11	275	98	274	100	86	271	99	98	266	97	98	249	91	97
12	279	98	277	99	86	276	99	98	267	96	98	254	91	96
13	281	97	280	100	86	276	98	98	270	96	98	256	91	96
17[a]	287	81	290	101	76	282	98	82	276	96	81	260	91	80
21	297	81	298	100	76	289	97	81	283	95	81	264	89	80
25	303	81	304	101	75	295	97	81	287	95	80	267	88	80
29	308	81	308	100	74	299	97	81	290	94	80	270	88	78
33[a]	314	65	321	102	64	307	98	65	299	95	64	270	86	61
37	316	65	322	102	64	308	97	65	300	95	64	267	84	61
41	325	65	330	102	64	312	96	64	301	93	63	269	83	61
45	329	65	338	103	63	314	96	64	305	93	63	269	82	61
49	338	65	338	100	62	320	95	64	307	91	61	271	80	60
53	341	64	343	101	61	324	95	64	309	91	60	271	79	60
57[a]	346	50	351	102	46	329	95	51	313	91	47	269	78	47
61	350	49	356	102	45	333	95	50	315	90	47	269	77	47
65	353	48	361	102	45	336	95	49	316	89	47	267	75	46
69	360	44	374	104	43	342	95	45	324	90	46	273	76	45
73	363	42	375	103	42	350	96	43	320	88	42	268	74	41
77	379	41	385	102	42	357	94	42	325	86	41	268	71	41
81	379	38	388	103	40	369	97	41	325	86	38	268	71	39
85	383	37	392	102	37	369	96	38	329	86	36	267	70	34
89	396	37	400	101	36	376	95	36	328	83	33	265	67	32
93	404	35	397	98	32	385	95	34	335	83	32	265	66	30
97	390	27	393	101	29	382	98	28	318	82	28	261	67	27
101	380	25	387	102	23	387	102	28	320	84	27	260	68	27
Mean for weeks														
1-13	247		247	100		245	99		243	98		237	96	
14-52	313		317	101		303	97		294	94		267	86	
53-101	371		377	102		357	96		321	87		267	72	

[a] Interim evaluations occurred during weeks 14, 31, and 53; number of survivors includes 5 (Group 2) or 17 (Groups 1, 3, 5, and 7) special study animals that were not evaluated as part of the core study.

Varying Ratio Mixture of PCB 126 and PCB 153

The mean body weights of Groups 4 and 5 were generally less than those of the vehicle controls after week 25, and those of Group 6 were less after week 12 (Figure 5 and Table 6). No clinical findings related to the administration of the binary mixture of PCB 126 and PCB 153 were observed.

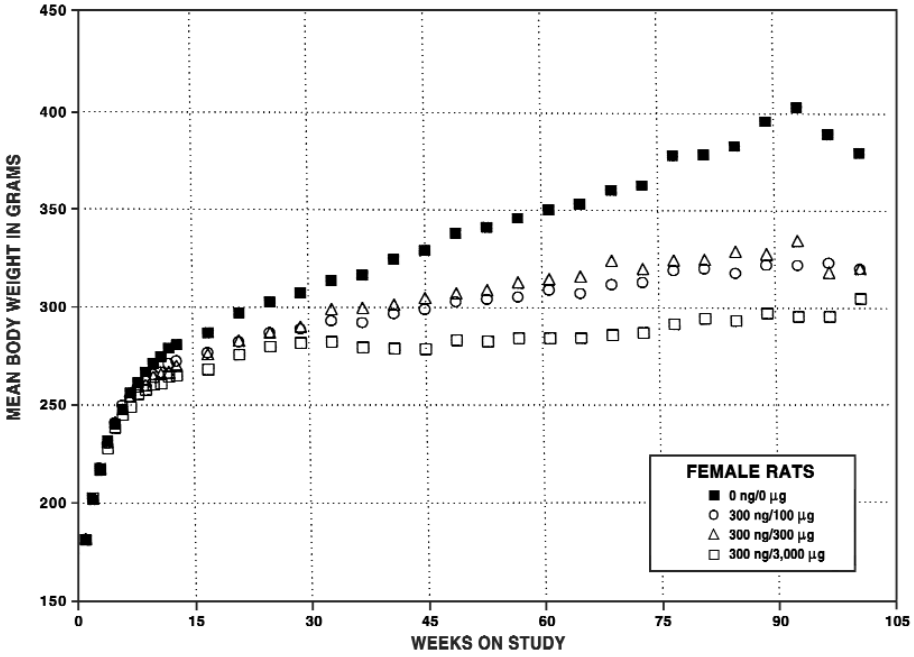


FIGURE 5
Growth Curves for Female Rats Administered a Binary Mixture of PCB 126 and PCB 153 by Gavage for 2 Years (Varying Ratio Mixtures)

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Weeks on Study	Group 1 Vehicle Control		Group 4 300 ng/kg:100 µg/kg			Group 5 300 ng/kg:300 µg/kg			Group 6 300 ng/kg:3,000 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	181	98	182	100	80	182	100	98	181	100	80
2	202	98	202	100	80	202	100	98	203	101	80
3	218	98	218	100	80	218	100	98	217	100	80
4	232	98	230	99	80	231	100	98	228	98	80
5	240	98	241	100	80	241	100	98	238	99	80
6	248	98	250	101	80	248	100	98	245	99	80
7	256	98	254	99	80	254	99	98	249	97	80
8	262	98	259	99	80	259	99	98	256	98	80
9	267	98	263	98	80	260	98	98	258	97	80
10	272	98	266	98	80	265	97	98	260	96	80
11	275	98	267	97	80	266	97	98	261	95	80
12	279	98	271	97	80	267	96	98	265	95	80
13	281	97	273	97	80	270	96	98	265	94	80
17[a]	287	81	277	96	70	276	96	81	268	94	70
21	297	81	282	95	70	283	95	81	276	93	70
25	303	81	287	95	70	287	95	80	280	93	70
29	308	81	289	94	70	290	94	80	282	92	70
33[a]	314	65	293	93	60	299	95	64	283	90	60
37	316	65	292	92	60	300	95	64	280	88	59
41	325	65	297	91	60	301	93	63	279	86	59
45	329	65	299	91	60	305	93	63	279	85	59
49	338	65	303	90	60	307	91	61	283	84	59
53	341	64	304	89	60	309	91	60	283	83	59
57[a]	346	50	306	88	50	313	91	47	284	82	50
61	350	49	309	88	50	315	90	47	284	81	49
65	353	48	307	87	49	316	89	47	285	81	49
69	360	44	312	87	48	324	90	46	286	79	46
73	363	42	313	86	43	320	88	42	287	79	44
77	379	41	319	84	43	325	86	41	292	77	44
81	379	38	320	85	42	325	86	38	294	78	43
85	383	37	318	83	40	329	86	36	293	77	40
89	396	37	322	81	37	328	83	33	297	75	37
93	404	35	322	80	32	335	83	32	296	73	33
97	390	27	323	83	30	318	82	28	296	76	30
101	380	25	320	84	28	320	84	27	305	80	30
Mean for weeks											
1-13	247		244	99		243	98		240	98	
14-52	313		291	93		294	94		279	89	
53-101	371		315	85		321	87		291	79	

[a] Interim evaluations occurred during weeks 14, 31, and 53; number of survivors includes 17 (Groups 1 and 5) special study animals that were not evaluated as part of the core study.

Constant Ratio Mixture of PCB 126 and PCB 153

Thyroid Hormone Concentrations

Assays for thyroid stimulating hormone (TSH), total triiodothyronine (T_3), total thyroxine (T_4), and free T_4 were conducted at the 14-, 31-, and 53-week interim evaluations. The dose-response effect of administration of the binary PCB mixture was evaluated via comparison of vehicle controls and the constant ratio Groups 2, 3, 5, and 7, which were exposed to a PCB 126:PCB 153 ratio of 1:1,000.

At 14 weeks, serum total T_4 was significantly lower in Groups 5 and 7 than in the vehicle control group (Table 7). Total T_4 concentrations in Groups 5 and 7 were 26.2% and 37.3% lower than in vehicle controls, respectively. Free T_4 was lower in Groups 5 and 7, but not significantly different than vehicle controls. Serum T_3 concentrations were significantly higher in Groups 5 and 7 than in vehicle controls; Groups 5 and 7 were 23.3% and 22.4% higher than vehicle controls, respectively. TSH was significantly higher in all dosed groups than in vehicle controls. The highest increase in TSH was observed in Group 7, which was 61.3% higher than in vehicle controls.

At the 31-week interim evaluation, serum total T_4 was significantly lower in Groups 3, 5, and 7, and free T_4 was significantly lower in Groups 5 and 7 than in vehicle controls. Serum total T_4 and free T_4 were dose-dependently decreased with the maximal reduction observed in Group 7, the highest dose group. In Group 7, total T_4 and free T_4 were 55.8% and 40.5% lower than in vehicle controls, respectively. There was an increasing dose-response trend for serum T_3 concentrations. Serum T_3 was significantly higher in Groups 3, 5, and 7 than in vehicle controls. T_3 concentrations were maximally induced by 40.1% in Group 7 compared to vehicle controls. No significant differences were observed in TSH concentrations between any of the treatment groups and vehicle controls.

At the 53-week interim evaluation, serum total T_4 was significantly lower in Groups 3, 5, and 7 than the vehicle control group. Total T_4 concentration in Group 7 was 40.6% lower than in vehicle controls. Free T_4 was significantly lower (31.4%) in Group 7 than vehicle controls. Serum T_3 was significantly higher in Groups 5 and 7 than vehicle controls. T_3 concentrations were 20.6% and 26.4% higher in Groups 5 and 7, respectively, compared to vehicle controls. TSH concentrations were significantly higher in Group 5 than vehicle controls. No significant differences were observed between any of the other treatment groups and vehicle controls.

TABLE 7
Serum Concentrations of Thyroid Hormones in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg	P Value[b]
Week 14						
n	10	10	10	10	10	
Total T_4 (µg/dL)	3.860 ± 0.173	4.070 ± 0.328	3.740 ± 0.375	2.850 ± 0.172**	2.420 ± 0.244**	<0.001N
Free T_4 (ng/dL)	1.508 ± 0.072	1.461 ± 0.095	1.566 ± 0.212	1.330 ± 0.110	1.093 ± 0.150	0.011N
Total T_3 (ng/dL)	150.8 ± 5.7	141.8 ± 9.4	174.8 ± 11.1	186.0 ± 9.1*	184.6 ± 7.9**	<0.001
TSH (ng/mL)	9.342 ± 0.522[c]	12.02 ± 0.74*	12.85 ± 0.55**[c]	11.22 ± 0.53**	15.07 ± 1.51**	0.004
Week 31						
n	10	10	10	10	10	
Total T_4 (µg/dL)	3.940 ± 0.196	3.670 ± 0.127	3.060 ± 0.163**	2.170 ± 0.102**	1.740 ± 0.229**	<0.001N
Free T_4 (ng/dL)	2.370 ± 0.091	2.172 ± 0.112	2.267 ± 0.096	1.880 ± 0.091**	1.410 ± 0.132**	<0.001N
Total T_3 (ng/dL)	132.4 ± 6.9	136.8 ± 7.7	155.7 ± 7.2*	179.3 ± 6.7**	185.5 ± 12.2**	<0.001
TSH (ng/mL)	12.91 ± 1.31	13.17 ± 0.95	13.20 ± 0.83	12.43 ± 1.07	14.34 ± 1.34	0.572
Week 53						
n	8	8	8	8	8	
Total T_4 (µg/dL	3.200 ± 0.174	3.225 ± 0.135	2.013 ± 0.123**	2.000 ± 0.140**	1.900 ± 0.145**	<0.001N
Free T_4 (ng/dL	1.435 ± 0.052	1.580 ± 0.095	1.280 ± 0.108	1.271 ± 0.067	0.984 ± 0.116**	0.001N
Total T_3 (ng/dL)	128.9 ± 6.5	120.1 ± 5.9	152.1 ± 11.4	155.4 ± 4.1*	162.9 ± 8.3*	<0.001
TSH (ng/mL)	15.73 ± 1.28	15.30 ± 1.38	18.46 ± 1.83	21.35 ± 0.98*	15.11 ± 0.88	0.865

* 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. T_4=thyroxine; T_3=triiodothyronine; TSH=thyroid stimulating hormone
[b] Probability of significant trend by Jonckheere's test. A negative trend is indicated by **N**.
[c] n=9

Hepatic Cell Proliferation Data

Hepatocellular proliferation in groups administered the constant ratio mixture of PCB 126 and PCB 153 was measured at the 14-, 31-, and 53-week interim evaluations. The consumption of the BrdU drinking water solution prior to each interim evaluation was similar across groups (data not shown).

At 14 weeks, no significant differences in hepatocellular labeling index were observed between vehicle controls and the groups administered the PCB mixture (Table 8).

At 31 and 53 weeks, hepatocellular labeling index was significantly higher in Group 7 than in vehicle controls. The labeling index was 3.4- and 12.9-fold higher in Group 7 than in vehicle controls at 31 and 53 weeks, respectively.

Cytochrome P450 Enzyme Activities

At each interim evaluation, liver and lung samples were collected for determinations of P450 enzyme activities. Microsomal suspensions were prepared from liver samples and were assayed for 7-ethoxyresorufin-*O*-deethylase (EROD, CYP1A1) activity, acetanilide-4-hydroxylase (A4H, CYP1A2) activity, and 7-pentoxyresorufin-*O*-deethylase (PROD, CYP2B) activity. Microsomal samples from lung were analyzed for EROD activity only. CYP1A1 and CYP1A2 are known to be inducible by aryl hydrocarbon receptor (AhR) agonists such as PCB 126. CYP2B is known to be induced by di-*ortho* substituted PCBs such as PCB 153.

Hepatic EROD, A4H, and PROD activities were significantly higher in all groups treated with the PCB mixture compared to vehicle controls at all of the interim evaluations (Table 9). In Groups 2, 3, 5, and 7, which were exposed to increasing concentrations of a constant ratio of 1:1,000 of PCB 126:PCB 153, there were increasing trends in hepatic EROD, A4H, and PROD activities with higher doses at all of the interim evaluations. Pulmonary EROD activity was also significantly higher in all groups treated with the PCB mixture compared to vehicle controls at all of the interim evaluations. In the dosed groups, there were increasing trends in pulmonary EROD with higher doses at all of the interim evaluations, with the maximal induction of EROD activity observed in Group 7.

TABLE 8
Hepatic Cell Proliferation Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg	P Value[b]
n						
Week 14	10	10	10	10	10	
Week 31	10	10	10	10	10	
Week 53	8	8	8	8	8	
Labeling index (%)						
Week 14	1.266 ± 0.248	1.194 ± 0.292	1.175 ± 0.088	1.727 ± 0.405	0.707 ± 0.170	0.281N
Week 31	0.942 ± 0.151	0.892 ± 0.145	0.861 ± 0.162	0.736 ± 0.136	3.217 ± 0.895*	0.058
Week 53	0.847 ± 0.088	0.794 ± 0.129	0.987 ± 0.073	0.766 ± 0.189	10.930 ± 1.661**	0.001

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] Probability of significant trend by Jonckheere's test. A negative trend is indicated by **N**.

TABLE 9
Liver and Lung Cytochrome P450 Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg	P Value[b]
n						
Week 14	10	10	10	10	10	
Week 31	10	10	10	10	10	
Week 53	8	8	8	8	8	
Liver microsomes						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Week 14	0.519 ± 0.026	0.586 ± 0.020*	1.728 ± 0.114**	2.548 ± 0.218**	2.474 ± 0.106**	<0.001
Week 31	0.433 ± 0.016	0.572 ± 0.023**	1.792 ± 0.088**	2.850 ± 0.240**	2.308 ± 0.147**	<0.001
Week 53	0.487 ± 0.036	0.647 ± 0.048*	1.952 ± 0.151**	4.387 ± 0.442**	3.461 ± 0.172**	<0.001
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	63.86 ± 2.36	200.77 ± 17.28**	1,905.6 ± 110.52**	2,726.4 ± 167.46**	2,779.5 ± 90.94**	<0.001
Week 31	68.83 ± 3.72	342.76 ± 23.32**	2,028.2 ± 125.46**	2,534.2 ± 81.62**	2,404.0 ± 146.79**	<0.001
Week 53	52.09 ± 3.88	258.56 ± 20.38**	1,859.9 ± 78.77**	3,078.2 ± 350.27**	3,219.5 ± 271.93**	<0.001
7-Pentoxyresorufin-*O*-deethylase (PROD) (pmol/minute per mg microsomal protein)						
Week 14	3.106 ± 0.116	6.232 ± 0.240**	22.871 ± 0.929**	30.552 ± 2.158**	37.729 ± 2.267**	<0.001
Week 31	4.322 ± 0.241	7.568 ± 0.365**	21.503 ± 0.784**	55.040 ± 4.219**	38.347 ± 2.378**	<0.001
Week 53	5.145 ± 0.313	8.991 ± 0.398**	24.797 ± 1.132**	104.628 ± 11.687**	53.409 ± 2.953**	<0.001
Lung microsomes						
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	2.557 ± 0.382[c]	8.505 ± 0.766**	55.773 ± 4.834**	57.351 ± 2.143**	62.796 ± 2.467**	<0.001
Week 31	2.721 ± 0.647	5.933 ± 0.605**	40.491 ± 2.785**	46.340 ± 3.471**	55.883 ± 4.249**	<0.001
Week 53	0.785 ± 0.050[d]	4.381 ± 0.203**	46.240 ± 3.588**	69.889 ± 2.847**	76.646 ± 4.982**	<0.001

* 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] Probability of significant positive trend by Jonckheere's test.
[c] n=9
[d] n=7

Varying Ratio Mixture of PCB 126 and PCB 153

Thyroid Hormone Concentrations

Assays for TSH, T_3, total T_4, and free T_4 were conducted at the 14-, 31-, and 53-week interim evaluations. Potential interactions between PCB 153 and PCB 126 were evaluated via comparison between the varying ratio Groups 4, 5, and 6, all of which were exposed to 300 ng/kg PCB 126 and either 100 (Group 4), 300 (Group 5), or 3,000 (Group 6) µg/kg PCB 153. These doses represent 1:333, 1:1,000, and 1:10,000 ratios of PCB 126:PCB 153 for Groups 4, 5, and 6, respectively.

At 14 weeks, there were no significant differences observed in serum total T_4, free T_4, T_3, or TSH concentrations between Groups 4, 5, and 6 (Table 10).

At 31 weeks, serum total T_4 and free T_4 were significantly lower in Group 6 compared to Groups 4 and 5, which were administered equal doses of PCB 126, but lower doses of PCB 153 than Group 6. With increasing doses of PCB 153, total T_4 and free T_4 were dose-dependently decreased. Total T_4 in Group 6 was 47.9% lower than in Group 5 and 60.2% lower than in Group 4. Similarly, free T_4 in Group 6 was 36.3% lower than in Group 5 and 47.0% lower than in Group 4. TSH was significantly higher in Group 6 than Group 5. There were no significant differences observed in serum T_3 concentrations between the three groups.

At 53 weeks, serum total T_4 and free T_4 were significantly lower in Group 6 compared to Groups 4 and 5. Total T_4 in Group 6 was 60.0% lower than in Group 5 and 48.4% lower than in Group 4. Similarly, free T_4 in Group 6 was 46.2% lower than in Group 5 and 32.9% lower than in Group 4. TSH was significantly higher in Group 5 than Group 4. There were no significant differences observed in serum T_3 concentrations between the three groups.

Hepatic Cell Proliferation Data

Hepatocellular proliferation in groups administered the varying ratio mixture of PCB 126 and PCB 153 was measured at the 14-, 31-, and 53-week interim evaluations. The consumption of the BrdU drinking water solution prior to each interim evaluation was similar across groups (data not shown).

At 53 weeks, the hepatocellular labeling index in Group 6 was 10-fold higher than in vehicle controls (Table 11). The significant increase in labeling index observed in Group 6, which received 300 ng/kg PCB 126 and 3,000 µg/kg PCB 153, was not observed in Groups 4 or 5, which received the same dose of PCB 126, but lesser doses (100 and 300 µg/kg, respectively) of PCB 153. The hepatocellular labeling index in Group 6 was significantly higher than that in Groups 4 and 5.

TABLE 10
Serum Concentrations of Thyroid Hormones in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg	P Value[b]
Week 14					
n	10	10	10	10	
Total T_4 (µg/dL)	3.860 ± 0.173	2.910 ± 0.375	2.850 ± 0.172	2.260 ± 0.184	0.1641N
Free T_4 (ng/dL)	1.508 ± 0.072	1.260 ± 0.176	1.330 ± 0.110	1.137 ± 0.082	0.9091N
Total T_3 (ng/dL)	150.8 ± 5.7	171.3 ± 9.4	186.0 ± 9.1	182.7 ± 8.2	0.2702
TSH (ng/mL)	9.342 ± 0.522[c]	13.771 ± 1.430[c]	11.220 ± 0.526	12.676 ± 0.784	0.7039N
Week 31					
n	10	10	10	10	
Total T_4 (µg/dL)	3.940 ± 0.196	2.840 ± 0.213▲▲	2.170 ± 0.102▲	1.130 ± 0.218▲,▲▲	<0.0001N
Free T_4 (ng/dL)	2.370 ± 0.091	2.259 ± 0.220▲▲	1.880 ± 0.091▲	1.197 ± 0.136▲,▲▲	<0.0001N
Total T_3 (ng/dL)	132.4 ± 6.9	188.4 ± 11.8	179.3 ± 6.7	151.2 ± 10.3	0.0166N
TSH (ng/mL)	12.91 ± 1.31	12.63 ± 0.83	12.43 ± 1.07▲	15.80 ± 1.09▲	0.0400
Week 53					
n	8	10	8	9	
Total T_4 (µg/dL)	3.200 ± 0.174	1.550 ± 0.108▲	2.000 ± 0.140▲▲	0.800 ± 0.133▲,▲▲	0.0271N
Free T_4 (ng/dL)	1.435 ± 0.052	1.020 ± 0.079▲	1.271 ± 0.067▲▲	0.684 ± 0.059▲,▲▲	0.0344N
Total T_3 (ng/dL)	128.9 ± 6.5	152.9 ± 7.5	155.4 ± 4.1	142.6 ± 6.4	0.2471N
TSH (ng/mL)	15.73 ± 1.28	14.21 ± 1.56▲▲	21.35 ± 0.98▲▲	15.38 ± 1.02[d]	0.3499

▲ For pairwise comparisons of Groups 4, 5, and 6, means that are in the same row and share this symbol are significantly different ($P \leq 0.05$) from each other by Dunn's test.
▲▲ $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
T_4=thyroxine; T_3=triiodothyronine; TSH=thyroid stimulating hormone
[b] Probability of significant trend by Jonckheere's test; the vehicle control group is excluded from the trend test. A negative trend is indicated by **N**.
[c] n=9
[d] n=8

TABLE 11
Hepatic Cell Proliferation Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg	P Value[b]
n					
Week 14	10	10	10	10	
Week 31	10	10	10	10	
Week 53	8	10	8	9	
Labeling index (%)					
Week 14	1.266 ± 0.248	1.066 ± 0.127	1.727 ± 0.405	0.890 ± 0.178	0.4937
Week 31	0.942 ± 0.151	1.083 ± 0.157	0.736 ± 0.136	3.323 ± 1.615	0.5684
Week 53	0.847 ± 0.088	0.862 ± 0.188▲▲	0.766 ± 0.189●●	8.720 ± 1.568▲▲,●●	0.0033

▲▲,●● For pairwise comparisons of Groups 4, 5, and 6, means that are in the same row and share these symbols are significantly different ($P \leq 0.01$) from each other by Dunn's test.
a Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
b Probability of significant positive trend by Jonckheere's test; the vehicle control group is excluded from the trend test.

Cytochrome P450 Enzyme Activities

At each interim evaluation, liver and lung samples were collected for determinations of P450 enzyme activities. Microsomal suspensions were prepared from liver samples and were assayed for EROD, A4H, and PROD activities. Microsomal samples from lung were analyzed for EROD activity only.

In Groups 4, 5, and 6, hepatic EROD, PROD, and A4H activities at 14 weeks were higher in groups receiving a greater proportion of PCB 153 in the PCB mixture (Table 12). For liver enzyme activity, an increase in the PCB 153 component of the mixture resulted in an increase in activity.

At 31 weeks, no significant differences in EROD activities were observed between Groups 4, 5, and 6. PROD activity was significantly higher in Group 5 than in Groups 4 and 6. Hepatic PROD activity was not significantly different between Groups 4 and 6. Hepatic A4H activity was lower in Group 6 than in Group 5, but not significantly different than Group 4.

At 53 weeks, EROD activity in Group 6 was significantly lower than in Group 5. EROD activity was 44- and 59-fold higher than vehicle controls in Groups 4 and 5, respectively, whereas EROD activity in Group 6 was 19-fold higher than vehicle controls. Hepatic PROD activity was similar in Groups 4 and 6 (approximately a 5-fold induction above vehicle controls). However, hepatic PROD activity in Group 5 was significantly higher than in Groups 4 and 6 and was induced 20-fold greater than in vehicle controls.

TABLE 12
Liver and Lung Cytochrome P450 Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg	P Value[b]
n					
Week 14	10	10	10	10	
Week 31	10	10	10	10	
Week 53	8	10	8	9	
Liver microsomes					
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)					
Week 14	0.519 ± 0.026	2.456 ± 0.230▲▲	2.548 ± 0.218▲	3.751 ± 0.214▲,▲▲	0.0021
Week 31	0.433 ± 0.016	2.387 ± 0.133	2.850 ± 0.240▲▲	1.431 ± 0.275▲▲	0.0365N
Week 53	0.487 ± 0.036	2.959 ± 0.115	4.387 ± 0.442▲▲	1.791 ± 0.302▲▲	0.1543N
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)					
Week 14	63.86 ± 2.36	2,514.3 ± 119.22▲▲	2,726.4 ± 167.46●●	3,744.9 ± 150.50▲▲,●●	<0.0001
Week 31	68.83 ± 3.72	2,453.6 ± 137.05	2,534.2 ± 81.62	1,942.4 ± 399.75	0.2385N
Week 53	52.09 ± 3.88	2,308.6 ± 113.05	3,078.2 ± 350.27▲▲	995.61 ± 319.31▲▲	0.0680N
7-Pentoxyresorufin-*O*-deethylase (PROD) (pmol/minute per mg microsomal protein)					
Week 14	3.106 ± 0.116	25.302 ± 1.295▲▲	30.552 ± 2.158●●	71.299 ± 4.155▲▲,●●	<0.0001
Week 31	4.322 ± 0.241	25.349 ± 1.094▲▲	55.040 ± 4.219▲▲,●●	29.615 ± 6.209●●	0.5945
Week 53	5.145 ± 0.313	28.793 ± 1.074▲	104.628 ± 11.687▲,▲▲	27.389 ± 6.544▲▲	0.3977N
Lung microsomes					
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)					
Week 14	2.557 ± 0.382[c]	60.356 ± 3.116	57.351 ± 2.143	54.227 ± 3.798	0.0624N
Week 31	2.721 ± 0.647	45.198 ± 2.499	46.340 ± 3.471	48.457 ± 2.850	0.4470
Week 53	0.785 ± 0.050[d]	71.987 ± 3.973	69.889 ± 2.847	80.661 ± 6.288	0.2126

▲ For pairwise comparisons of Groups 4, 5, and 6, means that are in the same row and share this symbol are significantly different (P≤0.05) from each other by Dunn's test.
▲▲,●● (P≤0.01)
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] Probability of significant trend by Jonckheere's test; the vehicle control group is excluded from the trend test. A negative trend is indicated by **N**.
[c] n=9
[d] n=7

Determinations of PCB 126 and PCB 153 Concentrations in Tissues

Concentrations of PCB 126 and PCB 153 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).

PCB 126 concentrations were below the limit of detection in all tissues of vehicle control animals (Table 13). In the constant ratio dose groups (Groups 2, 3, 5, and 7), the highest concentrations of PCB 126 were observed in the liver and fat, with lower levels detectable in the lung and blood. In general, tissue concentrations of PCB 126 in the constant ratio groups increased with increasing dose and duration of exposure to the PCB mixture with the highest concentrations being observed in Group 7. In this group, the highest concentrations in the liver, fat, and blood were observed at 53 weeks of exposure,

TABLE 13
Tissue Concentrations of PCB 126 in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Week 105	10	10	10	10	10
Fat					
Week 14	BLOQ	3 ± 0	17 ± 1	36 ± 1	98 ± 3
Week 31	BLOQ	5 ± 0	26 ± 1	58 ± 2	126 ± 5
Week 53	BLOQ	5 ± 0	25 ± 1	44 ± 6	136 ± 6
Week 105	BLOQ	9 ± 1	25 ± 3	56 ± 4	66 ± 8
Liver					
Week 14	BLOQ	2 ± 0	43 ± 4	130 ± 11	208 ± 17
Week 31	BLOQ	4 ± 1	59 ± 5	178 ± 9	429 ± 16
Week 53	BLOQ	6 ± 0	74 ± 3	209 ± 6[b]	605 ± 19
Week 105	BLOQ	10 ± 2	74 ± 5	202 ± 9[c]	290 ± 28
Lung					
Week 14	BLOQ	BLOQ	234 ± 55[d]	233 ± 37[e]	1,483 ± 260
Week 31	BLOQ	BLOQ	140 ± 11[c]	236 ± 21	439 ± 23[c]
Week 53	BLOQ	101[f]	113[f]	243 ± 19	398 ± 53
Week 105	BLOQ	206 ± 25[g]	492 ± 131[h]	459 ± 83[c]	479 ± 57
Blood					
Week 14	BLOQ	BLOQ	46 ± 3	95 ± 3	290 ± 19
Week 31	BLOQ	11 ± 1	66 ± 2	139 ± 3	372 ± 27
Week 53	BLOQ	13 ± 1	82 ± 9	143 ± 19	476 ± 49[b]
Week 105	BLOQ	30 ± 3	96 ± 3	196 ± 12	417 ± 56

[a] Data are given in ng/g tissue (fat, liver) or pg/g (lung, blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation. BLOQ=below the limit of quantitation; LOQ_{fat}=0.4 ng/g, LOQ_{liver}=0.4 ng/g, LOQ_{lung}=100 pg/g, LOQ_{blood}=10 pg/g
[b] n=7
[c] n=9
[d] n=5
[e] n=3
[f] n=1, standard error not calculated
[g] n=6
[h] n=8

whereas peak levels in the lung were observed at the 14-week interim time point.

Tissue concentrations of PCB 126 in the varying PCB 126:153 ratio groups (Groups 4, 5, and 6) showed a similar pattern of exposure with the highest concentrations being observed in the liver and fat, with lower levels detectable in the lung and blood (Table 14). To test for a potential interaction between PCB 153 on the tissue disposition of PCB 126, trend tests were conducted on data from these groups. In the liver, there was a significant negative interaction between PCB 153 and PCB 126 at the 31-, 53-, and 105-week time points, with lower concentrations of PCB 126 being observed in the liver with increasing proportions of PCB 153 in the mixture. In the fat of exposed animals, there was a significant

TABLE 14
Statistical Comparisons of PCB 126 Tissue Concentrations in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg	P-Value[b]
n				
Week 14	10	10	10	
Week 31	10	10	10	
Week 53	10	8	9	
Week 105	10	10	10	
Fat				
Week 14	41 ± 2▲	36 ± 1▲▲	49 ± 3▲,▲▲	0.07
Week 31	59 ± 3▲▲	58 ± 2●●	75 ± 4▲▲,●●	<0.01
Week 53	52 ± 2	44 ± 6	48 ± 4	0.15N
Week 105	57 ± 3	56 ± 4	63 ± 4	0.3
Liver				
Week 14	94 ± 10	130 ± 11	79 ± 10	0.36N
Week 31	185 ± 12▲	178 ± 9●	136 ± 14▲,●	0.013N
Week 53	220 ± 5▲▲	209 ± 6●●[c]	156 ± 17▲▲,●●	0.01N
Week 105	232 ± 17▲▲	202 ± 9●●[d]	125 ± 13▲▲,●●	<0.01N
Lung				
Week 14	234 ± 22[d]	233 ± 37[e]	528 ± 173[c]	0.47
Week 31	183 ± 19	236 ± 21	257 ± 32[d]	0.03
Week 53	280 ± 23	243 ± 19	209 ± 28	0.05N
Week 105	902 ± 111▲,●	459 ± 83▲[d]	478 ± 104●[d]	<0.01N
Blood				
Week 14	100 ± 5	95 ± 3▲	118 ± 7▲	0.07
Week 31	143 ± 6▲▲	139 ± 3●●	178 ± 9▲▲,●●	0.02
Week 53	139 ± 12▲▲	143 ± 19●●	240 ± 16▲▲,●●	<0.01
Week 105	175 ± 8▲▲	196 ± 12●●	325 ± 34▲▲,●●	<0.01

▲,● For pairwise comparisons of Groups 4, 5, and 6, means that are in the same row and share symbols are significantly different (P≤0.05) from each other by Scheffé's test.
▲▲,●● P≤0.01
a Data are given in ng/g tissue (fat, liver) or pg/g (lung, blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation. BLOQ=below the limit of quantitation; LOQ_{fat}=0.4 ng/g, LOQ_{liver}=0.4 ng/g, LOQ_{lung}=100 pg/g, LOQ_{blood}=10 pg/g
b Probability of significant trend by Jonckheere's test. For this analysis, log transformation was used to bring the tissue concentration data into closer conformance with normality assumptions. A negative trend is indicated by **N**.
c n=7
d n=9
e n=3

positive interaction of PCB 153 on PCB 126 levels at the 31-week time point, with higher levels being observed in the fat with higher proportions of PCB 153 in the mixture. A similar effect was seen in the blood at the 31-, 53-, and 105-week time points. In the lung, there was a marginally positive interaction at the 31-week time point and negative interaction at the 53- and 105-week time points.

PCB 153 was detectable in the fat of all vehicle control animals, albeit at levels at least 10-fold lower than those seen in animals exposed to the lowest dose of PCB 153

used in the mixture study (Table 15). This is consistent with the known presence of PCB 153 in rodent diet (Table E5). Similarly, PCB 153 was detected in the liver, lung, and blood of vehicle control animals at the later time points. Levels in vehicle control animals generally increased with length of time on study. In the constant ratio dose groups (Groups 2, 3, 5, and 7), the highest concentrations of PCB 153 were observed in the fat, with lower levels detectable in the liver, lung, and blood. In general, tissue concentrations of PCB 153 in the constant ratio groups increased with increasing dose and duration of exposure of the PCB mixture, with the highest concentrations being observed in Group 7 at the end of the 2-year study. Mean concentrations in the fat of dosed animals in this group at 105 weeks were 16-fold higher than those seen in the liver, and 95-fold higher than those in the lung. This pattern of distribution and accumulation is consistent with the known high lipophilicity of PCB 153.

In the varying ratio groups (Groups 4, 5, and 6), as expected, the highest concentrations of PCB 153 were also observed in the fat, with lower levels detectable in the liver, lung, and blood (Table 15). Tissue concentrations of PCB 153 increased with increasing proportions of PCB 153 in the mixture and duration of exposure. The highest concentrations of PCB 153 in the study were seen in tissues from Group 6 exposed to 3,000 µg/kg PCB 153, at the end of the 2-year study.

TABLE 15
Tissue Concentrations of PCB 153 in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Week 105	10	10	10	10	10
Fat					
Week 14	299 ± 35[b]	3,117 ± 161	27,080 ± 1,666	92,840 ± 6,303	307,900 ± 18,569
Week 31	256 ± 12[c]	6,657 ± 339	59,930 ± 2,870	201,000 ± 8,406	437,100 ± 14,241
Week 53	209 ± 2[d]	6,424 ± 415	54,088 ± 5,203	186,013 ± 35,995	681,500 ± 67,251
Week 105	801 ± 142	23,024 ± 3,467	134,580 ± 17,273	414,800 ± 15,937	1,553,000 ± 66,149
Liver					
Week 14	BLOQ	BLOQ	1,046 ± 84	3,663 ± 385	10,701 ± 1,849
Week 31	BLOQ	BLOQ	940 ± 96	5,458 ± 687	34,460 ± 4,728
Week 53	BLOQ	247 ± 24[d]	2,161 ± 150	13,014 ± 1,879	59,450 ± 7,990
Week 105	309 ± 9	724 ± 65	4,688 ± 564	25,700 ± 2,190	94,080 ± 10,711
Lung					
Week 14	51 ± 0[d]	124 ± 15[e]	418 ± 95	333 ± 86	6,281 ± 1,376
Week 31	101 ± 9[d]	71 ± 5	502 ± 47	900 ± 105	2,224 ± 153[e]
Week 53	BLOQ	105 ± 6[f]	212 ± 35	1,056 ± 44	2,037 ± 139[g]
Week 105	121 ± 7	504 ± 87[e]	1,922 ± 236[e]	5,217 ± 921[e]	16,308 ± 2,108
Blood					
Week 14	BLOQ	5 ± 0	55 ± 4	176 ± 12	606 ± 36
Week 31	BLOQ	10 ± 1	104 ± 6	394 ± 18	1,542 ± 168
Week 53	BLOQ	13 ± 1	183 ± 18	528 ± 36	3,213 ± 301
Week 105	4 ± 0	57 ± 9	348 ± 57[e]	1,663 ± 188	11,020 ± 664[e]

TABLE 15
Tissue Concentrations of PCB 153 in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
n			
Week 14	10	10	10
Week 31	10	10	10
Week 53	10	8	9
Week 105	10	10	10
Fat			
Week 14	30,000 ± 1,495	92,840 ± 6,303	1,118,300 ± 49,947
Week 31	67,490 ± 4,306	201,000 ± 8,406	1,855,556 ± 155,484[e]
Week 53	41,250 ± 4,882	186,013 ± 35,995	1,824,556 ± 189,588
Week 105	161,450 ± 14,407	414,800 ± 15,937	5,068,000 ± 630,854
Liver			
Week 14	1,087 ± 160	3,663 ± 385	34,010 ± 5,837
Week 31	1,677 ± 172	5,458 ± 687	107,600 ± 21,205
Week 53	2,705 ± 171	13,014 ± 1,879	125,189 ± 10,293
Week 105	7,908 ± 675	25,700 ± 2,190	290,100 ± 30,863
Lung			
Week 14	242 ± 27	333 ± 86	14,323 ± 4,835[h]
Week 31	238 ± 25	900 ± 105	7,512 ± 1,027[e]
Week 53	552 ± 63	1,056 ± 44	5,051 ± 533
Week 105	3,842 ± 519	5,217 ± 921[e]	67,510 ± 16,978
Blood			
Week 14	55 ± 5	176 ± 12	1,788 ± 158
Week 31	138 ± 4	394 ± 18	3,694 ± 161[e]
Week 53	195 ± 13	528 ± 36	6,535 ± 547
Week 105	573 ± 61	1,663 ± 188	35,310 ± 4,971

[a] Data are given in ng/g tissue as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation. BLOQ=below the limit of quantitation; LOQ_{fat}=200 ng/g, LOQ_{liver}=200 ng/g, LOQ_{lung}=50 ng/g, LOQ_{blood}=2 ng/g
[b] n=5
[c] n=4
[d] n=2
[e] n=9
[f] n=3
[g] n=7
[h] n=8

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the liver, lung, oral mucosa, pancreas, uterus, adrenal cortex, thyroid gland, thymus, kidney, nose, bone marrow, forestomach, mandibular lymph node, mammary gland, ovary, and pituitary gland (pars distalis). Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for Groups 1, 2, 3, 5, and 7 and in Appendix B for Groups 4, 5, and 6.

Constant Ratio Mixture of PCB 126 and PCB 153

Liver: At 14, 31, and 53 weeks, the absolute and relative liver weights of all dosed groups were significantly greater than those of vehicle controls except Group 2 at 53 weeks and absolute liver weights in Group 3 at 31 weeks (Table C1).

At 14 weeks, the incidences of several nonneoplastic lesions were increased compared to the vehicle controls (Tables 16 and A5a). The incidences of minimal to mild hepatocytic hypertrophy were significantly increased in most dosed groups. Significantly increased incidences of multinucleated hepatocytes (minimal) occurred in

TABLE 16
Incidences of Nonneoplastic Lesions of the Liver in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
14-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Hepatocyte, Hypertrophy[a]	0	4* (1.0)[b]	3 (1.0)	6** (1.0)	10** (2.2)
Hepatocyte, Multinucleated	0	0	0	0	7** (1.0)
Pigmentation	0	0	4* (1.0)	5* (1.0)	8** (1.0)
Fatty Change, Diffuse	0	0	0	1 (1.0)	6** (1.2)
31-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Hepatocyte, Hypertrophy	0	3 (1.0)	5* (1.0)	10** (1.0)	10** (3.2)
Hepatocyte, Multinucleated	0	0	0	0	9** (1.2)
Pigmentation	0	0	3 (1.0)	10** (1.0)	10** (1.2)
Fatty Change, Diffuse	0	0	0	1 (1.0)	10** (2.1)
Toxic Hepatopathy	0	0	0	0	5* (1.0)
53-Week Interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Hepatocyte, Hypertrophy	0	2 (1.0)	2 (1.0)	8** (1.0)	8** (3.3)
Hepatocyte, Multinucleated	0	0	0	2 (1.0)	8** (1.9)
Pigmentation	0	1 (1.0)	6** (1.0)	8** (1.4)	8** (2.1)
Fatty Change, Diffuse	0	0	0	3 (1.0)	8** (1.5)
Bile Duct, Hyperplasia	0	0	0	0	8** (1.8)
Eosinophilic Focus (includes multiple)	0	0	0	1	7**
Oval Cell, Hyperplasia	0	0	0	0	4* (1.3)
Toxic Hepatopathy	0	0	0	0	8** (1.6)
Cholangiofibrosis	0	0	0	0	1 (3.0)

* 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

Group 7. The incidences of pigmentation were significantly increased in Groups 3, 5, and 7, and the incidence of diffuse fatty change was significantly increased in Group 7.

At 31 weeks, significantly increased incidences of hepatocytic hypertrophy occurred and the severity was increased in Group 7 (Tables 16 and A5a). Significantly increased incidences of pigmentation occurred in Groups 5 and 7. Incidences of multinucleated hepatocytes, diffuse fatty change, and toxic hepatopathy were significantly increased in Group 7.

In Groups 5 and 7 at 53 weeks, hepatocytic hypertrophy occurred in all rats and the severity was increased in Group 7; multinucleated hepatocytes occurred in all rats in Group 7 (Tables 16 and A5a). The incidences of pigmentation were significantly increased in Groups 3, 5, and 7, and the severity of this lesion increased with increasing dose. Increased incidences of eosinophilic focus (single or multiple), diffuse fatty change, bile duct hyperplasia, oval cell hyperplasia, and toxic hepatopathy were significantly increased in Group 7. One animal in Group 7 had cholangiofibrosis.

At 2 years, the incidences of hepatocellular adenoma (single or multiple) and cholangiocarcinoma (single or multiple) in Groups 5 and 7 were significantly increased (Tables 17, A1b, and A3). The incidence of hepatocholangioma was significantly increased in Group 7. The incidences of these lesions in Groups 3, 5, and 7 generally exceeded the historical vehicle control ranges (Tables 17 and A4a). Two animals in Group 7 had hepatocellular carcinoma; no hepatocellular carcinomas have been seen in the historical vehicle controls (Tables 17 and A4a). The incidences of cholangiofibrosis were significantly increased in Groups 5 and 7 (Tables 17 and A5b).

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 (Plates 1 and 2). Adenoma was composed of a rather uniform population of mildly to moderately pleomorphic hepatocytes that generally were normal size or slightly larger than normal and were arranged in abnormal lobular patterns. The hepatic cords within an adenoma usually intersected the surrounding normal hepatic cords at an oblique angle or sometimes even at a right angle. A few small proliferating bile ducts or oval cells were sometimes seen but were not as numerous as in nodular hyperplasia. The uniform population of hepatocytes and lack of proliferating bile ducts were important features differentiating adenoma from nodular hyperplasia.

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.

Cholangiocarcinoma consisted of an irregular, relatively large, noncircumscribed lesion that replaced normal liver parenchyma. The lesion consisted of fibrous connective tissue stroma containing numerous atypical bile ducts, which frequently contained mucinous material and cellular debris. The epithelium forming the atypical bile ducts was often discontinuous, consisted of large atypical cells, and displayed degenerative changes. Mitotic figures and localized invasion of adjacent liver parenchyma were also observed (Plate 3). Cholangiofibrosis appeared relatively small in size and well demarcated and did not show invasion (Plate 4).

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.

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Number Examined Microscopically	53	53	52	52	51
Hepatocyte, Hypertrophy[a]	1 (1.0)[b]	7* (1.0)	17** (1.4)	33** (2.1)	50** (3.3)
Hepatocyte, Multinucleated	0	0	14** (1.4)	46** (1.6)	48** (1.9)
Pigmentation	2 (1.0)	5 (1.2)	38** (1.3)	50** (1.9)	50** (2.0)
Fatty Change, Diffuse	3 (1.7)	1 (1.0)	9 (1.7)	31** (1.5)	38** (1.6)
Fatty Change, Focal	3 (1.0)	4 (1.0)	7 (1.1)	1 (2.0)	12** (1.9)
Eosinophilic Focus (includes multiple)	14	16	30**	40**	18
Toxic Hepatopathy	0	2 (1.0)	34** (1.2)	48** (2.0)	49** (3.5)
Bile Duct, Cyst	4 (2.3)	3 (2.3)	1 (2.0)	5 (2.4)	23** (2.4)
Bile Duct, Hyperplasia	8 (1.3)	2 (1.5)	9 (1.6)	29** (1.4)	46** (2.0)
Necrosis	4 (1.8)	8 (2.0)	5 (2.0)	4 (1.0)	20** (2.3)
Oval Cell, Hyperplasia	2 (1.0)	2 (1.0)	15** (1.3)	39** (1.6)	46** (2.9)
Portal Fibrosis	0	0	0	7** (1.4)	34** (2.3)
Hyperplasia, Nodular	0	0	2	24**	42**
Cholangiofibrosis	0	1 (1.0)	0	7** (2.0)	39** (3.2)
Hepatocholangioma (includes multiple)[c]					
Overall rate[d]	0/53 (0%)	0/53 (0%)	0/52 (0%)	2/52 (4%)	6/51 (12%)
Adjusted rate[e]	0.0%	0.0%	0.0%	5.4%	16.6%
Terminal rate[f]	0/22 (0%)	0/21 (0%)	0/22 (0%)	2/24 (8%)	6/24 (25%)
First incidence (days)	—[h]	—	—	729 (T)	729 (T)
Poly-3 test[g]	P<0.001	—[i]	—	P=0.232	P=0.012
Hepatocellular Adenoma, Multiple	0	0	0	0	16**
Hepatocellular Adenoma (includes multiple)[j]					
Overall rate	0/53 (0%)	0/53 (0%)	3/52 (6%)	5/52 (10%)	27/51 (53%)
Adjusted rate	0.0%	0.0%	7.7%	13.3%	67.7%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	4/24 (17%)	18/24 (75%)
First incidence (days)	—	—	654	684	479
Poly-3 test	P<0.001	—	P=0.122	P=0.028	P<0.001
Hepatocellular Carcinoma[c]	0	0	0	0	2
Cholangiocarcinoma, Multiple	0	0	1	5*	21**
Cholangiocarcinoma (includes multiple)[c]					
Overall rate	0/53 (0%)	0/53 (0%)	1/52 (2%)	9/52 (17%)	30/51 (59%)
Adjusted rate	0.0%	0.0%	2.6%	23.7%	75.5%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	7/24 (29%)	20/24 (83%)
First incidence (days)	—	—	729 (T)	603	479
Poly-3 test	P<0.001	—	P=0.503	P<0.001	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal 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: 0/371
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. 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.
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed.
[j] Historical incidence (mean ± standard deviation): 4/371 (1.1% ± 1.5%), range 0%-4%

At 2 years, the incidences of minimal to moderate hepatocyte hypertrophy, multinucleated hepatocytes, pigmentation, toxic hepatopathy, and oval cell hyperplasia were significantly increased in Groups 3, 5, and 7; hepatocyte hypertrophy was also significantly increased in Group 2 (Tables 17 and A5b). The incidences of diffuse fatty change, bile duct hyperplasia, portal fibrosis, and nodular hyperplasia were significantly increased in Groups 5 and 7. Increased incidences of eosinophilic focus (single or multiple) occurred in Groups 3 and 5. Increased incidences of focal fatty change, bile duct cyst, and hepatocellular necrosis occurred in Group 7.

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 nuclei (more than 2 and often 4 to 6). The presence of binucleated hepatocytes was not sufficient to make this diagnosis.

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

Eosinophilic foci were characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic focus was composed of cells with eosinophilic cytoplasm. 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. If two or more foci of a given type were present in a liver, it was diagnosed as multiple. The treatment-related foci were eosinophilic, and often differed somewhat from those in vehicle control animals. Foci in vehicle control animals consisted of hepatocytes that were generally somewhat larger than normal but appeared otherwise normal and were arranged in a relatively normal lobular pattern. The hepatic cords at the periphery of these foci generally merged imperceptibly with the surrounding normal liver resulting in an indistinct border and little or no compression of the adjacent liver parenchyma. In contrast, foci in treated animals often had a more definite border, the cords within the focus often were not smoothly continuous with those in the surrounding parenchyma, and the foci consisted of cells that were often prominently enlarged with abundant eosinophilic or clear vacuolated cytoplasm. If more than 20% of the cells were vacuolated, the focus was classified as mixed cell type, otherwise it was classified as an eosinophilic focus. In addition, some larger foci caused varying degrees of compression of the surrounding hepatic parenchyma. The cells were arranged in a relatively normal lobular pattern and foci sometimes contained large blood vessels and/or portal areas.

Necrosis consisted of scattered necrotic areas of hepatic parenchyma that were distributed randomly, or, in more severe cases, diffusely. Focal or diffuse fatty change was generally a minimal to mild change consisting of discrete clear vacuoles (consistent with lipid) in the cytoplasm of hepatocytes and involving either foci of hepatocytes (focal fatty change) or scattered diffusely throughout the liver (diffuse fatty change).

Bile duct hyperplasia consisted of increased numbers of bile duct nuclei within portal areas. Oval cell hyperplasia consisted of small ovoid cells with basophilic cytoplasm and 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. Portal fibrosis consisted of fibrous connective tissue accumulation that extended between adjacent portal areas.

Nodular hyperplasia was characterized by few to numerous, small to large, nodular foci generally composed of hepatocytes that were considerably larger than normal hepatocytes (hepatocytic hypertrophy) sometimes mixed with areas of increased numbers of small hepatocytes (hepatocytic hyperplasia) (Plates 5 and 6). Areas of nodular hyperplasia blended with the surrounding parenchyma, although often they had a distinct border. Large, focal to multifocal areas of nodular hyperplasia were sometimes seen that caused compression of surrounding tissue, and/or bulging of the capsular surface. 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 varying degrees of cytoplasmic vacuolization. In a few areas of nodular hyperplasia, however, the cells were of more normal size or sometimes slightly smaller than normal. The cells

appeared to be arranged in normal cords, but the cells often were so large as to obscure the sinusoids between the cords giving the appearance of solid sheets of hepatocytes. Bile duct hyperplasia and portal areas were usually present within nodular hyperplasia. Blood vessels and/or central veins were also sometimes seen within areas of nodular hyperplasia, usually when hepatocytes were not so hypertrophic as to obscure completely the normal architecture. The presence of hypertrophic, vacuolated hepatocytes together with proliferating bile ducts was considered to be characteristic of nodular hyperplasia and was considered to be useful in the diagnosis of nodular hyperplasia. Since this lesion is included as part of toxic hepatopathy, which is graded, there was no need to grade the severity of nodular hyperplasia.

Toxic hepatopathy included all nonneoplastic liver changes under one overall term. The severity of toxic hepatopathy was graded in order to give one overall severity grade for the degree of toxicity in a liver. This was to allow for easier comparison of the degree of toxic change among different dosed groups than would be possible if the severities of all the individual nonneoplastic changes were compared among the different groups. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. The changes included under the diagnosis were hepatocyte hypertrophy, pigmentation, inflammation, multinucleated hepatocytes, diffuse fatty change, bile duct hyperplasia, oval cell hyperplasia, nodular hyperplasia, focal cellular alteration, cholangiofibrosis, bile duct cyst, necrosis, portal fibrosis, and centrilobular degeneration. Some dosed animals occasionally had just a few of these changes present but this was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy.

Lung: At 2 years, incidences of cystic keratinizing epithelioma (single or multiple) occurred in Groups 5 and 7, and the increase was significant in Group 7 (Tables 18, A1b, and A3). One incidence of squamous cell carcinoma occurred in Groups 5 and 7. These lesions have not been seen in historical vehicle controls (Tables 18 and A4b).

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 irregular walls of highly keratinized stratified squamous epithelium and a center filled with keratin (Plates 7 and 8). The outer portion of the lesion grew by expansion into the adjacent lung but evidence of invasion was not observed. Squamous cell carcinoma was composed of numerous irregular clusters and cords of keratinizing stratified squamous epithelium with a scant to modest amount of dense fibrous tissue stroma. Localized invasive growth into the adjacent lung was present. Squamous cell carcinoma was distinguished from cystic keratinzing epithelioma by the presence of areas of solid growth and evidence of invasion into the surrounding lung parenchyma.

At 2 years, dose-related increased incidences and severities of alveolar squamous metaplasia occurred in Groups 3, 5, and 7 (Tables 18 and A5b). Significantly increased incidences of alveolar epithelium bronchiolar metaplasia occurred in all dosed groups, with the greatest increase in Group 5. Significantly decreased incidences of alveolar epithelial hyperplasia occurred in Groups 5 and 7; this was not considered dose related.

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. The lesion generally diffusely affected the epithelium located at the bronchiolar-alveolar junction and adjacent alveoli. Aggregates of large alveolar macrophages were sometimes present in areas of bronchiolar metaplasia. This change was differentiated from alveolar epithelial hyperplasia. In alveolar epithelial hyperplasia, alveoli were lined by bronchiolar epithelium and unlike bronchiolar metaplasia in treated animals, prominent mucus production was not observed in alveolar epithelial hyperplasia. Very prominent inflammatory cell infiltrate, consisting of large aggregates of alveolar macrophages commonly mixed with focal aggregates of neutrophils, was usually associated with the affected areas. Squamous metaplasia of the alveolar epithelium was generally a minor change consisting of one or more small, irregular foci of keratinizing stratified squamous epithelium that had replaced the normal alveolar epithelium.

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Number Examined Microscopically	53	53	52	53	52
Metaplasia, Squamous[a]	0	0	1 (1.0)[b]	2 (1.5)	11** (2.1)
Alveolar Epithelial, Hyperplasia	23 (1.2)	20 (1.4)	17 (1.1)	5** (1.4)	5** (1.4)
Alveolar Epithelium, Metaplasia, Bronchiolar	0	6* (1.5)	23** (1.4)	34** (1.7)	32** (1.9)
Cystic Keratinizing Epithelioma, Multiple	0	0	0	0	8**
Cystic Keratinizing Epithelioma (includes multiple)[c]					
Overall rate[d]	0/53 (0%)	0/53 (0%)	0/52 (0%)	1/53 (2%)	11/52 (21%)
Adjusted rate[e]	0.0%	0.0%	0.0%	2.7%	29.4%
Terminal rate[f]	0/22 (0%)	0/21 (0%)	0/22 (0%)	1/24 (4%)	7/24 (29%)
First incidence (days)	—[h]	—	—	729 (T)	606
Poly-3 test[g]	P<0.001	—[i]	—	P=0.496	P<0.001
Squamous Cell Carcinoma[c]	0	0	0	1	1

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤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: 0/371
[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. 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.
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed.

Oral Mucosa (Gingival): At 2 years, significantly increased incidences of squamous cell carcinoma of the oral mucosa occurred in Groups 5 and 7 (Tables 19, A1b, and A3). The incidences that occurred in Groups 3, 5, and 7 exceeded the historical vehicle control range (Tables 19 and A4c).

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

At 2 years, dose-related increased incidences of squamous cell hyperplasia occurred in Groups 3, 5, and 7 (Tables 19 and A5b). Increased incidences of inflammation of the periodontal tissue occurred in Groups 3, 5, and 7.

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

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Oral Mucosa in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Oral Mucosa[a]	12	11	25	30	36
Gingival, Hyperplasia, Squamous[b]	8 (1.3)[c]	8 (1.0)	18 (1.6)	22 (1.6)	24 (1.8)
Squamous Cell Carcinoma (Gingival) (includes multiple)[d]					
Overall rate[e]	0/53 (0%)	0/53 (0%)	2/53 (4%)	5/53 (9%)	9/53 (17%)
Adjusted rate[f]	0.0%	0.0%	5.0%	12.9%	22.7%
Terminal rate[g]	0/22 (0%)	0/21 (0%)	0/22 (0%)	1/24 (4%)	0/24 (0%)
First incidence (days)	—[i]	—	491	479	563
Poly-3 test[h]	P<0.001	—[j]	P=0.247	P=0.031	P=0.002

[a] Number of animals with oral mucosa 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
[d] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 4/371 (1.1% ± 1.0%), range 0%-2%
[e] Number of animals with neoplasm per number of animals necropsied
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

Pancreas: At 53 weeks, the incidence of acinar cytoplasmic vacuolization was significantly increased in Group 7 (Tables 20 and A5a). At 2 years, sporadic incidences of exocrine adenomas and carcinomas occurred in dosed groups, with the highest incidence observed in Group 5 (Tables 20, A1b, and A3). The incidences of exocrine adenoma in Group 5 and exocrine adenoma or carcinoma (combined) in Groups 5 and 7 exceeded the historical vehicle control ranges (Tables 20 and A4d).

Adenoma of the acinar cells was characterized microscopically by a discrete mass consisting of tubular and acinar structures composed of small acinar cells with brightly eosinophilic cytoplasm and lacking zymogen granules. Carcinoma was a large, multinodular lesion, with moderate amounts of dense fibrous stroma. Carcinomas were composed of densely packed clusters of poorly formed acinar structures consisting of small acinar cells with prominent vesicular nuclei and small amounts of eosinophilic cytoplasm with indistinct borders. Scattered solid areas composed of densely packed, highly pleomorphic, round to ovoid acinar cells with large vesicular nuclei and scant cytoplasm were also seen.

At 2 years, incidences of exocrine acinar atrophy occurred in treated groups, with the highest incidence observed in Group 7 (Tables 20 and A5b). Increased incidences and severities of exocrine acinar vacuolization occurred in Groups 5 and 7.

Atrophy was a focal to multifocal to diffuse change consisting of a reduction in the amount of acinar tissue with an associated increase in stromal fibrous connective tissue. Chronic active inflammation was generally associated with atrophy and consisted of an infiltrate of mononuclear cells with occasional neutrophils within the stroma. Cytoplasmic vacuolization consisted of small, clear, discrete intracytoplasmic vacuoles within pancreatic acinar cells. Sometimes these vacuoles coalesced to form larger single vacuoles. The severity of the change was determined by the degree of vacuolization per cell and the amount of tissue involved.

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
53-Week interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Acinus, Vacuolization Cytoplasmic[a]	0	0	0	0	7** (1.0)[b]
2-Year Study					
Number Examined Microscopically	53	53	52	52	50
Acinus, Vacuolization Cytoplasmic	0	0	0	7** (1.0)	40** (1.9)
Acinus, Atrophy	0	2 (1.5)	1 (2.0)	1 (1.0)	8** (1.6)
Adenoma[c]					
Overall rate[d]	0/53 (0%)	1/53 (2%)	1/52 (2%)	3/52 (6%)	1/50 (2%)
Adjusted rate[e]	0.0%	2.7%	2.6%	8.0%	2.8%
Terminal rate[f]	0/22 (0%)	0/21 (0%)	1/22 (5%)	3/24 (13%)	0/24 (0%)
First incidence (days)	—[h]	698	729 (T)	729 (T)	654
Poly-3 test[g]	P=0.494	P=0.496	P=0.503	P=0.114	P=0.489
Adenoma or Carcinoma[c]					
Overall rate	0/53 (0%)	1/53 (2%)	1/52 (2%)	4/52 (8%)	2/50 (4%)
Adjusted rate	0.0%	2.7%	2.6%	10.7%	5.5%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	4/24 (17%)	1/24 (4%)
First incidence (days)	—	698	729 (T)	729 (T)	654
Poly-3 test	P=0.226	P=0.496	P=0.503	P=0.056	P=0.224

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test (53-week interim evaluation) or the Poly-3 test (2-year study)
(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 ± standard deviation): 1/366 (0.3% ± 0.7%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with pancreas examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group

Uterus: At 2 years, the incidence of uterine squamous cell carcinoma was increased in Group 5; single incidences of this lesion occurred in Groups 1, 2, and 3 (Tables 21, A1b, and A3). The incidence of uterine squamous cell carcinoma in Group 5 exceeded the historical vehicle control range (Tables 21 and A4e). Squamous cell carcinoma occurred on the endometrial surface and was characterized by irregular cords and clusters of atypical stratified squamous epithelial cells that invaded the underlying myometrium.

Adrenal Cortex: At 2 years, one incidence each of cortical adenoma occurred in Groups 5 and 7 (Tables 22 and A1b); the incidences were within the historical vehicle control range (Tables 22 and A4f).

Cortical adenoma was a large, discrete lesion that replaced glandular parenchyma and caused compression of the remaining normal tissue. Adenoma was distinguished from hypertrophy or hyperplasia by the fact that adenoma consisted of somewhat atypical cortical cells that were arranged in abnormal patterns, rather than consisting of normal-appearing cells arranged in the normal cord pattern as seen with hypertrophy and hyperplasia. Large adenomas replaced much of the gland and caused enlargement of the gland. In contrast, cortical carcinoma was larger than adenoma, and consisted of highly atypical cells arranged in highly abnormal patterns. Invasion through the capsule into adjacent tissue was also present. Carcinomas replaced much of the gland and caused enlargement of the gland.

At 2 years, the incidence of cortical atrophy was significantly increased in Group 7 (Tables 22 and A5b). Significantly increased incidences of cortical hyperplasia occurred in Groups 3 and 5. Significantly increased incidences of cortical angiectasis occurred in Groups 2 and 3 and the incidence was decreased in Group 7.

Cortical atrophy was a locally extensive to diffuse change characterized by loss of cortical epithelial cells within the zona fasiculata 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 thickened capsule (Plates 9 and 10). Cortical hyperplasia was a focal to multifocal change, generally located in the zona fasiculata, consisting of a discrete area containing increased numbers of cortical cells. The hyperplastic cells were the same size or somewhat smaller than surrounding normal cortical cells, and had slightly basophilic cytoplasm. In some

TABLE 21
Incidences of Neoplasms of the Uterus in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Squamous Cell Carcinoma[a]					
Overall rate[b]	1/53 (2%)	1/53 (2%)	1/53 (2%)	4/53 (8%)	0/53 (0%)
Adjusted rate[c]	2.6%	2.7%	2.6%	10.7%	0.0%
Terminal rate[d]	1/22 (5%)	1/21 (5%)	1/22 (5%)	2/24 (8%)	0/24 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	715	—[f]
Poly-3 test[e]	P=0.397N	P=0.757	P=0.757N	P=0.171	P=0.509N

[a] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 1/371 (0.3% ± 0.7%), range 0%-2%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] 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 dosed group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group

TABLE 22
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control		Group 2 10 ng/kg: 10 µg/kg		Group 3 100 ng/kg: 100 µg/kg		Group 5 300 ng/kg: 300 µg/kg		Group 7 1,000 ng/kg: 1,000 µg/kg	
14-Week Interim Evaluation										
Thyroid Gland[a]	10		10		10		10		10	
Follicular Cell, Hypertrophy[b]	3	(1.0)[c]	3	(1.0)	4	(1.0)	8*	(1.4)	9**	(1.3)
Thymus	10		10		10		10		10	
Atrophy	0		4*	(1.0)	1	(1.0)	1	(2.0)	5*	(1.2)
31-Week Interim Evaluation										
Thyroid Gland	10		10		10		10		10	
Follicular Cell, Hypertrophy	0		5*	(1.0)	5*	(1.0)	7**	(1.0)	8**	(1.4)
Thymus	10		10		10		10		10	
Atrophy	6	(1.2)	5	(1.2)	6	(1.0)	7	(1.0)	10*	(1.9)
53-Week Interim Evaluation										
Thyroid Gland	8		8		8		8		8	
Follicular Cell, Hypertrophy	0		2	(1.0)	4*	(1.0)	6**	(1.2)	4*	(1.0)
2-Year Study										
Adrenal Cortex	53		53		52		52		51	
Atrophy	0		0		0		3	(3.0)	35**	(2.6)
Hyperplasia	11	(2.2)	18	(2.2)	23*	(2.2)	25**	(2.6)	18	(2.6)
Angiectasis	17	(1.4)	26*	(1.7)	33**	(1.6)	23	(1.7)	5*	(1.8)
Adenoma[d]	0		0		0		1		1	
Thyroid Gland	53		53		51		52		52	
Follicular Cell, Hypertrophy	14	(1.3)	17	(1.3)	34**	(1.4)	35**	(1.5)	42**	(1.9)
Thymus	53		50		48		50		51	
Atrophy	33	(2.3)	33	(2.5)	43**	(2.8)	42**	(3.8)	49**	(3.6)
Kidney	53		53		52		52		51	
Nephropathy	29	(1.2)	22	(1.0)	29	(1.1)	34	(1.3)	43**	(2.2)
Pigmentation	0		1	(2.0)	3	(1.0)	7**	(1.3)	35**	(2.0)
Transitional Epithelium, Hyperplasia	2	(2.0)	2	(1.0)	4	(1.8)	11**	(1.9)	6	(2.2)
Nose	53		53		53		53		53	
Inflammation	22	(1.5)	13	(1.2)	13*	(1.2)	13	(1.6)	31*	(1.3)
Respiratory Epithelium, Hyperplasia	10	(2.4)	5	(1.8)	7	(2.0)	11	(2.1)	20*	(2.6)
Olfactory Epithelium, Metaplasia	4	(2.3)	3	(2.0)	5	(2.0)	6	(2.2)	15**	(2.2)
Bone Marrow	53		53		53		53		53	
Hyperplasia	39	(3.2)	38	(3.0)	42	(2.9)	48*	(2.8)	49**	(2.7)
Forestomach	53		53		52		52		51	
Hyperplasia, Squamous	1	(2.0)	1	(1.0)	2	(1.5)	7*	(2.0)	8*	(1.8)
Lymph Node, Mandibular	53		51		52		50		51	
Ectasia	0		3	(2.0)	6*	(1.7)	3	(1.7)	6*	(2.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)
** $P \leq 0.01$
[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
[d] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 2/369 (0.5% ± 0.9%), range 0%-2%

cases, especially with large lesions, there was compression of the surrounding tissue. However, these were distinguishable as hyperplasia by the fact that the cells still formed normal cords, particularly in the upper zona fasiculata. Cortical hypertrophy and hyperplasia frequently occurred in the same gland. Angiectasis consisted of dilated vascular spaces.

Thyroid Gland: At 31 weeks, absolute thyroid gland weights of all dosed groups except Group 2 were significantly decreased (Table C1).

At 14, 31, and 53 weeks and 2 years, increased incidences of follicular cell hypertrophy occurred in most dosed groups (Tables 22, A5a, and A5b). The increases were significant in Groups 3, 5, and 7 at all time points (except Group 3 at 14 weeks).

Follicular cell hypertrophy was a localized to diffuse change, characterized by follicles that were decreased in size and contained decreased amounts of colloid in which aggregates of amphophilic, flocculant appearing material were often present. The affected follicles were lined by large, prominent cuboidal follicular epithelial cells that were approximately two to three times normal size, usually with abundant pale cytoplasm containing small, clear, vacuoles. Since some degree of this change can occur spontaneously, the severity grade of minimal was recorded when 50% to 60% of the follicles were involved, mild severity 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: At 14 weeks, the absolute and relative thymus weights of Group 7 were significantly lower than those of the vehicle controls (Table C1). Significantly increased incidences of atrophy occurred in Group 2 at 14 weeks and in Group 7 at 14 and 31 weeks (Tables 22 and A5a).

At 2 years, significantly increased incidences of atrophy occurred in all dosed groups except Group 2 (Tables 22 and A5b). The severity of atrophy was increased in all groups with significantly increased incidences. Atrophy consisted of varying degrees of loss of lymphoid cells from the cortex resulting in reduction of cortical thickness.

Kidney: At 2 years, the severity of nephropathy increased with increasing dose, and the incidence was significantly increased in Group 7 (Tables 22 and A5b). Significantly increased incidences of pigmentation and hyperplasia of the transitional epithelium occurred in Group 5. The incidence of pigmentation was significantly increased in Group 7.

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

Pigmentation was characterized by small to moderate amounts of yellow-brown, granular material within the cytoplasm of renal tubular epithelial cells in the outer cortex. A slight amount of similar appearing pigment was seen scattered in the cortex of vehicle controls and 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.

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

Nose: At 2 years, significantly increased incidences of inflammation, hyperplasia of the respiratory epithelium, and metaplasia of the olfactory epithelium occurred in Group 7 (Tables 22 and A5b). Inflammation was usually seen in section III and was generally characterized by

accumulation of varying numbers of neutrophils mixed with mucus and debris within the nasal cavity. Olfactory epithelial metaplasia, consisting of replacement of normal olfactory epithelium by respiratory-type epithelium, and respiratory epithelial hyperplasia, consisting of varying degrees of respiratory epithelium due to an increase in the number of epithelial cells, were generally seen in association with inflammation and appeared to be secondary to the inflammation (Plates 11 and 12).

Bone Marrow: At 2 years, significantly increased incidences of bone marrow hyperplasia occurred in Groups 5 and 7 (Tables 22 and A5b). The severity of bone marrow hyperplasia was graded as follows: marked was used when the entire marrow cavity was filled with dense marrow; moderate hyperplasia was recorded when marrow elements composed about 90% of the cavity (the remaining 10% was fat); mild hyperplasia was recorded when marrow elements composed approximately 60% to 90% of the marrow cavity; and minimal hyperplasia was rarely recorded because of the normal variation in the amount of bone marrow. Normal bone marrow was used when the distal end of the femur section contained 20% to 60% marrow.

Forestomach: At 2 years, significantly increased incidences of squamous hyperplasia of the forestomach occurred in Groups 5 and 7 (Tables 22 and A5b). Squamous hyperplasia of the forestomach epithelium was generally a minimal to mild, focal, or occasionally multifocal change characterized by varying degrees of thickening of the stratified squamous epithelium up to approximately five times normal thickness in more severe cases (Plates 13 and 14). Sometimes the hyperplasia occurred around a focal ulcer, although most cases occurred without the presence of an apparent ulcer.

Lymph Node (Mandibular): At 2 years, some incidences of ectasia occurred in each treated group, with no incidences within the vehicle controls. The incidences in Groups 3 and 7 were significantly increased (Tables 22 and A5b). Ectasia consisted of mild to moderate, focal to multifocal dilatation of medullary sinuses (lymphangiectasis).

Mammary Gland: At 2 years, there were significant negative trends in the incidences of fibroadenoma and carcinoma. The incidence of fibroadenoma (Group 1, 40/53; Group 2, 39/53; Group 3, 40/53; Group 5, 34/53; Group 7, 12/53) and carcinoma (8/53, 4/53, 3/53, 2/53, 0/53) were significantly decreased in Group 7 (Table A3).

Pituitary Gland (Pars Distalis): At 2 years, there was a significant negative trend in the incidences of adenoma and the incidence was significantly decreased in Group 7 (22/53, 21/53, 17/53, 17/52, 1/52; Table A3).

Varying Ratio Mixture of PCB 126 and PCB 153

Liver: At 14, 31, and 53 weeks, the absolute and relative liver weights of all dosed groups were greater than those of vehicle controls (Table C2). At 14 weeks, the absolute liver weight of Group 6 was significantly greater than those of Groups 4 and 5. At 31 weeks, the absolute liver weights of Groups 4 and 6 were significantly greater than those of Group 5.

At 14 weeks, the incidences of hepatocytic hypertrophy and fatty change increased with increasing concentrations of PCB 153 (Tables 23 and B4a). The incidence of diffuse fatty change was increased in Group 6.

At 31 weeks, hepatocytic hypertrophy and pigmentation were present in most dosed animals (Tables 23 and B4a). The incidence of diffuse fatty change was increased in Group 6.

At 53 weeks, hepatocytic hypertrophy and pigmentation occurred in all dosed rats, with the greatest severities observed in Group 6 (Tables 23 and B4a). Increased incidences of multinucleated hepatocytes, diffuse fatty change, bile duct hyperplasia, and toxic hepatopathy occurred in Group 6.

TABLE 23
Incidences of Nonneoplastic Lesions of the Liver in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 4 300 ng/kg: 100 µg/kg		Group 5 300 ng/kg: 300 µg/kg		Group 6 300 ng/kg: 3,000 µg/kg		P Value[a]
14-Week Interim Evaluation							
Number Examined Microscopically	10		10		10		
Hepatocyte, Hypertrophy[b]	3	(1.0)[c]	6	(1.0)	10	(2.0)	0.001
Pigmentation	7	(1.0)	5	(1.0)	5	(1.0)	0.282N
Fatty Change, Diffuse	0		1	(1.0)	8	(1.6)	<0.0001
31-Week Interim Evaluation							
Number Examined Microscopically	10		10		10		
Hepatocyte, Hypertrophy	10	(1.0)	10	(1.0)	10	(1.9)	—[d]
Pigmentation	10	(1.0)	10	(1.0)	8	(1.5)	0.019N
Fatty Change, Diffuse	0		1	(1.0)	7	(2.0)	<0.0001
53-Week Interim Evaluation							
Number Examined Microscopically	10		8		9		
Hepatocyte, Hypertrophy	10	(1.0)	8	(1.0)	9	(2.4)	—
Hepatocyte, Multinucleated	3	(1.0)	2	(1.0)	7	(1.1)	0.007
Pigmentation	10	(1.4)	8	(1.4)	9	(2.1)	—
Fatty Change, Diffuse	1	(1.0)	3	(1.0)	9	(1.7)	<0.0001
Bile Duct, Hyperplasia	2	(1.0)	0		5	(1.4)	0.008
Toxic Hepatopathy	3	(1.0)	0		6	(1.0)	0.006

[a] Probability of significant trend by Cochran-Armitage test. A negative trend is indicated by **N**.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Statistic cannot be calculated

At 2 years, the incidences of hepatocellular adenoma (single or multiple) and cholangiocarcinoma (single or multiple) occurred with positive trends (Tables 24, B1b, and B3). Hepatocholangiomas occurred in Groups 5 and 6. The incidences of cholangiofibrosis occurred with a positive trend (Tables 24 and B4b).

At 2 years, the incidences of mild to moderate hepatocyte hypertrophy, diffuse and focal fatty change, basophilic focus, eosinophilic focus (single or multiple), clear cell focus, bile duct hyperplasia, and hematopoietic cell proliferation occurred with positive trends (Tables 24 and B4b).

Eosinophilic, basophilic, and clear cell foci appeared similar and were characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic focus was composed of cells with eosinophilic cytoplasm. Basophilic focus consisted of hepatocytes with basophilic cytoplasm, occasionally with basophilic linear (tigroid) intracytoplasmic aggregates. Clear cell focus was composed of cells having clear cytoplasm. 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. If two or more foci of a given type were present in a liver, they were diagnosed as multiple. The treatment-related foci were of eosinophilic and mixed cell type, and often differed somewhat from those in vehicle control animals. Foci in vehicle control animals consisted of hepatocytes that were generally somewhat larger than normal but appeared otherwise normal and were arranged in a relatively normal lobular pattern. The hepatic cords at the periphery of these foci generally merged imperceptibly with the surrounding normal liver resulting in an indistinct border and little or no

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg	P Value[a]
Number Examined Microscopically	50	52	51	
Hepatocyte, Hypertrophy[b]	22 (2.1)[c]	33 (2.1)	47 (3.0)	P<0.001
Pigmentation	50 (1.9)	50 (1.9)	44 (1.8)	P<0.001N
Fatty Change, Diffuse	28 (1.1)	31 (1.5)	47 (1.5)	P<0.001
Fatty Change, Focal	4 (1.3)	1 (2.0)	11 (1.9)	P=0.002
Basophilic Focus	5	3	18	P<0.001
Eosinophilic Focus (includes multiple)	27	40	45	P<0.001
Clear Cell Focus	5	3	11	P=0.019
Bile Duct, Hyperplasia	20 (1.2)	29 (1.4)	40 (1.7)	P<0.001
Hematopoietic Cell Proliferation	18 (1.1)	19 (1.1)	29 (1.1)	P=0.011
Cholangiofibrosis	5 (2.4)	7 (2.0)	13 (2.2)	P=0.026
Hepatocholangioma (includes multiple)	0	2	2	
Hepatocellular Adenoma, Multiple	2	0	7	
Hepatocellular Adenoma (includes multiple)				
Overall rate[d]	2/50 (4%)	5/52 (10%)	21/51 (41%)	P<0.001
Adjusted rate[e]	5.1%	13.3%	49.6%	
Terminal rate[f]	2/28 (7%)	4/24 (17%)	14/27 (52%)	
First incidence (days)	729 (T)	684	491	
Cholangiocarcinoma, Multiple	1	5	13	
Cholangiocarcinoma (includes multiple)				
Overall rate	7/50 (14%)	9/52 (17%)	25/51 (49%)	P<0.001
Adjusted rate	17.3%	23.7%	59.5%	
Terminal rate	4/28 (14%)	7/24 (29%)	18/27 (67%)	
First incidence (days)	603	603	588	

(T) Terminal sacrifice
[a] Probability of significant trend by the Poly-3 test. A negative trend is indicated by **N**.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[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

compression of the adjacent liver parenchyma. In contrast, foci in treated animals often had a more definite border, the cords within the focus often were not smoothly continuous with those in the surrounding parenchyma, and the foci consisted of cells that were often prominently enlarged with abundant eosinophilic or clear vacuolated cytoplasm. If more than 20% of the cells were vacuolated, the focus was classified as mixed cell type, otherwise it was classified as an eosinophilic focus. In addition, some larger foci caused varying degrees of compression of the surrounding hepatic parenchyma. The cells were arranged in a relatively normal lobular pattern and foci sometimes contained large blood vessels and/or portal areas. The presence of proliferating bile ducts or oval cells was not considered characteristic of a focus. Bile duct hyperplasia consisted of increased numbers of bile duct nuclei within portal areas.

Cholangiofibrosis appeared relatively small and well demarcated, without evidence for local invasion. The lesion consisted of fibrous connective tissue stroma

containing atypical bile ducts, which frequently contained mucinous material and cellular debris. Liver hematopoietic cell proliferation consisted of varying numbers of scattered, small clusters of small, deeply basophilic hematopoietic cells.

Lung: At 2 years, the incidences of bronchiolar metaplasia of alveolar epithelium occurred with a negative trend (Group 4, 39/50; Group 5, 34/53; Group 6, 30/50; Table B4b).

Pancreas: At 53 weeks and 2 years, the incidences of exocrine acinar cytoplasmic vacuolization occurred with a positive trend (Tables 25, B4a, and B4b).

Thyroid Gland: At 31 weeks, the absolute thyroid gland weights of all dosed groups were decreased (Table C2). At 31 weeks and 2 years, the incidences of follicular cell hypertrophy occurred with a positive trend (Tables 25, B4a, and B4b).

Kidney: At 14, 31, and 53 weeks, kidney weights were generally greater than those of the vehicle controls (Table C2). At 14 weeks, the absolute kidney weight of Group 6 was significantly greater than that of Group 5. At 31 weeks, the absolute kidney weights of Groups 4 and 6 were significantly greater than that of Group 5. At 2 years, the incidences of pigmentation and pelvic inflammation occurred with positive trends (Tables 25 and B4b). Inflammation of the renal pelvis consisted of a multifocal to diffuse infiltrate of small to moderate numbers of inflammatory cells, primarily neutrophils, within the renal pelvis.

Ovary: At 2 years, there was a positive trend in the incidences of chronic inflammation (Tables 25 and B4b).

Pituitary Gland (Pars Distalis): At 2 years, there was a negative trend in the incidences of adenoma (Group 4, 19/50; Group 5, 17/52; Group 6, 9/51; Table B3).

TABLE 25
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 4 300 ng/kg: 100 µg/kg		Group 5 300 ng/kg: 300 µg/kg		Group 6 300 ng/kg: 3,000 µg/kg		P Value[a]
31-Week Interim Evaluation							
Thyroid Gland[b]	10		10		10		
Follicular Cell, Hypertrophy[c]	6	(1.2)[d]	7	(1.0)	10	(1.1)	0.037
53-Week Interim Evaluation							
Pancreas	10		8		9		
Acinus, Vacuolization Cytoplasmic	0		0		6	(1.0)	<0.001
2-Year Study							
Pancreas	49		52		49		
Acinus, Vacuolization Cytoplasmic	3	(1.0)	7	(1.0)	44	(1.5)	<0.001
Thyroid Gland	49		52		50		
Follicular Cell, Hypertrophy	28	(1.6)	35	(1.5)	44	(1.8)	<0.001
Kidney	48		52		51		
Pigmentation	2	(1.5)	7	(1.3)	17	(1.5)	<0.001
Pelvis, Inflammation	1	(2.0)	3	(2.3)	8	(2.3)	0.011
Ovary	48		52		50		
Inflammation, Chronic Active	0		0		4		0.009

[a] Probability of significant trend by the Poly-3 test
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

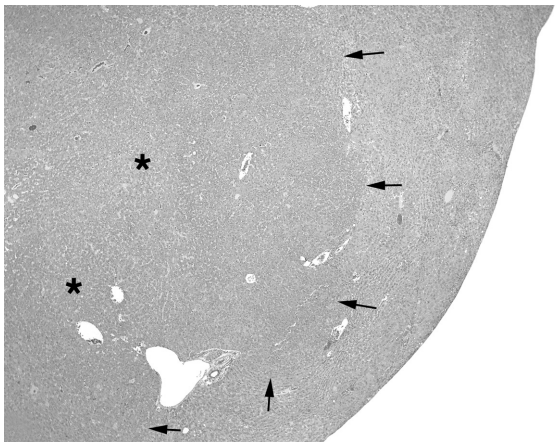


PLATE 1
Hepatocellular adenoma (asterisks) in the liver of a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. The hepatocellular neoplasm has a distinct border, producing compression of surrounding normal parenchyma (arrows). H&E; 2.5×

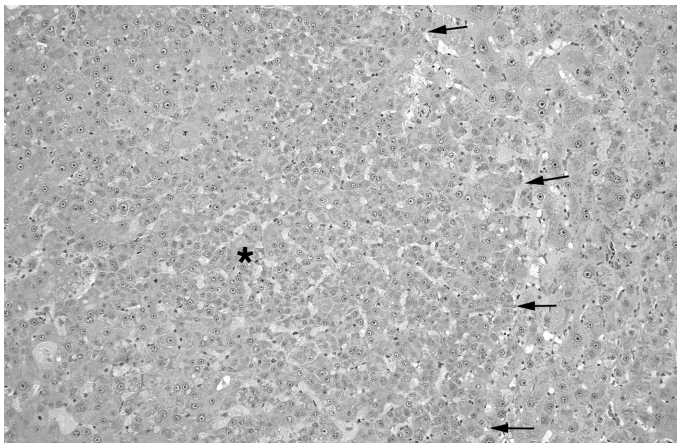


PLATE 2
Hepatocellular adenoma (asterisk) in the liver of a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. The hepatocellular adenoma is composed of a rather uniform population of mildly pleomorphic hepatocytes that are slightly smaller in size than normal and are arranged in abnormal lobular patterns. Arrows indicate the margin of the adenoma. H&E; 12.5×

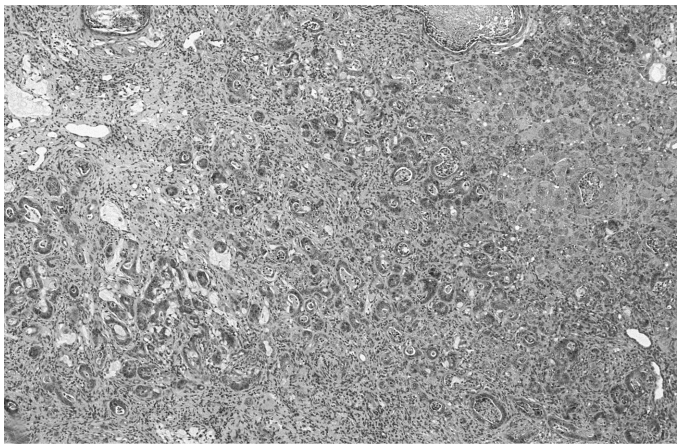


PLATE 3
Cholangiocarcinoma in the liver of a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. Note the invasion of the neoplastic tissue into the surrounding hepatic tissue. H&E; 16×

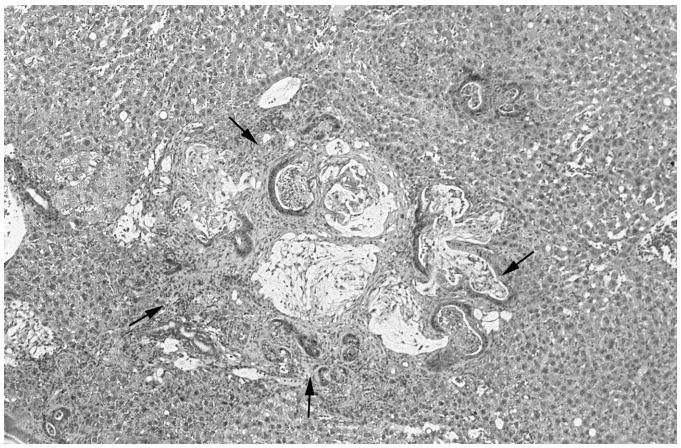


PLATE 4
Cholangiofibrosis in the liver of a Group 4 (300 ng/kg:100 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. Note the relatively smaller size (arrows) of this lesion compared to the cholangiocarcinoma presented in Plate 3. H&E; 16×

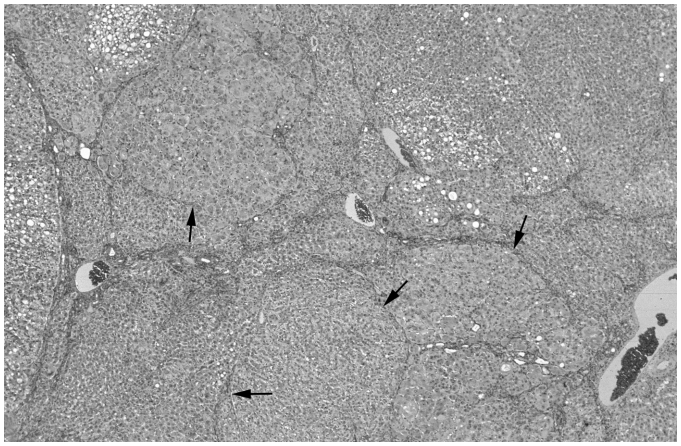


PLATE 5
Nodular hyperplasia in the liver of a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. Note the multiple nodules of different sizes (arrows). H&E; 6×

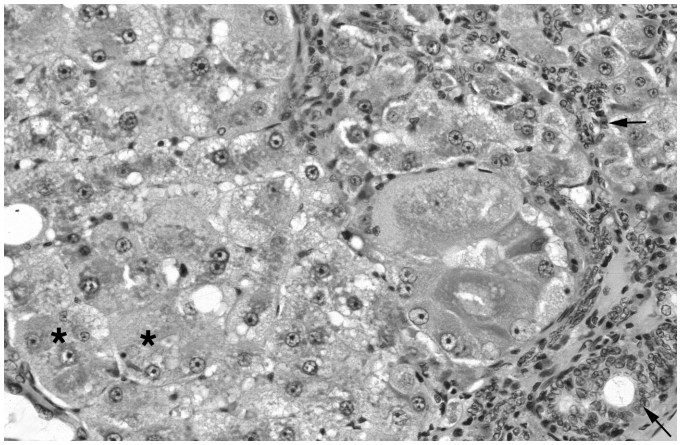


PLATE 6
Higher magnification of Plate 5. Note that the nodule is composed of hepatocytes that are considerably larger (hepatocyte hypertrophy, asterisks) than normal hepatocytes, with adjacent bile duct hyperplasia (arrows). H&E; 66×

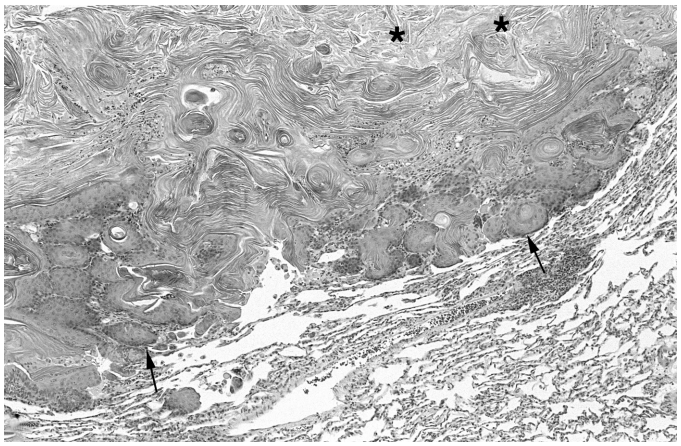


PLATE 7
Cystic keratinizing epithelioma in the lung of a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. Note the cystic structure consisting of an irregular wall (arrows) of highly keratinized stratified squamous epithelium and a center filled with keratin (asterisks). The outer portion of the lesion grows by expansion into the adjacent lung but there is no evidence of invasion. H&E; 16×

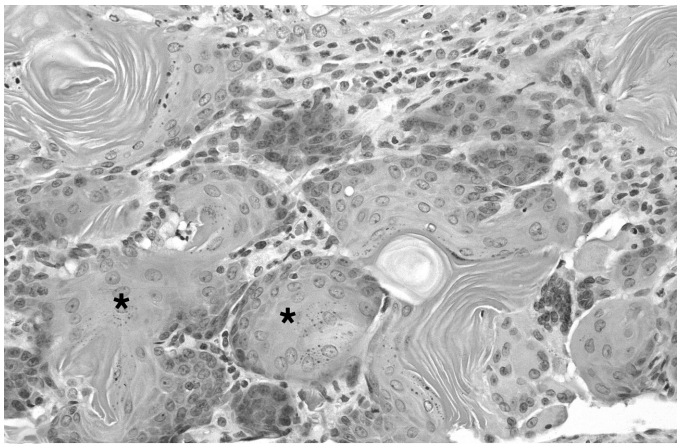


PLATE 8
Higher magnification of Plate 7. Note the highly irregular wall of keratinized stratified squamous epithelium (asterisks). H&E; 66×

PLATE 9
Normal size and aspect of the cortex of the adrenal gland (arrows) in a Group 1 (vehicle control) female rat from the 2-year gavage study of a binary mixture of PCB 126 and PCB 153. H&E; 5×

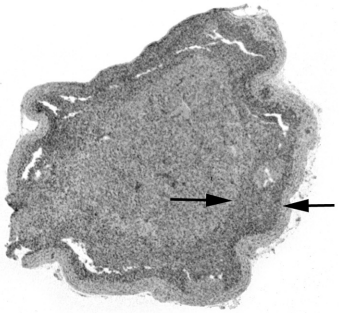


PLATE 10
Diffuse adrenal cortical atrophy (arrows) in a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. Compare with Plate 9. H&E; 5×

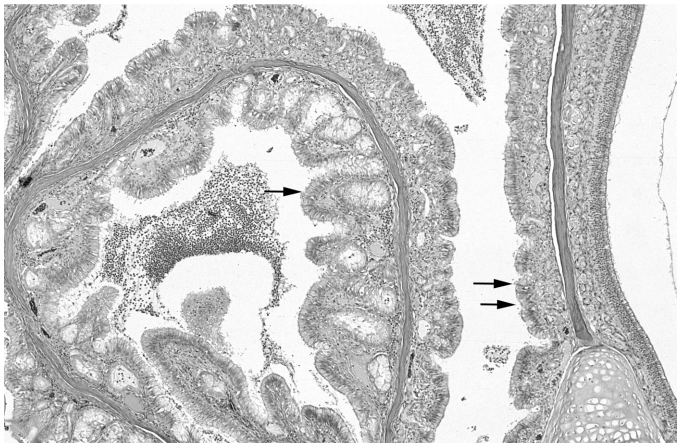


PLATE 11
Respiratory metaplasia and moderate hyperplasia (arrow) in the olfactory epithelium in the ethmoid turbinates at level III of the nasal passages in a Group 7 (1,000 ng/kg:1,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. The prominent hyperplasia of the respiratory epithelium, rich in goblet cells, is forming papillary projections and "crypt-like" invagination. There is also respiratory metaplasia of the olfactory epithelium and minimal hyperplasia of the epithelium lining the nasal septum (two arrows). H&E; 16×

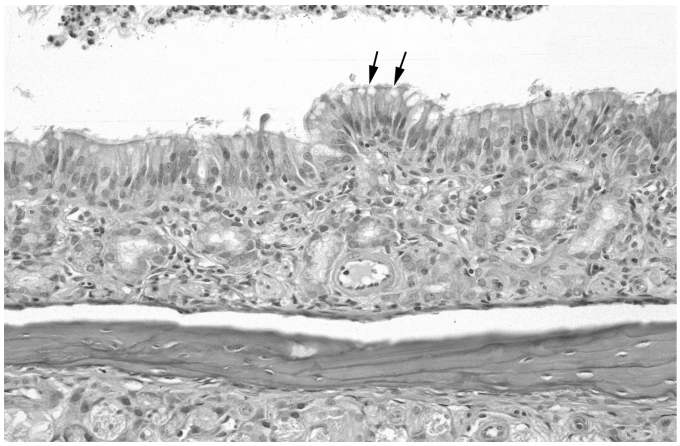


PLATE 12
Higher magnification of Plate 11; note respiratory metaplasia of the olfactory epithelium and minimal hyperplasia of the epithelium lining the nasal septum (two arrows). H&E; 66×

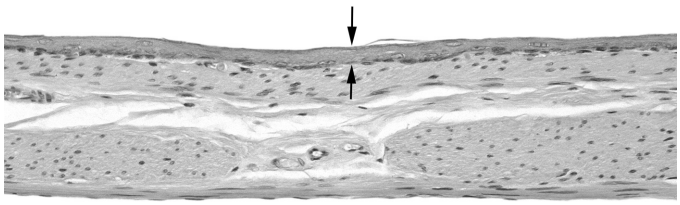


Plate 13
Normal thickness of the squamous epithelium of the forestomach (arrows) in a Group 1 (vehicle control) female rat from the 2-year gavage study of a binary mixture of PCB 126 and PCB 153. H&E; 66×

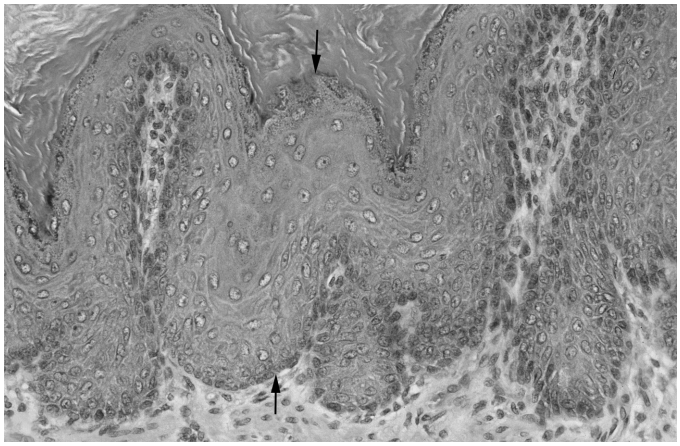


Plate 14
Squamous hyperplasia of the forestomach epithelium (arrows) in a Group 6 (300 ng/kg:3,000 µg/kg) female rat administered a binary mixture of PCB 126 and PCB 153 by gavage for 2 years. H&E; 66×

DISCUSSION AND CONCLUSIONS

This 2-year study of the chronic toxicity and carcinogenicity of a binary mixture of PCB 126 and PCB 153 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). The primary goal of the current study was to assess the carcinogenic activity of dioxin-like PCB 126 in the presence of non-dioxin-like PCB 153. Data from this study were used to test two specific hypotheses. Is the potency of carcinogenicity of a constant ratio mixture of PCB 126 and PCB 153 different from that of PCB 126 alone, and does altering the ratio of PCB 126 and PCB 153 in the mixture affect the carcinogenic activity of PCB 126? Toxicology and carcinogenicity study results of the binary mixture of PCB 126 and PCB 153 are described in this Technical Report. Where appropriate, qualitative comparisons are made to other studies conducted as part of the dioxin toxic equivalency factor (TEF) evaluation. A quantitative comparative analysis of the effects observed in this study compared to responses observed with PCB 126 alone or to other compounds studied as part of the dioxin TEF evaluation will be presented elsewhere.

PCB 126 and PCB 153 are persistent, environmentally relevant compounds with widespread chronic human exposure. PCB 126 is the most potent coplanar TCDD-like PCB. PCB 153, the most abundant PCB in human tissue samples on a molar basis, is a di-*ortho*-substituted nonplanar PCB that does not exhibit dioxin-like activity (McFarland and Clarke, 1989; Schecter *et al.*, 1994; Heudorf *et al.*, 2002; Ayotte *et al.*, 2003; Chu *et al.*, 2003). Several studies have demonstrated an interaction between exposure to PCB 153 or other di-*ortho*-substituted PCBs with regard to tissue concentrations and biochemical and biological effects induced by DLCs. This study provides the opportunity to investigate the carcinogenicity of a binary mixture of an environmentally relevant ratio of dioxin-like and non-dioxin-like PCBs. This study also provides the opportunity to investigate potential interactions between PCB 153, a di-*ortho*-substituted nonplanar PCB that lacks dioxin-like activity, and PCB 126, a coplanar dioxin-like PCB that induces neoplasms in the same target organs as TCDD (NTP, 2006a).

Dose selection of PCB 126 for this study was based on TCDD-induced increases in liver adenomas at doses of 10 and 100 ng/kg in a 2-year carcinogenicity study in Spartan Sprague-Dawley rats (Kociba *et al.*, 1978). Given the World Health Organization's (WHO) TCDD TEF for PCB 126 of 0.1, the dose range for the present study was selected as 10 to 1,000 ng PCB 126/kg body weight per day. The dosages of PCB 153 were selected based on those used in previous tumor promotion studies, and are similar to those used in another study as part of the dioxin TEF evaluation of PCB 153; these results are reported in a separate Technical Report (NTP, 2006e). For the current study, mixtures with a 1:1,000 ratio of PCB 126 to PCB 153 were used to provide information on the shape of the dose response curve. The doses for the constant ratio of PCB 126 (ng/kg) and PCB 153 (µg/kg) were 0:0, 10:10, 100:100, 300:300, and 1,000:1,000 and appear in this report as Groups 1, 2, 3, 5, and 7. Mixtures with a varying ratio (1:333, 1:1,000, and 1:3,000) of PCB 126 and PCB 153 were used to investigate potential interactions between PCB 153 and PCB 126. The doses for the varying ratio mixtures of PCB 126 (ng/kg) and PCB 153 (µg/kg) were 300:100, 300:300, 300:3,000 and are referred to in this report as Groups 4, 5, and 6.

Constant Ratio Mixture of PCB 126 and PCB 153

In the current study, administration of a constant ratio mixture of PCB 126 and PCB 153 had no effect on survival. At higher doses, treatment resulted in decreased body weight gain. Mean body weights in Group 5 were maximally reduced to 82% that of the vehicle control group. Mean body weights in Group 7 were maximally reduced to 67% of the vehicle control group. The decreased body weight gains in Groups 5 and 7 were comparable to those previously observed with PCB 126 alone at similar doses (NTP, 2006a). The reduction of body weight gain is a characteristic toxic response to DLCs.

The principal findings of this study were increased incidences of benign and malignant neoplasms in several organs, specifically in the liver (cholangiocarcinoma, hepatocholangioma, hepatocellular adenoma, and hepatocellular carcinoma), lung (predominantly cystic keratinizing epithelioma, but squamous cell carcinomas were also seen), and oral mucosa (gingival squamous cell carcinoma). The highest neoplastic response was in the liver (cholangiocarcinoma) with an adjusted incidence rate of 75.5%.

The principal nonneoplastic finding in this study was a significant increase in the incidence and severity of hepatotoxicity in the liver at 14, 31, and 53 weeks and 2 years. In addition, numerous organs exhibited increased incidences of nonneoplastic lesions; notably in the lung, pancreas, adrenal cortex, thyroid gland, thymus, kidney, nose, and forestomach at 14, 31, and 53 weeks and/or 2 years.

Hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) and acetanilide-4-hydroxylase (A4H) activities were significantly induced in all groups treated with the constant ratio mixture compared to vehicle controls at all interim evaluations in the current study. The degree of hepatic EROD and A4H induction was comparable to that observed in the study of PCB 126 as part of the TEF evaluation (NTP, 2006a). The induction of CYP1A1 (EROD) and CYP1A2 (A4H) activities by dioxin-like PCB congeners and other DLCs are characteristic responses in liver and are directly linked to binding and activation of the aryl hydrocarbon receptor (AhR) by DLCs (Whitlock, 1993). PCB 126 has the highest affinity of coplanar PCBs for the AhR. In a study of PCB 153 as part of the TEF evaluation, EROD and A4H activities were only slightly induced by PCB 153 at 14 and 31 weeks (NTP, 2006e). Therefore, the induction of EROD and A4H activities by the binary mixture of PCBs is probably due to PCB 126. Increased pulmonary EROD activity was observed at all interim evaluations. These increases were consistent with increases in pulmonary EROD activity by PCB 126 and other DLCs evaluated in the other studies of the dioxin TEF evaluation (NTP, 2006a,c,d,e).

In the current study, administration of the constant ratio mixture induced hepatic 7-pentoxyresorufin-*O*-deethylase (PROD) activity at all time points. Previous studies in rodents have demonstrated that PROD activity, a measure of CYP2B expression, is induced by PCB 153 (Luotamo *et al.*, 1991; Li *et al.*, 1994; Bouwman *et al.*, 1999; Craft *et al.*, 2002). The induction of PROD in Group 7 (9- to 12-fold increase) was lower than that observed in the PCB 153 study at doses of 1,000 µg/kg PCB 153 (36- to 91-fold increase) alone (NTP, 2006e). Comparable increases in PROD activities were observed in the study of PCB 126 alone at similar doses (NTP, 2006a). This suggests that PCB 126 may be interfering with the induction of PCB 153 PROD activity.

Numerous toxicity studies of DLCs and PCBs have demonstrated that the liver is a principal target organ for the action of these compounds. In the current study, the incidence and pattern of hepatic toxicity in the constant ratio mixture exhibited a clear dose and duration dependence, preceding neoplastic effects in the liver. There was a significant increase in hepatic toxicity with increases in severity occurring at higher doses and longer durations of treatment. Hepatic toxicity was characterized by foci of cellular alteration, multinucleated hepatocytes, diffuse fatty change, necrosis, pigmentation, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, hepatocyte hypertrophy, oval cell hyperplasia, and portal fibrosis. A comprehensive term of toxic hepatopathy was also used to reflect the overall severity of the nonneoplastic effects, allowing for an easier comparison of the toxic changes among different dose groups than a comparison of individual nonneoplastic changes. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. Some treated animals occasionally had a few of these changes, but this was not considered sufficient liver involvement to warrant a diagnosis of toxic hepatopathy.

The hepatotoxicity observed in the current study was consistent with results for PCB 126 (NTP, 2006a). Although hepatocyte hypertrophy, diffuse fatty change, bile duct hyperplasia, pigmentation, and oval cell hyperplasia were induced by PCB 153 (NTP, 2006e), the spectrum and severity of lesions induced by the constant ratio PCB mixture more closely reflected those induced by PCB 126, rather than PCB 153. The broad-spectrum nonneoplastic liver effects that defined the toxic hepatopathy diagnosis are not induced by PCB 153 alone. In general, di-*ortho*-substituted PCB congeners are not as hepatotoxic as the coplanar, dioxin-like congeners (Safe, 1994).

The spectrum of hepatocellular proliferative lesions observed in the present study is consistent with the dioxin TEF evaluation studies of TCDD and PCB 126

(Hailey *et al*., 2005; NTP, 2006a,b). There were significant increases in the incidences of hepatocholangioma in Group 7 and hepatocellular adenoma and cholangiocarcinoma in Groups 5 and 7. Two hepatocellular carcinomas were observed in Group 7. The increased incidences of hepatocellular neoplasms are consistent with previously observed effects of TCDD, PCB 126, and Aroclor mixtures of PCBs (Kociba *et al.*, 1978; NTP, 1982a,b; Goodman and Sauer, 1992; Mayes *et al.*, 1998). In initiation-promotion models of carcinogenesis, mixtures of PCB 126 and PCB 153 at various ratios also induce the development of altered hepatocellular foci (AHF), which are believed to progress and develop into hepatocyte-derived neoplastic lesions such as hepatocellular adenomas and possibly carcinomas (Bager *et al.*, 1995; Dean *et al.*, 2002). Hepatocellular carcinomas were observed in previous studies of Aroclors and TCDD (Kociba *et al.*, 1978; Mayes *et al.*, 1998). However, there were no increases in the incidences of carcinomas in the studies of PCB 126 and TCDD conducted as part of the dioxin TEF evaluation (NTP, 2006a,b).

The principal hepatic neoplasm observed in the current study was cholangiocarcinoma. The induction of cholangiocarcinomas in the current study is consistent with the results from the dioxin TEF evaluation study of PCB 126 (NTP, 2006a). In that study, significant increases in the incidences of cholangiocarcinoma were observed in the 300, 550, and 1,000 ng/kg groups. In the current study, increased incidences of cholangiocarcinoma occurred in Groups 5 and 7, for which the PCB 126 dose component of the mixture was 300 and 1,000 ng/kg PCB 126, respectively. The increased incidences of cholangiocarcinoma are also consistent with the effects seen in the dioxin TEF evaluation of TCDD (NTP, 2006b). In the dioxin TEF evaluation study of PCB 153, no cholangiocarcinomas were observed (NTP, 2006e). Therefore, the induction of cholangiocarcinomas in the current study is likely due to the PCB 126 component. Cholangiocarcinomas were rarely seen in previous studies of DLCs and PCBs despite data showing that bile ducts are targets for DLCs. In an initiation-promotion study, cholangiocarcinoma was seen in one of 14 DEN-initiated female rats exposed to 100 ng TCDD/kg body weight per day for 60 weeks (Walker *et al.*, 2000). No cholangiocarcinomas were observed in a 2-year bioassay of Aroclor 1254 (Mayes *et al.*, 1998) or in the TCDD feed study by Kociba *et al.* (1978).

Initial analysis of this study suggests that there is a positive interactive effect between PCB 126 and PCB 153 on the induction of cholangiocarcinoma and hepatocellular adenoma, and also hepatocholangioma. The significant increased incidence of hepatocholangioma in Group 7 in the current study was not observed in the dioxin TEF evaluation study of PCB 153 (NTP, 2006e). By comparison, three hepatocholangiomas were observed in rats exposed to 1,000 ng/kg PCB 126 in a study of PCB 126 alone (NTP, 2006a). No hepatocholangiomas have been observed in the vehicle controls from any of the other dioxin TEF evaluation studies (NTP, 2006a,b,c,d,e,f). A significantly increased incidence of hepatocellular adenoma was observed in the PCB 126 study at 1,000 ng/kg (NTP, 2006a). The incidence in that study was considerably lower than the incidence seen in the current study in Group 7 rats. A single hepatocellular adenoma was observed in the PCB 153 study in the 3,000 µg/kg group (NTP, 2006e). The mechanisms by which interactions occur between PCB 153 and DLCs are not clearly understood. Further investigation and an in-depth analysis regarding the interactive effects between PCB 126 and PCB 153 are in progress and will be reported elsewhere.

Several studies have reported an interaction between PCB 126 and PCB 153 on the promotion of preneoplastic lesions. These studies demonstrate that mixtures of PCB 153 and PCB 126 antagonize the PCB 126-induced development of AHF, expressing the placental form of glutathione-S-transferase (Haag-Grönlund *et al.*, 1998; Dean *et al.*, 2002), and mediate a more than additive effect on the development of AHF expressing *gamma*-glutamyltranspeptidase (Bager *et al.*, 1995). These interactions of PCB 153 on preneoplastic hepatic lesion development have also been demonstrated in interactions with other DLCs and for other toxic responses, including altered development and immunotoxicity (Biegel *et al.*, 1989; Morrissey *et al.*, 1992; Berberian *et al.*, 1995; Wölfle, 1998).

PCB 153 promotes the development of preneoplastic AHF and decreases apoptosis in focal hepatocytes without inducing focal cell proliferation (Buchmann *et al.*, 1986; Hemming *et al.*, 1993; Bager *et al.*, 1995; Tharappel *et al.*, 2002). Since preneoplastic AHF may potentially progress and develop into neoplasms, it would be expected that chronic exposure to PCB 153 would induce hepatocellular neoplasms. However,

hepatocellular neoplasms were not observed following administration of PCB 153 for 2 years (NTP, 2006e). It may be possible that the tumor promoting activity of PCB 153 alone is not robust enough to induce an observable neoplastic response at the doses used. However, in the presence of PCB 126, proliferation of focal hepatocytes or other biological effects of PCB 126 may contribute to an increase in hepatocellular neoplasms and the hepatocellular component of hepatocholangiomas.

In the higher dose animals with more severe toxic hepatopathy, there was evidence of hepatocyte degeneration and loss, and a regenerative response by the damaged liver. The term "nodular hyperplasia" was selected as the inclusive term, and was characterized by areas of focal hypertrophy and hyperplasia of hepatocytes that contained proliferating biliary epithelium. Nodular hyperplasia varied in size, but generally appeared morphologically similar whether in a high dose group animal with severe toxic hepatopathy or in a lower dose group animal where the toxic hepatopathy was minimal to nonexistent. In the dioxin TEF evaluation studies, nodular hyperplasia was seen in higher dose groups with prominent toxic changes (NTP, 2006a,b,c,d,e,f). However, a lesser degree of nodular hyperplasia was sometimes seen in lower dose animals where the only evidence of liver pathology was 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 stimulus, in addition to regeneration secondary to degeneration and necrosis and toxic hepatopathy 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 treatment-related hepatocellular change seen in these studies, noted at the 14-, 31-, and 53-week interim evaluations, was a diffuse hepatocyte hypertrophy (NTP, 2006a,b,c,d,e,f). With continued dosing, poorly demarcated foci of prominent hypertrophic, often vacuolated hepatocytes, resembling those seen in foci and nodular hyperplasia, were seen superimposed on the background of diffuse hypertrophy. It appeared that with continued dosing, the poorly demarcated foci of hypertrophic cells grew, giving rise to lesions diagnosed as foci, and with continued dosing, in some instances aided by toxic changes, may have progressed to nodular hyperplasia.

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

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

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

in the scirrhous reaction that may be observed with spontaneous cholangiocarcinoma. The lesions seen in this study were sometimes large, effacing an entire liver lobe. Cholangiofibrosis was the term used to describe small lesions that 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. As a result, the most appropriate classification scheme for these lesions is somewhat uncertain and controversial. While the characteristic of malignancy, distant metastasis, was not observed in any animals in the present study, other characteristics of malignancy were present such as atypical appearance of the epithelial cells and apparent localized invasion. It was clear that some of these cholangiolar lesions were small and very benign appearing and warranted a non-neoplastic diagnosis, and there were lesions at the other end of the spectrum that appeared aggressive. While there were specific diagnostic criteria for cholangiofibrosis versus cholangiocarcinoma, some of the lesions did not readily fit the criteria and posed a diagnostic challenge.

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

Spontaneous hepatocholangiomas are rare and did not occur in 371 vehicle control animals from this study and the six other dioxin TEF evaluation studies (NTP, 2006a,b,c,d,e,f). Hepatocholangiomas were mixed neoplasms with areas of hepatocytes that appeared identical to hepatocellular adenoma and areas of ductular structures lined by biliary epithelium that appeared identical to cholangioma. The pluripotent nature of these neoplasms was demonstrated by occasional ductular structures lined by cells resembling both hepatocytes and biliary epithelium. In contrast to the cholangiofibrosis and cholangiocarcinomas, a scirrhous response was not present within these neoplasms. While the histogenesis of hepatocholangioma is not clear, there was evidence of proliferation of hepatocytes, biliary epithelium and oval cells within these studies.

The mechanism underlying the increased incidences of cholangiocarcinoma is likely multifactorial. There was clearly an effect on bile duct proliferation in this study. This may be an indirect response to the hepatocellular toxicity or due to a direct effect on the biliary cells themselves. Tritscher *et al.* (1995) showed a high degree of staining for TGF alpha in bile duct cells after TCDD administration in female rats. The observed bile duct proliferation may represent a process of excessive and long term repair, following specific damage to hepatocytes, 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 also be a direct stimulatory effect on the oval cells themselves. This is supported by the observed increased incidence of oval cell hyperplasia in the present study. Since oval cells may differentiate into both 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 in the liver. There is a general scientific consensus that almost all responses of TCDD and related compounds require initial binding to the AhR. Recent data indicate that the acute toxic responses (including hepatotoxicity) to TCDD require AhR binding and nuclear localization (Bunger *et al.*, 2003). In addition, transgenic mouse studies indicate that constitutive activation of the AhR alone can lead to an induction of stomach tumors (Andersson *et al.*, 2002).

While the dioxin-like effects of PCB 126 are likely AhR-mediated, the mechanism of toxic effects of PCB 153 are

not clearly understood. Due to the lack of direct genotoxicity, the action of PCB 126 and PCB 153 is likely as tumor promoters. There are essentially three potential modes of action via the AhR: increased numbers of initiated cells capable of undergoing promotion, increased net growth rate of initiated cells due to selective growth advantage, or decreased rate of cell death via suppression of apoptosis. Studies have shown a suppression of apoptosis by TCDD and PCBs, including suppression of apoptosis in preneoplastic foci by PCB 153 (Stinchcombe *et al.*, 1995; Worner and Schrenk, 1996; Bohnenberger *et al.*, 2001; Tharappel *et al.*, 2002). TCDD also significantly increases hepatocyte replication as determined by BrdU labeling (Maronpot *et al.*, 1993; Walker *et al.*, 1998; Wyde *et al.*, 2001a). In addition, altered growth regulation may be due to alterations in intercellular communication, which have also been observed in the livers of rats exposed to DLCs (Baker *et al.*, 1995; Warngard *et al.*, 1996; Bager *et al.*, 1997). While DLCs are not direct-acting genotoxic agents, there are data indicating that persistent AhR-active compounds may be indirectly genotoxic. This may contribute to an increase in the number of cells within the liver capable of undergoing promotion (Moolgavkar *et al.*, 1996; Portier *et al.*, 1996). It is hypothesized that the indirect genotoxicity occurs via an AhR-dependent induction of CYP1 family cytochromes P450 that leads to an induction of oxidative stress either by 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 demonstrated that high dose acute exposure to TCDD induces oxidative stress and DNA damage (Stohs *et al.*, 1990). The induction of lipid peroxidation and single-strand DNA breaks was also observed in tissues from the present study (Hassoun *et al.*, 2000). Other studies on the female-specific tumor promotion response in rats have shown an induction of oxidative DNA damage and hepatocyte replication by TCDD that is female specific and estrogen dependent (Lucier *et al.*, 1991; Tritscher *et al.*, 1996; Wyde *et al.*, 2001a,b).

In the current study, there was a significant increase in the incidence of lung cystic keratinizing epithelioma (CKE) in Group 7 at 2 years. Histopathologically, these lesions varied in size and number and appeared as cystic structures consisting of an irregular wall of highly keratinized stratified squamous epithelium, with a center filled with keratin. In the 2-year feed study of TCDD, conducted by Kociba *et al.* (1978), an increased incidence of keratinizing squamous cell carcinoma of the lung was observed following exposure to 100 ng TCDD/kg body weight per day. In the present study as well as the NTP study of PCB 126 alone, squamous cell carcinomas were identified and distinguished from CKE by the presence of areas of solid growth and evidence of invasion. 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 likely that these are similar lesions. CKE was not a diagnostic term consistently used at the time of the Kociba *et al.* (1978) evaluation. Diagnostic criteria for classification of CKE as a lesion distinct from squamous cell carcinoma were later developed at a workshop held in the mid 1990s (Boorman *et al.*, 1996). In contrast to the present study, a recent study of the carcinogenicity of the high toxic equivalents (TEQ) PCB mixture Aroclor 1254 demonstrated no increases in the incidences of any type of lung tumor (Mayes *et al.*, 1998). While Aroclor 1254 contains a significant TEQ contribution by PCB 126, this mixture also contains mono-*ortho* and di-*ortho* PCBs, including PCB 153. No squamous cell carcinomas were observed in the PCB 153 study (NTP, 2006e).

The incidences of CKE in the current study were 1/53 (2.7% adjusted rate) in Group 5 and 11/52 (29.4% adjusted rate) in Group 7. In the study of PCB 126 alone, the incidences of lung CKE were 1/53 (2.7% adjusted rate) and 35/51 (83.5% adjusted rate) at doses of 300 and 1,000 ng/kg, respectively (NTP, 2006a); the incidence of CKEs (11/51, 26.0% adjusted rate) were significantly increased in the PCB 126 study at 550 ng/kg. No CKEs were observed in the PCB 153 study (NTP, 2006e). An initial analysis of these studies suggests there may be a less than additive effect between PCB 126 and PCB 153 on the induction of lung CKEs. The mechanism for these effects is not clear and requires further investigation and a more in-depth analysis of the results from these studies.

In the current study at 2 years, there were significant increases in the incidences of bronchiolar metaplasia of the alveolar epithelium in all dosed groups. The incidence of alveolar squamous metaplasia was increased in Group 7. These findings are consistent with prior observations of increased incidences of alveolar-bronchiolar metaplasia following exposure to TCDD in a two stage initiation-promotion model in Sprague-Dawley rat lung (Tritscher *et al.*, 2000).

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

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

Another possible mechanism for the action of DLCs on the lung may be an indirect effect due to the disruption of retinoid homeostasis in the liver. It is known that in rodents, mobilization of retinoid stores by TCDD and DLCs leads to a disruption in retinoid homeostasis and vitamin A deficiency (Van Birgelen *et al.*, 1994, 1995b; Fiorella *et al.*, 1995; Fattore *et al.*, 2000; Schmidt *et al.*, 2003). A characteristic of retinoid deficiency is abnormal epithelial differentiation to a keratinized squamous phenotype (Lancillotti *et al.*, 1992; Lotan, 1994). The action of DLCs may therefore be a disruption of retinoid action leading to altered growth and differentiation of the lung epithelium resulting in squamous metaplasia and ultimately neoplasia. The mechanisms by which coexposure to PCB 153 in the mixture may alter the incidence of CKEs in the lung are not clear and require further mechanistic investigation.

The incidences of gingival squamous cell carcinoma of the oral mucosa were significantly increased in Groups 5 and 7 at 2 years. Similarly, in the PCB 126 gavage study conducted as part of the dioxin TEF evaluation, there was a significant increase in the incidence of gingival squamous cell carcinoma of the oral mucosa in the high dose group (NTP, 2006a). Gingival squamous cell carcinoma of the oral mucosa was not observed in the study of PCB 153 (NTP, 2006e). There were increased incidences of stratified squamous cell carcinoma of the hard palate/nasal turbinates in both male and female rats in the TCDD feed study by Kociba *et al.* (1978). Similarly, in the TCDD gavage study conducted as part of the dioxin TEF evaluation, the incidence of this lesion was significantly increased at 100 ng/kg (NTP, 2006b). The location of the squamous cell carcinomas in the present study was adjacent to the molars and invaded into the hard palate/nasal turbinate areas. This suggests that the lesions seen in the NTP (2006b) and Kociba *et al.* (1978) TCDD studies are similar and that the development of this lesion is an effect induced by exposure to DLCs.

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

2003). In addition, as noted above for the effects of PCB 126 on the lung, the squamous lesions in the oral cavity may also be related to the alteration in retinoid homeostasis that is known to be induced by PCB 126.

In the current study, increased incidences of acinar cytoplasmic vacuolization occurred at 53 weeks in Group 7, and at 2 years, increased incidences of acinar cytoplasmic vacuolization occurred in Groups 5 and 7 and the incidence of acinar atrophy was increased in Group 7. The pancreatic effects were similar to those observed in the study of PCB 126, where increased incidences of acinar cytoplasmic vacuolization occurred at doses as low as 300 ng/kg, and there were increased incidences of pancreatic inflammation and arterial inflammation (NTP, 2006a). No significant incidences of pancreatic lesions were observed in the study of PCB 153 (NTP, 2006e).

Acinar atrophy of the pancreas may be related to the down-regulation of cholecystokinin (CCK). As shown by Lee *et al.* (2000) in samples from the PCB 126 study conducted as part of the dioxin TEF evaluation, intestinal CCK is reduced by PCB 126 exposure. Down-regulation of CCK is likely due to a general endocrine effect as a result of the reduction in body weight gain following exposure to PCB 126 as was observed in the high dose group. 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). Therefore, the PCB 126 is likely responsible for the endocrine effects observed in the current study of the PCB mixture.

In the constant ratio groups in the current study, there was an increased incidence of adrenal cortical atrophy in Group 7 at 2 years, and there was a single incidence of adenoma in Groups 5 and 7. These findings are consistent with sporadic cases of adenoma observed in the dioxin TEF evaluation TCDD study, a single incidence in the 300 µg/kg group in the PCB 153 study, and the equivocal evidence of treatment-related increases in the PCB 126 study (NTP, 2006a,b,e). The cortical atrophy observed was a prominent effect in Group 7 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 case of chemically induced damage or atrophy of the adrenal cortex, focal regenerative hyperplasia has been reported in rats (Yarrington and Reindel, 1996). However, in the current study, increased incidences of hyperplasia occurred in Groups 3 and 5 in the absence of increases in cortical atrophy. Likewise, cortical atrophy was increased in Group 7 without significant increases in hyperplasia.

In the current study, the incidence of kidney nephropathy was significantly increased in Group 7 at 2 years. The incidences of pigmentation were increased in Groups 5 and 7, and the incidence of hyperplasia of the transitional epithelium was significantly increased in Group 5. There were no treatment-related effects in the PCB 153 study on nephropathy, but there were increased incidences in the PCB 126 study at 550 and 1,000 ng/kg (NTP, 2006a,e). There were no incidences of pigmentation in either study. Increased incidences of hyperplasia of the transitional epithelium occurred in all dose groups in the PCB 153 study and several dose groups in the PCB 126 study, but no significant treatment-related effects were observed. While it is known that the kidney is directly responsive to the AhR agonist TCDD, the kidney historically has not been a target for DLC-induced neoplasia.

The heart is a target for TCDD and related DLCs in both rodents and humans (Peterson *et al.*, 1993; Flesch-Janys *et al.*, 1995; Walker and Catron, 2000; Heid *et al.*, 2001). Administration of PCB 126 to female Harlan Sprague-Dawley rats significantly increased the incidences, but not the severity, of cardiomyopathy in a dose-related manner (Jokinen *et al.*, 2003; NTP, 2006a). Cardiomyopathy is a common, spontaneously occurring degenerative change of myocardial fibers of unknown etiology that is seen in rats as they age. The age of onset and severity of this lesion are affected by diet, environment, and stress. In the PCB 126 study, there were significant increases in the incidences of cardiomyopathy at 300 ng/kg or greater. In the current binary mixture study of PCB 126 and PCB 153, these effects were not observed.

In the current study, significantly increased incidences of thymic atrophy occurred at 14 weeks in Groups 2 and 7, at 31 weeks in Group 7, and at 2 years in Groups 3, 5, and 7. Thymic atrophy is one of the hallmark immunotoxic responses to DLCs (Poland and Knutson, 1982) and is due to an AhR-mediated alteration in lymphocyte growth and differentiation (Staples *et al.*, 1998; Gasiewicz *et al.*, 2000). Thymic atrophy and other

hematopoietic changes may be related in part to the reduction in body weight gain observed in these animals as seen in short term feed restriction studies (Levin *et al.*, 1993).

Decreases in thyroxine (T_4) levels observed at all interim evaluations are consistent with previously observed effects on serum thyroid hormones (Ness *et al.*, 1993; Morse *et al.*, 1996). Alteration in thyroid hormone homeostasis by DLCs, including dioxin-like PCBs, may be due to increased T_4 glucuronidation as a result of increased UDP-GT expression (Van Birgelen *et al.*, 1994,1995a; Schmidt *et al.*, 2003). Subsequently, a decreased negative feedback inhibition of the thyroid gland may lead to overexpression of thyroid stimulating hormone (TSH) (Curran and DeGroot, 1991). It has been hypothesized that overstimulation of the thyroid gland by TSH may be involved in the mechanism of follicular cell carcinogenesis (Hill *et al.*, 1989). In the current study, the decrease in T_4 was only accompanied by an increase in TSH at 14 weeks. There was, however, one follicular cell adenoma in Group 5. Since TSH levels were not evaluated in this group beyond the 14-week interim evaluation and elevations were observed in other groups without induction of follicular cell adenoma, it cannot be determined if the increase in TSH promoted this follicular cell neoplasm. There were increased incidences of thyroid gland follicular cell hypertrophy in many of the dosed groups at each of the interim evaluations and at the end of the 2-year study.

At 2 years in the current study, there was a significantly lower adjusted incidence of mammary gland neoplasms following administration of the PCB mixture. Fibroadenoma is a spontaneous lesion in female Sprague-Dawley rats and occurred at the highest incidence (40/53) in vehicle controls. The incidence of fibroadenoma in Group 7 was 12/53. The incidence of mammary gland carcinoma in vehicle control animals was 8/53. The incidences of this lesion in Groups 2, 3, 5, and 7 were 4/53, 3/52, 2/53, 0/53, respectively. In addition, there was a significantly lower incidence of spontaneous pituitary gland (pars distalis) adenoma in Group 7 following exposure to the PCB mixture. In vehicle control animals, 22/53 exhibited pituitary gland neoplasms, but the incidence generally decreased with increasing dose. The incidence in Group 7 was 1/52.

It is believed that the lower incidences of mammary gland and pituitary gland neoplasms in dosed rodents are related to a general endocrine effect as a result of reductions in body weight gain associated with treatment. A significant association between reduced body weight gain and lower incidence of mammary gland and pituitary gland neoplasms has been observed in many NTP studies (Seilkop, 1995). Significantly lower incidences of mammary gland and pituitary gland neoplasms were also observed in animals exposed to 100 ng TCDD/kg body weight in the 2-year feed study of Kociba *et al.* (1978). Similarly, there were significantly lower incidences of spontaneous mammary gland and pituitary gland neoplasms in the dioxin TEF evaluation studies of both TCDD and PCB 126 (NTP, 2006a,b).

Reductions in IGF-1 may underlie the inhibitory effect of reduced body weight gain on tumor development. It is known that caloric restriction leads to lower levels of IGF-1 and 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 dioxin TEF evalaution 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.

Varying Ratio Mixture of PCB 126 and PCB 153

Human exposure to PCBs occurs as a mixture of PCB congeners and other structurally related compounds such as PCDDs and PCDFs. Several studies have demonstrated an interaction between exposure to non-dioxin-like, *ortho*-substituted PCBs and DLCs with regard to tissue concentrations and biochemical and biological effects. As discussed in this report, administration of an environmentally relevant ratio of PCB 126 and PCB 153 (1:1,000) increased the incidences of a spectrum of neoplastic lesions similar to those observed following administration of PCB 126 or TCDD (NTP, 2006a,b). Initial analysis of these data for the constant ratio dose groups suggests an interaction between PCB 126 and PCB 153 on the incidences of liver and lung neoplasms when compared to the induction of these lesions by the individual congeners. An additional objective of the current study was to evaluate the effect of varying concentrations of PCB 153 on the PCB 126-induced

responses. Doses for the varying ratio mixture were based on a constant concentration of 300 ng/kg PCB 126 with three concentrations of PCB 153 (µg/kg) and are referred to in this report as Groups 4 (300:100), 5 (300:300), and 6 (300:3,000). Although an initial analysis of the data is presented in this report, a more detailed analysis of the results from the current study and those from the studies of the individual congeners is in progress and will be presented elsewhere.

The liver was the primary target organ for the induction of neoplasms by the binary mixture of PCB 126 and PCB 153. The increases in the incidences of hepatocholangioma, hepatocellular adenoma, and cholangiocarcinoma at 2 years in the current study were consistent with the effects of PCB 126 (NTP, 2006a). Although PCB 153 alone does not induce these lesions (NTP, 2006e), there was a positive trend in the incidence of hepatocellular adenoma and cholangiocarcinoma with an increasing ratio of the PCB 153 component of the mixture. The incidences of hepatocellular adenoma were 2/50, 5/52, and 21/51 in Groups 4, 5, and 6, respectively. In the current study, incidences of hepatocellular adenoma (21/51) were higher in Group 6 rats than those in the 300 ng/kg group in the PCB 126 study (2/53) (NTP, 2006a). Similarly, the incidences of cholangiocarcinoma in the current study were 7/50, 9/52, and 25/51 in Groups 4, 5, and 6, respectively. Although the incidences of cholangiocarcinoma were similar in Group 4 compared to the incidences of 5/53 induced by the individual PCB 126 congener at the 300 ng/kg dose, administration of the mixture with higher concentrations of PCB 153 induced greater incidences of cholangiocarcinomas. Moreover, cholangiocarcinoma was not observed in the study of PCB 153 alone. These data demonstrate a positive interaction between PCB 153 and PCB 126 on liver neoplasms.

Similar effects were observed in the induction of nonneoplastic liver lesions. There was a positive trend in the incidences of diffuse and focal fatty change, hematopoietic cell proliferation, hepatocyte hypertrophy, cholangiofibrosis, bile duct hyperplasia, and basophilic, clear cell, and eosinophilic foci at 2 years. The incidences of several nonneoplastic liver lesions also occurred with positive trends at 14, 31, and 53 weeks. These interactions demonstrate a clear effect on hepatocellular and biliary nonneoplastic responses. A positive trend in the incidences of toxic hepatopathy did not occur. This lack of interaction was consistent with the results from the PCB 153 study, in which the spectrum and severity of hepatotoxicity was not sufficient to use the diagnosis of toxic hepatopathy (NTP, 2006e).

The principal lung neoplasm induced by exposure to the individual PCB 126 congener was CKE (NTP, 2006a). As previously discussed, the incidence of this lesion was considerably higher at PCB 126 doses of 1,000 ng/kg than with the same dose of PCB 126 when administered in combination with 1,000 µg/kg PCB 153. These data suggest an inhibitory or negative effect of PCB 153 on induction of the incidence of CKE by PCB 126. However, no significant differences in the incidences of CKEs were observed between the varying ratio groups. These data are not surprising, considering that a dose of 300 ng PCB 126/kg did not significantly increase the incidence of CKEs (1/53). Given the incidences of CKEs from the constant ratio mixture compared to those induced by PCB 126 alone, it would be expected that an interaction would be observed following administration of a mixture containing concentrations of PCB 126 that significantly induce CKEs.

Gingival squamous cell carcinomas of the oral mucosa have been consistently observed in treated animals from other studies of DLCs in the dioxin TEF evaluation (NTP, 2006a,b,c,d,e,f). A positive trend in the incidences of gingival squamous cell carcinoma of the oral mucosa did not occur although the survival-adjusted incidence of this neoplasm was marginally higher (7.4%, 12.9% and 14.4%) than that seen in the study of PCB 126 alone at 300 ng/kg (5.4%) (NTP, 2006a). These data suggest that PCB 153 has a minimal effect on the induction of these lesions by PCB 126.

In the current study of the constant ratio mixture of PCB 126 and PCB 153, acinar cytoplasmic vacuolation of the pancreas was observed at 53 weeks in Group 7, and at 2 years in Groups 5 and 7. This lesion was also significantly and dose-dependently increased at doses of 300 ng/kg or greater in the study of PCB 126 (NTP, 2006a). In the varying ratio groups, there was a positive trend in the incidence of acinar cytoplasmic vacuolation in groups administered increasing concentrations of PCB 153 at 2 years. The incidences were 3/49, 7/52, and 44/49 in Groups 4, 5, and 6, respectively. These data suggest a positive interaction between PCB 153 and PCB 126 for the induction of acinar cytoplasmic vacuolation. However, there were no significant treatment-related effects observed in the PCB 153 study (NTP, 2006e).

In the varying ratio groups, there was a positive trend in the incidence of thyroid gland follicular cell hypertrophy at 2 years. The incidences of follicular cell hypertrophy increased with increasing concentrations of PCB 153. Similar increases in the incidences of follicular cell hypertrophy were also observed in the current study in the constant ratio groups and in both studies of the individual PCB congeners (NTP, 2006a,e).

In the varying ratio groups, there was a negative trend in the incidence of pituitary gland (pars distalis) adenoma at 2 years. In the constant ratio groups, the incidences of spontaneous pituitary gland (pars distalis) adenoma decreased with increasing doses of PCB 153. A similar treatment-related decrease was observed in the PCB 126 study, and no treatment-related effects on this lesion were observed in PCB 153 study (NTP, 2006a,e). As previously discussed, the lower incidences of pituitary gland neoplasms may be related to a general endocrine effect as a result of reductions in body weight gain associated with treatment.

In the current study, there were no significant effects of treatment in the constant ratio groups on inflammation of the ovary or the uterus compared to vehicle controls. There was a positive trend in the incidence of chronic active inflammation of the ovary with mixtures containing greater concentrations of PCB 153 at 2 years. These results are consistent with PCB 153-induced increases in inflammation of the ovary, oviduct, and uterus observed in the PCB 153 study (NTP, 2006e). In that study, the incidences for these lesions were increased in the 1,000 and 3,000 µg/kg groups. The estrogenic potential of PCB 153 may contribute to the induction of these effects, some of which have been observed following exposure to the synthetic estrogen diethylstilbesterol (McLachlan *et al.*, 1980; Newbold, 1995).

Tissue Dosimetry

The observations of an interactive effect of PCB 153 on the carcinogenicity of PCB 126 may be due to a pharmacokinetic and/or pharmacodynamic interaction. PCB 126 and PCB 153 concentrations were analyzed in multiple tissues in this study.

Chronic administration led to significant accumulation of PCB 126 and PCB 153 in liver, fat, lung, and blood; these results are consistent with the persistent and lipophilic nature of these compounds. Previous studies of DLCs, including PCB 126, indicate that the liver and fat are the main targets in rodents and comprise approximately 70% to 80% of the total body burden in rodents (DeVito *et al.*, 1995). The levels of PCB 126 in liver were two- to fourfold higher than those in fat on a wet weight basis. This hepatic sequestration is characteristic of persistent dioxin-like compounds such as TCDD and PCB 126, and is believed to be a result of the compound binding to CYP1A2, whose expression can be induced by DLCs in the liver (Diliberto *et al.*, 1997). By comparison, PCB 153 concentrations in liver were generally less than 10% of the concentrations seen in fat, indicating minimal CYP1A2-mediated sequestration in the liver. Rather, PCB 153 distribution is determined by the lipophilic nature of the compound and the fat component of the tissue of concern.

The PCB 126 concentrations observed in the present study at lower doses were generally similar to those seen at comparable doses in the NTP study of PCB 126 alone, although concentrations at higher doses tended to be lower (NTP, 2006a). In the current study, at 2 years, the PCB 126 mean liver burden in Groups 3 and 7 was 74 ng/g and 290 ng/g, respectively. In the study of PCB 126 alone, PCB 126 mean liver concentrations were 91 ng/g and 536 ng/g in the 100 ng/kg and 1,000 ng/kg groups, respectively. In the current study, in fat, PCB 126 concentrations were 25 ng/g and 66 ng/g in Group 3 and Group 7, respectively. In the study of PCB 126 alone, terminal fat concentrations were 35 ng/g and 130 ng/g, respectively.

At 2 years, PCB 153 was detected in vehicle control animals; this is consistent with the concentrations of PCB 153 present in the animal diet (Table E5). In the current study, PCB 153 concentrations in treated groups were similar to those at comparable doses in the study of PCB 153 alone (NTP, 2006e). In the current study, at 2 years, the PCB 153 mean liver burdens were 4,688 ng/g and 94,080 ng/g in Groups 3 and 7, respectively. In the study of PCB 153 alone, PCB 153 mean liver concentrations were 3,699 ng/g and 42,664 ng/g in the 100 ng/kg and 1,000 ng/kg groups, respectively (NTP, 2006e). In the current study, the fat PCB 153 concentrations were 135 µg/g and 1,553 µg/g in Groups 3 and 7, respectively. In the study of PCB 153 alone, PCB 153 terminal fat concentrations were 158 µg/g and 1,557 µg/g (NTP, 2006e).

In the varying ratio groups, trend test analyses showed that the most notable and consistent effect was an antagonism of PCB 126 accumulation in the liver with

increasing concentrations of PCB 153. This was supported by the observation that the levels of PCB 126 in Group 6 were lower than that seen in the comparable dose group in the study of PCB 126 alone (NTP, 2006a). This phenomenon of reduced liver accumulation by PCB 153 is consistent with prior observations of decreases in the retention of TCDD in the liver of rats subchronically exposed to TCDD and PCB 153 (van der Kolk *et al*., 1992). This negative effect of PCB 153 on concentrations of PCB 126 suggests that the positive effect of PCB 153 on the carcinogenicity of PCB 126 at the 300 ng/kg dose is not due to enhanced PCB 126 accummulation. An evaluation of the mechanism of decreased PCB 126 accumulation in the liver was made using a PBPK model developed to explain the tissue dosimetry of PCB 126 and PCB 153. The model predicted decreases in PCB 126 liver accumulation at higher coadministered doses of PCB 153; this was accompanied by a decrease in CYP1A2-associated A4H activity. From this evaluation, it was concluded that the decrease in PCB 126 was likely due to an interference of CYP1A2-dependent hepatic sequestration by PCB 153.

CONCLUSIONS

Under the conditions of this 2-year gavage study 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. Increased incidences of pancreatic acinar neoplasms were also considered to be related to administration of the binary mixture of PCB 126 and PCB 153. The 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 caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, pancreas, adrenal cortex, thyroid gland, thymus, kidney, nose, and forestomach.

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

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APPENDIX A
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF A BINARY MIXTURE OF PCB 126 AND PCB 153: GROUPS 1, 2, 3, 5, 7

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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 a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Disposition Summary					
Animals initially in study	28	28	28	28	28
14-Week interim evaluation	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8
Animals examined microscopically	28	28	28	28	28
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					
Urinary System					
Kidney				(1)	
Nephroblastoma				1 (100%)	
Systems Examined at 31 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					
53-Week Interim Evaluation					
Endocrine System					
Thyroid gland	(8)	(8)	(8)	(8)	(8)
C-cell, adenoma	2 (25%)			1 (13%)	2 (25%)

Table A1a
Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Integumentary System					
Mammary gland	(8)	(2)		(5)	(1)
Carcinoma				1 (20%)	
Fibroadenoma				2 (40%)	1 (100%)
Fibroadenoma, multiple				1 (20%)	
Systems Examined at 53 Weeks with No Neoplasms Observed					
Alimentary System					
Cardiovascular System					
General Body System					
Genital System					
Hematopoietic System					
Musculoskeletal System					
Nervous System					
Respiratory System					
Special Senses System					
Urinary System					
Neoplasm Summary					
Total animals with primary neoplasms[b]					
31-Week interim evaluation				1	
53-Week interim evaluation	2			4	3
Total primary neoplasms					
31-Week interim evaluation				1	
53-Week interim evaluation	2			5	3
Total animals with benign neoplasms					
53-Week interim evaluation	2			3	3
Total benign neoplasms					
53-Week interim evaluation	2			4	3
Total animals with malignant neoplasms					
31-Week interim evaluation				1	
53-Week interim evaluation				1	
Total malignant neoplasms					
31-Week interim evaluation				1	
53-Week interim evaluation				1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Disposition Summary					
Animals initially in study	53	53	53	53	53
Early deaths					
Accidental deaths	1	1	1		2
Moribund	22	19	24	19	20
Natural deaths	8	12	6	10	7
Survivors					
Terminal sacrifice	22	21	22	24	24
Animals examined microscopically	53	53	53	53	53
Alimentary System					
Intestine large, colon	(53)	(53)	(53)	(52)	(49)
Intestine large, rectum	(53)	(53)	(53)	(53)	(50)
Schwannoma malignant, metastatic, vagina		1 (2%)		1 (2%)	
Intestine large, cecum	(53)	(53)	(53)	(52)	(49)
Intestine small, duodenum	(53)	(53)	(52)	(52)	(50)
Leiomyoma				2 (4%)	
Intestine small, jejunum	(53)	(53)	(52)	(52)	(49)
Leiomyosarcoma	1 (2%)				
Intestine small, ileum	(53)	(53)	(52)	(52)	(49)
Liver	(53)	(53)	(52)	(52)	(51)
Cholangiocarcinoma				4 (8%)	9 (18%)
Cholangiocarcinoma, multiple			1 (2%)	5 (10%)	21 (41%)
Hemangioma	1 (2%)				
Hepatocellular carcinoma					2 (4%)
Hepatocellular adenoma			3 (6%)	5 (10%)	11 (22%)
Hepatocellular adenoma, multiple					16 (31%)
Hepatocholangioma				2 (4%)	5 (10%)
Hepatocholangioma, multiple					1 (2%)
Mesentery	(47)	(47)	(46)	(47)	(42)
Nephroblastoma, metastatic, kidney		1 (2%)			
Oral mucosa	(12)	(11)	(25)	(30)	(36)
Gingival, squamous cell carcinoma			2 (8%)	5 (17%)	8 (22%)
Gingival, squamous cell carcinoma, multiple					1 (3%)
Pancreas	(53)	(53)	(52)	(52)	(50)
Acinus, adenoma		1 (2%)	1 (2%)	3 (6%)	1 (2%)
Acinus, carcinoma				1 (2%)	1 (2%)
Salivary glands	(53)	(51)	(52)	(50)	(52)
Stomach, forestomach	(53)	(53)	(52)	(52)	(51)
Stomach, glandular	(53)	(53)	(52)	(52)	(51)
Cardiovascular System					
Blood vessel	(53)	(53)	(52)	(53)	(52)
Heart	(53)	(52)	(52)	(53)	(52)
Fibrosarcoma, metastatic, lung				1 (2%)	
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)				
Schwannoma malignant		1 (2%)	1 (2%)		

Table A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Endocrine System					
Adrenal cortex	(53)	(53)	(52)	(52)	(51)
Adenoma				1 (2%)	1 (2%)
Adrenal medulla	(52)	(53)	(52)	(52)	(51)
Pheochromocytoma benign	2 (4%)	1 (2%)	4 (8%)	1 (2%)	
Bilateral, pheochromocytoma benign	1 (2%)				1 (2%)
Islets, pancreatic	(53)	(53)	(52)	(52)	(50)
Adenoma	1 (2%)		1 (2%)		
Parathyroid gland	(45)	(47)	(46)	(47)	(46)
Adenoma			1 (2%)		
Pituitary gland	(53)	(53)	(53)	(52)	(52)
Carcinoma	1 (2%)				
Pars distalis, adenoma	22 (42%)	21 (40%)	17 (32%)	17 (33%)	1 (2%)
Pars distalis, carcinoma		1 (2%)			
Pars intermedia, adenoma			2 (4%)		
Thyroid gland	(53)	(53)	(51)	(52)	(52)
Bilateral, C-cell, adenoma	2 (4%)	4 (8%)	4 (8%)	3 (6%)	
C-cell, adenoma	8 (15%)	10 (19%)	12 (24%)	12 (23%)	7 (13%)
C-cell, carcinoma	4 (8%)		1 (2%)	1 (2%)	
Follicular cell, adenoma				1 (2%)	
General Body System					
None					
Genital System					
Clitoral gland	(53)	(53)	(53)	(53)	(50)
Adenoma	1 (2%)				
Carcinoma		1 (2%)			
Ovary	(53)	(53)	(52)	(52)	(49)
Granulosa cell tumor malignant	1 (2%)			1 (2%)	
Granulosa cell tumor benign				1 (2%)	
Uterus	(53)	(53)	(53)	(52)	(50)
Adenoma					1 (2%)
Carcinoma		1 (2%)			
Fibrosarcoma				1 (2%)	
Polyp stromal	5 (9%)	7 (13%)	5 (9%)	6 (12%)	3 (6%)
Polyp stromal, multiple	3 (6%)		2 (4%)		
Sarcoma			1 (2%)		
Sarcoma stromal			1 (2%)		
Schwannoma malignant				2 (4%)	
Schwannoma malignant, metastatic, vagina				1 (2%)	
Squamous cell carcinoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)	
Cervix, granular cell tumor benign				1 (2%)	
Cervix, squamous cell carcinoma				2 (4%)	
Vagina	(1)	(1)	(1)	(1)	
Schwannoma malignant		1 (100%)		1 (100%)	
Squamous cell carcinoma	1 (100%)				

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Hematopoietic System					
Bone marrow	(53)	(53)	(53)	(53)	(53)
Lymph node	(4)	(7)	(2)	(7)	(14)
Nephroblastoma, metastatic, kidney		1 (14%)			
Pancreatic, nephroblastoma, metastatic, kidney		1 (14%)			
Lymph node, mandibular	(53)	(51)	(52)	(50)	(51)
Lymph node, mesenteric	(53)	(53)	(52)	(52)	(49)
Spleen	(53)	(53)	(52)	(52)	(50)
Thymus	(53)	(50)	(48)	(50)	(51)
Squamous cell carcinoma, metastatic, lung				1 (2%)	
Integumentary System					
Mammary gland	(53)	(53)	(52)	(53)	(52)
Adenoma	2 (4%)	1 (2%)			
Carcinoma	6 (11%)	4 (8%)	2 (4%)	1 (2%)	
Carcinoma, multiple	2 (4%)		1 (2%)	1 (2%)	
Fibroadenoma	27 (51%)	22 (42%)	25 (48%)	25 (47%)	11 (21%)
Fibroadenoma, multiple	13 (25%)	17 (32%)	15 (29%)	9 (17%)	1 (2%)
Skin	(53)	(53)	(53)	(53)	(53)
Basal cell carcinoma		2 (4%)			1 (2%)
Fibroma	2 (4%)	1 (2%)	3 (6%)	1 (2%)	
Fibrosarcoma		1 (2%)			
Fibrous histiocytoma			1 (2%)		
Keratoacanthoma			1 (2%)		
Lipoma				1 (2%)	1 (2%)
Liposarcoma		1 (2%)			
Sarcoma		1 (2%)			1 (2%)
Schwannoma malignant		1 (2%)			
Musculoskeletal System					
Bone	(53)	(53)	(53)	(53)	(53)
Skeletal muscle	(1)	(1)		(1)	(1)
Fibrosarcoma, metastatic, lung				1 (100%)	
Fibrous histiocytoma	1 (100%)				
Hemangiosarcoma		1 (100%)			
Nervous System					
Brain	(53)	(53)	(53)	(52)	(52)
Astrocytoma malignant		1 (2%)			
Carcinoma, metastatic, pituitary gland	1 (2%)	1 (2%)			
Granular cell tumor malignant				1 (2%)	

Table A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Respiratory System					
Lung	(53)	(53)	(52)	(53)	(52)
Carcinoma, metastatic, mammary gland	1 (2%)				
Cystic keratinizing epithelioma				1 (2%)	3 (6%)
Cystic keratinizing epithelioma, multiple					8 (15%)
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)				
Schwannoma malignant, metastatic, skin		1 (2%)			
Squamous cell carcinoma				1 (2%)	1 (2%)
Mediastinum, fibrosarcoma				1 (2%)	
Nose	(53)	(53)	(53)	(53)	(53)
Special Senses System					
Eye	(53)	(53)	(53)	(52)	(52)
Harderian gland	(53)	(53)	(53)	(52)	(52)
Squamous cell carcinoma, metastatic, oral mucosa				1 (2%)	3 (6%)
Zymbal's gland		(1)		(1)	
Adenoma				1 (100%)	
Carcinoma		1 (100%)			
Urinary System					
Kidney	(53)	(53)	(52)	(52)	(51)
Nephroblastoma		1 (2%)		1 (2%)	1 (2%)
Renal tubule, adenoma	1 (2%)				
Ureter	(1)	(1)		(1)	(1)
Urinary bladder	(53)	(53)	(53)	(52)	(50)
Nephroblastoma, metastatic, kidney		1 (2%)			
Papilloma	1 (2%)				1 (2%)
Systemic Lesions					
Multiple organs[b]	(53)	(53)	(53)	(53)	(53)
Lymphoma malignant		1 (2%)	2 (4%)	2 (4%)	2 (4%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	51	49	50	50	46
Total primary neoplasms	110	106	110	126	121
Total animals with benign neoplasms	49	43	48	46	36
Total benign neoplasms	92	85	96	93	73
Total animals with malignant neoplasms	14	18	14	23	37
Total malignant neoplasms	18	21	14	33	48
Total animals with metastatic neoplasms	3	5		3	3
Total metastatic neoplasms	4	8		6	3

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	085	345	377	418	426	449	451	451	451	484	499	533	556	556	556	556	581	636	640	647	654	654	664	664	664	664
Carcass ID Number	042	005	022	088	081	032	012	025	097	031	093	009	010	015	041	065	044	020	024	086	089	008	014	036	039
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Mesentery	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Oral mucosa							+						+				+	+		+			+	+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth							+	+					+				+			+	+		+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skeletal muscle			X																						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																								X	
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	M	M	+	+	M	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Pars distalis, adenoma				X	X											X		X			X	X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma					X																				X
C-cell, carcinoma					X																				
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	670	684	688	708	714	719	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	067	063	087	091	011	061	001	007	018	021	034	063	069	082	092	094	003	040	083	004	013	043	064	066	068	070	090	096	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leiomyosarcoma																								X					1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hemangioma			X																										1
Mesentery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	M	47
Oral mucosa				+	+								+				+				+								12
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth			+	+	+	+	+	+				+	+	+	+		+				+	+				+			23
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibrous histiocytoma, metastatic, skeletal muscle																													1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	52
Pheochromocytoma benign																									X				2
Bilateral, pheochromocytoma benign																	X												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma		X																											1
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma					X																								1
Pars distalis, adenoma	X					X	X		X	X		X	X		X			X	X			X	X	X	X	X			22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma		X										X																	2
C-cell, adenoma									X						X						X	X				X	X		8
C-cell, carcinoma	X																			X					X				4
General Body System																													
None																													

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	085	345	377	418	426	449	451	451	451	484	499	533	556	556	556	581	636	640	647	654	654	664	664	664	664
Carcass ID Number	042	005	022	088	081	032	012	025	097	031	093	009	010	015	041	065	044	020	024	086	089	008	014	036	039
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																									
Polyp stromal, multiple																		X							
Squamous cell carcinoma																									
Vagina																									
Squamous cell carcinoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+		+									+								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma						X																			
Carcinoma, multiple																									
Fibroadenoma		X					X	X	X	X			X			X			X	X	X	X	X		X
Fibroadenoma, multiple											X			X	X		X	X						X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																						X			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						
Fibrous histiocytoma			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland																									
Fibrous histiocytoma, metastatic, skeletal muscle			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	670	684	688	708	714	719	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	067	063	087	091	011	061	001	007	018	021	034	063	069	082	092	094	003	040	083	004	013	043	064	066	068	070	090	096	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																													1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granulosa cell tumor malignant																				X									1
Oviduct													+																1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp stromal					X						X			X						X								X	5
Polyp stromal, multiple						X																			X				3
Squamous cell carcinoma							X																						1
Vagina																								+					1
Squamous cell carcinoma																								X					1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																								+					4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma												X												X					2
Carcinoma	X									X			X												X			X	6
Carcinoma, multiple									X													X							2
Fibroadenoma	X		X	X			X		X	X	X		X		X				X		X	X		X	X				27
Fibroadenoma, multiple		X			X			X				X				X		X					X						13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma						X																							2
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																													1
Fibrous histiocytoma																													1
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, pituitary gland					X																								1
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, mammary gland																									X				1
Fibrous histiocytoma, metastatic, skeletal muscle																													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	085	345	377	418	426	449	451	451	451	484	499	533	556	556	556	581	636	640	647	654	654	664	664	664	664
Carcass ID Number	042	005	022	088	081	032	012	025	097	031	093	009	010	015	041	065	044	020	024	086	089	008	014	036	039
Special Senses System																									
Ear																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Ureter																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma																	X								
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	670	684	688	708	714	719	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	067	063	087	091	011	061	001	007	018	021	034	063	069	082	092	094	003	040	083	004	013	043	064	066	068	070	090	096		Total Tissues/ Tumors
Special Senses System																														
Ear																								+						1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Urinary System																														
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Renal tubule, adenoma																									X					1
Ureter																								+						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Papilloma																														1
Systemic Lesions																														
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 2 10 ng/kg:10 µg/kg

Number of Days on Study	167	178	296	330	337	367	378	407	469	469	499	534	534	563	580	589	603	636	636	639	643	661	661	664	680
Carcass ID Number	108	181	163	161	167	131	123	151	132	165	124	112	120	147	183	146	148	113	127	118	155	115	154	117	133
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, vagina																									
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma, metastatic, kidney	X																								
Oral mucosa											+			+						+	+			+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth											+	+		+						+				+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aorta, lymphoma malignant, metastatic, lymph node, mesenteric						X																			
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Schwannoma malignant																			X						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma														X	X			X		X		X	X		X
Pars distalis, carcinoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																		X							
C-cell, adenoma											X				X				X						
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 2 10 ng/kg:10 µg/kg

Number of Days on Study	689	689	689	694	698	720	727	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731
Carcass ID Number	111	114	152	185	104	119	101	125	126	128	129	134	142	144	149	164	186	121	141	143	103	105	116	122	145	162	182	184	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant, metastatic, vagina										X																			1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Mesentery	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	47
Nephroblastoma, metastatic, kidney																													1
Oral mucosa						+	+									+	+			+	+								11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Acinus, adenoma					X																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth						+		+						+	+	+	+		+	+	+								14
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Aorta, lymphoma malignant, metastatic, lymph node, mesenteric																													1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Schwannoma malignant																													1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign																	X												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Parathyroid gland	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma	X	X	X		X			X		X		X		X			X			X	X		X		X		X		21
Pars distalis, carcinoma																X													1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma								X	X							X													4
C-cell, adenoma	X	X											X				X		X				X		X				10
General Body System																													
None																													

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 2 10 ng/kg:10 µg/kg

Number of Days on Study	167	178	296	330	337	367	378	407	469	469	499	534	534	563	580	589	603	636	636	639	643	661	661	664	680
Carcass ID Number	108	181	163	161	167	131	123	151	132	165	124	112	120	147	183	146	148	113	127	118	155	115	154	117	133
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								X	
Polyp stromal													X	X											
Squamous cell carcinoma																									
Vagina																									
Schwannoma malignant																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+				+	+				+															
Nephroblastoma, metastatic, kidney	X																								
Pancreatic, nephroblastoma, metastatic, kidney	X																								
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X																				
Fibroadenoma			X	X								X	X		X	X			X	X				X	
Fibroadenoma, multiple										X	X			X				X					X		X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																	X								
Fibroma																									
Fibrosarcoma																									
Liposarcoma																									
Sarcoma								X																	
Schwannoma malignant																									X
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Hemangiosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Carcinoma, metastatic, pituitary gland																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 2 10 ng/kg:10 µg/kg

Number of Days on Study	689	689	689	694	698	720	727	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	
Carcass ID Number	1114	1114	1582	1885	1004	1119	1001	1225	1226	1228	1229	1334	1442	1444	1449	1664	1886	1221	1441	1443	1003	1005	1116	1222	1445	1662	1882	1884		Total Tissues/ Tumors
Genital System																														
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Carcinoma												X																		1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Carcinoma																														1
Polyp stromal			X								X			X			X						X							7
Squamous cell carcinoma																											X			1
Vagina										+																				1
Schwannoma malignant										X																				1
Hematopoietic System																														
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Lymph node										+	+													+						7
Nephroblastoma, metastatic, kidney																														1
Pancreatic, nephroblastoma, metastatic, kidney																														1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+		50
Integumentary System																														
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Adenoma																				X										1
Carcinoma																	X							X		X				4
Fibroadenoma		X	X		X	X					X		X	X		X				X		X			X	X	X			22
Fibroadenoma, multiple	X			X			X	X	X	X					X						X		X	X				X		17
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Basal cell carcinoma																								X						2
Fibroma																				X										1
Fibrosarcoma				X																										1
Liposarcoma												X																		1
Sarcoma																														1
Schwannoma malignant																														1
Musculoskeletal System																														
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Skeletal muscle																						+								1
Hemangiosarcoma																						X								1
Nervous System																														
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Astrocytoma malignant																X														1
Carcinoma, metastatic, pituitary gland																X														1

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 2 10 ng/kg:10 µg/kg

Number of Days on Study	167	178	296	330	337	367	378	407	469	469	499	534	534	563	580	589	603	636	636	639	643	661	661	664	680
Carcass ID Number	108	181	163	161	167	131	123	151	132	165	124	112	120	147	183	146	148	113	127	118	155	115	154	117	133
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, skin																									X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland									+																
Carcinoma									X																
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma	X																								
Ureter						+																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma, metastatic, kidney	X																								
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant						X																			

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 2 10 ng/kg:10 µg/kg

Number of Days on Study	689	689	689	694	698	720	727	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731
Carcass ID Number	111	114	152	185	104	119	101	125	126	128	129	134	142	144	149	164	186	121	141	143	103	105	116	122	145	162	182	184	Total Tissues/ Tumors
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant, metastatic, skin																													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Zymbal's gland																													1
Carcinoma																													1
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nephroblastoma																													1
Ureter																													1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nephroblastoma, metastatic, kidney																													1
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymphoma malignant																													1

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 3 100 ng/kg:100 µg/kg

Number of Days on Study	125	254	407	433	458	461	469	469	491	499	531	556	581	588	589	603	603	636	638	654	654	654	659	664	664
Carcass ID Number	262	229	245	269	283	287	224	242	210	289	250	285	281	211	279	208	263	264	271	268	282	288	267	248	280
Alimentary System																									
Esophagus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma, multiple																									
Hepatocellular adenoma																				X					
Mesentery	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+
Oral mucosa							+		+			+	+	+			+		+	+		+	+		+
Gingival, squamous cell carcinoma									X										X						
Pancreas	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+		+	+	+					+	+	+			+			+		+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Endocrine System																									
Adrenal cortex	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																		X							
Islets, pancreatic	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	M	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X				X				X		X			X		
Pars intermedia, adenoma						X																			
Thyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma																	X		X	X					
C-cell, carcinoma																									
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 3 100 ng/kg:100 µg/kg

Number of Days on Study	705	715	715	715	720	722	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	731	731	731	731	731	731		
Carcass ID Number	241	203	213	273	251	266	206	207	222	247	252	270	277	278	290	296	202	205	209	225	243	297	201	244	246	274	286	298	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Cholangiocarcinoma, multiple																X													1
Hepatocellular adenoma				X																				X					3
Mesentery	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	46
Oral mucosa			+	+		+	+			+	+		+	+		+				+		+	+	+				+	25
Gingival, squamous cell carcinoma																													2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Acinus, adenoma							X																						1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Tooth	+			+	+	+	+			+	+	+	+	+		+	+		+	+	+	+	+	+		+		+	33
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Schwannoma malignant		X																											1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pheochromocytoma benign						X	X													X									4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma	X																												1
Parathyroid gland	+	M	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	46
Adenoma										X																			1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma				X			X					X		X		X	X		X		X	X		X		X		X	17
Pars intermedia, adenoma	X																												2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Bilateral, C-cell, adenoma		X				X		X																X					4
C-cell, adenoma												X	X			X		X	X	X		X			X	X			12
C-cell, carcinoma																	X												1
General Body System																													
None																													

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 3 100 ng/kg:100 µg/kg

Number of Days on Study	125	254	407	433	458	461	469	469	491	499	531	556	581	588	589	603	603	636	638	654	654	654	659	664	664
Carcass ID Number	262	229	245	269	283	287	224	242	210	289	250	285	281	211	279	208	263	264	271	268	282	288	267	248	280
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oviduct																+									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																									
Polyp stromal, multiple																							X		
Sarcoma																								X	
Sarcoma stromal					X																				
Squamous cell carcinoma																									
Vagina																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+													+							
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma						X																			
Carcinoma, multiple																									
Fibroadenoma		X					X	X		X	X		X		X		X		X		X			X	
Fibroadenoma, multiple												X		X				X				X	X		X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																								X	
Fibrous histiocytoma																									
Keratoacanthoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 3 100 ng/kg:100 µg/kg

Number of Days on Study	705	715	715	715	720	722	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	731	731	731	731	731	731	731
Carcass ID Number	241	203	213	273	251	266	206	207	222	247	252	270	277	278	290	296	302	205	209	225	243	297	201	244	246	274	286	298	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Oviduct																													1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp stromal	X							X			X			X							X								5
Polyp stromal, multiple																							X						2
Sarcoma																													1
Sarcoma stromal																													1
Squamous cell carcinoma																										X			1
Vagina																										+			1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																													2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	+	+	+	+	M	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma																					X								2
Carcinoma, multiple														X															1
Fibroadenoma					X	X		X	X	X				X	X	X	X					X		X	X	X		X	25
Fibroadenoma, multiple	X	X	X	X														X	X	X			X				X		15
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma				X			X																						3
Fibrous histiocytoma																											X		1
Keratoacanthoma																													1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 3 100 ng/kg:100 µg/kg

Number of Days on Study	125	254	407	433	458	461	469	469	491	499	531	556	581	588	589	603	603	636	638	654	654	654	659	664	664
Carcass ID Number	262	229	245	269	283	287	224	242	210	289	250	285	281	211	279	208	263	264	271	268	282	288	267	248	280
Urinary System																									
Kidney	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 3 100 ng/kg:100 µg/kg

Number of Days on Study	705	715	715	715	720	722	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	731	731	731	731	731	731		
Carcass ID Number	241	203	213	273	251	266	206	207	222	247	252	270	277	278	290	296	302	305	309	225	243	297	201	244	246	274	286	298	Total Tissues/ Tumors
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymphoma malignant	X																					X							2

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	096	142	254	310	330	363	451	479	499	505	505	506	539	552	556	580	583	603	603	612	618	650	654	659	665
Carcass ID Number	466	486	490	443	402	427	412	498	410	414	488	497	411	485	417	442	456	421	425	405	457	496	409	481	418
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, vagina														X											
Intestine large, cecum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Intestine small, jejunum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																			X						
Cholangiocarcinoma, multiple																									
Hepatocellular adenoma																									
Hepatocholangioma																									
Mesentery	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+
Oral mucosa							+	+					+	+	+	+	+		+	+	+	+	+		
Gingival, squamous cell carcinoma								X								X									
Pancreas	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Acinus, carcinoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Stomach, forestomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+		+		+	+	+			+		+	+		+		+		+	+	+	+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, lung																			X						
Endocrine System																									
Adrenal cortex	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pars distalis, adenoma												X		X			X							X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma									X						X	X									X
C-cell, carcinoma																									
Follicular cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	684	710	715	719	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	493	420	484	430	401	407	413	416	428	445	465	482	487	494	404	406	408	423	460	462	476	480	495	424	426	461	491	492	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant, metastatic, vagina																													1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Leiomyoma		X																								X			2
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Cholangiocarcinoma							X	X					X																4
Cholangiocarcinoma, multiple	X								X											X		X			X				5
Hepatocellular adenoma	X						X					X	X											X					5
Hepatocholangioma																			X	X									2
Mesentery	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	47
Oral mucosa		+	+	+			+	+	+		+	+	+	+		+	+			+		+	+		+		+	+	30
Gingival, squamous cell carcinoma		X		X																X									5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Acinus, adenoma						X										X										X			3
Acinus, carcinoma										X																			1
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Tooth	+	+	+	+		+	+	+	+		+	+	+	+		+	+	+	+	+		+	+		+			+	35
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibrosarcoma, metastatic, lung																													1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma																													1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pheochromocytoma benign																									X				1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pars distalis, adenoma			X							X	X		X	X	X	X					X	X	X			X	X	X	17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Bilateral, C-cell, adenoma																							X	X				X	3
C-cell, adenoma	X						X		X	X					X						X	X				X			12
C-cell, carcinoma																		X											1
Follicular cell, adenoma																					X								1

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	096	142	254	310	330	363	451	479	499	505	505	506	539	552	556	580	583	603	603	612	618	650	654	659	665
Carcass ID Number	466	486	490	443	402	427	412	498	410	414	488	497	411	485	417	442	456	421	425	405	457	496	409	481	418
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Granulosa cell tumor benign																									
Oviduct													+											+	
Uterus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Polyp stromal																									
Schwannoma malignant								X					X												
Schwannoma malignant, metastatic, vagina														X											
Squamous cell carcinoma																									
Cervix, granular cell tumor benign																									
Cervix, squamous cell carcinoma																									
Vagina														+											
Schwannoma malignant														X											
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+			+				+		+							+				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Squamous cell carcinoma, metastatic,lung																			X						
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma							X																		
Carcinoma, multiple																									
Fibroadenoma			X		X				X		X	X					X	X	X	X	X	X			
Fibroadenoma, multiple										X			X		X								X		X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																								X	
Lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																			+						
Fibrosarcoma, metastatic, lung																			X						

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	684	710	715	719	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731
Carcass ID Number	493	420	484	430	401	407	413	416	428	445	465	482	487	494	404	406	408	423	460	462	476	480	495	424	426	461	491	492	Total Tissues/ Tumors
General Body System																													
None																													
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Granulosa cell tumor malignant										X																			1
Granulosa cell tumor benign							X																						1
Oviduct																													2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Fibrosarcoma																						X							1
Polyp stromal			X				X		X										X	X		X							6
Schwannoma malignant																													2
Schwannoma malignant, metastatic, vagina																													1
Squamous cell carcinoma													X										X						2
Cervix, granular cell tumor benign																						X							1
Cervix, squamous cell carcinoma			X	X																									2
Vagina																													1
Schwannoma malignant																													1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node									+																	+			7
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, lung																													1
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																													1
Carcinoma, multiple																					X								1
Fibroadenoma		X		X	X	X			X		X		X			X	X			X	X			X		X		X	25
Fibroadenoma, multiple			X												X			X							X				9
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma																													1
Lipoma																					X								1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																													1
Fibrosarcoma, metastatic, lung																													1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	096	142	254	310	330	363	451	479	499	505	505	506	539	552	556	580	583	603	603	612	618	650	654	659	665
Carcass ID Number	466	486	490	443	402	427	412	498	410	414	488	497	411	485	417	442	456	421	425	405	457	496	409	481	418
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Granular cell tumor malignant																										
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystic keratinizing epithelioma																										
Squamous cell carcinoma																			X							
Mediastinum, fibrosarcoma																			X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear																		+			+					
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Squamous cell carcinoma, metastatic, oral mucosa																X										
Zymbal's gland																										
Adenoma																										
Urinary System																										
Kidney	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma		X																								
Ureter																					+					
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																				X						

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	684	710	715	719	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	493	420	484	430	401	407	413	416	428	445	465	482	487	494	404	406	408	423	460	462	476	480	495	424	426	461	491	492		Total Tissues/ Tumors
Nervous System																														
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Granular cell tumor malignant																		X												1
Respiratory System																														
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Cystic keratinizing epithelioma												X																		1
Squamous cell carcinoma																														1
Mediastinum, fibrosarcoma																														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Special Senses System																														
Ear																														2
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Squamous cell carcinoma, metastatic, oral mucosa																														1
Zymbal's gland												+																		1
Adenoma												X																		1
Urinary System																														
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Nephroblastoma																														1
Ureter																														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Systemic Lesions																														
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Lymphoma malignant																						X								2

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 7 1,000 ng/kg:1,000 µg/kg

Number of Days on Study	071	076	169	181	206	314	426	477	479	485	491	499	540	558	563	563	574	580	581	595	606	619	640	647	654
Carcass ID Number	682	629	627	662	684	670	680	650	692	674	676	649	630	667	622	683	602	671	624	661	607	641	663	640	643
Alimentary System																									
Esophagus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	M	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	M	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	M	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Liver	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma									X															X	
Cholangiocarcinoma, multiple													X		X					X		X			X
Hepatocellular carcinoma																									
Hepatocellular adenoma									X					X		X					X	X	X		X
Hepatocellular adenoma, multiple																								X	
Hepatocholangioma																									
Hepatocholangioma, multiple																									
Mesentery	M	M	+	+	M	+	M	M	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+
Oral mucosa									+	+	+		+	+	+		+	+	+	+	+	+	+	+	+
Gingival, squamous cell carcinoma																	X	X		X		X	X	X	
Gingival, squamous cell carcinoma, multiple															X										
Pancreas	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									X
Acinus, carcinoma																									
Salivary glands	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+						+	+	+	+	+							+	+		+		+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, pheochromocytoma benign						X																			
Islets, pancreatic	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	M	+	M	+	+	+	M	+	+	M	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma										X													X		
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 7 1,000 ng/kg:1,000 µg/kg

Number of Days on Study	665	706	714	714	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	694	685	603	604	601	609	625	626	638	669	678	691	695	696	623	637	639	647	677	698	628	648	664	666	672	673	679	693	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cholangiocarcinoma			X	X					X		X							X		X							X		9
Cholangiocarcinoma, multiple		X			X	X	X	X					X		X	X	X		X		X		X	X	X	X		X	21
Hepatocellular carcinoma												X																X	2
Hepatocellular adenoma										X	X	X				X													11
Hepatocellular adenoma, multiple		X				X	X	X					X		X			X	X	X	X			X	X	X	X	X	16
Hepatocholangioma						X														X	X		X			X			5
Hepatocholangioma, multiple																											X		1
Mesentery	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	42
Oral mucosa	+	+	+	+	+	+	+	+		+	+	+		+	+	+				+	+	+	+	+	+			+	36
Gingival, squamous cell carcinoma		X	X																										8
Gingival, squamous cell carcinoma, multiple																													1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																													1
Acinus, carcinoma																		X											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Tooth	+	+	+	+	+		+			+	+	+		+	+	+	+			+	+	+	+	+	+	+		+	31
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenoma																								X					1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Bilateral, pheochromocytoma benign																													1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pars distalis, adenoma				X																									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
C-cell, adenoma		X								X										X						X	X		7
General Body System																													
None																													

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 7 1,000 ng/kg:1,000 µg/kg

Number of Days on Study	071	076	169	181	206	314	426	477	479	485	491	499	540	558	563	563	574	580	581	595	606	619	640	647	654
Carcass ID Number	682	629	627	662	684	670	680	650	692	674	676	649	630	667	622	683	602	671	624	661	607	641	663	640	643
Genital System																									
Clitoral gland	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	M	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Oviduct										+	+														
Uterus	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Polyp stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+				+		+						+											+
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	M	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																			X						X
Fibroadenoma, multiple									X																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma				X																					
Lipoma																									
Sarcoma					X																				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Nervous System																									
Brain	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																									+
Spinal cord																									+
Respiratory System																									
Lung	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystic keratinizing epithelioma																					X		X		
Cystic keratinizing epithelioma, multiple																						X			
Squamous cell carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 7 1,000 ng/kg:1,000 µg/kg

Number of Days on Study	665	706	714	714	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731
Carcass ID Number	694	685	603	604	601	609	625	626	638	669	678	691	695	696	623	637	639	647	677	698	628	648	664	666	672	673	679	693	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Oviduct											+																		3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																													1
Polyp stromal	X								X		X																		3
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node					+					+	+	+						+	+			+	+		+				14
Lymph node, mandibular	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Fibroadenoma	X			X	X	X	X		X			X			X										X				11
Fibroadenoma, multiple																													1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Basal cell carcinoma																													1
Lipoma				X																									1
Sarcoma																													1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																													1
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Peripheral nerve																													1
Spinal cord																													1
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Cystic keratinizing epithelioma				X																									3
Cystic keratinizing epithelioma, multiple							X		X		X											X	X	X		X			8
Squamous cell carcinoma																							X						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 7 1,000 ng/kg:1,000 µg/kg

Number of Days on Study	071	076	169	181	206	314	426	477	479	485	491	499	540	558	563	563	574	580	581	595	606	619	640	647	654
Carcass ID Number	682	629	627	662	684	670	680	650	692	674	676	644	630	667	622	683	602	671	624	661	607	641	663	640	643
Special Senses System																									
Ear																									
Eye	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, oral mucosa															X			X						X	
Urinary System																									
Kidney	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma			X																						
Ureter												+													
Urinary bladder	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Papilloma																X									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																									

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 7 1,000 ng/kg:1,000 µg/kg

Number of Days on Study	665	706	714	714	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	
Carcass ID Number	694	685	603	604	601	609	625	626	638	669	678	691	695	696	623	637	639	647	677	698	628	648	664	666	672	673	679	693		Total Tissues/ Tumors
Special Senses System																														
Ear																								+						1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Squamous cell carcinoma, metastatic, oral mucosa																														3
Urinary System																														
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		51
Nephroblastoma																														1
Ureter																														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Papilloma																														1
Systemic Lesions																														
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Lymphoma malignant																			X							X				2

TABLE A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Adrenal Medulla: Pheochromocytoma Benign					
Overall rate[a]	3/52 (6%)	1/53 (2%)	4/52 (8%)	1/52 (2%)	1/51 (2%)
Adjusted rate[b]	8.0%	2.7%	10.2%	2.7%	2.7%
Terminal rate[c]	2/21 (10%)	1/21 (5%)	2/22 (9%)	1/24 (4%)	0/24 (0%)
First incidence (days)	664	729 (T)	636	729 (T)	314
Poly-3 test[d]	P=0.255N	P=0.304N	P=0.525	P=0.304N	P=0.306N
Liver: Hepatocholangioma					
Overall rate	0/53 (0%)	0/53 (0%)	0/52 (0%)	2/52 (4%)	6/51 (12%)
Adjusted rate	0.0%	0.0%	0.0%	5.4%	16.6%
Terminal rate	0/22 (0%)	0/21 (0%)	0/22 (0%)	2/24 (8%)	6/24 (25%)
First incidence (days)	—[e]	—	—	729 (T)	729 (T)
Poly-3 test	P<0.001	—[f]	—	P=0.232	P=0.012
Liver: Hepatocellular Adenoma					
Overall rate	0/53 (0%)	0/53 (0%)	3/52 (6%)	5/52 (10%)	27/51 (53%)
Adjusted rate	0.0%	0.0%	7.7%	13.3%	67.7%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	4/24 (17%)	18/24 (75%)
First incidence (days)	—	—	654	684	479
Poly-3 test	P<0.001	—	P=0.122	P=0.028	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	0/53 (0%)	0/53 (0%)	3/52 (6%)	5/52 (10%)	27/51 (53%)
Adjusted rate	0.0%	0.0%	7.7%	13.3%	67.7%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	4/24 (17%)	18/24 (75%)
First incidence (days)	—	—	654	684	479
Poly-3 test	P<0.001	—	P=0.122	P=0.028	P<0.001
Liver: Cholangiocarcinoma					
Overall rate	0/53 (0%)	0/53 (0%)	1/52 (2%)	9/52 (17%)	30/51 (59%)
Adjusted rate	0.0%	0.0%	2.6%	23.7%	75.5%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	7/24 (29%)	20/24 (83%)
First incidence (days)	—	—	729 (T)	603	479
Poly-3 test	P<0.001	—	P=0.503	P<0.001	P<0.001
Lung: Cystic Keratinizing Epithelioma					
Overall rate	0/53 (0%)	0/53 (0%)	0/52 (0%)	1/53 (2%)	11/52 (21%)
Adjusted rate	0.0%	0.0%	0.0%	2.7%	29.4%
Terminal rate	0/22 (0%)	0/21 (0%)	0/22 (0%)	1/24 (4%)	7/24 (29%)
First incidence (days)	—	—	—	729 (T)	606
Poly-3 test	P<0.001	—	—	P=0.496	P<0.001
Mammary Gland: Fibroadenoma					
Overall rate	40/53 (75%)	39/53 (74%)	40/53 (75%)	34/53 (64%)	12/53 (23%)
Adjusted rate	83.1%	85.1%	84.2%	73.5%	31.4%
Terminal rate	16/22 (73%)	17/21 (81%)	17/22 (77%)	15/24 (63%)	7/24 (29%)
First incidence (days)	345	296	254	254	479
Poly-3 test	P<0.001N	P=0.510	P=0.555	P=0.178N	P<0.001N

TABLE A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Mammary Gland: Fibroadenoma or Adenoma					
Overall rate	40/53 (75%)	39/53 (74%)	40/53 (75%)	34/53 (64%)	12/53 (23%)
Adjusted rate	83.1%	85.1%	84.2%	73.5%	31.4%
Terminal rate	16/22 (73%)	17/21 (81%)	17/22 (77%)	15/24 (63%)	7/24 (29%)
First incidence (days)	345	296	254	254	479
Poly-3 test	P<0.001N	P=0.510	P=0.555	P=0.178N	P<0.001N
Mammary Gland: Carcinoma					
Overall rate	8/53 (15%)	4/53 (8%)	3/53 (6%)	2/53 (4%)	0/53 (0%)
Adjusted rate	20.4%	10.4%	7.5%	5.2%	0.0%
Terminal rate	6/22 (27%)	3/21 (14%)	2/22 (9%)	1/24 (4%)	0/24 (0%)
First incidence (days)	449	337	461	451	—
Poly-3 test	P=0.010N	P=0.182N	P=0.089N	P=0.046N	P=0.005N
Mammary Gland: Adenoma or Carcinoma					
Overall rate	10/53 (19%)	5/53 (9%)	3/53 (6%)	2/53 (4%)	0/53 (0%)
Adjusted rate	25.5%	13.1%	7.5%	5.2%	0.0%
Terminal rate	8/22 (36%)	4/21 (19%)	2/22 (9%)	1/24 (4%)	0/24 (0%)
First incidence (days)	449	337	461	451	—
Poly-3 test	P=0.003N	P=0.132N	P=0.029N	P=0.013N	P<0.001N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma					
Overall rate	42/53 (79%)	41/53 (77%)	42/53 (79%)	35/53 (66%)	12/53 (23%)
Adjusted rate	85.9%	87.7%	87.1%	74.5%	31.4%
Terminal rate	17/22 (77%)	18/21 (86%)	18/22 (82%)	15/24 (63%)	7/24 (29%)
First incidence (days)	345	296	254	254	479
Poly-3 test	P<0.001N	P=0.518	P=0.555	P=0.114N	P<0.001N
Oral Mucosa (Gingival): Squamous Cell Carcinoma					
Overall rate	0/53 (0%)	0/53 (0%)	2/53 (4%)	5/53 (9%)	9/53 (17%)
Adjusted rate	0.0%	0.0%	5.0%	12.9%	22.7%
Terminal rate	0/22 (0%)	0/21 (0%)	0/22 (0%)	1/24 (4%)	0/24 (0%)
First incidence (days)	—	—	491	479	563
Poly-3 test	P<0.001	—	P=0.247	P=0.031	P=0.002
Pancreas: Adenoma					
Overall rate	0/53 (0%)	1/53 (2%)	1/52 (2%)	3/52 (6%)	1/50 (2%)
Adjusted rate	0.0%	2.7%	2.6%	8.0%	2.8%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	3/24 (13%)	0/24 (0%)
First incidence (days)	—	698	729 (T)	729 (T)	654
Poly-3 test	P=0.494	P=0.496	P=0.503	P=0.114	P=0.489
Pancreas: Adenoma or Carcinoma					
Overall rate	0/53 (0%)	1/53 (2%)	1/52 (2%)	4/52 (8%)	2/50 (4%)
Adjusted rate	0.0%	2.7%	2.6%	10.7%	5.5%
Terminal rate	0/22 (0%)	0/21 (0%)	1/22 (5%)	4/24 (17%)	1/24 (4%)
First incidence (days)	—	698	729 (T)	729 (T)	654
Poly-3 test	P=0.226	P=0.496	P=0.503	P=0.056	P=0.224

TABLE A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	22/53 (42%)	21/53 (40%)	17/53 (32%)	17/52 (33%)	1/52 (2%)
Adjusted rate	52.8%	52.0%	41.3%	43.8%	2.8%
Terminal rate	13/22 (59%)	10/21 (48%)	11/22 (50%)	12/24 (50%)	0/24 (0%)
First incidence (days)	418	563	499	506	714
Poly-3 test	P<0.001N	P=0.560N	P=0.195N	P=0.271N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma					
Overall rate	23/53 (43%)	22/53 (42%)	17/53 (32%)	17/52 (33%)	1/52 (2%)
Adjusted rate	55.2%	54.5%	41.3%	43.8%	2.8%
Terminal rate	13/22 (59%)	11/21 (52%)	11/22 (50%)	12/24 (50%)	0/24 (0%)
First incidence (days)	418	563	499	506	714
Poly-3 test	P<0.001N	P=0.567N	P=0.139N	P=0.203N	P<0.001N
Skin: Fibroma					
Overall rate	2/53 (4%)	1/53 (2%)	3/53 (6%)	1/53 (2%)	0/53 (0%)
Adjusted rate	5.2%	2.7%	7.6%	2.7%	0.0%
Terminal rate	0/22 (0%)	1/21 (5%)	1/22 (5%)	0/24 (0%)	0/24 (0%)
First incidence (days)	664	729 (T)	664	659	—
Poly-3 test	P=0.157N	P=0.510N	P=0.510	P=0.507N	P=0.248N
Skin: Fibroma, Fibrosarcoma, or Sarcoma					
Overall rate	2/53 (4%)	3/53 (6%)	4/53 (8%)	1/53 (2%)	1/53 (2%)
Adjusted rate	5.2%	7.8%	10.2%	2.7%	2.7%
Terminal rate	0/22 (0%)	1/21 (5%)	2/22 (9%)	0/24 (0%)	0/24 (0%)
First incidence (days)	664	407	664	659	206
Poly-3 test	P=0.208N	P=0.499	P=0.346	P=0.507N	P=0.509N
Thyroid Gland (C-Cell): Adenoma					
Overall rate	10/53 (19%)	14/53 (26%)	16/51 (31%)	15/52 (29%)	7/52 (13%)
Adjusted rate	25.4%	35.4%	40.1%	38.5%	18.7%
Terminal rate	7/22 (32%)	8/21 (38%)	11/22 (50%)	10/24 (42%)	4/24 (17%)
First incidence (days)	426	499	603	499	485
Poly-3 test	P=0.077N	P=0.231	P=0.118	P=0.151	P=0.333N
Thyroid Gland (C-Cell): Carcinoma					
Overall rate	4/53 (8%)	0/53 (0%)	1/51 (2%)	1/52 (2%)	0/52 (0%)
Adjusted rate	10.2%	0.0%	2.6%	2.7%	0.0%
Terminal rate	2/22 (9%)	0/21 (0%)	1/22 (5%)	1/24 (4%)	0/24 (0%)
First incidence (days)	426	—	729 (T)	729 (T)	—
Poly-3 test	P=0.152N	P=0.065N	P=0.180N	P=0.196N	P=0.069N
Thyroid Gland (C-Cell): Adenoma or Carcinoma					
Overall rate	13/53 (25%)	14/53 (26%)	17/51 (33%)	16/52 (31%)	7/52 (13%)
Adjusted rate	32.8%	35.4%	42.6%	41.1%	18.7%
Terminal rate	9/22 (41%)	8/21 (38%)	12/22 (55%)	11/24 (46%)	4/24 (17%)
First incidence (days)	426	499	603	499	485
Poly-3 test	P=0.036N	P=0.498	P=0.246	P=0.294	P=0.121N

Table A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Uterus: Stromal Polyp					
Overall rate	8/53 (15%)	7/53 (13%)	7/53 (13%)	6/53 (11%)	3/53 (6%)
Adjusted rate	20.7%	18.1%	17.8%	16.0%	8.2%
Terminal rate	5/22 (23%)	4/21 (19%)	5/22 (23%)	5/24 (21%)	2/24 (8%)
First incidence (days)	640	534	659	715	665
Poly-3 test	P=0.087N	P=0.498N	P=0.483N	P=0.407N	P=0.109N
Uterus: Stromal Polyp or Stromal Sarcoma					
Overall rate	8/53 (15%)	7/53 (13%)	8/53 (15%)	6/53 (11%)	3/53 (6%)
Adjusted rate	20.7%	18.1%	19.9%	16.0%	8.2%
Terminal rate	5/22 (23%)	4/21 (19%)	5/22 (23%)	5/24 (21%)	2/24 (8%)
First incidence (days)	640	534	458	715	665
Poly-3 test	P=0.078N	P=0.498N	P=0.577N	P=0.407N	P=0.109N
Uterus: Squamous Cell Carcinoma					
Overall rate	1/53 (2%)	1/53 (2%)	1/53 (2%)	4/53 (8%)	0/53 (0%)
Adjusted rate	2.6%	2.7%	2.6%	10.7%	0.0%
Terminal rate	1/22 (5%)	1/21 (5%)	1/22 (5%)	2/24 (8%)	0/24 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	715	—
Poly-3 test	P=0.397N	P=0.757	P=0.757N	P=0.171	P=0.509N
All Organs: Benign Neoplasms					
Overall rate	49/53 (92%)	43/53 (81%)	48/53 (91%)	46/53 (87%)	36/53 (68%)
Adjusted rate	98.5%	93.3%	97.3%	96.4%	84.7%
Terminal rate	22/22 (100%)	20/21 (95%)	22/22 (100%)	23/24 (96%)	22/24 (92%)
First incidence (days)	345	296	254	254	314
Poly-3 test	P<0.001N	P=0.157N	P=0.684N	P=0.494N	P=0.005N
All Organs: Malignant Neoplasms					
Overall rate	14/53 (26%)	18/53 (34%)	14/53 (26%)	23/53 (43%)	37/53 (70%)
Adjusted rate	34.2%	41.8%	33.4%	53.8%	83.7%
Terminal rate	9/22 (41%)	8/21 (38%)	7/22 (32%)	11/24 (46%)	21/24 (88%)
First incidence (days)	377	167	458	142	169
Poly-3 test	P<0.001	P=0.308	P=0.560N	P=0.049	P<0.001
All Organs: Benign or Malignant Neoplasms					
Overall rate	51/53 (96%)	49/53 (92%)	50/53 (94%)	50/53 (94%)	46/53 (87%)
Adjusted rate	99.2%	96.4%	98.5%	99.6%	95.6%
Terminal rate	22/22 (100%)	20/21 (95%)	22/22 (100%)	24/24 (100%)	23/24 (96%)
First incidence (days)	345	167	254	142	169
Poly-3 test	P=0.252N	P=0.359N	P=0.798N	P=0.973	P=0.254N

(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, 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 dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

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

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

[a] Data as of February 27, 2005

TABLE A4b
Historical Incidence of Lung Neoplasms in Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls	
	Cystic Keratinizing Epithelioma	Squamous Cell Carcinoma
PCB 126	0/53	0/53
TCDD	0/53	0/53
PeCDF	0/53	0/53
TEF Dioxin Mixture	0/53	0/53
PCB 153	0/53	0/53
Binary Mixture PCB 126/PCB 153	0/53	0/53
PCB Mixture PCB 126/PCB 118	0/53	0/53
Overall Historical Incidence		
Total	0/371	0/371

[a] Data as of February 27, 2005

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

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

[a] Data as of February 27, 2005

TABLE A4d
Historical Incidence of Pancreas Neoplasms in Vehicle Control Female Sprague-Dawley Rats[a]

	Incidence in Controls	
Study	Adenoma	Adenoma or Carcinoma
PCB 126	1/51	1/51
TCDD	0/51	0/51
PeCDF	0/53	0/53
TEF Dioxin Mixture	0/52	0/52
PCB 153	0/53	0/53
Binary Mixture PCB 126/PCB 153	0/53	0/53
PCB Mixture PCB 126/PCB 118	0/53	0/53
Overall Historical Incidence		
Total (%)	1/366 (0.3%)	1/366 (0.3%)
Mean ± standard deviation	0.3% ± 0.7%	0.3% ± 0.7%
Range	0%-2%	0%-2%

[a] Data as of February 27, 2005

TABLE A4e
Historical Incidence of Squamous Cell Carcinoma in the Uterus in Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls
PCB 126	0/53
TCDD	0/53
PeCDF	0/53
TEF Dioxin Mixture	0/53
PCB 153	0/53
Binary Mixture PCB 126/PCB 153	1/53
PCB Mixture PCB 126/PCB 118	0/53
Overall Historical Incidence	
Total (%)	1/371 (0.3%)
Mean ± standard deviation	0.3% ± 0.7%
Range	0%-2%

[a] Data as of February 27, 2005

TABLE A4f
Historical Incidence of Adrenal Cortex Neoplasms in Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls	
	Adenoma	Carcinoma
PCB 126	0/52	0/52
TCDD	1/53	0/53
PeCDF	1/53	1/53
TEF Dioxin Mixture	0/52	0/52
PCB 153	0/53	0/53
Binary Mixture PCB 126/PCB 153	0/53	0/53
PCB Mixture PCB 126/PCB 118	0/53	1/53
Overall Historical Incidence		
Total (%)	2/369 (0.5%)	2/369 (0.5%)
Mean ± standard deviation	0.5% ± 0.9%	0.5% ± 0.9%
Range	0%-2%	0%-2%

[a] Data as of February 27, 2005

TABLE A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Disposition Summary					
Animals initially in study	28	28	28	28	28
14-Week interim evaluation	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8
Animals examined microscopically	28	28	28	28	28
14-Week Interim Evaluation					
Alimentary System					
Liver	(10)	(10)	(10)	(10)	(10)
Fatty change, diffuse				1 (10%)	6 (60%)
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus		1 (10%	1 (10%)		1 (10%)
Necrosis					1 (10%)
Pigmentation			4 (40%)	5 (50%)	8 (80%)
Hepatocyte, hypertrophy		4 (40%)	3 (30%)	6 (60%)	10 (100%)
Hepatocyte, multinucleated					7 (70%)
Pancreas	(10)	(10)	(10)	(10)	(10)
Basophilic focus				2 (20%)	
Acinus, atrophy		1 (10%)			1 (10%)
Endocrine System					
Adrenal cortex	(10)	(10)	(10)	(10)	(10)
Hypertrophy		1 (10%)		1 (10%)	1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy	3 (30%)	3 (30%)	4 (40%)	8 (80%)	9 (90%)
Genital System					
Ovary	(10)	(10)	(10)	(10)	(10)
Atrophy	4 (40%)	2 (20%)	4 (40%)	3 (30%	3 (30%))
Uterus	(10)	(10)	(10)	(10)	(10)
Metaplasia, squamous	2 (20%)	1 (10%)	3 (30%)	1 (10%)	1 (10%)
Hematopoietic System					
Spleen	(10)				
Pigmentation	10 (100%)				
Thymus	(10)	(10)	(10)	(10)	(10)
Atrophy		4 (40%)	1 (10%)	1 (10%)	5 (5%)
Respiratory System					
Lung	(10)	(10)	(10)	(10)	(10)
Hemorrhage				1 (10%)	
Inflammation		1 (10%)		1 (10%)	1 (10%)

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

Table A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Systems Examined at 14 Weeks with No Nonneoplastic Lesions Observed					
Cardiovascular System					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					
Urinary System					
31-Week Interim Evaluation					
Alimentary System					
Liver	(10)	(10)	(10)	(10)	(10)
Clear cell focus		1 (10%)			
Fatty change, diffuse				1 (10%)	10 (100%)
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus	1 (10%)		2 (20%)	3 (30%)	2 (20%)
Mixed cell focus, multiple	1 (10%)	1 (10%)	1 (10%)	3 (30%)	3 (30%)
Pigmentation			3 (30%)	10 (100%)	10 (100%)
Toxic hepatopathy					5 (50%)
Bile duct, hyperplasia				1 (10%)	2 (20%)
Bile duct, inflammation, chronic active, focal					1 (10%)
Hepatocyte, hypertrophy		3 (30%)	5 (50%)	10 (100%)	10 (100%)
Hepatocyte, multinucleated					9 (90%)
Pancreas	(10)	(10)	(10)	(10)	(10)
Basophilic focus				1 (10%)	
Inflammation, chronic active	1 (10%)	1 (10%)		1 (10%)	
Acinus, atrophy	1 (10%)	1 (10%)		1 (10%)	
Acinus, vacuolization cytoplasmic					3 (30%)
Endocrine System					
Adrenal cortex	(10)	(10)	(10)	(10)	(10)
Degeneration, cystic		1 (10%)	1 (10%)		
Hyperplasia				1 (10%)	2 (20%)
Hypertrophy	3 (30%)	2 (20%)	2 (20%)	4 (40%)	1 (10%)
Vacuolization cytoplasmic					1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy		5 (50%)	5 (50%)	7 (70%)	8 (80%)
Genital System					
Ovary	(10)	(10)	(10)	(10)	(10)
Atrophy	6 (60%)	7 (70%)	9 (90%)	10 (100%)	6 (60%)
Uterus	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative	1 (10%)	1 (10%)			
Metaplasia, squamous	6 (60%)	7 (70%)	5 (50%)	9 (90%)	4 (40%)
Endometrium, hyperplasia, cystic		1 (10%)	1 (10%)		

Table A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Hematopoietic System					
Lymph node			(1)		
Mediastinal, inflammation, granulomatous			1 (100%)		
Spleen	(10)				
Pigmentation	10 (100%)				
Thymus	(10)	(10)	(10)	(10)	(10)
Atrophy	6 (60%)	5 (50%)	6 (60%)	7 (70%)	10 (100%)
Respiratory System					
Lung	(10)	(10)	(10)	(10)	(10)
Hemorrhage				1 (10%)	
Infiltration cellular, histiocyte	2 (20%)	1 (10%)		1 (10%)	4 (40%)
Inflammation		1 (10%)			
Systems Examined at 31 Weeks with No Nonneoplastic Lesions Observed					
Cardiovascular System					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					
Urinary System					
53-Week Interim Evaluation					
Alimentary System					
Liver	(8)	(8)	(8)	(8)	(8)
Basophilic focus		3 (38%)	1 (13%)		1 (13%)
Cholangiofibrosis					1 (13%)
Eosinophilic focus				1 (13%)	
Eosinophilic focus, multiple					7 (88%)
Fatty change, diffuse				3 (38%)	8 (100%)
Fatty change, focal					1 (13%)
Hyperplasia, nodular					2 (25%)
Infiltration cellular, histiocyte			1 (13%)	1 (13%)	
Inflammation	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Mixed cell focus	1 (13%)	1 (13%)	4 (50%)		
Mixed cell focus, multiple	4 (50%)	7 (88%)	2 (25%)	2 (25%)	1 (13%)
Necrosis					1 (13%)
Pigmentation		1 (13%)	6 (75%)	8 (100%)	8 (100%)
Toxic hepatopathy					8 (100%)
Bile duct, fibrosis					1 (13%)
Bile duct, hyperplasia					8 (100%)
Hepatocyte, hypertrophy		2 (25%)	2 (25%)	8 (100%)	8 (100%)
Hepatocyte, multinucleated				2 (25%)	8 (100%)
Oval cell, hyperplasia					4 (50%)
Pancreas	(8)	(8)	(8)	(8)	(8)
Inflammation, chronic active		1 (13%)			1 (13%)
Acinus, atrophy		1 (13%)			1 (13%)
Acinus, vacuolization cytoplasmic					7 (88%)

Table A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Cardiovascular System					
Heart					(1)
Pericardium, inflammation, chronic active					1 (100%)
Endocrine System					
Adrenal cortex	(8)	(8)	(8)	(8)	(8)
Degeneration, cystic	2 (25%)	1 (13%)		1 (13%)	
Hyperplasia	1 (13%)	4 (50%)	1 (13%)	1 (13%)	1 (13%)
Hypertrophy	4 (50%)	4 (50%)	5 (63%)	5 (63%)	2 (25%)
Vacuolization cytoplasmic			1 (13%)		1 (13%)
Thyroid gland	(8)	(8)	(8)	(8)	(8)
C-cell, hyperplasia	2 (25%)	1 (13%)			
Follicular cell, hypertrophy		2 (25%)	4 (50%)	6 (75%)	4 (50%)
Genital System					
Ovary	(8)	(8)	(8)	(8)	(8)
Atrophy	7 (88%)	6 (75%)	7 (88%)	7 (88%)	6 (75%)
Cyst	1 (13%)			1 (13%)	1 (13%)
Uterus	(8)	(8)	(8)	(8)	(8)
Inflammation, suppurative					1 (13%)
Metaplasia, squamous	7 (88%)	6 (75%)	7 (88%)	7 (88%)	3 (38%)
Endometrium, hyperplasia, cystic	5 (63%)				
Hematopoietic System					
Spleen	(8)				
Pigmentation	8 (100%)				
Thymus	(8)	(8)	(8)	(8)	(8)
Atrophy	5 (63%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Integumentary System					
Mammary gland	(8)	(2)		(5)	(1)
Cyst	1 (13%)	2 (100%)		2 (40%)	
Hyperplasia	4 (50%)	2 (100%)		1 (20%)	
Inflammation, granulomatous				1 (20%)	
Respiratory System					
Lung	(8)	(8)	(8)	(8)	(8)
Hemorrhage					1 (13%)
Infiltration cellular, histiocyte	4 (50%)	5 (63%)	5 (63%)	2 (25%)	1 (13%)
Alveolar epithelium, metaplasia, bronchiolar				1 (13%)	3 (38%)

Systems Examined at 53 Weeks with No Nonneoplastic Lesions Observed
General Body System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Disposition Summary					
Animals initially in study	53	53	53	53	53
Early deaths					
Accidental deaths	1	1	1		2
Moribund	22	19	24	19	20
Natural deaths	8	12	6	10	7
Survivors					
Terminal sacrifice	22	21	22	24	24
Animals examined microscopically	53	53	53	53	53
Alimentary System					
Esophagus	(53)	(53)	(52)	(53)	(52)
Cyst					1 (2%)
Perforation			1 (2%)		2 (4%)
Muscularis, inflammation	3 (6%)	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Periesophageal tissue, hemorrhage					1 (2%)
Periesophageal tissue, inflammation			1 (2%)		1 (2%)
Intestine large, colon	(53)	(53)	(53)	(52)	(49)
Hyperplasia, lymphoid			1 (2%)		
Parasite metazoan		2 (4%)			
Intestine large, rectum	(53)	(53)	(53)	(53)	(50)
Metaplasia, squamous					1 (2%)
Parasite metazoan	2 (4%)			1 (2%)	3 (6%)
Intestine large, cecum	(53)	(53)	(53)	(52)	(49)
Mineralization			1 (2%)		
Intestine small, jejunum	(53)	(53)	(52)	(52)	(49)
Hyperplasia, lymphoid			1 (2%)	2 (4%)	1 (2%)
Ulcer				1 (2%)	
Intestine small, ileum	(53)	(53)	(52)	(52)	(49)
Hyperplasia, lymphoid	1 (2%)				
Liver	(53)	(53)	(52)	(52)	(51)
Angiectasis	3 (6%)	1 (2%)	1 (2%)	2 (4%)	5 (10%)
Basophilic focus	9 (17%)	7 (13%)	8 (15%)	2 (4%)	3 (6%)
Basophilic focus, multiple	13 (25%)	15 (28%)	6 (12%)	1 (2%)	3 (6%)
Cholangiofibrosis		1 (2%)		7 (13%)	39 (76%)
Clear cell focus	3 (6%)	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Clear cell focus, multiple	6 (11%)	1 (2%)	1 (2%)		
Eosinophilic focus	7 (13%)	8 (15%)	4 (8%)	7 (13%)	1 (2%)
Eosinophilic focus, multiple	7 (13%)	8 (15%)	26 (50%)	33 (63%)	17 (33%)
Fatty change, diffuse	3 (6%)	1 (2%)	9 (17%)	31 (60%)	38 (75%)
Fatty change, focal	3 (6%)	4 (8%)	7 (13%)	1 (2%)	12 (24%)
Hematopoietic cell proliferation	27 (51%)	29 (55%)	30 (58%)	19 (37%)	11 (22%)
Hepatodiaphragmatic nodule		2 (4%)			
Hyperplasia, nodular			2 (4%)	24 (46%)	42 (82%)
Inflammation	44 (83%)	41 (77%)	46 (88%)	48 (92%)	46 (90%)
Metaplasia					1 (2%)
Mixed cell focus	7 (13%)	3 (6%)	4 (8%)	2 (4%)	1 (2%)
Mixed cell focus, multiple	19 (36%)	19 (36%)	26 (50%)	24 (46%)	2 (4%)
Necrosis	4 (8%)	8 (15%)	5 (10%)	4 (8%)	20 (39%)
Pigmentation	2 (4%)	5 (9%)	38 (73%)	50 (96%)	50 (98%)

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

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Alimentary System (continued)					
Liver (continued)	(53)	(53)	(52)	(52)	(51)
Toxic hepatopathy		2 (4%)	34 (65%)	48 (92%)	49 (96%)
Bile duct, cyst	4 (8%)	3 (6%)	1 (2%)	5 (10%)	23 (45%)
Bile duct, dilatation		1 (2%)			
Bile duct, fibrosis	1 (2%)	3 (6%)	3 (6%)	4 (8%)	
Bile duct, hyperplasia	8 (15%)	2 (4%)	9 (17%)	29 (56%)	46 (90%)
Centrilobular, degeneration	5 (9%)	7 (13%)	2 (4%)	4 (8%)	3 (6%)
Centrilobular, fibrosis				1 (2%)	1 (2%)
Hepatocyte, hypertrophy	1 (2%)	7 (13%)	17 (33%)	33 (63%)	50 (98%)
Hepatocyte, multinucleated			14 (27%)	46 (88%)	48 (94%)
Oval cell, hyperplasia	2 (4%)	2 (4%)	15 (29%)	39 (75%)	46 (90%)
Portal, fibrosis				7 (13%)	34 (67%)
Serosa, inflammation, chronic active	1 (2%)				
Mesentery	(47)	(47)	(46)	(47)	(42)
Artery, inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	2 (4%)	4 (10%)
Fat, necrosis		2 (4%)	2 (4%)	1 (2%)	
Oral mucosa	(12)	(11)	(25)	(30)	(36)
Gingival, hyperplasia, squamous	8 (67%)	8 (73%)	18 (72%)	22 (73%)	24 (67%)
Pharyngeal, inflammation					1 (3%)
Pancreas	(53)	(53)	(52)	(52)	(50)
Inflammation, chronic active		3 (6%)	3 (6%)	1 (2%)	4 (8%)
Acinus, atrophy		2 (4%)	1 (2%)	1 (2%)	8 (16%)
Acinus, hyperplasia	2 (4%)			1 (2%)	
Acinus, vacuolization cytoplasmic				7 (13%)	40 (80%)
Artery, inflammation, chronic active			1 (2%)	2 (4%)	2 (4%)
Duct, dilatation					1 (2%)
Salivary glands	(53)	(51)	(52)	(50)	(52)
Inflammation, chronic active			1 (2%)		
Stomach, forestomach	(53)	(53)	(52)	(52)	(51)
Hyperkeratosis			1 (2%)	2 (4%)	
Hyperplasia, squamous	1 (2%)	1 (2%)	2 (4%)	7 (13%)	8 (16%)
Inflammation			1 (2%)	1 (2%)	
Mineralization	1 (2%)	1 (2%)			
Ulcer			1 (2%)	2 (4%)	1 (2%)
Stomach, glandular	(53)	(53)	(52)	(52)	(51)
Cyst					1 (2%)
Ectopic tissue				1 (2%)	
Erosion				1 (2%)	2 (4%)
Inflammation, chronic active			1 (2%)		
Mineralization	4 (8%)	3 (6%)	3 (6%)	1 (2%)	
Ulcer			1 (2%)		
Tooth	(23)	(14)	(33)	(35)	(31)
Peridontal tissue, inflammation	23 (100%)	14 (100%)	33 (100%)	35 (100%)	31 (100%)

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Cardiovascular System					
Blood vessel	(53)	(53)	(52)	(53)	(52)
Aorta, mineralization				1 (2%)	
Heart	(53)	(52)	(52)	(53)	(52)
Cardiomyopathy	22 (42%)	19 (37%)	22 (42%)	26 (49%)	15 (29%)
Fibrosis					1 (2%)
Hemorrhage					1 (2%)
Inflammation, chronic active	1 (2%)				1 (2%)
Thrombosis					1 (2%)
Coronary artery, inflammation, chronic active			1 (2%)		6 (12%)
Pericardium, necrosis				1 (2%)	
Endocrine System					
Adrenal cortex	(53)	(53)	(52)	(52)	(51)
Angiectasis	17 (32%)	26 (49%)	33 (63%)	23 (44%)	5 (10%)
Atrophy				3 (6%)	35 (69%)
Degeneration, cystic	13 (25%)	12 (23%)	13 (25%)	14 (27%)	16 (31%)
Hematopoietic cell proliferation	1 (2%)				
Hyperplasia	11 (21%)	18 (34%)	23 (44%)	25 (48%)	18 (35%)
Hypertrophy	47 (89%)	38 (72%)	44 (85%)	41 (79%)	32 (63%)
Necrosis	1 (2%)				1 (2%)
Vacuolization cytoplasmic	11 (21%)	12 (23%)	14 (27%)	10 (19%)	13 (25%)
Adrenal medulla	(52)	(53)	(52)	(52)	(51)
Hyperplasia	15 (29%)	13 (25%)	14 (27%)	13 (25%)	3 (6%)
Islets, pancreatic	(53)	(53)	(52)	(52)	(50)
Hyperplasia			1 (2%)		
Parathyroid gland	(45)	(47)	(46)	(47)	(46)
Hyperplasia		1 (2%)			
Pituitary gland	(53)	(53)	(53)	(52)	(52)
Angiectasis	17 (32%)	20 (38%)	15 (28%)	17 (33%)	1 (2%)
Cyst	1 (2%)	3 (6%)			
Cytoplasmic alteration			3 (6%)		
Inflammation				1 (2%)	
Vacuolization cytoplasmic				1 (2%)	
Pars distalis, hyperplasia	13 (25%)	13 (25%)	19 (36%)	20 (38%)	13 (25%)
Pars intermedia, hyperplasia					2 (4%)
Thyroid gland	(53)	(53)	(51)	(52)	(52)
C-cell, hyperplasia	15 (28%)	19 (36%)	22 (43%)	12 (23%)	15 (29%)
Follicle, cyst	2 (4%)				
Follicular cell, hypertrophy	14 (26%)	17 (32%)	34 (67%)	35 (67%)	42 (81%)
General Body System					
None					

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Genital System					
Clitoral gland	(53)	(53)	(53)	(53)	(50)
Inflammation	45 (85%)	49 (92%)	46 (87%)	40 (75%)	23 (46%)
Duct, cyst	38 (72%)	40 (75%)	48 (91%)	43 (81%)	43 (86%)
Ovary	(53)	(53)	(52)	(52)	(49)
Atrophy	45 (85%)	44 (83%)	45 (87%)	43 (83%)	16 (33%)
Cyst	14 (26%)	7 (13%)	17 (33%)	14 (27%)	13 (27%)
Inflammation, chronic active	1 (2%)	1 (2%)			1 (2%)
Inflammation, suppurative				1 (2%)	
Oviduct	(1)		(1)	(2)	(3)
Cyst	1 (100%)		1 (100%)	2 (100%)	
Inflammation, chronic active			1 (100%)		3 (100%)
Uterus	(53)	(53)	(53)	(52)	(50)
Adenomyosis			1 (2%)		1 (2%)
Hemorrhage					1 (2%)
Hyperplasia		1 (2%)			
Inflammation, chronic active	3 (6%)		1 (2%)	7 (13%)	2 (4%)
Inflammation, suppurative	3 (6%)	5 (9%)	8 (15%)	10 (19%)	5 (10%)
Metaplasia, squamous	27 (51%)	30 (57%)	35 (66%)	36 (69%)	14 (28%)
Ulcer					1 (2%)
Endometrium, hyperplasia, cystic	28 (53%)	23 (43%)	34 (64%)	18 (35%)	12 (24%)
Vagina	(1)	(1)	(1)	(1)	
Inflammation			1 (100%)		
Hematopoietic System					
Bone marrow	(53)	(53)	(53)	(53)	(53)
Atrophy		1 (2%)			
Degeneration			1 (2%)		
Hyperplasia	39 (74%)	38 (72%)	42 (79%)	48 (91%)	49 (92%)
Lymph node	(4)	(7)	(2)	(7)	(14)
Angiectasis				1 (14%)	1 (7%)
Inguinal, hyperplasia, plasma cell				1 (14%)	
Lumbar, ectasia	4 (100%)	4 (57%)		1 (14%)	
Lumbar, hemorrhage		2 (29%)			
Lumbar, hyperplasia		1 (14%)			
Lumbar, hyperplasia, plasma cell	4 (100%)	1 (14%)			
Mediastinal, ectasia					3 (21%)
Mediastinal, hemorrhage				1 (14%)	2 (14%)
Mediastinal, hyperplasia, histiocytic		1 (14%)		1 (14%)	3 (21%)
Mediastinal, hyperplasia, lymphoid		1 (14%)			
Mediastinal, hyperplasia, plasma cell					2 (14%)
Pancreatic, hyperplasia, histiocytic				1 (14%)	3 (21%)
Pancreatic, hyperplasia, plasma cell					1 (7%)
Pancreatic, pigmentation				1 (14%)	1 (7%)
Renal, ectasia		1 (14%)	1 (50%)	1 (14%)	1 (7%)
Renal, hemorrhage			1 (50%)		
Renal, hyperplasia, histiocytic				1 (14%)	2 (14%)
Renal, hyperplasia, lymphoid					1 (7%)
Renal, hyperplasia, plasma cell		1 (14%)		1 (14%)	

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Hematopoietic System (continued)					
Lymph node, mandibular	(53)	(51)	(52)	(50)	(51)
Congestion				1 (2%)	
Ectasia		3 (6%)	6 (12%)	3 (6%)	6 (12%)
Hemorrhage		1 (2%)			1 (2%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	3 (6%)	2 (4%)	5 (10%)
Hyperplasia, plasma cell	37 (70%)	37 (73%)	40 (77%)	36 (72%)	31 (61%)
Lymph node, mesenteric	(53)	(53)	(52)	(52)	(49)
Hemorrhage				1 (2%)	
Hyperplasia, histiocytic				1 (2%)	2 (4%)
Hyperplasia, lymphoid			1 (2%)		1 (2%)
Inflammation, chronic active			1 (2%)		
Spleen	(53)	(53)	(52)	(52)	(50)
Fibrosis				1 (2%)	
Hematopoietic cell proliferation	51 (96%)	51 (96%)	49 (94%)	47 (90%)	44 (88%)
Hyperplasia			1 (2%)		
Pigmentation	47 (89%)	46 (87%)	43 (83%)	51 (98%)	46 (92%)
Lymphoid follicle, atrophy			2 (4%)	4 (8%)	5 (10%)
Red pulp, atrophy	1 (2%)		1 (2%)		5 (10%)
Thymus	(53)	(50)	(48)	(50)	(51)
Atrophy	33 (62%)	33 (66%)	43 (90%)	42 (84%)	49 (96%)
Cyst				1 (2%)	
Hemorrhage	1 (2%)			3 (6%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)				
Inflammation					1 (2%)
Epithelial cell, hyperplasia				1 (2%)	
Integumentary System					
Mammary gland	(53)	(53)	(52)	(53)	(52)
Cyst	3 (6%)	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Hyperplasia	29 (55%)	29 (55%)	25 (48%)	22 (42%)	3 (6%)
Inflammation, granulomatous	5 (9%)		4 (8%)	3 (6%)	
Duct, cyst	1 (2%)				
Skin	(53)	(53)	(53)	(53)	(53)
Angiectasis	1 (2%)				
Cyst epithelial inclusion				1 (2%)	
Fibrosis	1 (2%)				
Inflammation			1 (2%)		1 (2%)
Ulcer			2 (4%)		1 (2%)
Dermis, fibrosis			1 (2%)		
Epidermis, hyperplasia			1 (2%)		
Subcutaneous tissue, inflammation			1 (2%)		
Musculoskeletal System					
Skeletal muscle	(1)	(1)		(1)	(1)
Hemorrhage					1 (100%)
Inflammation					1 (100%)

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Nervous System					
Brain	(53)	(53)	(53)	(52)	(52)
Hemorrhage			1 (2%)	1 (2%)	
Hydrocephalus	1 (2%)				
Respiratory System					
Lung	(53)	(53)	(52)	(53)	(52)
Hemorrhage			1 (2%)		
Infiltration cellular, histiocyte	47 (89%)	41 (77%)	47 (90%)	46 (87%)	45 (87%)
Inflammation	8 (15%)	5 (9%)	2 (4%)	4 (8%)	1 (2%)
Metaplasia, squamous			1 (2%)	2 (4%)	11 (21%)
Mineralization		1 (2%)		1 (2%)	
Alveolar epithelium, hyperplasia	23 (43%)	20 (38%)	17 (33%)	5 (9%)	5 (10%)
Alveolar epithelium, metaplasia, bronchiolar		6 (11%)	23 (44%)	34 (64%)	32 (62%)
Bronchiole, hyperplasia					1 (2%)
Mediastinum, hemorrhage					1 (2%)
Mediastinum, necrosis				1 (2%)	
Serosa, inflammation				1 (2%)	
Nose	(53)	(53)	(53)	(53)	(53)
Hyperplasia					1 (2%)
Inflammation	22 (42%)	13 (25%)	13 (25%)	13 (25%)	31 (58%)
Glands, hyperplasia					8 (15%)
Olfactory epithelium, degeneration	1 (2%)				2 (4%)
Olfactory epithelium, metaplasia	4 (8%)	3 (6%)	5 (9%)	6 (11%)	15 (28%)
Respiratory epithelium, hyperplasia	10 (19%)	5 (9%)	7 (13%)	11 (21%)	20 (38%)
Respiratory epithelium, metaplasia	1 (2%)				
Respiratory epithelium, vacuolization cytoplasmic		1 (2%)			
Trachea	(53)	(53)	(52)	(53)	(52)
Inflammation	1 (2%)				
Peritracheal tissue, hemorrhage					1 (2%)
Peritracheal tissue, inflammation					1 (2%)
Special Senses System					
Eye	(53)	(53)	(53)	(52)	(52)
Anterior chamber, ciliary body, cornea, inflammation				1 (2%)	3 (6%)
Cornea, inflammation				1 (2%)	
Retina, atrophy	1 (2%)	3 (6%)	4 (8%)	2 (4%)	5 (10%)
Harderian gland	(53)	(53)	(53)	(52)	(52)
Inflammation	20 (38%)	14 (26%)	16 (30%)	14 (27%)	16 (31%)

Table A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
Urinary System					
Kidney	(53)	(53)	(52)	(52)	(51)
Accumulation, hyaline droplet	2 (4%)		1 (2%)	1 (2%)	1 (2%)
Calculus gross observation		1 (2%)			
Calculus microscopic observation only	7 (13%)	4 (8%)	1 (2%)	4 (8%)	1 (2%)
Casts protein	2 (4%)	1 (2%)			
Cyst		1 (2%)	1 (2%)	1 (2%)	
Infarct			1 (2%)		1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Inflammation, suppurative	5 (9%)	3 (6%)	1 (2%)	5 (10%)	1 (2%)
Mineralization	42 (79%)	38 (72%)	39 (75%)	42 (81%)	35 (69%)
Necrosis			1 (2%)		
Nephropathy	29 (55%)	22 (42%)	29 (56%)	34 (65%)	43 (84%)
Pigmentation		1 (2%)	3 (6%)	7 (13%)	35 (69%)
Pelvis, dilatation	1 (2%)			2 (4%)	1 (2%)
Pelvis, inflammation	3 (6%)		4 (8%)	3 (6%)	5 (10%)
Renal tubule, degeneration			1 (2%)	2 (4%)	1 (2%)
Transitional epithelium, hyperplasia	2 (4%)	2 (4%)	4 (8%)	11 (21%)	6 (12%)
Ureter	(1)	(1)		(1)	(1)
Inflammation	1 (100%)			1 (100%)	
Mineralization					1 (100%)
Transitional epithelium, hyperplasia	1 (100%)				1 (100%)
Urinary bladder	(53)	(53)	(53)	(52)	(50)
Inflammation	7 (13%)	8 (15%)	5 (9%)	7 (13%)	
Transitional epithelium, hyperplasia				1 (2%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF A BINARY MIXTURE OF PCB 126 AND PCB 153: GROUPS 1, 4, 5, 6

TABLE B1a Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 154

TABLE B1b Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 156

TABLE B2 Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 160

TABLE B3 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 184

TABLE B4a Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 188

TABLE B4b Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 193

TABLE B1a
Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Disposition Summary				
Animals initially in study	28	30	28	29
14-Week interim evaluation	10	10	10	10
31-Week interim evaluation	10	10	10	10
53-Week interim evaluation	8	10	8	9
Animals examined microscopically	28	30	28	29
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)			(10)
Fibroadenoma				1 (10%)
Urinary System				
Kidney			(1)	
Nephroblastoma			(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				

TABLE B1a
Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
53-Week Interim Evaluation				
Endocrine System				
Thyroid gland	(8)	(10)	(8)	(9)
C-cell, adenoma	2 (25%)		1 (13%)	
Integumentary System				
Mammary gland	(8)	(2)	(5)	(9)
Carcinoma			1 (20%)	
Fibroadenoma		2 (100%)	2 (40%)	1 (11%)
Fibroadenoma, multiple			1 (20%)	
Systems Examined at 53 Weeks with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
General Body System				
Genital System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[b]				
31-Week interim evaluation			1	1
53-Week interim evaluation	2	2	4	1
Total primary neoplasms				
31-Week interim evaluation			1	1
53-Week interim evaluation	2	2	5	1
Total animals with benign neoplasms				
31-Week interim evaluation				1
53-Week interim evaluation	2	2	3	1
Total benign neoplasms				
31-Week interim evaluation				1
53-Week interim evaluation	2	2	4	1
Total animals with malignant neoplasms				
31-Week interim evaluation			1	
53-Week interim evaluation			1	
Total malignant neoplasms				
31-Week interim evaluation			1	
53-Week interim evaluation			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Disposition Summary				
Animals initially in study	53	50	53	51
Early deaths				
Accidental deaths	1			1
Moribund	22	10	19	13
Natural deaths	8	12	10	10
Survivors				
Terminal sacrifice	22	28	24	27
Animals examined microscopically	53	50	53	51
Alimentary System				
Intestine large, colon	(53)	(50)	(52)	(50)
Intestine large, rectum	(53)	(50)	(53)	(51)
Schwannoma malignant, metastatic, vagina			1 (2%)	
Intestine small, duodenum	(53)	(50)	(52)	(50)
Leiomyoma			2 (4%)	
Intestine small, jejunum	(53)	(50)	(52)	(51)
Fibrosarcoma				1 (2%)
Leiomyosarcoma	1 (2%)			
Intestine small, ileum	(53)	(50)	(52)	(50)
Liver	(53)	(50)	(52)	(51)
Carcinoma, metastatic, uterus				1 (2%)
Cholangiocarcinoma		6 (12%)	4 (8%)	12 (24%)
Cholangiocarcinoma, multiple		1 (2%)	5 (10%)	13 (25%)
Hemangioma	1 (2%)			
Hepatocellular adenoma			5 (10%)	14 (27%)
Hepatocellular adenoma, multiple		2 (4%)		7 (14%)
Hepatocholangioma			2 (4%)	1 (2%)
Hepatocholangioma, multiple				1 (2%)
Mesentery	(47)	(31)	(47)	(47)
Oral mucosa	(12)	(28)	(30)	(41)
Gingival, squamous cell carcinoma		3 (11%)	5 (17%)	6 (15%)
Pancreas	(53)	(49)	(52)	(49)
Carcinoma, metastatic, uterus				1 (2%)
Acinus, adenoma			3 (6%)	1 (2%)
Acinus, carcinoma			1 (2%)	1 (2%)
Cardiovascular System				
Heart	(53)	(50)	(53)	(50)
Fibrosarcoma, metastatic, lung			1 (2%)	
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)			
Schwannoma malignant		1 (2%)		

TABLE B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Endocrine System				
Adrenal cortex	(53)	(49)	(52)	(51)
Adenoma			1 (2%)	
Carcinoma				1 (2%)
Adrenal medulla	(52)	(49)	(52)	(49)
Pheochromocytoma benign	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Bilateral, pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(53)	(49)	(52)	(49)
Adenoma	1 (2%)			1 (2%)
Pituitary gland	(53)	(50)	(52)	(51)
Carcinoma	1 (2%)			
Pars distalis, adenoma	22 (42%)	19 (38%)	17 (33%)	9 (18%)
Pars distalis, carcinoma		1 (2%)		
Thyroid gland	(53)	(49)	(52)	(50)
Bilateral, C-cell, adenoma	2 (4%)		3 (6%)	1 (2%)
C-cell, adenoma	8 (15%)	6 (12%)	12 (23%)	7 (14%)
C-cell, carcinoma	4 (8%)	1 (2%)	1 (2%)	
Follicular cell, adenoma		1 (2%)	1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(53)	(49)	(53)	(50)
Adenoma	1 (2%)			
Ovary	(53)	(48)	(52)	(50)
Granulosa cell tumor malignant	1 (2%)		1 (2%)	
Granulosa cell tumor benign			1 (2%)	
Luteoma				1 (2%)
Uterus	(53)	(50)	(52)	(50)
Carcinoma		1 (2%)		
Carcinoma, multiple				1 (2%)
Fibrosarcoma			1 (2%)	
Polyp stromal	5 (9%)	3 (6%)	6 (12%)	
Polyp stromal, multiple	3 (6%)	1 (2%)		1 (2%)
Schwannoma malignant		1 (2%)	2 (4%)	2 (4%)
Schwannoma malignant, metastatic, vagina			1 (2%)	
Squamous cell carcinoma	1 (2%)	1 (2%)	2 (4%)	
Cervix, granular cell tumor benign			1 (2%)	
Cervix, squamous cell carcinoma		1 (2%)	2 (4%)	
Vagina	(1)	(1)	(1)	
Sarcoma		1 (100%)		
Schwannoma malignant			1 (100%)	
Squamous cell carcinoma	1 (100%)			

TABLE B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Hematopoietic System				
Lymph node	(4)	(2)	(7)	(5)
Mediastinal, carcinoma, metastatic, uterus				1 (20%)
Lymph node, mesenteric	(53)	(49)	(52)	(49)
Spleen	(53)	(49)	(52)	(49)
Thymus	(53)	(48)	(50)	(47)
Squamous cell carcinoma, metastatic, lung			1 (2%)	
Integumentary System				
Mammary gland	(53)	(50)	(53)	(51)
Adenoma	2 (4%)	1 (2%)		1 (2%)
Carcinoma	6 (11%)	1 (2%)	1 (2%)	4 (8%)
Carcinoma, multiple	2 (4%)		1 (2%)	
Fibroadenoma	27 (51%)	21 (42%)	25 (47%)	17 (33%)
Fibroadenoma, multiple	13 (25%)	8 (16%)	9 (17%)	12 (24%)
Skin	(53)	(50)	(53)	(51)
Fibroma	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Fibrosarcoma		1 (2%)		
Lipoma			1 (2%)	
Neural crest tumor		1 (2%)		
Squamous cell papilloma				1 (2%)
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(1)	(3)
Carcinoma, metastatic, uterus				1 (33%)
Fibrosarcoma, metastatic, lung			1 (100%)	
Fibrous histiocytoma	1 (100%)			
Nervous System				
Brain	(53)	(50)	(52)	(51)
Carcinoma, metastatic, pituitary gland	1 (2%)	1 (2%)		
Granular cell tumor malignant			1 (2%)	
Granular cell tumor benign		1 (2%)		
Respiratory System				
Lung	(53)	(50)	(53)	(50)
Carcinoma, metastatic, mammary gland	1 (2%)			
Carcinoma, metastatic, uterus				1 (2%)
Cystic keratinizing epithelioma		1 (2%)	1 (2%)	1 (2%)
Cystic keratinizing epithelioma, multiple				1 (2%)
Fibrous histiocytoma, metastatic, skeletal muscle	1 (2%)			
Squamous cell carcinoma			1 (2%)	
Mediastinum, fibrosarcoma			1 (2%)	

TABLE B1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Special Senses System				
Harderian gland	(53)	(49)	(52)	(51)
Squamous cell carcinoma, metastatic, oral mucosa			1 (2%)	
Zymbal's gland			(1)	
Adenoma			1 (100%)	
Urinary System				
Kidney	(53)	(48)	(52)	(51)
Carcinoma, metastatic, uterus				1 (2%)
Nephroblastoma			1 (2%)	
Renal tubule, adenoma	1 (2%)			
Urinary bladder	(53)	(49)	(52)	(50)
Carcinoma, metastatic, uterus				1 (2%)
Papilloma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(53)	(50)	(53)	(51)
Lymphoma malignant		1 (2%)	2 (4%)	1 (2%)
Mesothelioma malignant		1 (2%)		
Neooplasm Summary				
Total animals with primary neoplasms[c]	51	44	50	47
Total primary neoplasms	110	90	126	122
Total animals with benign neoplasms	49	39	46	40
Total benign neoplasms	92	67	93	80
Total animals with malignant neoplasms	14	16	23	35
Total malignant neoplasms	18	22	33	42
Total animals with metastatic neoplasms	3	1	3	1
Total metastatic neoplasms	4	1	6	7
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 B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	085	345	377	418	426	449	451	451	451	484	499	533	556	556	556	581	636	640	647	654	654	664	664	664	664
Carcass ID Number	042	005	022	088	081	032	012	025	097	031	093	009	010	015	041	065	044	020	024	086	089	008	014	036	039
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Mesentery	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Oral mucosa							+						+				+	+		+			+	+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth							+	+					+				+			+	+		+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skeletal muscle			X																						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																								X	
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	M	M	+	+	M	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Pars distalis, adenoma				X	X											X		X			X	X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma					X																				X
C-cell, carcinoma					X																				
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	670	684	688	708	714	719	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	067	063	087	091	011	061	001	007	018	021	034	063	069	082	092	094	003	040	083	004	013	043	064	066	068	070	090	096	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leiomyosarcoma																								X					1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hemangioma			X																										1
Mesentery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	M	47
Oral mucosa				+	+								+				+				+								12
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth			+	+	+	+	+	+				+	+	+	+		+				+	+				+			23
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibrous histiocytoma, metastatic, skeletal muscle																													1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	52
Pheochromocytoma benign																									X				2
Bilateral, pheochromocytoma benign																	X												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma		X																											1
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma					X																								1
Pars distalis, adenoma	X					X	X		X	X		X	X		X			X	X			X	X	X	X	X			22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma		X										X																	2
C-cell, adenoma									X						X						X	X				X	X		8
C-cell, carcinoma	X																			X					X				4
General Body System																													
None																													

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	085	345	377	418	426	449	451	451	451	484	499	533	556	556	556	581	636	640	647	654	654	664	664	664	664
Carcass ID Number	042	005	022	088	081	032	012	025	097	031	093	009	010	015	041	065	044	020	024	086	089	008	014	036	039
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																									
Polyp stromal, multiple																		X							
Squamous cell carcinoma																									
Vagina																									
Squamous cell carcinoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+		+									+								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma						X																			
Carcinoma, multiple																									
Fibroadenoma		X					X	X	X	X			X			X			X	X	X	X	X		X
Fibroadenoma, multiple											X			X	X		X	X						X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																						X			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						
Fibrous histiocytoma			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland																									
Fibrous histiocytoma, metastatic, skeletal muscle			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	670	684	688	708	714	719	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	067	063	087	091	011	061	001	007	018	021	034	063	069	082	092	094	003	040	083	004	013	043	064	066	068	070	090	096	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																													1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granulosa cell tumor malignant																				X									1
Oviduct													+																1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp stromal					X						X			X						X								X	5
Polyp stromal, multiple						X																			X				3
Squamous cell carcinoma							X																						1
Vagina																								+					1
Squamous cell carcinoma																								X					1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																								+					4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma												X												X					2
Carcinoma	X									X			X												X			X	6
Carcinoma, multiple									X													X							2
Fibroadenoma	X		X	X			X		X	X	X		X		X				X		X	X		X	X				27
Fibroadenoma, multiple		X			X			X				X				X		X					X						13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma						X																							2
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																													1
Fibrous histiocytoma																													1
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, pituitary gland					X																								1
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, mammary gland																									X				1
Fibrous histiocytoma, metastatic, skeletal muscle																													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	085	345	377	418	426	449	451	451	451	484	499	533	556	556	556	581	636	640	647	654	654	664	664	664	664
Carcass ID Number	042	005	022	088	081	032	012	025	097	031	093	009	010	015	041	065	044	020	024	086	089	008	014	036	039
Special Senses System																									
Ear																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Ureter																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma																	X								
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 1 Vehicle Control

Number of Days on Study	670	684	688	708	714	719	729	729	729	729	729	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	067	063	087	091	011	061	001	007	018	021	034	063	069	082	092	094	003	040	083	004	013	043	064	066	068	070	090	096		Total Tissues/ Tumors
Special Senses System																														
Ear																								+						1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Urinary System																														
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Renal tubule, adenoma																									X					1
Ureter																								+						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Papilloma																														1
Systemic Lesions																														
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 4 300 ng/kg:100 µg/kg

Number of Days on Study	441	462	478	484	491	491	494	534	567	569	603	612	615	625	633	636	640	643	653	664	689	701	729	729	729
Carcass ID Number	358	322	344	304	310	359	345	365	336	356	377	327	379	343	323	308	305	306	361	309	337	307	303	328	329
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma											X							X		X					
Cholangiocarcinoma, multiple																									
Hepatocellular adenoma, multiple																								X	
Mesentery	+	M	+	+	+	M	+	+	+	+	+	M	M	+	M	M	+	+	M	+	M	+	+	M	M
Oral mucosa				+	+		+		+		+	+	+	+		+	+		+	+	+		+	+	+
Gingival, squamous cell carcinoma				X							X														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+		+		+				+		+		+			+	+		+	+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pheochromocytoma benign																		X							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	M	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X							X			X	X	X	X	X			X	X	X	
Pars distalis, carcinoma																									X
Thyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma																									
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 4 300 ng/kg:100 µg/kg

Number of Days on Study	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	338	351	355	360	362	364	380	301	302	324	325	330	340	342	352	353	357	363	376	378	321	326	339	341	354	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma						X									X				X							6
Cholangiocarcinoma, multiple														X												1
Hepatocellular adenoma, multiple																						X				2
Mesentery	+	+	+	M	M	+	M	M	+	M	+	+	M	+	M	+	+	+	+	M	+	+	+	+	M	31
Oral mucosa	+	+		+		+					+	+			+		+		+		+		+	+		28
Gingival, squamous cell carcinoma						X																				3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+		+			+	+	+	+	+		+		+		+		+		+		+	+		25
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																		X								1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma							X		X	X	X		X		X				X				X		X	19
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma			X				X	X		X			X										X			6
C-cell, carcinoma																				X						1
Follicular cell, adenoma													X													1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 4 300 ng/kg:100 µg/kg

Number of Days on Study	441	462	478	484	491	491	494	534	567	569	603	612	615	625	633	636	640	643	653	664	689	701	729	729	729
Carcass ID Number	358	322	344	304	310	359	445	365	336	356	377	327	379	343	323	308	305	306	361	309	337	307	303	328	329
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Polyp stromal																							X		
Polyp stromal, multiple																									
Schwannoma malignant																				X					
Squamous cell carcinoma															X										
Cervix, squamous cell carcinoma																									
Vagina																									
Sarcoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+																							
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Carcinoma							X																		
Fibroadenoma	X		X	X	X				X		X	X				X	X	X			X				
Fibroadenoma, multiple								X						X										X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																				X	X				
Fibrosarcoma						X																			
Neural crest tumor																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle				+																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																									X
Granular cell tumor benign																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystic keratinizing epithelioma														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 4 300 ng/kg:100 µg/kg

Number of Days on Study	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	338	351	355	360	362	364	380	301	302	324	325	330	340	342	352	353	357	363	376	378	321	326	339	341	354	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Oviduct																		+								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma						X																				1
Polyp stromal								X					X													3
Polyp stromal, multiple																						X				1
Schwannoma malignant																										1
Squamous cell carcinoma																										1
Cervix, squamous cell carcinoma																			X							1
Vagina																					+					1
Sarcoma																					X					1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node															+											2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																										1
Fibroadenoma	X							X	X		X					X		X		X		X	X	X		21
Fibroadenoma, multiple		X								X				X	X		X									8
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma																										2
Fibrosarcoma																										1
Neural crest tumor											X															1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pituitary gland																										1
Granular cell tumor benign																								X		1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystic keratinizing epithelioma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 4 300 ng/kg:100 µg/kg

Number of Days on Study	441	462	478	484	491	491	494	534	567	569	603	612	615	625	633	636	640	643	653	664	689	701	729	729	729
Carcass ID Number	358	322	344	304	310	359	445	465	363	356	377	327	379	343	323	308	305	306	361	309	337	307	303	328	329
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant												X													
Mesothelioma malignant				X																					

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 4 300 ng/kg:100 µg/kg

Number of Days on Study	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	338	351	355	360	362	364	380	301	302	324	325	330	340	442	452	453	457	463	476	478	321	326	339	341	354	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																										1
Mesothelioma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	096	142	254	310	330	363	451	479	499	505	505	506	539	552	556	580	583	603	603	612	618	650	654	659	665
Carcass ID Number	466	486	490	443	402	427	412	498	410	414	488	497	411	485	417	442	456	421	425	405	457	496	409	481	418
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, vagina														X											
Intestine large, cecum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Intestine small, jejunum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																			X						
Cholangiocarcinoma, multiple																									
Hepatocellular adenoma																									
Hepatocholangioma																									
Mesentery	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+
Oral mucosa							+	+					+	+	+	+	+		+	+	+	+	+		
Gingival, squamous cell carcinoma								X								X									
Pancreas	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Acinus, carcinoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Stomach, forestomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+		+		+	+	+			+		+	+		+		+		+	+	+	+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, lung																			X						
Endocrine System																									
Adrenal cortex	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pars distalis, adenoma												X		X			X							X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma									X						X	X									X
C-cell, carcinoma																									
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	684	710	715	719	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	493	420	484	430	401	407	413	416	428	445	465	482	487	494	404	406	408	423	460	462	476	480	495	424	426	461	491	492		Total Tissues/ Tumors
Alimentary System																														
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Schwannoma malignant, metastatic, vagina																														1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Leiomyoma		X																								X				2
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Cholangiocarcinoma							X	X					X																	4
Cholangiocarcinoma, multiple	X								X											X		X			X					5
Hepatocellular adenoma	X						X					X	X											X						5
Hepatocholangioma																			X	X										2
Mesentery	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+		47
Oral mucosa		+	+	+			+	+	+		+	+	+	+		+	+			+		+	+		+		+	+		30
Gingival, squamous cell carcinoma		X		X																X										5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Acinus, adenoma						X										X										X				3
Acinus, carcinoma										X																				1
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Tooth	+	+	+	+		+	+	+	+		+	+	+	+		+	+	+	+	+		+	+		+			+		35
Cardiovascular System																														
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Fibrosarcoma, metastatic, lung																														1
Endocrine System																														
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Adenoma																														1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Pheochromocytoma benign																									X					1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+	+	+	+	+	+		47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Pars distalis, adenoma			X							X	X		X	X	X	X					X	X	X			X	X	X		17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Bilateral, C-cell, adenoma																							X	X				X		3
C-cell, adenoma	X						X		X	X					X						X	X				X				12
C-cell, carcinoma																		X												1
Follicular cell, adenoma																					X									1
General Body System																														
None																														

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	096	142	254	310	330	363	451	479	499	505	505	506	539	552	556	580	583	603	603	612	618	650	654	659	665
Carcass ID Number	466	486	490	443	402	427	412	498	410	414	488	497	411	485	417	442	456	421	425	405	457	496	409	481	418
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Granulosa cell tumor benign																									
Oviduct													+											+	
Uterus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Polyp stromal																									
Schwannoma malignant								X					X												
Schwannoma malignant, metastatic, vagina														X											
Squamous cell carcinoma																									
Cervix, granular cell tumor benign																									
Cervix, squamous cell carcinoma																									
Vagina														+											
Schwannoma malignant														X											
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+			+				+		+							+				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Squamous cell carcinoma,metastatic, lung																			X						
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma							X																		
Carcinoma, multiple																									
Fibroadenoma			X		X				X		X	X					X	X	X	X	X	X			
Fibroadenoma, multiple										X			X		X								X		X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																								X	
Lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																			+						
Fibrosarcoma, metastatic, lung																			X						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Granular cell tumor malignant																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	684	710	715	719	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731
Carcass ID Number	493	420	484	430	401	407	413	416	428	445	465	482	487	494	404	406	408	423	460	462	476	480	495	424	426	461	491	492	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Granulosa cell tumor malignant										X																			1
Granulosa cell tumor benign							X																						1
Oviduct																													2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Fibrosarcoma																						X							1
Polyp stromal			X				X		X										X	X		X							6
Schwannoma malignant																													2
Schwannoma malignant, metastatic, vagina																													1
Squamous cell carcinoma													X										X						2
Cervix, granular cell tumor benign																						X							1
Cervix, squamous cell carcinoma			X	X																									2
Vagina																													1
Schwannoma malignant																													1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node									+																	+			7
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, lung																													1
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																													1
Carcinoma, multiple																					X								1
Fibroadenoma		X		X	X	X			X		X		X			X	X			X	X			X		X		X	25
Fibroadenoma, multiple			X												X			X							X				9
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma																													1
Lipoma																					X								1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																													1
Fibrosarcoma, metastatic, lung																													1
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Granular cell tumor malignant																		X											1

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	096	142	254	310	330	363	451	479	499	505	505	506	539	552	556	580	583	603	603	612	618	650	654	659	665
Carcass ID Number	466	486	490	443	402	427	412	498	410	414	488	497	411	485	417	442	456	421	425	405	457	496	409	481	418
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystic keratinizing epithelioma																									
Squamous cell carcinoma																			X						
Mediastinum, fibrosarcoma																			X						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																		+			+				
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Squamous cell carcinoma, metastatic, oral mucosa																X									
Zymbal's gland																									
Adenoma																									
Urinary System																									
Kidney	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma		X																							
Ureter																					+				
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																				X					

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 5 300 ng/kg:300 µg/kg

Number of Days on Study	684	710	715	719	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	493	420	484	430	401	407	413	416	428	445	465	482	487	494	404	406	408	423	460	462	476	480	495	424	426	461	491	492		Total Tissues/ Tumors
Respiratory System																														
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Cystic keratinizing epithelioma												X																		1
Squamous cell carcinoma																														1
Mediastinum, fibrosarcoma																														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Special Senses System																														
Ear																														2
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Squamous cell carcinoma, metastatic, oral mucosa																														1
Zymbal's gland												+																		1
Adenoma												X																		1
Urinary System																														
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Nephroblastoma																														1
Ureter																														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Systemic Lesions																														
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Lymphoma malignant																						X								2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 6 300 ng/kg:3,000 µg/kg

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 6 300 ng/kg:3,000 µg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731
Carcass ID Number	519	523	531	532	533	537	563	565	574	503	504	513	522	536	538	564	568	569	571	502	505	521	524	534	558	573	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Fibrosarcoma																X											1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, uterus								X																			1
Cholangiocarcinoma							X		X												X	X	X	X		X	12
Cholangiocarcinoma, multiple		X		X		X		X					X		X		X	X	X	X					X		13
Hepatocellular adenoma		X		X		X			X						X		X			X	X			X			14
Hepatocellular adenoma, multiple			X		X					X															X		7
Hepatocholangioma																			X								1
Hepatocholangioma, multiple					X																						1
Mesentery	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	M	+	+	+	47
Oral mucosa	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+		+	+	41
Gingival, squamous cell carcinoma										X							X										6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, metastatic, uterus								X																			1
Acinus, adenoma					X																						1
Acinus, carcinoma																								X			1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Tooth	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	42
Cardiovascular System																											
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma																											1
Adrenal medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign					X																						2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																											1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	M	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Pars distalis, adenoma						X		X				X				X		X				X			X		9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma																	X										1
C-cell, adenoma			X		X	X					X					X								X	X		7
General Body System																											
None																											

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 6 300 ng/kg:3,000 µg/kg

Number of Days on Study	2	4	4	4	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7
	3	0	5	6	6	9	9	5	8	8	8	8	9	0	0	2	3	3	4	4	6	1	1	2	2	9
	3	9	8	2	3	0	1	6	8	8	8	8	6	1	2	2	2	6	3	7	8	3	2	4	0	9
Carcass ID Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0	7	2	1	5	4	6	6	1	3	7	6	6	6	1	5	1	1	6	3	7	0	5	2	1	1
	7	5	0	7	7	0	0	7	8	5	2	1	2	1	6	6	4	2	6	9	0	1	9	5	1	5
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Luteoma																										
Oviduct																								+		+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Carcinoma, multiple																										
Polyp stromal, multiple																										
Schwannoma malignant																		X		X						
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node														+												
Mediastinal, carcinoma, metastatic, uterus																										
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+
Thymus	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Carcinoma										X		X			X											
Fibroadenoma			X		X		X	X	X			X					X							X		X
Fibroadenoma, multiple										X	X					X					X		X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																										
Squamous cell papilloma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																				+					
Carcinoma, metastatic, uterus																										
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																										
Cystic keratinizing epithelioma																										
Cystic keratinizing epithelioma, multiple																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 6 300 ng/kg:3,000 µg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	519	523	531	532	533	537	563	565	574	503	504	513	522	536	538	564	568	569	571	502	505	521	524	534	558	573	Total Tissues/ Tumors
Genital System																											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Luteoma									X																		1
Oviduct																					+						3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, multiple								X																			1
Polyp stromal, multiple													X														1
Schwannoma malignant																											2
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node		+			+			+															+				5
Mediastinal, carcinoma, metastatic, uterus								X																			1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenoma					X																						1
Carcinoma						X																					4
Fibroadenoma		X				X	X						X	X	X								X	X			17
Fibroadenoma, multiple	X		X							X	X								X		X					X	12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Fibroma					X																						1
Squamous cell papilloma																							X				1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Skeletal muscle								+																			3
Carcinoma, metastatic, uterus								X																			1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, uterus								X																			1
Cystic keratinizing epithelioma														X													1
Cystic keratinizing epithelioma, multiple					X																						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 6 300 ng/kg:3,000 µg/kg

Number of Days on Study	2	4	4	4	4	4	4	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7
	3	0	5	6	6	9	9	5	8	8	8	9	0	0	2	3	3	4	4	6	7	1	1	2	2	2
	3	9	8	2	3	0	1	6	8	8	8	6	1	2	2	2	6	3	7	8	3	2	4	0	9	9
Carcass ID Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0	7	2	1	5	4	6	6	1	3	7	6	6	1	5	1	1	6	3	7	0	5	2	1	1	1
	7	5	0	7	7	0	0	7	8	5	2	1	2	6	6	4	2	6	9	0	1	9	5	1	5	5
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																										
Ureter																				+						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Carcinoma, metastatic, uterus																										
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153: Group 6 300 ng/kg:3,000 µg/kg

Number of Days on Study	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731		
Carcass ID Number	519	523	531	532	533	537	563	565	574	503	504	513	522	536	538	564	568	569	571	502	505	521	524	534	558	573	Total Tissues/ Tumors
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, uterus								X																			1
Ureter																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, uterus								X																			1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymphoma malignant		X																									1

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Adrenal Medulla: Pheochromocytoma Benign				
Overall rate[a]	3/52 (6%)	1/49 (2%)	1/52 (2%)	2/49 (4%)
Adjusted rate[b]	8.0%	2.6%	2.7%	5.1%
Terminal rate[c]	2/21 (10%)	0/28 (0%)	1/24 (4%)	1/26 (4%)
First incidence (days)	664	643	729 (T)	588
Poly-3 test[d]		P=0.428		
Liver: Hepatocellular Adenoma				
Overall rate	0/53 (0%)	2/50 (4%)	5/52 (10%)	21/51 (41%)
Adjusted rate	0.0%	5.1%	13.3%	49.6%
Terminal rate	0/22 (0%)	2/28 (7%)	4/24 (17%)	14/27 (52%)
First incidence (days)	—[e]	729 (T)	684	491
Poly-3 test		P<0.001		
Liver: Cholangiocarcinoma				
Overall rate	0/53 (0%)	7/50 (14%)	9/52 (17%)	25/51 (49%)
Adjusted rate	0.0%	17.3%	23.7%	59.5%
Terminal rate	0/22 (0%)	4/28 (14%)	7/24 (29%)	18/27 (67%)
First incidence (days)	—	603	603	588
Poly-3 test		P<0.001		
Mammary Gland: Fibroadenoma				
Overall rate	40/53 (75%)	29/50 (58%)	34/53 (64%)	29/51 (57%)
Adjusted rate	83.1%	63.1%	73.5%	63.5%
Terminal rate	16/22 (73%)	16/28 (57%)	15/24 (63%)	16/27 (59%)
First incidence (days)	345	441	254	458
Poly-3 test		P=0.377N		
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	40/53 (75%)	29/50 (58%)	34/53 (64%)	30/51 (59%)
Adjusted rate	83.1%	63.1%	73.5%	65.7%
Terminal rate	16/22 (73%)	16/28 (57%)	15/24 (63%)	17/27 (63%)
First incidence (days)	345	441	254	458
Poly-3 test		P=0.480N		
Mammary Gland: Carcinoma				
Overall rate	8/53 (15%)	1/50 (2%)	2/53 (4%)	4/51 (8%)
Adjusted rate	20.4%	2.5%	5.2%	9.7%
Terminal rate	6/22 (27%)	0/28 (0%)	1/24 (4%)	1/27 (4%)
First incidence (days)	449	494	451	588
Poly-3 test		P=0.167		
Mammary Gland: Adenoma or Carcinoma				
Overall rate	10/53 (19%)	2/50 (4%)	2/53 (4%)	5/51 (10%)
Adjusted rate	25.5%	4.9%	5.2%	12.1%
Terminal rate	8/22 (36%)	0/28 (0%)	1/24 (4%)	2/27 (7%)
First incidence (days)	449	494	451	588
Poly-3 test		P=0.145		

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	42/53 (79%)	30/50 (60%)	35/53 (66%)	31/51 (61%)
Adjusted rate	85.9%	64.3%	74.5%	67.2%
Terminal rate	17/22 (77%)	16/28 (57%)	15/24 (63%)	17/27 (63%)
First incidence (days)	345	441	254	458
Poly-3 test		P=0.501N		
Oral Mucosa (Gingival): Squamous Cell Carcinoma				
Overall rate	0/53 (0%)	3/50 (6%)	5/53 (9%)	6/51 (12%)
Adjusted rate	0.0%	7.4%	12.9%	14.4%
Terminal rate	0/22 (0%)	1/28 (4%)	1/24 (4%)	2/27 (7%)
First incidence (days)	—	484	479	490
Poly-3 test		P=0.308		
Pancreas: Adenoma				
Overall rate	0/53 (0%)	0/49 (0%)	3/52 (6%)	1/49 (2%)
Adjusted rate	0.0%	0.0%	8.0%	2.6%
Terminal rate	0/22 (0%)	0/28 (0%)	3/24 (13%)	1/27 (4%)
First incidence (days)	—	—	729 (T)	729 (T)
Poly-3 test		P=0.626N		
Pancreas: Adenoma or Carcinoma				
Overall rate	0/53 (0%)	0/49 (0%)	4/52 (8%)	2/49 (4%)
Adjusted rate	0.0%	0.0%	10.7%	5.2%
Terminal rate	0/22 (0%)	0/28 (0%)	4/24 (17%)	2/27 (7%)
First incidence (days)	—	—	729 (T)	729 (T)
Poly-3 test		P=0.595		
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/53 (42%)	19/50 (38%)	17/52 (33%)	9/51 (18%)
Adjusted rate	52.8%	44.8%	43.8%	22.2%
Terminal rate	13/22 (59%)	11/28 (39%)	12/24 (50%)	7/27 (26%)
First incidence (days)	418	491	506	588
Poly-3 test		P=0.011N		
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	23/53 (43%)	20/50 (40%)	17/52 (33%)	9/51 (18%)
Adjusted rate	55.2%	47.2%	43.8%	22.2%
Terminal rate	13/22 (59%)	12/28 (43%)	12/24 (50%)	7/27 (26%)
First incidence (days)	418	491	506	588
Poly-3 test		P=0.007N		
Skin: Fibroma or Fibrosarcoma				
Overall rate	2/53 (4%)	3/50 (6%)	1/53 (2%)	1/51 (2%)
Adjusted rate	5.2%	7.4%	2.7%	2.5%
Terminal rate	0/22 (0%)	0/28 (0%)	0/24 (0%)	1/27 (4%)
First incidence (days)	664	491	659	729 (T)
Poly-3 test		P=0.376N		

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Thyroid Gland (C-Cell): Adenoma				
Overall rate	10/53 (19%)	6/49 (12%)	15/52 (29%)	8/50 (16%)
Adjusted rate	25.4%	15.3%	38.5%	20.2%
Terminal rate	7/22 (32%)	6/28 (21%)	10/24 (42%)	8/27 (30%)
First incidence (days)	426	729 (T)	499	729 (T)
Poly-3 test		P=0.340N		
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	13/53 (25%)	7/49 (14%)	16/52 (31%)	8/50 (16%)
Adjusted rate	32.8%	17.9%	41.1%	20.2%
Terminal rate	9/22 (41%)	7/28 (25%)	11/24 (46%)	8/27 (30%)
First incidence (days)	426	729 (T)	499	729 (T)
Poly-3 test		P=0.238N		
Uterus: Stromal Polyp				
Overall rate	8/53 (15%)	4/50 (8%)	6/53 (11%)	1/51 (2%)
Adjusted rate	20.7%	10.1%	16.0%	2.5%
Terminal rate	5/22 (23%)	4/28 (14%)	5/24 (21%)	1/27 (4%)
First incidence (days)	640	729 (T)	715	729 (T)
Poly-3 test		P=0.071N		
Uterus: Squamous Cell Carcinoma				
Overall rate	1/53 (2%)	2/50 (4%)	4/53 (8%)	0/51 (0%)
Adjusted rate	2.6%	5.0%	10.7%	0.0%
Terminal rate	1/22 (5%)	1/28 (4%)	2/24 (8%)	0/27 (0%)
First incidence (days)	729 (T)	633	715	—
Poly-3 test		P=0.097N		
All Organs: Benign Neoplasms				
Overall rate	49/53 (92%)	39/50 (78%)	46/53 (87%)	40/51 (78%)
Adjusted rate	98.5%	83.1%	96.4%	87.6%
Terminal rate	22/22 (100%)	22/28 (79%)	23/24 (96%)	27/27 (100%)
First incidence (days)	345	441	254	458
Poly-3 test		P=0.516N		
All Organs: Malignant Neoplasms				
Overall rate	14/53 (26%)	16/50 (32%)	23/53 (43%)	35/51 (69%)
Adjusted rate	34.2%	36.8%	53.8%	77.9%
Terminal rate	9/22 (41%)	8/28 (29%)	11/24 (46%)	20/27 (74%)
First incidence (days)	377	484	142	490
Poly-3 test		P<0.001		

Table B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	51/53 (96%)	44/50 (88%)	50/53 (94%)	47/51 (92%)
Adjusted rate	99.2%	91.0%	99.6%	97.9%
Terminal rate	22/22 (100%)	25/28 (89%)	24/24 (100%)	27/27 (100%)
First incidence (days)	345	441	142	458
Poly-3 test		P=0.301		

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, pancreas, 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 Group 4 (300 ng/kg:100 µg/kg) incidence is the P value associated with the trend test for Groups 4, 5, and 6; Group 1 (Vehicle Control) is not included in the trend test. A negative trend is indicated by **N.**
[e] Not applicable; no neoplasms in animal group

TABLE B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Disposition Summary				
Animals initially in study	28	30	28	29
14-Week interim evaluation	10	10	10	10
31-Week interim evaluation	10	10	10	10
53-Week interim evaluation	8	10	8	9
Animals examined microscopically	28	30	28	29
14-Week Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Fatty change, diffuse			1 (10%)	8 (80%)
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus		1 (10%)		
Pigmentation		7 (70%)	5 (50%)	5 (50%)
Hepatocyte, hypertrophy		3 (30%)	6 (60%)	10 (100%)
Pancreas	(10)	(10)	(10)	(10)
Basophilic focus			2 (20%)	1 (10%)
Acinus, atrophy		1 (10%)		1 (10%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy			1 (10%)	3 (30%)
Thyroid gland	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy	3 (30%)	6 (60%)	8 (80%)	6 (60%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Atrophy	4 (40%)	1 (10%)	3 (30%)	4 (40%)
Uterus	(10)	(10)	(10)	(10)
Metaplasia, squamous	2 (20%)	1 (10%)	1 (10%)	3 (30%)
Endometrium, hyperplasia, cystic				1 (10%)
Hematopoietic System				
Spleen	(10)			(10)
Pigmentation	10 (100%)			10 (100%)
Thymus	(10)	(10)	(10)	(10)
Atrophy			1 (10%)	3 (30%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage			1 (10%)	
Inflammation			1 (10%)	2 (20%)

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

Table B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Systems Examined at 14 Weeks with No Nonneoplastic Lesions Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
31-Week Interim Evaluation				
Alimentary System				
Esophagus				(1)
Periesophageal tissue, pigmentation				1 (100%)
Liver	(10)	(10)	(10)	(10)
Eosinophilic focus, multiple				3 (30%)
Fatty change, diffuse			1 (10%)	7 (70%)
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus	1 (10%)	2 (20%)	3 (30%)	2 (20%)
Mixed cell focus, multiple	1 (10%)		3 (30%)	
Necrosis				1 (10%)
Pigmentation		10 (100%)	10 (100%)	8 (80%)
Toxic hepatopathy				3 (30%)
Bile duct, hyperplasia			1 (10%)	1 (10%)
Bile duct, inflammation, chronic active, focal				1 (10%)
Hepatocyte, hypertrophy		10 (100%)	10 (100%)	10 (100%)
Pancreas	(10)	(10)	(10)	(10)
Basophilic focus			1 (10%)	
Inflammation, chronic active	1 (10%)	2 (20%)	1 (10%)	
Acinus, atrophy	1 (10%)	1 (10%)	1 (10%)	
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hyperplasia			1 (10%)	1 (10%)
Hypertrophy	3 (30%)	5 (50%)	4 (40%)	2 (20%)
Thyroid gland	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy		6 (60%)	7 (70%)	10 (100%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Atrophy	6 (60%)	8 (80%)	10 (100%)	10 (100%)
Uterus	(10)	(10)	(10)	(10)
Inflammation, suppurative	1 (10%)			
Metaplasia, squamous	6 (60%)	8 (80%)	9 (90%)	9 (90%)
Vagina	(10)			(10)
Inflammation, suppurative				1 (10%)

Table B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Hematopoietic System				
Spleen	(10)			(10)
Pigmentation	10 (100%)			10 (100%)
Thymus	(10)	(10)	(10)	(10)
Atrophy	6 (60%)	6 (60%)	7 (70%)	6 (60%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage			1 (10%)	
Infiltration cellular, histiocyte	2 (20%)		1 (10%)	2 (20%)
Inflammation		2 (20%)		
Inflammation, granulomatous				1 (10%)
Systems Examined at 31 Weeks with No Nonneoplastic Lesions Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
53-Week Interim Evaluation				
Alimentary System				
Liver	(8)	(10)	(8)	(9)
Basophilic focus		1 (10%)		1 (11%)
Eosinophilic focus		1 (10%)	1 (13%)	1 (11%)
Eosinophilic focus, multiple		1 (10%)		
Fatty change, diffuse		1 (10%)	3 (38%)	9 (100%)
Fatty change, focal		1 (10%)		
Hepatodiaphragmatic nodule		1 (10%)		
Infiltration cellular, histiocyte			1 (13%)	
Inflammation	8 (100%)	10 (100%)	8 (100%)	9 (100%)
Mixed cell focus	1 (13%)	1 (10%)		3 (33%)
Mixed cell focus, multiple	4 (50%)	8 (80%)	2 (25%)	3 (33%)
Pigmentation		10 (100%)	8 (100%)	9 (100%)
Toxic hepatopathy		3 (30%)		6 (67%)
Bile duct, fibrosis		1 (10%)		
Bile duct, hyperplasia		2 (20%)		5 (56%)
Hepatocyte, hypertrophy		10 (100%)	8 (100%)	9 (100%)
Hepatocyte, multinucleated		3 (30%)	2 (25%)	7 (78%)
Oval cell, hyperplasia		1 (10%)		2 (22%)
Pancreas	(8)	(10)	(8)	(9)
Acinus, atrophy				1 (11%)
Acinus, hyperplasia				1 (11%)
Acinus, vacuolization cytoplasmic				6 (67%)

Table B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Endocrine System				
Adrenal cortex	(8)	(10)	(8)	(9)
Degeneration, cystic	2 (25%)	1 (10%)	1 (13%)	1 (11%)
Hyperplasia	1 (13%)	3 (30%)	1 (13%)	1 (11%)
Hypertrophy	4 (50%)	5 (50%)	5 (63%)	3 (33%)
Vacuolization cytoplasmic				1 (11%)
Thyroid gland	(8)	(10)	(8)	(9)
C-cell, hyperplasia	2 (25%)			1 (11%)
Follicular cell, hypertrophy		7 (70%)	6 (75%)	6 (67%)
Genital System				
Ovary	(8)	(10)	(8)	(9)
Atrophy	7 (88%)	8 (80%)	7 (88%)	8 (89%)
Cyst	1 (13%)		1 (13%)	
Inflammation, suppurative				1 (11%)
Uterus	(8)	(10)	(8)	(9)
Inflammation, suppurative				6 (67%)
Metaplasia, squamous	7 (88%)	9 (90%)	7 (88%)	7 (78%)
Endometrium, hyperplasia, cystic	5 (63%)			2 (22%)
Hematopoietic System				
Spleen	(8)			(9)
Pigmentation	8 (100%)			9 (100%)
Thymus	(8)	(10)	(8)	(9)
Atrophy	5 (63%)	10 (100%)	8 (100%)	9 (100%)
Integumentary System				
Mammary gland	(8)	(2)	(5)	(9)
Cyst	1 (13%)		2 (40%)	
Hyperplasia	4 (50%)		1 (20%)	
Inflammation, granulomatous			1 (20%)	
Respiratory System				
Lung	(8)	(10)	(8)	(9)
Infiltration cellular, histiocyte	4 (50%)		2 (25%)	4 (44%)
Alveolar epithelium, hyperplasia		1 (10%)		
Alveolar epithelium, metaplasia, bronchiolar		3 (30%)	1 (13%)	
Urinary System				
Kidney		(1)		
Mineralization		1 (100%)		
Nephropathy		1 (100%)		

Table B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Systems Examined at 53 Weeks with No Nonneoplastic Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				

Table B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Disposition Summary				
Animals initially in study	53	50	53	51
Early deaths				
Accidental deaths	1			1
Moribund	22	10	19	13
Natural deaths	8	12	10	10
Survivors				
Terminal sacrifice	22	28	24	27
Animals examined microscopically	53	50	53	51
Alimentary System				
Esophagus	(53)	(50)	(53)	(50)
Perforation				1 (2%)
Muscularis, inflammation	3 (6%)		3 (6%)	
Periesophageal tissue, hemorrhage				1 (2%)
Periesophageal tissue, inflammation		1 (2%)		1 (2%)
Intestine large, rectum	(53)	(50)	(53)	(51)
Parasite metazoan	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Artery, inflammation, chronic active		1 (2%)		2 (4%)
Intestine large, cecum	(53)	(50)	(52)	(50)
Edema				1 (2%)
Inflammation		1 (2%)		
Artery, inflammation		1 (2%)		
Artery, inflammation, chronic active				1 (2%)
Artery, thrombosis		1 (2%)		
Lymphoid tissue, hyperplasia		1 (2%)		
Intestine small, jejunum	(53)	(50)	(52)	(51)
Hyperplasia, lymphoid			2 (4%)	
Ulcer			1 (2%)	
Intestine small, ileum	(53)	(50)	(52)	(50)
Hyperplasia, lymphoid	1 (2%)			
Liver	(53)	(50)	(52)	(51)
Angiectasis	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Basophilic focus	9 (17%)	4 (8%)	2 (4%)	8 (16%)
Basophilic focus, multiple	13 (25%)	1 (2%)	1 (2%)	10 (20%)
Cholangiofibrosis		5 (10%)	7 (13%)	13 (25%)
Clear cell focus	3 (6%)	1 (2%)	3 (6%)	5 (10%)
Clear cell focus, multiple	6 (11%)	4 (8%)		6 (12%)
Eosinophilic focus	7 (13%)	6 (12%)	7 (13%)	
Eosinophilic focus, multiple	7 (13%)	21 (42%)	33 (63%)	45 (88%)
Fatty change, diffuse	3 (6%)	28 (56%)	31 (60%)	47 (92%)
Fatty change, focal	3 (6%)	4 (8%)	1 (2%)	11 (22%)
Hematopoietic cell proliferation	27 (51%)	18 (36%)	19 (37%)	29 (57%)
Hemorrhage				1 (2%)
Hyperplasia, nodular		20 (40%)	24 (46%)	21 (41%)

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

Table B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Alimentary System (continued)				
Liver (continued)	(53)	(50)	(52)	(51)
Inflammation	44 (83%)	47 (94%)	48 (92%)	46 (90%)
Mixed cell focus	7 (13%)		2 (4%)	1 (2%)
Mixed cell focus, multiple	19 (36%)	27 (54%)	24 (46%)	26 (51%)
Necrosis	4 (8%)	6 (12%)	4 (8%)	7 (14%)
Pigmentation	2 (4%)	50 (100%)	50 (96%)	44 (86%)
Toxic hepatopathy		44 (88%)	48 (92%)	48 (94%)
Bile duct, cyst	4 (8%)	4 (8%)	5 (10%)	6 (12%)
Bile duct, fibrosis	1 (2%)		4 (8%)	5 (10%)
Bile duct, hyperplasia	8 (15%)	20 (40%)	29 (56%)	40 (78%)
Centrilobular, degeneration	5 (9%)	6 (12%)	4 (8%)	2 (4%)
Centrilobular, fibrosis		1 (2%)	1 (2%)	
Hepatocyte, hypertrophy	1 (2%)	22 (44%)	33 (63%)	47 (92%)
Hepatocyte, multinucleated		42 (84%)	46 (88%)	44 (86%)
Oval cell, hyperplasia	2 (4%)	33 (66%)	39 (75%)	43 (84%)
Portal, fibrosis		3 (6%)	7 (13%)	10 (20%)
Serosa, inflammation, chronic active	1 (2%)			
Mesentery	(47)	(31)	(47)	(47)
Artery, inflammation, chronic active	1 (2%)	1 (3%)	2 (4%)	1 (2%)
Fat, necrosis			1 (2%)	
Oral mucosa	(12)	(28)	(30)	(41)
Gingival, hyperplasia, squamous	8 (67%)	21 (75%)	22 (73%)	29 (71%)
Pancreas	(53)	(49)	(52)	(49)
Inflammation, chronic active		5 (10%)	1 (2%)	5 (10%)
Necrosis				1 (2%)
Acinus, atrophy		4 (8%)	1 (2%)	5 (10%)
Acinus, hyperplasia	2 (4%)		1 (2%)	1 (2%)
Acinus, vacuolization cytoplasmic		3 (6%)	7 (13%)	44 (90%)
Artery, inflammation, chronic active		4 (8%)	2 (4%)	3 (6%)
Stomach, forestomach	(53)	(50)	(52)	(51)
Cyst		1 (2%)		
Hyperkeratosis			2 (4%)	2 (4%)
Hyperplasia, squamous	1 (2%)	6 (12%)	7 (13%)	9 (18%)
Inflammation			1 (2%)	6 (12%)
Mineralization	1 (2%)			
Ulcer		1 (2%)	2 (4%)	1 (2%)
Artery, inflammation, chronic active				2 (4%)
Stomach, glandular	(53)	(50)	(52)	(51)
Ectopic tissue			1 (2%)	
Erosion		3 (6%)	1 (2%)	
Mineralization	4 (8%)	1 (2%)	1 (2%)	
Tooth	(23)	(25)	(35)	(42)
Periodontal tissue, inflammation	23 (100%)	25 (100%)	35 (100%)	42 (100%)

Table B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Cardiovascular System				
Blood vessel	(53)	(50)	(53)	(51)
Aorta, mineralization			1 (2%)	
Heart	(53)	(50)	(53)	(50)
Cardiomyopathy	22 (42%)	18 (36%)	26 (49%)	25 (50%)
Inflammation, chronic active	1 (2%)			
Mineralization		1 (2%)		
Thrombosis		1 (2%)		
Coronary artery, inflammation, chronic active		1 (2%)		
Endocardium, hyperplasia		1 (2%)		
Pericardium, necrosis			1 (2%)	
Endocrine System				
Adrenal cortex	(53)	(49)	(52)	(51)
Angiectasis	17 (32%)	28 (57%)	23 (44%)	7 (14%)
Atrophy		2 (4%)	3 (6%)	5 (10%)
Degeneration, cystic	13 (25%)	14 (29%)	14 (27%)	18 (35%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia	11 (21%)	17 (35%)	25 (48%)	21 (41%)
Hypertrophy	47 (89%)	42 (86%)	41 (79%)	40 (78%)
Necrosis	1 (2%)	3 (6%)		2 (4%)
Vacuolization cytoplasmic	11 (21%)	15 (31%)	10 (19%)	13 (25%)
Adrenal medulla	(52)	(49)	(52)	(49)
Hyperplasia	15 (29%)	8 (16%)	13 (25%)	6 (12%)
Islets, pancreatic	(53)	(49)	(52)	(49)
Hyperplasia		1 (2%)		
Pituitary gland	(53)	(50)	(52)	(51)
Angiectasis	17 (32%)	14 (28%)	17 (33%)	7 (14%)
Cyst	1 (2%)			
Cytoplasmic alteration		1 (2%)		2 (4%)
Inflammation			1 (2%)	
Necrosis				1 (2%)
Vacuolization cytoplasmic			1 (2%)	2 (4%)
Pars distalis, hyperplasia	13 (25%)	17 (34%)	20 (38%)	21 (41%)
Thyroid gland	(53)	(49)	(52)	(50)
Fibrosis				1 (2%)
Inflammation, chronic		1 (2%)		
C-cell, hyperplasia	15 (28%)	16 (33%)	12 (23%)	17 (34%)
Follicle, cyst	2 (4%)			
Follicular cell, hyperplasia		1 (2%)		
Follicular cell, hypertrophy	14 (26%)	28 (57%)	35 (67%)	44 (88%)
General Body System				
None				

Table B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Genital System				
Clitoral gland	(53)	(49)	(53)	(50)
Hyperplasia, squamous		1 (2%)		
Inflammation	45 (85%)	38 (78%)	40 (75%)	40 (80%)
Duct, cyst	38 (72%)	41 (84%)	43 (81%)	41 (82%)
Ovary	(53)	(48)	(52)	(50)
Atrophy	45 (85%)	46 (96%)	43 (83%)	36 (72%)
Cyst	14 (26%)	16 (33%)	14 (27%)	19 (38%)
Inflammation, chronic active	1 (2%)			4 (8%)
Inflammation, granulomatous		1 (2%)		
Inflammation, suppurative			1 (2%)	
Oviduct	(1)	(1)	(2)	(3)
Cyst	1 (100%)		2 (100%)	
Inflammation, chronic active				3 (100%)
Metaplasia, squamous		1 (100%)		
Uterus	(53)	(50)	(52)	(50)
Adenomyosis		1 (2%)		
Hemorrhage		1 (2%)		
Inflammation, chronic active	3 (6%)	4 (8%)	7 (13%)	8 (16%)
Inflammation, suppurative	3 (6%)	2 (4%)	10 (19%)	8 (16%)
Metaplasia, squamous	27 (51%)	27 (54%)	36 (69%)	34 (68%)
Cervix, cyst		1 (2%)		
Endometrium, hyperplasia, cystic	28 (53%)	25 (50%)	18 (35%)	15 (30%)
Hematopoietic System				
Bone marrow	(53)	(50)	(53)	(51)
Hyperplasia	39 (74%)	38 (76%)	48 (91%)	39 (76%)
Lymph node	(4)	(2)	(7)	(5)
Angiectasis			1 (14%)	
Inguinal, hyperplasia, plasma cell			1 (14%)	
Lumbar, ectasia	4 (100%)		1 (14%)	1 (20%)
Lumbar, hemorrhage				1 (20%)
Lumbar, hyperplasia, plasma cell	4 (100%)			
Mediastinal, ectasia				2 (40%)
Mediastinal, hemorrhage			1 (14%)	2 (40%)
Mediastinal, hyperplasia, histiocytic			1 (14%)	1 (20%)
Mediastinal, hyperplasia, lymphoid		1 (50%)		
Mediastinal, hyperplasia, plasma cell		1 (50%)		1 (20%)
Pancreatic, hyperplasia, histiocytic			1 (14%)	
Pancreatic, pigmentation			1 (14%)	
Renal, ectasia			1 (14%)	
Renal, hyperplasia, histiocytic			1 (14%)	
Renal, hyperplasia, plasma cell			1 (14%)	
Lymph node, mandibular	(53)	(49)	(50)	(49)
Congestion			1 (2%)	
Ectasia			3 (6%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)		2 (4%)	2 (4%)
Hyperplasia, plasma cell	37 (70%)	33 (67%)	36 (72%)	32 (65%)
Lymph node, mesenteric	(53)	(49)	(52)	(49)
Hemorrhage			1 (2%)	
Hyperplasia, histiocytic			1 (2%)	
Hyperplasia, lymphoid		1 (2%)		
Hyperplasia, plasma cell		1 (2%)		2 (4%)

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Hematopoietic System (continued)				
Spleen	(53)	(49)	(52)	(49)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	51 (96%)	41 (84%)	47 (90%)	45 (92%)
Pigmentation	47 (89%)	48 (98%)	51 (98%)	49 (100%)
Lymphoid follicle, atrophy		5 (10%)	4 (8%)	2 (4%)
Red pulp, atrophy	1 (2%)	1 (2%)		1 (2%)
Thymus	(53)	(48)	(50)	(47)
Atrophy	33 (62%)	48 (100%)	42 (84%)	45 (96%)
Cyst			1 (2%)	
Hemorrhage	1 (2%)		3 (6%)	
Hyperplasia, lymphoid	2 (4%)			
Epithelial cell, hyperplasia			1 (2%)	
Integumentary System				
Mammary gland	(53)	(50)	(53)	(51)
Cyst	3 (6%)	2 (4%)	3 (6%)	2 (4%)
Hyperplasia	29 (55%)	14 (28%)	22 (42%)	15 (29%)
Inflammation, granulomatous	5 (9%)	2 (4%)	3 (6%)	
Duct, cyst	1 (2%)			
Skin	(53)	(50)	(53)	(51)
Angiectasis	1 (2%)			
Cyst epithelial inclusion			1 (2%)	
Edema				1 (2%)
Fibrosis	1 (2%)			
Inflammation				2 (4%)
Necrosis				1 (2%)
Subcutaneous tissue, edema				1 (2%)
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(1)	(3)
Inflammation				1 (33%)
Necrosis				1 (33%)
Nervous System				
Brain	(53)	(50)	(52)	(51)
Hemorrhage		1 (2%)	1 (2%)	
Hydrocephalus	1 (2%)	1 (2%)		1 (2%)
Cerebellum, hemorrhage		1 (2%)		

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Respiratory System				
Lung	(53)	(50)	(53)	(50)
Hemorrhage		1 (2%)		
Infiltration cellular, histiocyte	47 (89%)	49 (98%)	46 (87%)	40 (80%)
Inflammation	8 (15%)	2 (4%)	4 (8%)	2 (4%)
Metaplasia, squamous		3 (6%)	2 (4%)	6 (12%)
Mineralization			1 (2%)	
Alveolar epithelium, hyperplasia	23 (43%)	5 (10%)	5 (9%)	8 (16%)
Alveolar epithelium, metaplasia, bronchiolar		39 (78%)	34 (64%)	30 (60%)
Mediastinum, necrosis		1 (2%)	1 (2%)	
Serosa, inflammation			1 (2%)	
Nose	(53)	(50)	(53)	(51)
Hyperplasia				1 (2%)
Inflammation	22 (42%)	17 (34%)	13 (25%)	15 (29%)
Nerve, degeneration				1 (2%)
Olfactory epithelium, degeneration	1 (2%)	1 (2%)		
Olfactory epithelium, dysplasia				1 (2%)
Olfactory epithelium, metaplasia	4 (8%)	5 (10%)	6 (11%)	5 (10%)
Respiratory epithelium, hyperplasia	10 (19%)	14 (28%)	11 (21%)	12 (24%)
Respiratory epithelium, metaplasia	1 (2%)			
Trachea	(53)	(50)	(53)	(50)
Inflammation	1 (2%)			
Peritracheal tissue, inflammation				1 (2%)
Special Senses System				
Eye	(53)	(50)	(52)	(51)
Anterior chamber, ciliary body, cornea, inflammation		2 (4%)	1 (2%)	
Cornea, inflammation		1 (2%)	1 (2%)	
Retina, atrophy	1 (2%)		2 (4%)	5 (10%)
Harderian gland	(53)	(49)	(52)	(51)
Inflammation	20 (38%)	15 (31%)	14 (27%)	17 (33%)
Urinary System				
Kidney	(53)	(48)	(52)	(51)
Accumulation, hyaline droplet	2 (4%)		1 (2%)	2 (4%)
Calculus microscopic observation only	7 (13%)	6 (13%)	4 (8%)	6 (12%)
Casts protein	2 (4%)			
Cyst		1 (2%)	1 (2%)	
Hydronephrosis				2 (4%)
Infarct				2 (4%)
Inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Inflammation, suppurative	5 (9%)	6 (13%)	5 (10%)	3 (6%)
Mineralization	42 (79%)	39 (81%)	42 (81%)	33 (65%)
Nephropathy	29 (55%)	30 (63%)	34 (65%)	34 (67%)
Pigmentation		2 (4%)	7 (13%)	17 (33%)
Pelvis, dilatation	1 (2%)	4 (8%)	2 (4%)	
Pelvis, inflammation	3 (6%)	1 (2%)	3 (6%)	8 (16%)
Renal tubule, degeneration			2 (4%)	
Renal tubule, hyperplasia				1 (2%)
Transitional epithelium, hyperplasia	2 (4%)	6 (13%)	11 (21%)	9 (18%)

Table B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
Urinary System (continued)				
Ureter	(1)		(1)	(1)
Inflammation	1 (100%)		1 (100%)	1 (100%)
Transitional epithelium, hyperplasia	1 (100%)			1 (100%)
Urinary bladder	(53)	(49)	(52)	(50)
Inflammation	7 (13%)	3 (6%)	7 (13%)	8 (16%)
Transitional epithelium, hyperplasia			1 (2%)	1 (2%)

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

TABLE C1 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 13-, 14-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 (Groups 1, 2, 3, 5, 7)** **202**

TABLE C2 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 13-, 14-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 (Groups 1, 4, 5, 6)** **204**

Table C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 13-, 41-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 (Groups 1, 2, 3, 5, 7)[a]

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Necropsy body wt					
Week 14	268 ± 7	281 ± 4	281 ± 8	272 ± 6	261 ± 5
Week 31	310 ± 7	306 ± 10	296 ± 6	281 ± 3**	265 ± 8**
Week 53	340 ± 12	337 ± 9	325 ± 7	304 ± 8**	265 ± 8**
L. Kidney					
Week 14					
Absolute	0.746 ± 0.015	0.799 ± 0.014	0.774 ± 0.020	0.734 ± 0.017	0.716 ± 0.011
Relative	2.794 ± 0.034	2.855 ± 0.069	2.768 ± 0.059	2.703 ± 0.050	2.752 ± 0.038
Week 31					
Absolute	0.828 ± 0.012	0.846 ± 0.019	0.804 ± 0.022	0.819 ± 0.011	0.783 ± 0.026
Relative	2.681 ± 0.054	2.778 ± 0.055	2.725 ± 0.073	2.915 ± 0.047**	2.951 ± 0.031**
Week 53					
Absolute	0.931 ± 0.031	0.943 ± 0.023	0.960 ± 0.009	0.961 ± 0.030	0.878 ± 0.023
Relative	2.747 ± 0.075	2.802 ± 0.076	2.957 ± 0.052*	3.159 ± 0.036**	3.328 ± 0.090**
Liver					
Week 14					
Absolute	8.167 ± 0.447	9.897 ± 0.317**	9.889 ± 0.403**	10.219 ± 0.271**	11.394 ± 0.303**
Relative	30.342 ± 0.988	35.216 ± 0.753**	35.178 ± 0.731**	37.606 ± 0.585**	43.719 ± 0.926**
Week 31					
Absolute	9.010 ± 0.256	10.389 ± 0.284*	9.711 ± 0.289	10.473 ± 0.195**	13.134 ± 0.611**
Relative	29.099 ± 0.515	34.066 ± 0.563**	32.934 ± 0.989**	37.255 ± 0.579**	49.586 ± 1.934**
Week 53					
Absolute	10.64 ± 0.59	10.73 ± 0.31	12.21 ± 0.34*	13.11 ± 0.48**	14.31 ± 0.51**
Relative	31.227 ± 1.093	31.899 ± 1.029	37.544 ± 0.802**	43.064 ± 0.619**	54.037 ± 0.795**
Lung					
Week 14					
Absolute	1.766 ± 0.66	1.892 ± 0.059	1.890 ± 0.056	1.920 ± 0.071	1.775 ± 0.051
Relative	6.602 ± 0.178	6.762 ± 0.250	6.763 ± 0.214	7.081 ± 0.273	6.827 ± 0.224
Week 31					
Absolute	1.667 ± 0.061	2.007 ± 0.077*	1.592 ± 0.070	1.633 ± 0.040	1.638 ± 0.065
Relative	5.393 ± 0.187	6.617 ± 0.314**	5.392 ± 0.207	5.819 ± 0.178	6.190 ± 0.221
Week 53					
Absolute	2.139 ± 0.111	2.220 ± 0.077	2.180 ± 0.124	1.993 ± 0.060	2.170 ± 0.093
Relative	6.309 ± 0.309	6.619 ± 0.323	6.752 ± 0.482	6.593 ± 0.280	8.229 ± 0.362**
L. Ovary					
Week 14					
Absolute	0.055 ± 0.004	0.065 ± 0.004	0.059 ± 0.003	0.056 ± 0.004	0.055 ± 0.002
Relative	0.205 ± 0.014	0.232 ± 0.013	0.209 ± 0.009	0.205 ± 0.014	0.212 ± 0.009
Week 31					
Absolute	0.062 ± 0.005	0.062 ± 0.004	0.050 ± 0.002	0.050 ± 0.002	0.055 ± 0.006
Relative	0.198 ± 0.012	0.203 ± 0.008	0.168 ± 0.007	0.178 ± 0.008	0.206 ± 0.018
Week 53					
Absolute	0.059 ± 0.006	0.065 ± 0.005	0.065 ± 0.006	0.063 ± 0.006	0.052 ± 0.006
Relative	0.172 ± 0.015	0.193 ± 0.014	0.198 ± 0.014	0.208 ± 0.016	0.196 ± 0.023

Table C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 13-, 41-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 (Groups 1, 2, 3, 5, 7)

	Group 1 Vehicle Control	Group 2 10 ng/kg: 10 µg/kg	Group 3 100 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 7 1,000 ng/kg: 1,000 µg/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Necropsy body wt					
Week 14	268 ± 7	281 ± 4	281 ± 8	272 ± 6	261 ± 5
Week 31	310 ± 7	306 ± 10	296 ± 6	281 ± 3**	265 ± 8**
Week 53	340 ± 12	337 ± 9	325 ± 7	304 ± 8**	265 ± 8**
Spleen					
Week 14					
Absolute	0.521 ± 0.024	0.610 ± 0.023*	0.564 ± 0.018	0.544 ± 0.023	0.475 ± 0.021
Relative	1.944 ± 0.068	2.173 ± 0.075	2.014 ± 0.047	1.999 ± 0.059	1.826 ± 0.087
Week 31					
Absolute	0.568 ± 0.021	0.581 ± 0.017	0.528 ± 0.015	0.485 ± 0.021**	0.436 ± 0.023**
Relative	1.837 ± 0.063	1.907 ± 0.037	1.791 ± 0.057	1.728 ± 0.083	1.644 ± 0.069*
Week 53					
Absolute	0.572 ± 0.030	0.548 ± 0.026	0.488 ± 0.014*	0.491 ± 0.031*	0.425 ± 0.021**
Relative	1.677 ± 0.44	1.622 ± 0.52	1.498 ± 0.029	1.610 ± 0.080	1.602 ± 0.052
Thymus					
Week 14					
Absolute	0.241 ± 0.013	0.238 ± 0.017	0.221 ± 0.011	0.207 ± 0.015	0.174 ± 0.007**
Relative	0.905 ± 0.053	0.850 ± 0.064	0.790 ± 0.041	0.763 ± 0.053	0.670 ± 0.029**
Thyroid Gland					
Week 14					
Absolute	0.023 ± 0.002	0.023 ± 0.001	0.023 ± 0.001	0.023 ± 0.002	0.023 ± 0.001
Relative	0.086 ± 0.007	0.081 ± 0.005	0.081 ± 0.003	0.086 ± 0.008	0.089 ± 0.002
Week 31					
Absolute	0.33 ± 0.001	0.029 ± 0.002	0.026 ± 0.001*	0.028 ± 0.002*	0.024 ± 0.001**
Relative	0.107 ± 0.003	0.094 ± 0.007	0.090 ± 0.006	0.099 ± 0.006	0.091 ± 0.005
Week 53					
Absolute	0.024 ± 0.002	0.025 ± 0.001	0.025 ± 0.001	0.024 ± 0.002	0.022 ± 0.001
Relative	0.071 ± 0.005	0.073 ± 0.004	0.078 ± 0.003	0.080 ± 0.006	0.084 ± 0.004

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

Table C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 13-, 41-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 (Groups 1, 4, 5, 6)[a]

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
n				
Week 14	10	10	10	10
Week 31	10	10	10	9
Week 53	8	10	8	9
Necropsy body wt				
Week 14	268 ± 7	272 ± 5	272 ± 6	269 ± 6
Week 31	310 ± 7	300 ± 4▲,•	281 ± 3▲	275 ± 5•
Week 53	340 ± 12	304 ± 9	304 ± 8	289 ± 15
L. Kidney				
Week 14				
Absolute	0.746 ± 0.015	0.783 ± 0.012	0.734 ± 0.017▲▲	0.844 ± 0.013▲▲
Relative	2.794 ± 0.034	2.887 ± 0.048	2.703 ± 0.050	3.151 ± 0.068
Week 31				
Absolute	0.828 ± 0.012	0.891 ± 0.011▲▲	0.819 ± 0.011▲▲,••	0.929 ± 0.021••
Relative	2.681 ± 0.054	2.972 ± 0.032	2.915 ± 0.047	3.390 ± 0.075
Week 53				
Absolute	0.931 ± 0.031	0.954 ± 0.036	0.961 ± 0.030	0.926 ± 0.026
Relative	2.747 ± 0.075	3.145 ± 0.093	3.159 ± 0.036	3.256 ± 0.148
Liver				
Week 14				
Absolute	8.167 ± 0.447	10.459 ± 0.224▲	10.219 ± 0.271••	11.858 ± 0.312▲,••
Relative	30.342 ± 0.988	38.501 ± 0.526	37.606 ± 0.585	44.168 ± 0.870
Week 31				
Absolute	9.010 ± 0.256	12.539 ± 0.293▲▲	10.473 ± 0.195▲▲,••	12.613 ± 0.434••
Relative	29.099 ± 0.515	41.783 ± 0.510	37.255 ± 0.579	45.859 ± 0.874
Week 53				
Absolute	10.64 ± 0.59	12.36 ± 0.55	13.11 ± 0.48	12.76 ± 0.30
Relative	31.227 ± 1.093	40.739 ± 1.477	43.064 ± 0.619	44.677 ± 1.407
Lung				
Week 14				
Absolute	1.766 ± 0.66	1.742 ± 0.081	1.920 ± 0.071	1.901 ± 0.123
Relative	6.602 ± 0.178	6.393 ± 0.204	7.081 ± 0.273	7.082 ± 0.425
Week 31				
Absolute	1.667 ± 0.061	1.910 ± 0.097	1.633 ± 0.040	1.848 ± 0.095
Relative	5.393 ± 0.187	6.365 ± 0.302	5.819 ± 0.178	6.730 ± 0.315
Week 53				
Absolute	2.139 ± 0.111	1.934 ± 0.085	1.993 ± 0.060	1.801 ± 0.067
Relative	6.309 ± 0.309	6.412 ± 0.334	6.593 ± 0.280	6.331 ± 0.320
L. Ovary				
Week 14				
Absolute	0.055 ± 0.004	0.064 ± 0.005	0.056 ± 0.004	0.056 ± 0.003
Relative	0.205 ± 0.014	0.234 ± 0.015	0.205 ± 0.014	0.207 ± 0.009
Week 31				
Absolute	0.062 ± 0.005	0.055 ± 0.002▲	0.050 ± 0.002	0.047 ± 0.003▲
Relative	0.198 ± 0.012	0.185 ± 0.006	0.178 ± 0.008	0.171 ± 0.010
Week 53				
Absolute	0.059 ± 0.006	0.058 ± 0.004	0.063 ± 0.006	0.057 ± 0.004
Relative	0.172 ± 0.015	0.192 ± 0.013	0.208 ± 0.016	0.199 ± 0.012

Table C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 13-, 41-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153 (Groups 1, 4, 5, 6)

	Group 1 Vehicle Control	Group 4 300 ng/kg: 100 µg/kg	Group 5 300 ng/kg: 300 µg/kg	Group 6 300 ng/kg: 3,000 µg/kg
n				
Week 14	10	10	10	10
Week 31	10	10	10	9
Week 53	8	10	8	9
Necropsy body wt				
Week 14	268 ± 7	272 ± 5	272 ± 6	269 ± 6
Week 31	310 ± 7	300 ± 4▲,●	281 ± 3▲	275 ± 5●
Week 53	340 ± 12	304 ± 9	304 ± 8	289 ± 15
Spleen				
Week 14				
Absolute	0.521 ± 0.024	0.528 ± 0.010	0.544 ± 0.023	0.526 ± 0.019
Relative	1.944 ± 0.068	1.943 ± 0.019	1.999 ± 0.059	1.956 ± 0.049
Week 31				
Absolute	0.568 ± 0.021	0.511 ± 0.011▲▲	0.485 ± 0.021	0.436 ± 0.013▲▲
Relative	1.837 ± 0.063	1.704 ± 0.029	1.728 ± 0.083	1.586 ± 0.028
Week 53				
Absolute	0.572 ± 0.030	0.461 ± 0.015	0.491 ± 0.031	0.431 ± 0.021
Relative	1.677 ± 0.44	1.526 ± 0.059	1.610 ± 0.080	1.498 ± 0.052
Thymus				
Week 14				
Absolute	0.241 ± 0.013	0.202 ± 0.007	0.207 ± 0.015	0.227 ± 0.011
Relative	0.905 ± 0.053	0.748 ± 0.034	0.763 ± 0.053	0.847 ± 0.046
Thyroid Gland				
Week 14				
Absolute	0.023 ± 0.002	0.021 ± 0.002	0.023 ± 0.002	0.022 ± 0.001
Relative	0.086 ± 0.007	0.077 ± 0.006	0.086 ± 0.008	0.083 ± 0.004
Week 31				
Absolute	0.33 ± 0.001	0.030 ± 0.002	0.028 ± 0.002	0.025 ± 0.001
Relative	0.107 ± 0.003	0.100 ± 0.006	0.099 ± 0.006	0.091 ± 0.003
Week 53				
Absolute	0.024 ± 0.002	0.022 ± 0.001	0.024 ± 0.002	0.021 ± 0.001
Relative	0.071 ± 0.005	0.074 ± 0.003	0.080 ± 0.006	0.072 ± 0.004

▲,● Means that are in the same row and share symbols are significantly different ($P \leq 0.05$) from each other by Dunn's test
▲▲,●● $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error)

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization

Reports on analyses performed in support of the study of a binary mixture of PCB 126 and PCB 153 are on file at the National Institute of Environmental Health Sciences.

PCB 126

PCB 126 was obtained from AccuStandard, Inc. (New Haven, CT), in one lot (130494) that was used in the 2-year study. One additional lot (DK-130) was procured by Midwest Research Institute (Kansas City, MO) from Cambridge Isotope Laboratories, Inc. (Andover, MA), solely for dose formulation stability studies and was not used in the 2-year animal study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Chemistry Support Services) (Columbus, OH), and the study laboratory (Battelle Columbus Operations, Columbus, OH).

Lot 130494 of the chemical, a white powder, was identified as PCB 126 by proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and melting point determination. All spectra were consistent with the structure of a pentachlorobiphenyl, and determination of the melting point (156.9° C) by differential scanning calorimetry agreed with the literature (Bolgar *et al*., 1995). Proton and carbon-13 NMR spectra are presented in Figures D1 and D2.

The purity of lot 130494 was determined by the analytical chemistry laboratory using gas chromatography (GC) coupled to a high resolution mass spectrometer (MS) by system A (Table D1) and by the study laboratory using GC by system B. The purity profile obtained by system A detected four impurities with a combined relative area of 0.49%. Two impurities were tetrachlorinated biphenyls and one was a pentachlorinated biphenyl. One impurity was not identified, but was determined not to be a dioxin, dibenzofuran, or PCB. GC by system B indicated a purity of 100.3% ± 0.7% for lot 130494 relative to the reference sample. The overall purity of lot 130494 was determined to be greater than 99%.

PCB 153

PCB 153 was obtained from Radian International LLC (Austin, TX) by Midwest Research Institute and provided to the study laboratory in one lot (31532-78) that was used in the 2-year study. Additional lots (HE-553, HF-440, and HD-175) were procured by Midwest Research Institute from Cambridge Isotope Laboratories, Inc., solely for dose formulation stability studies and were not used in the 2-year animal study. Identity and purity analyses were conducted by the analytical chemistry laboratory and the study laboratory.

Lot 31532-78 of the chemical, a white powder, was identified as PCB 153 by the analytical chemistry laboratory using proton and carbon-13 NMR spectroscopy. In addition, identity analysis was conducted by the study laboratory using proton NMR; spectra of a purity analysis sample and a frozen reference sample were compared to each other and to the spectrum of the same lot previously reported by the analytical chemistry laboratory. All spectra were consistent with the structure of PCB 153. Proton and carbon-13 NMR spectra are presented in Figures D3 and D4.

The purity of lot 31532-78 was determined by the analytical chemistry laboratory to be approximately 99.8% using GC/MS system A. The purity profile detected two significant impurities: 0.21% of the test article was identified as a pentachlorobiphenyl and 0.002% of the test article was identified as a heptachlorobiphenyl. Standards of the possible impurities were obtained by the analytical chemistry laboratory from Cambridge Isotope Laboratories,

Inc., and analyzed using GC/MS system A; the pentachlorobiphenyl impurity was identified as 2,2′,4,5,5′-pentachlorobiphenyl (PCB 101), and the heptachlorobiphenyl impurity was identified as 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180).

Additional evaluations of the purity of lot 31532-78 were performed by the study laboratory. Initial evaluation using flame ionization by system B indicated an average purity of 96.1% for the test article relative to that of a frozen reference sample supplied by the analytical chemistry laboratory. To resolve the discrepancy in the purity estimates for the test article by the analytical chemistry and study laboratories, additional purity studies were conducted by the study laboratory. A new frozen reference sample of the same lot was obtained from the analytical chemistry laboratory, and comparative purity analysis using GC system B indicated that the relative purity of the test article was 101.1%. Subsequent analyses of these samples using GC/MS system A detected single impurities in each sample with peak areas of 0.5% relative to the major peak areas. The overall purity of lot 31532-78 was determined to be greater than 99%.

Formulation Materials

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

Preparation of Stock Samples of PCB 126

Lot 130494 of PCB 126 was dissolved in acetone and prealiquotted for use as analytical stock or formulation stock in the study because of the very small amount of chemical that was required to prepare the dose formulations at the intended concentrations. An analytical stock solution was prepared at a target concentration of 100 µg/mL by dissolving 10 mg of accurately weighed PCB 126 in 100 mL of acetone. A formulation stock solution was prepared at a target concentration of 125 µg/mL by dissolving 250 mg of accurately weighed PCB 126 in 2,000 mL of acetone. Following analysis to confirm proper concentration, these solutions were used to prepare analytical standard stocks of 50 and 100 µg, frozen reference stocks and chemical reference stocks of 100 µg for periodic purity determinations, and dose formulation working stocks. They were prepared by transferring the required volumes of respective solutions into appropriately sized glass containers and evaporating the solvent. The test article was stored at room temperature (approximately 25° C), protected from light in amber glass bottles sealed with Teflon®-lined lids. Purity was monitored by periodic reanalysis. No degradation was observed during the course of the study.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving the PCB 126 working stocks in acetone and diluting in the corn oil vehicle that contained either an aliquot of a PCB 153 working stock (for the 4 ng/mL PCB 126:4 µg/mL PCB 153 dose formulation only) or neat PCB 153 (Table D2). The final dose formulations contained 1% acetone and were stored at room temperature in amber glass bottles with minimal headspeace, sealed with Teflon®-lined lids for up to 35 days with four exceptions. Formulations prepared on December 17, 1999, March 10, 2000, and June 2, 2000, were used for 41, 38, and 40 days after formulating, respectively, pending completion of analysis of subsequent sets of formulations. Formulations prepared on September 1, 1998, were used 2 days after expiration due to an oversight.

Homogeneity of 4 ng/mL PCB 126:4 µg/mL PCB 153 and 120 ng/mL:1,200 µg/mL dose formulations and gavageability of a 120 ng/mL:1,200 µg/mL dose formulation were confirmed by the study laboratory using GC/MS system D for PCB 126 and GC system E for PCB 153. Stability studies of a 4 ng/mL:4 µg/mL formulation of lots DK-130 (PCB 126) and HE-553, HF-440, or HD-175 (PCB 153) with 0.04% hexane and 0.08% isooctane were conducted by Midwest Research Institute using GC/MS system F for PCB 126 and GC system G for PCB 153. Stability was confirmed for at least 35 days for the formulations stored in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids at 5° C and room temperature, and for 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of the binary mixture of PCB 126 and PCB 153 were conducted by the study laboratory using GC/MS similar to system D for PCB 126 concentrations and GC system E for PCB 153 concentrations. During the 2-year study, the dose formulations were analyzed at least every 3 months to determine the concentrations of PCB 126 and PCB 153 in the binary mixture (Tables D3 and D4, respectively). For the dose formulations analyzed and used in the study, 80% (44/55) and 98% (54/55) were within 10% of the target concentrations for PCB 126 and PCB 153, respectively; all were within 15% of target. Of the animal room samples, 64% (16/25) for PCB 126 and all 25 for PCB 153 were within 10% of the target concentrations; all PCB 126 concentrations were within 14% of target.

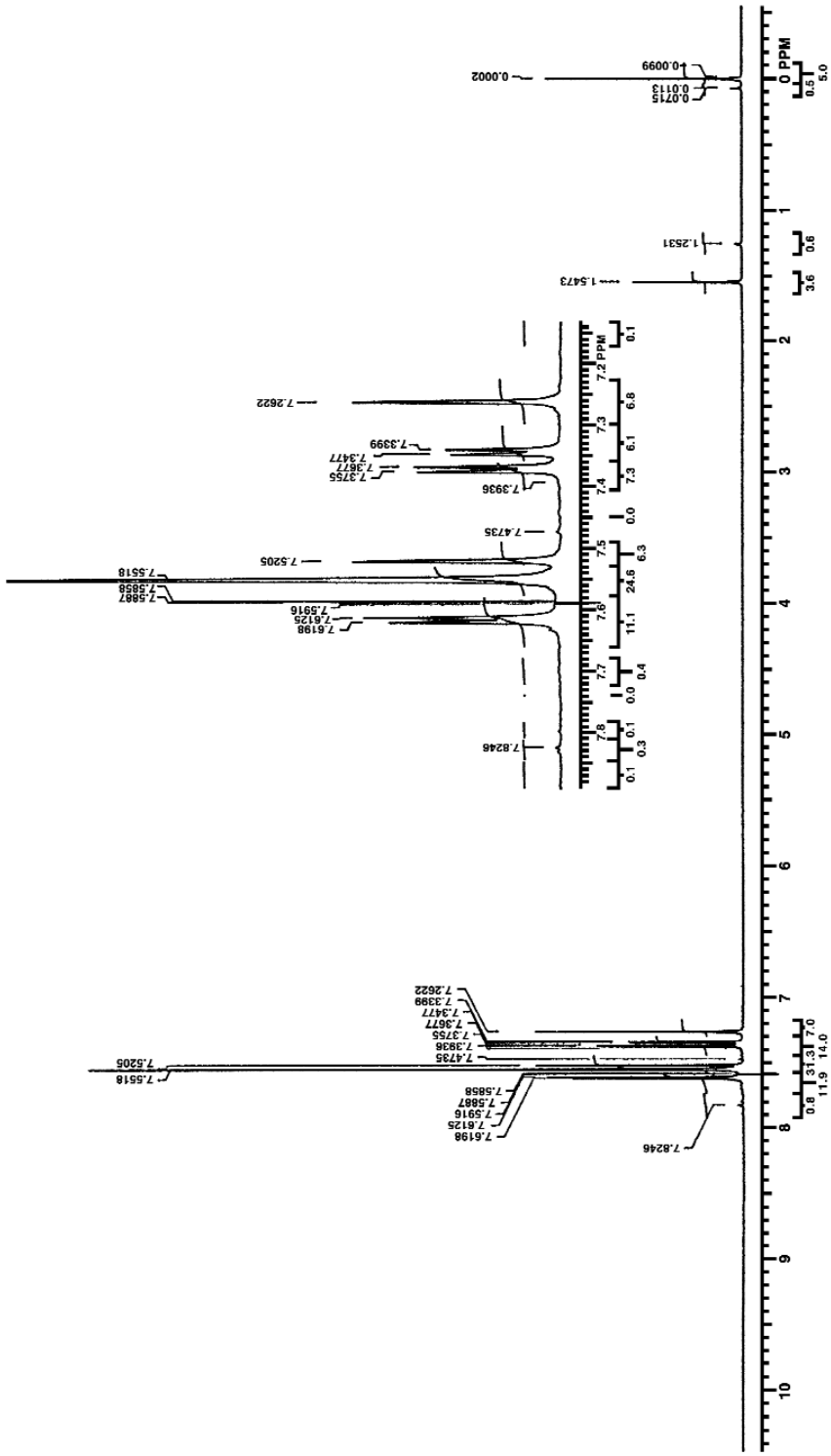

FIGURE D1
Proton Nuclear Magnetic Resonance Spectrum of PCB 126

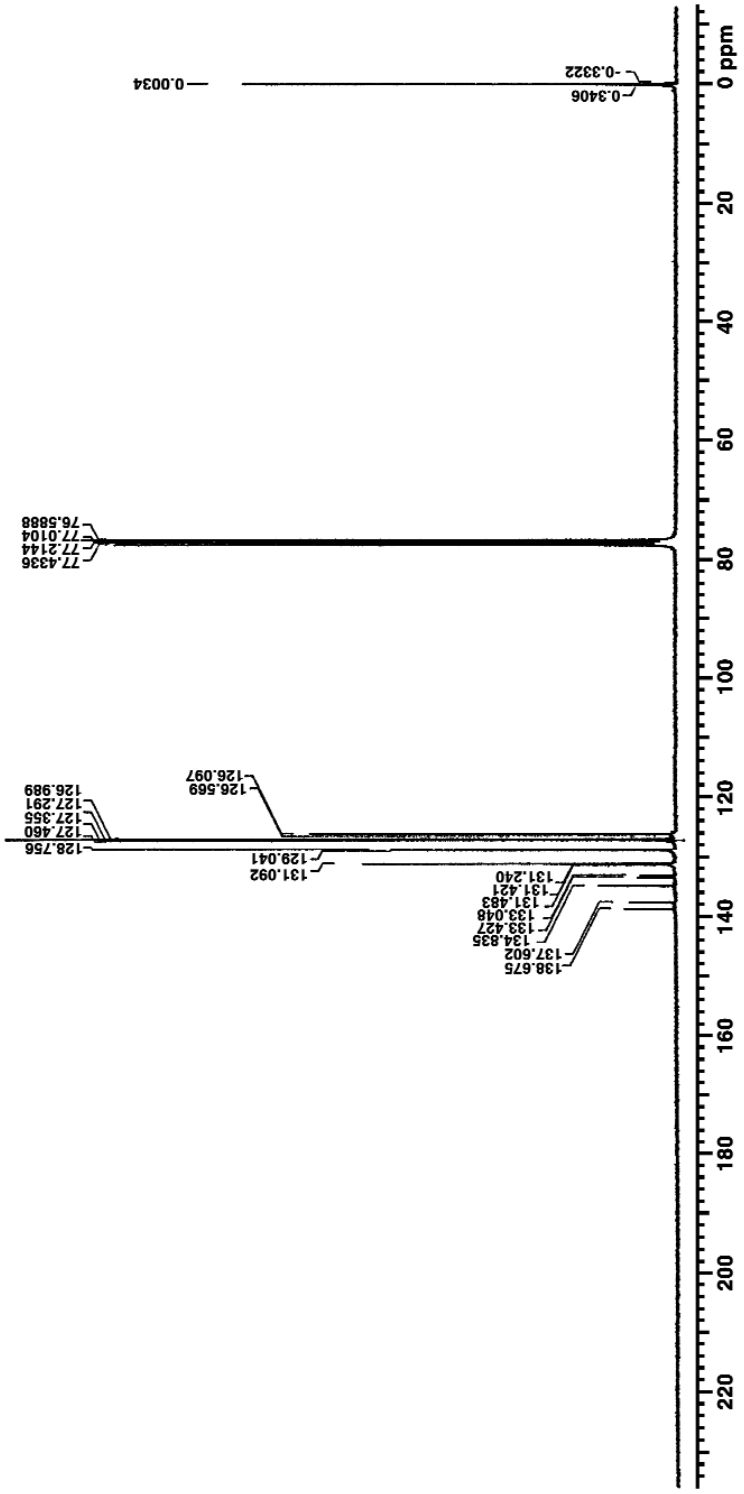

FIGURE D2
Carbon-13 Nuclear Magnetic Resonance Spectrum of PCB 126

TABLE D1
Gas Chromatography Systems Used in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
High resolution mass spectrometry	J&W DB-5 MS, 15 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific, Folsom, CA)	Helium at 6 mL/minute (PCB 126) or 4 psi (PCB 153)	50° C for 1 minute, then 10° C/minute (PCB 126) or 8° C/minute (PCB 153) to 300° C, held for 10 minutes
System B			
Flame ionization	Supelco PTE-5 (QTM), 15 m × 0.53 mm, 0.5-µm film thickness (Supelco, Inc., Bellefonte, PA)	Helium at ~5 psi	45° C for 5 minutes, then 15° C/minute to 300° C
System C			
Flame ionization	Supelco 20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcoport, 2.4 m × 2 mm (Supelco, Inc.)	Helium at 17 mL/minute	40° C for 4 minutes, then 10° C/minute to 170° C
System D			
High resolution mass spectrometry	J&W DB-5 MS, 15 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium, ultrahigh purity at ~6 psi	100° C for 1 minute, then 15° C/minute to 240° C, then 40° C/minute to 285° C, held for 2 minutes
System E			
Electron capture	Supelco PTE-5, 15 m × 0.53 mm, 0.5-µm film thickness (Supelco, Inc.)	Helium at ~17 mL/minute	150° C for 4 minutes, then 8° C/minute to 255° C, then 70° C/minute to 320° C, held for 1 minute
System F			
High resolution mass spectrometry	J&W DB-5 MS, 60 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium at 40 mL/minute	150° C for 2 minutes, then 50° C/minute to 230° C, held for 2 minutes, then 1° C/minute to 235° C, held for 2 minutes, then 15° C/minute to 320° C, held for 3 minutes
System G			
Electron capture	Restek RTX-5, 60 m × 0.25 mm, 0.25-µm film thickness (Restek, Bellefonte, PA)	Helium at 0.8 mL/minute	60° C for 2 minutes, then 50° C/minute to 220° C, held for 1 minute, then 8° C/minute to 310° C, held for 5 minutes

[a] The gas chromatographs were manufactured by Hewlett Packard (Palo Alto, CA) (systems A, B, C, E, and G) or Carlo Erba/Fisons, Ltd. (Systems D and F) (Valencia, CA). The mass spectrometers used in systems A, D, and F were manufactured by VG (Cheshire, UK).

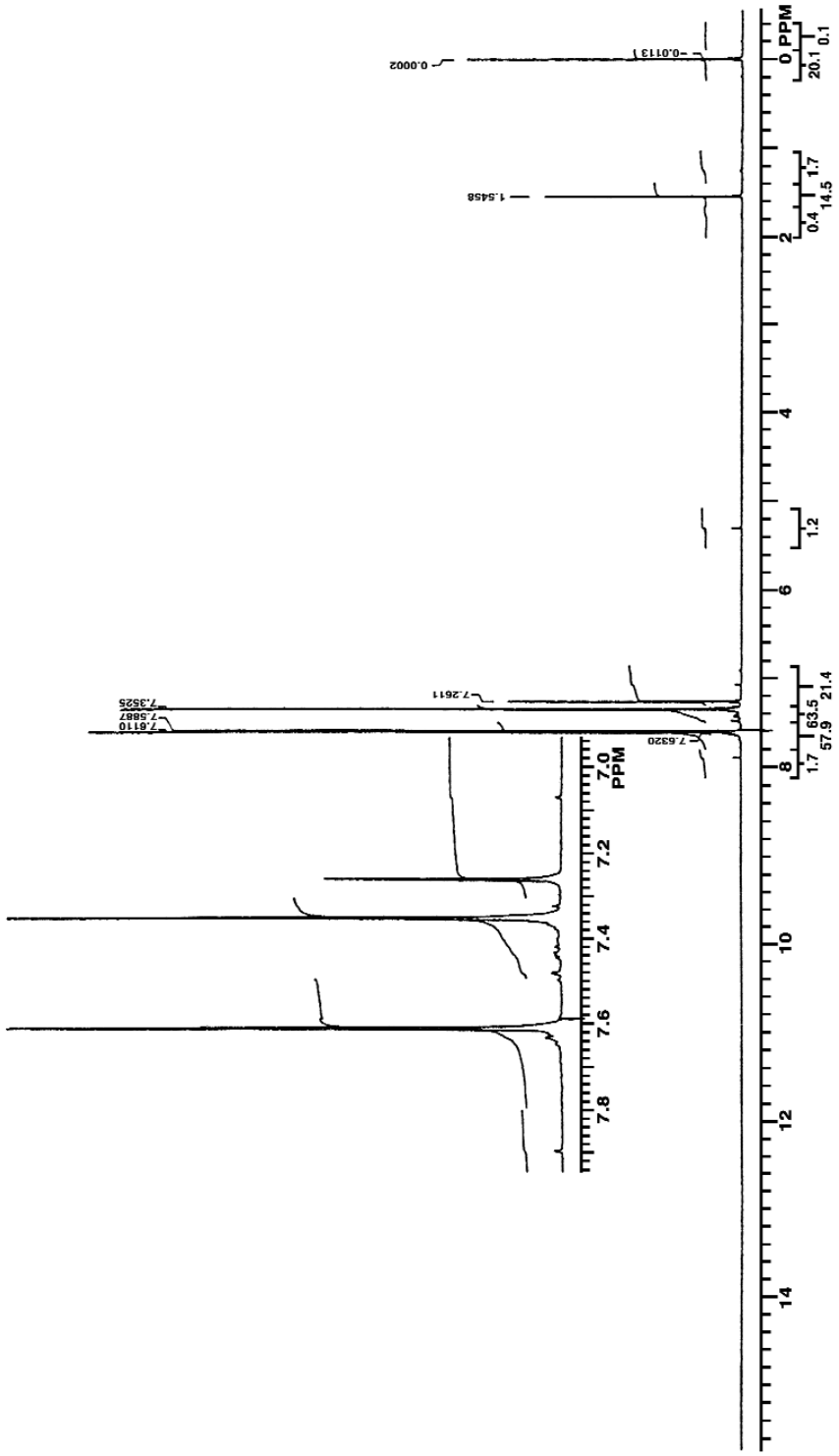

FIGURE D3
Proton Nuclear Magnetic Resonance Spectrum of PCB 153

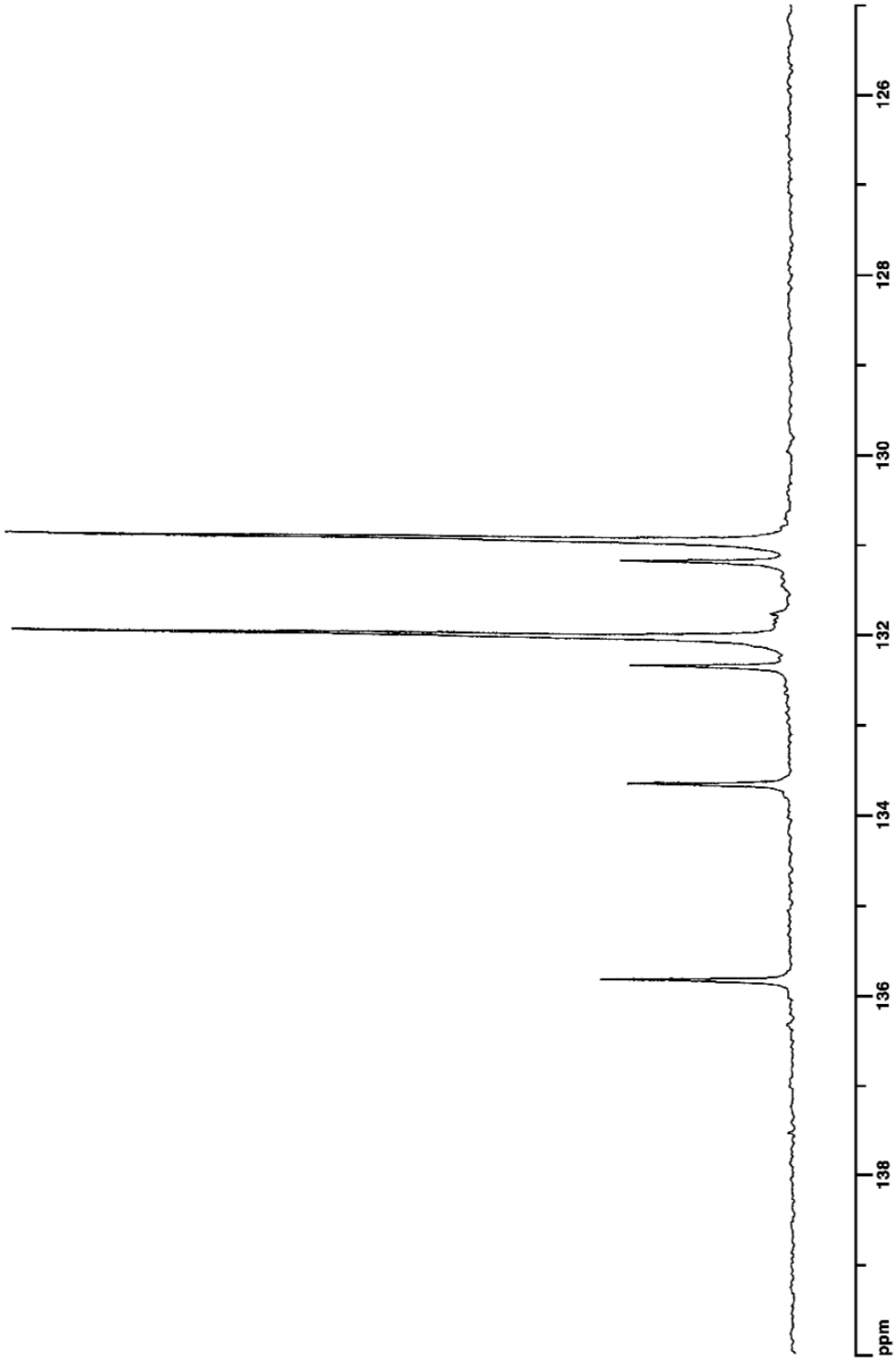

FIGURE D4
Expanded Carbon-13 Nuclear Magnetic Resonance Spectrum of PCB 153

Table D2
Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Preparation
Dose formulation working stocks of PCB 126 were prepared by transferring the appropriate volumes of a 125 µg/mL formulation stock solution into 15 mL amber glass bottles, evaporating the acetone, and sealing the bottles with Teflon® -lined lids.

A single dose formulation working stock of PCB 153 was prepared by dissolving the chemical in acetone to yield a solution with a concentration of 8 mg/mL.

To prepare the 4 ng/mL PCB 126:4µg/mL PCB 153 dose formulation of the binary mixture, 9 mL of acetone was added to the appropriate dose formulation working stock bottle of PCB 126, and the contents were vortexed for approximately 2 minutes, sonicated for approximately 30 minutes in an ice-cooled water bath, and transferred to a 2-L volumetric flask containing 1 L of corn oil. The dose formulation working stock bottle was rinsed twice with 5 mL of acetone; each acetone rinse was added to the volumetric flask after approximately 2 minutes of vortexing. One mL of the 8 mg/mL PCB 153 dose formulation working stock solution was pipetted into the 2-L volumetric flask and the flask was sealed and shaken vigorously. The contents of the volumetric flask were diluted to volume with corn oil and the flask was capped, shaken, and stirred on a stirplate for approximately 3 or 24 hours, with vigorous shaking done at least eight times over the stirring period.

The five higher dose formulations of the binary mixture were prepared by combining reconstituted PCB 126 dose formulation working stocks (reconstituted in 10 mL acetone) with the appropriate quantity of neat PCB 153 in a 2-L volumetric flask half filled with corn oil; all other steps of dose formulation preparation for the higher doses were as described above for the 4 ng/mL PCB 126:4 ug/mL PCB 153 dose formulation.

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

Chemical Lot Numbers
PCB 126: 130494
PCB 153: 31532-78

Maximum Storage Time
35 days

Storage Conditions
Dose formulation working stocks of PCB 126 were stored in 15 mL amber glass vials, sealed with Teflon®-lined lids at room temperature (approximately 25° C).

Dose formulations were stored in 120 mL amber glass screw-cap bottles with Teflon®-lined lids at room temperature.

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

TABLE D3
Results of Analyses of PCB 126 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Date Prepared	Date Analyzed	Group Number[a]	Target Concentration (ng/mL)	Determined Concentration[b] (ng/mL)	Difference from Target (%)
September 1, 1998	September 3-4, 1998	2	4	3.605	–10
		3	40	35.55[c]	–11
		4	120	109.6	–9
		5	120	109.9	–8
		6	120	107.0[c]	–11
		7	400	347.6	–13
		7	400	364.6	–9
	October 1-2, 1998[d]	2	4	3.751	–6
		3	40	37.99	–5
		4	120	116.6	–3
		5	120	113.2	–6
		6	120	109.4	–9
		7	400	352.4	–12
		7	400	356.6	–11
November 16, 1998	November 18-19, 1998	2	4	5.779[e]	+44
		3	40	39.01	–2
		4	120	114.8	–4
		5	120	109.1	–9
		6	120	111.3	–7
		7	400	354.2[c]	–11
November 19, 1998	Novemer 24-25, 1998	2	4	3.943[f]	–1
February 8, 1999	February 13-15, 1999	2	4	3.637	–9
		3	40	39.41	–1
		4	120	113.4	–6
		5	120	112.5	–6
		6	120	110.5	–8
		7	400	319.7 ± 16.5[e]	–20
	March 25-26, 1999[d]	2	4	3.546	–11
		3	40	36.59	–9
		4	120	105.2	–12
		5	120	109.6	–9
		6	120	103.3	–14
February 16, 1999	February 18, 1999	7	400	363.5[f]	–9
	March 25-26, 1999[d]	7	400	356.7	–11
May 3, 1999	May 7-8, 1999	2	4	3.945	–1
		3	40	36.94	–8
		4	120	118.1	–2
		5	120	115.0	–4
		6	120	114.8	–4
		7	400	366.3	–8
July 26, 1999	July 30, 1999-August 5, 1999	2	4	3.652	–9
		3	40	39.14	–2
		4	120	120.2	0
		5	120	122.5	+2
		6	120	114.3	–5
		7	400	366.1	–8

TABLE D3
Results of Analyses of PCB 126 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Date Prepared	Date Analyzed	Group Number	Target Concentration (ng/mL)	Determined Concentration (ng/mL)	Difference from Target (%)
October 25, 1999	October 28-November 1, 1999	2	4	3.685	–8
		3	40	38.77	–3
		4	120	110.8	–8
		5	120	110.4	–8
		6	120	114.2	–5
		7	400	382.3	–4
	November 28, 1999[d]	2	4	4.274	+7
		3	40	38.54	–4
		4	120	105.4	–12
		5	120	105.5	–12
		6	120	106.3	–11
		7	400	362.5	–9
January 10, 2000	January 17-20, 2000	2	4	3.449 ± 0.030	–14
		3	40	35.63 ± 1.63	–11
		4	120	102.6 ± 2.6	–15
		5	120	108.9 ± 2.4	–9
		6	120	104.8 ± 2.1	–13
		7	400	353.8 ± 6.0	–12
January 17, 2000	January 25, 2000	5	120	110.9 ± 3.3[g]	–8
April 12, 2000	April 14-15, 2000	2	4	3.980 ± 0.058	–1
		3	40	44.74 ± 2.41	+12
		4	120	137.2 ± 6.3	+14
		5	120	128.1 ± 12.7	+7
		6	120	122.4 ± 1.8	+2
		7	400	418.9 ± 26.0	+5
June 26, 2000	July 6-7, 2000	2	4	3.679 ± 0.027	–8
		3	40	31.37 ± 0.54[e]	–22
		4	120	109.5 ± 1.2	–9
		5	120	112.5 ± 1.0	–6
		6	120	113.3 ± 1.1	–6
		7	400	370.8 ± 4.2	–7
	August 22-23, 2000[d]	2	4	3.867 ± 0.162	–3
		4	120	112.7 ± 4.8	–6
		5	120	111.1 ± 1.9	–7
		6	120	112.7 ± 7.2	–6
		7	400	361.2 ± 6.0	–10
July 8, 2000	July 11-12, 2000	3	40	38.82 ± 1.02[f]	–3
	August 22-23, 2000[d]	3	40	42.45 ± 3.58	+6

[a] Group 1: Vehicle control; Group 2: 10 ng/kg PCB 126 and 10 µg/kg PCB 153; Group 3: 100 ng/kg PCB 126 and 100 µg/kg PCB 153
Group 4: 300 ng/kg PCB 126 and 100 µg/kg PCB 153; Group 5: 300 ng/kg PCB 126 and 300 µg/kg PCB 153
Group 6: 300 ng/kg PCB 126 and 3,000 µg/kg PCB 153; Group 7: 1,000 ng/kg PCB 126 and 1,000 µg/kg PCB 153
[b] Reported value is the average of duplicate analyses or the average ± standard deviation of triplicate or quadruplicate analyses.
Dosing volume=2.5 mL/kg; 4 ng/mL=10 ng/kg, 40 ng/mL=100 ng/kg, 120 ng/mL=300 ng/kg, 400 ng/mL=1,000 ng/kg
[c] Formulation was outside the acceptable range of ± 10% of target concentration, but was used at NTP's direction.
[d] Animal room samples
[e] Remixed, not used in study
[f] Results of remix
[g] Not used in study

TABLE D4
Results of Analyses of PCB 153 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Date Prepared	Date Analyzed	Group Number[a]	Target Concentration (µg/mL)	Determined Concentration[b] (µg/mL)	Difference from Target (%)
September 1, 1998	September 3-8, 1998	2	4	3.531 ± 0.148[c]	–12
		3	40	39.70 ± 1.85	–1
		4	40	39.47 ± 1.66	–1
		5	120	111.1 ± 5.5	–7
		6	1,200	1,143 ± 58	–5
		7	400	377.5 ± 14.4	–6
		7	400	380.2 ± 26.4	–5
	September 30-October 1, 1998[d]	2	4	3.794	–5
		3	40	40.37	+1
		4	40	41.06	+3
		5	120	131.9	+10
		6	1,200	1,223	+2
		7	400	379.0	–5
		7	400	395.1	–1
November 16, 1998	November 18-19, 1998	2	4	4.433[e]	+11
		3	40	41.54	+4
		4	40	40.78	+2
		5	120	126.3	+5
		6	1,200	1,259	+5
		7	400	389.9	–3
November 19, 1998	November 23-24, 1998	2	4	4.378[f]	+9
February 8, 1999	February 11-12, 1999	2	4	3.739	–7
		3	40	37.50	–6
		4	40	40.27	+1
		5	120	115.0	–4
		6	1,200	1,210	+1
		7	400	284.6[e]	–29
	March 17-19, 1999[d]	2	4	3.761	–6
		3	40	37.59	–6
		4	40	40.63	+2
		5	120	124.1	+3
		6	1,200	1,189	–1
February 16, 1999	February 18, 1999	7	400	407.5[f]	+2
	March 17-19, 1999[d]	7	400	375.0	–6
May 3, 1999	May 6-10, 1999	2	4	3.677 ± 0.032	–8
		3	40	38.41	–4
		4	40	40.01	0
		5	120	111.4	–7
		6	1,200	1,158	–4
		7	400	360.7	–10

TABLE D4
Results of Analyses of PCB 153 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Date Prepared	Date Analyzed	Group Number	Target Concentration (µg/mL)	Determined Concentration (µg/mL)	Difference from Target (%)
July 26, 1999	July 29-30, 1999	2	4	4.014	0
		3	40	39.29	–2
		4	40	38.47	–4
		5	120	118.5	–1
		6	1,200	1,167	–3
		7	400	383.4	–4
October 25, 1999	October 29-30, 1999	2	4	3.618	–10
		3	40	38.72	–3
		4	40	37.09	–7
		5	120	111.4	–7
		6	1,200	1,144	–5
		7	400	383.3	–4
	November 23-27, 1999[d]	2	4	3.762	–6
		3	40	40.51	+1
		4	40	38.56	–4
		5	120	116.6	–3
		6	1,200	1,196	0
		7	400	424.7	+6
January 10, 2000	January 12-19, 2000	2	4	4.053	+1
		3	40	41.25	+3
		4	40	38.80	–3
		5	120	120.0	0
		6	1,200	1,186	–1
		7	400	393.0	–2
January 17, 2000	January 25-26, 2000	5	120	121.6 ± 0.8[g]	+1
April 12, 2000	April 17-18, 2000	2	4	3.600 ± 0.135	–10
		3	40	38.45 ± 0.94	–4
		4	40	36.61 ± 0.84	–8
		5	120	109.6 ± 3.9	–9
		6	1,200	1,136 ± 30	–5
		7	400	380.4 ± 9.9	–5
June 26, 2000	June 29-July 1, 2000	2	4	3.835 ± 0.020	–4
		3	40	36.70 ± 0.66[e]	–8
		4	40	36.75 ± 0.29	–8
		5	120	112.1 ± 1.3	–7
		6	1,200	1,206 ± 96	+1
		7	400	362.5 ± 1.5	–9
	August 24-25, 2000[d]	2	4	4.164 ± 0.003	+4
		4	40	39.21 ± 0.41	–2
		5	120	120.9 ± 0.7	+1
		6	1,200	1,235 ± 42	+3
		7	400	395.0 ± 0.8	–1

TABLE D4
Results of Analyses of PCB 153 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153

Date Prepared	Date Analyzed	Group Number	Target Concentration (µg/mL)	Determined Concentration (µg/mL)	Difference from Target (%)
July 8, 2000	July 11-12, 2000	3	40	42.56 ± 0.15[f]	+6
	August 24-25, 2000[d]	3	40	41.81 ± 0.15	+5

[a] Group 1: Vehicle control; Group 2: 10 ng/kg PCB 126 and 10 µg/kg PCB 153; Group 3: 100 ng/kg PCB 126 and 100 µg/kg PCB 153
Group 4: 300 ng/kg PCB 126 and 100 µg/kg PCB 153; Group 5: 300 ng/kg PCB 126 and 300 µg/kg PCB 153
Group 6: 300 ng/kg PCB 126 and 3,000 µg/kg PCB 153; Group 7: 1,000 ng/kg PCB 126 and 1,000 µg/kg PCB 153
[b] Reported value is the average of duplicate analyses or the average ± standard deviation of triplicate or quadruplicate analyses.
Dosing volume = 2.5 mL/kg; 4 µg mL=10 µg/kg, 40 µg/mL=100 µg/kg, 120 µg/mL=300 µg/kg, 400 µg/mL=1,000 µg/kg, 1,200 µg/mL=3,000 µg/kg.
[c] Formulation was outside the acceptable range of ± 10% of target concentration, but was used at NTP's direction.
[d] Animal room samples
[e] Remixed, not used in study
[f] Results of remix
[g] Not used in study

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

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TABLE E1
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 E2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

Table E3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.6 ± 0.45	12.8 – 14.5	25
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.6	25
Crude fiber (% by weight)	9.1 ± 0.63	7.9 – 10.5	25
Ash (% by weight)	5.0 ± 0.20	4.7 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.748 ± 0.053	0.670 – 0.850	12
Cystine	0.223 ± 0.027	0.150 – 0.250	12
Glycine	0.702 ± 0.043	0.620 – 0.750	12
Histidine	0.343 ± 0.023	0.310 – 0.390	12
Isoleucine	0.534 ± 0.041	0.430 – 0.590	12
Leucine	1.078 ± 0.059	0.960 – 1.140	12
Lysine	0.729 ± 0.065	0.620 – 0.830	12
Methionine	0.396 ± 0.053	0.260 – 0.460	12
Phenylalanine	0.611 ± 0.038	0.540 – 0.660	12
Threonine	0.492 ± 0.045	0.430 – 0.590	12
Tryptophan	0.129 ± 0.016	0.110 – 0.160	12
Tyrosine	0.378 ± 0.054	0.280 – 0.460	12
Valine	0.658 ± 0.049	0.550 – 0.710	12
Essential Fatty Acids (% of total diet)			
Linoleic	3.89 ± 0.278	3.49 – 4.54	12
Linolenic	0.30 ± 0.038	0.21 – 0.35	12
Vitamins			
Vitamin A (IU/kg)	5,436 ± 1,047	3,460 – 7,790	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.3 ± 17.06	52.0 – 110.0	12
Thiamine (ppm)[b]	7.9 ± 0.81	6.3 – 9.3	25
Riboflavin (ppm)	6.4 ± 2.11	4.20 – 11.20	12
Niacin (ppm)	78.6 ± 10.86	66.4 – 98.2	12
Pantothenic acid (ppm)	23.1 ± 3.61	17.4 – 29.1	12
Pyridoxine (ppm)[b]	8.88 ± 2.05	6.4 – 12.4	12
Folic acid (ppm)	1.84 ± 0.56	1.26 – 3.27	12
Biotin (ppm)	0.337 ± 0.13	0.225 – 0.704	12
Vitamin B_{12} (ppb)	64.8 ± 50.9	18.3 – 174.0	12
Choline (ppm)[b]	3,094 ± 292	2,700 – 3,790	12
Minerals			
Calcium (%)	1.005 ± 0.045	0.903 – 1.090	25
Phosphorus (%)	0.571 ± 0.025	0.517 – 0.618	25
Potassium (%)	0.668 ± 0.023	0.627 – 0.694	12
Chloride (%)	0.368 ± 0.033	0.300 – 0.423	12
Sodium (%)	0.189 ± 0.016	0.160 – 0.212	12
Magnesium (%)	0.200 ± 0.009	0.185 – 0.217	12
Sulfur (%)	0.176 ± 0.026	0.116 – 0.209	12
Iron (ppm)	177 ± 46.2	135 – 311	12
Manganese (ppm)	53.4 ± 6.42	42.1 – 63.1	12
Zinc (ppm)	52.5 ± 6.95	43.3 – 66.0	12
Copper (ppm)	6.64 ± 1.283	5.08 – 9.92	12
Iodine (ppm)	0.535 ± 0.242	0.233 – 0.972	12
Chromium (ppm)	0.545 ± 0.125	0.330 – 0.751	12
Cobalt (ppm)	0.23 ± 0.041	0.20 – 0.30	12

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

Table E4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.17 ± 0.073	0.10 – 0.37	25
Cadmium (ppm)	0.04 ± 0.007	0.04 – 0.07	25
Lead (ppm)	0.11 ± 0.104	0.05 – 0.54	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.19 ± 0.034	0.14 – 0.28	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	10.8 ± 3.00	9.04 – 21.1	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 2	10 – 20	25
Coliform (MPN/g)	0.7 ± 1.5	0.0 – 3.6	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.5 ± 1.34	2.1 – 7.5	25
N-Nitrosodimethylamine (ppb)[e]	1.7 ± 0.53	1.0 – 3.0	25
N-Nitrosopyrrolidine (ppb)[e]	2.8 ± 1.04	1.0 – 5.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.156 ± 0.119	0.023 – 0.499	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.219 ± 0.184	0.020 – 0.826	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 E5
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 E5
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 E5
Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration

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

TABLE E5
Concentrations of PCBs and Dioxins in NTP-2000 Rat and Mouse Ration

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

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

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

APPENDIX F
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; six sentinal male rats at 6 months; five sentinel male rats at 12 and 18 months; and five 1,000 ng/kg plus 1,000 µg/kg females at the end of the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corp. (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	1, 6, 12, and 18 months, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, study termination

RESULTS

All serology tests were negative.

APPENDIX G
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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model for the mixture of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) was developed in support of the dioxin toxic equivalency factor (TEF) evaluation studies. The model is based on a PBPK model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Estimates of parameters for PCB 126 and PCB 153 were made from fits to data from individual studies and the binary mixture study. 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 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 and its metabolites, and subsequent molecular/biochemical responses may provide insights into the factors governing the dose response of toxicity, site specificity, and mode of action of the compound under study.

In general, PBPK models have been validated in the observable response range for numerous compounds in both animals and humans, making them useful for risk assessment, especially for cross-species extrapolation. They also aid in extrapolation from one chemical to other structurally related chemicals because many of the components of the 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, tissue volumes, etc.). Physicochemical parameters are used that are often specific to a given compound but can be measured experimentally and may be available in the literature. Some of these parameters may not be available *a priori* and so have to be determined within the framework of the model by an iterative process of changing the parameter, fitting the model to a given dataset and evaluating the goodness of the fit of the model to the data. Careful evaluation of any PBPK model must involve the adequacy of its fit to the data, the relationship of its structure to the underlying biology, and the mathematical details linking these two. In addition, the biological plausibility of optimized parameters needs to be considered. Validation of the model using datasets that were not used in its construction lends more credence to the predictive power of 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 (ADME). These processes for TCDD and related dioxin-like compounds 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 PBPK model is a mathematical structure that describes the relationship between these factors and ADME. This model describes the pharmacokinetics of a compound by a series of mass-balance differential equations in which the state variables represent the concentration of the compound in anatomically distinct regions, "compartments" of the body. These tissue compartments are linked by a physiologically realistic pattern of blood perfusion and flow through the different tissue compartments.

A model for the mixture of PCB 126 and PCB 153 was built from a model for TCDD. Separate models for PCB 126 and PCB 153 are linked as a mixture model by having both chemicals bind to the AhR and cytochrome P450 1A2 (CYP1A2). Data and models for chronic exposure of female Sprague-Dawley rats to PCB 126 or

PCB 153 as individual chemicals were available to aid the model development. Except for data where PCB 153 exposure is extremely high (1,000,000 ng/kg per day or more), the PCB 126 data in the mixture are predicted by a PCB 126-only model, suggesting that the pharmacokinetics of PCB 126 are not affected by the presence of PCB 153. The PCB 153 data in the mixture are predicted by a PCB 153-only model, suggesting that the pharmacokinetics of PCB 153 are not affected by the presence of PCB 126. Thus, interaction between PCB 126 and PCB 153 only occurs when extreme concentrations of PCB 153 are present, and interaction appears to be in the AhR pathway.

Model Development

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

Kohn's model includes compartments for fat, liver, kidney, gastrointestinal tract, muscle, and viscera with blood distributed among arterial, venous, and tissue capillary spaces. The model includes equations for the amounts of 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 the 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; transthyretin (also known as prealbumin) can bind hydroxylated polychlorinated dibenzodioxins, and single doses of TCDD can cause prolonged decreases in this protein. Accordingly, a dose-dependent decrease of blood protein was included in the model. This protein-bound TCDD cannot enter the tissues in the model but may become free in the blood by dissociation or proteolysis. To fit data at both low and high doses, the model includes loss of TCDD from the liver by lysis of dead cells (as a result of hepatotoxicity) where the rate of cell death was assumed to increase as a hyperbolic function of the cumulative amount of unbound hepatic TCDD.

There were several steps to building a PBPK model for the dioxin TEF evaluation binary mixture study. Adding a lung compartment, converting the body weight function, functional linking of model protein levels and activity data, and linking the mixtures together. A lung compartment was added to the model because the NTP data for the TEF studies include lung tissue concentrations. The lung compartment is diffusion limited and includes the same equations used in the liver for 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 data (liver, lung, fat, and blood).

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

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

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

Partition coefficients for the chemicals in the binary mixture were based on the partition coefficients in Kohn's TCDD model. Kohn fit the TCDD tissue permeability, but used the tissue:blood partition coefficients that were determined experimentally (Murphy *et al*., 1995, Kohn *et al*., 2001). Assuming that the permeability is the same for TCDD, PCB 126, and PCB 153, the values from Kohn's model can be used, and only partition coefficients are needed for PCB 126 and PCB 153. The ratios of partition coefficients (*n*-octanol:water; log P) were used to scale the TCDD partition coefficients to the partition coefficients of the binary mixture chemicals. Tissue partition coefficients (PC) of TCDD were multiplied by the ratio of log P values, e.g.,

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

While in such a large model many model parameters might be different for each dioxin-like chemical, only a small subset of parameters was found to be chemical dependent. The parameters for binding to the AhR, CYP1A2, and blood protein and metabolism, absorption, and hepatotoxicity consisted of 11 chemical-specific parameters. The binding, metabolic, absorption, and hepatotoxicity parameters were estimated by fitting the model predictions to logarithmic values of liver EROD and A4H activities and tissue concentration data (liver, fat, blood, and lung). Two parameters describing hepatotoxicity, k_{lysis} and $k_{recovery}$, are included in the optimizations because they are multipliers of the chemical concentration in the cytotoxicity equations (Kohn *et al.*, 2001). Thus, the model can represent the differences in the amount of chemical causing liver tissue damage among the dioxin-like chemicals.

The mixture model was constructed by modifying Kohn's model to include the appropriate number of compounds. Each compound in the binary mixture has unique binding constants. Binding to blood protein, the AhR, and CYP1A2 is represented as noncompetitive binding. The metabolism of each compound is assumed to occur independently. Hepatotoxicity constants and partition coefficients are unique for each compound. All of the other model parameters are kept as constants from Kohn's model. Background concentrations of PCB 126 were computed from measured concentrations in NTP-2000 feed. Since data were not initially available for background concentrations of PCB 153, the background value was set to several different values during optimizations. The model was written in Simulink and all optimizations were run in Matlab.

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

Results and Discussion

PCB 126 and PCB 153 parameters were previously estimated by fitting test chemical tissue concentration and cytochrome P450 enzyme activity data when the test chemicals were administered alone (Table G1). For PCB 153, multiple sets of parameter estimates gave similar fits to the data, suggesting that the model does not have a unique set of parameter estimates for this chemical, and that care needs to be taken to select sets of parameter estimates that are biologically relevant. Having additional data available, such as binding constants, would help to determine unique estimates for the parameters. Although several PCB 153 parameter estimates were tried for the current analysis, only one set was chosen because of its fit to the mixture data.

Using the previous estimates as initial input into the optimization, parameter estimates were determined by fitting the individual chemical data and binary mixture data simultaneously; derived estimates for the binary mixture are similar to the original estimates for the studies on the individual chemicals (Table G1). The model output agrees with the measured tissue concentration and liver cytochrome P450 enzyme activity data for the binary mixture and

with the model output from administering the chemicals alone, except at the highest doses (Figures G1 to G16). These results suggest that there is no interaction between PCB 126 and PCB 153 toxicokinetics except at the highest doses.

In the varying ratio Group 6 (300 ng/kg:3,000 µg/kg), the mixture model predicts decreases in liver concentrations of PCB 126 compared to those predicted when PCB 126 is modeled alone if the binding constant for PCB 153 is 0.0001 or larger (Figure G11). The mixture model also predicts decreases in liver A4H activities compared to those predicted when PCB 126 is modeled alone (Figure G16). Together, these results suggest that the interaction between PCB 126 and PCB 153 toxicokinetics at high concentrations appears to be in the liver AhR pathway; in particular, a decreased amount of PCB 126 binds to CYP1A2.

Since concentration data of PCB 153 in NTP-2000 feed were not available at the time of model development, the background PCB 153 value was set to several different values during the optimizations.

While dioxin-like chemicals are found ubiquitously as mixtures, only limited analyses have previously been performed on dioxin mixtures. In one study, acute effects of a TCDD/PCB 153 mixture were determined, and interactive pharmacokinetic effects occurred only at high doses (Van Birgelen *et al*., 1996). Another acute study demonstrated that a PCB 126/PCB 153 mixture caused increases in liver concentrations and decreases in fat concentrations of PCB 153 (Lee *et al.*, 2002). In this study, the PBPK model of PCB 153 was modified to include a time-dependent increase in the liver partition coefficient and a decrease in the fat diffusion permeation constant; these modeling changes were incorporated to represent the PCB 126-induced increase in liver lipid content and inhibition of fat lipoprotein lipase activity, respectively.

In contrast to the model by Lee *et al.* (2002), no changes were made to the structure of the current model. The current model predicts the effect of PCB 153 on PCB 126 toxicokinetics at high doses to be decreased liver concentrations and CYP1A2 binding of PCB 126. The effect of PCB 126 on PCB 153 toxicokinetics at high doses is currently being investigated.

Table G1
Model Parameters and Partition Coefficients for the PBPK Model of the Binary Mixture of PCB 126 and PCB 153

	TCDD[a]	PCB 126[a]	PCB 126[b]	PCB 153[a]	PCB 153[b]	Unit
Model Parameters						
Background	0.082	0.88	0.88	0.88	700	ng/kg per day
$Kd_{protein}$	10	572	530	999	985	nM
K_{AhR}	0.27	5.47	5.47	4.39	4.39	nM
K_{CYP1A2}	30	19.88	19.85	1.1	1.1	nM
$V_{metabolism}$	9.12	1.85	1.85	4.74	4.66	nmole/L per day
$K_{metabolism}$	0.968	31.45	31.10	2.31	2.30	nM
$n_{metabolism}$	1.12	—	—	—	—	—
$k_{absorption}$	4.8	1.08	1.08	—	4.77	$kg^{0.75}$/day
$k_{binding}$	1,000	38.96	39.03	0.0001	0.0001	/nmole per day
k_{lysis}	200	20.86	20.83	7.07	7.12	/day
$critical_{accumulation}$	0.6	0.16	0.16	—	0.6	nmole
$k_{recovery}$	0.01	0.13	0.13	—	0.01	/day
$critical_{concentration}$	2	101.8	100.0	—	2	nM
Partition Coefficients						
Fat	187.0	188.62		206.04		
Muscle	4.48	4.52		4.94		
Viscera	3.35	3.38		3.69		
Liver	4.60	4.64		5.07		
Kidney	3.35	3.38		3.69		
Gastrointestinal tract	3.35	3.38		3.69		
Lung	4.57	4.64		5.07		

[a] Test chemical administered alone
[b] Test chemical administered as a binary mixture of PCB 126 and PCB 153

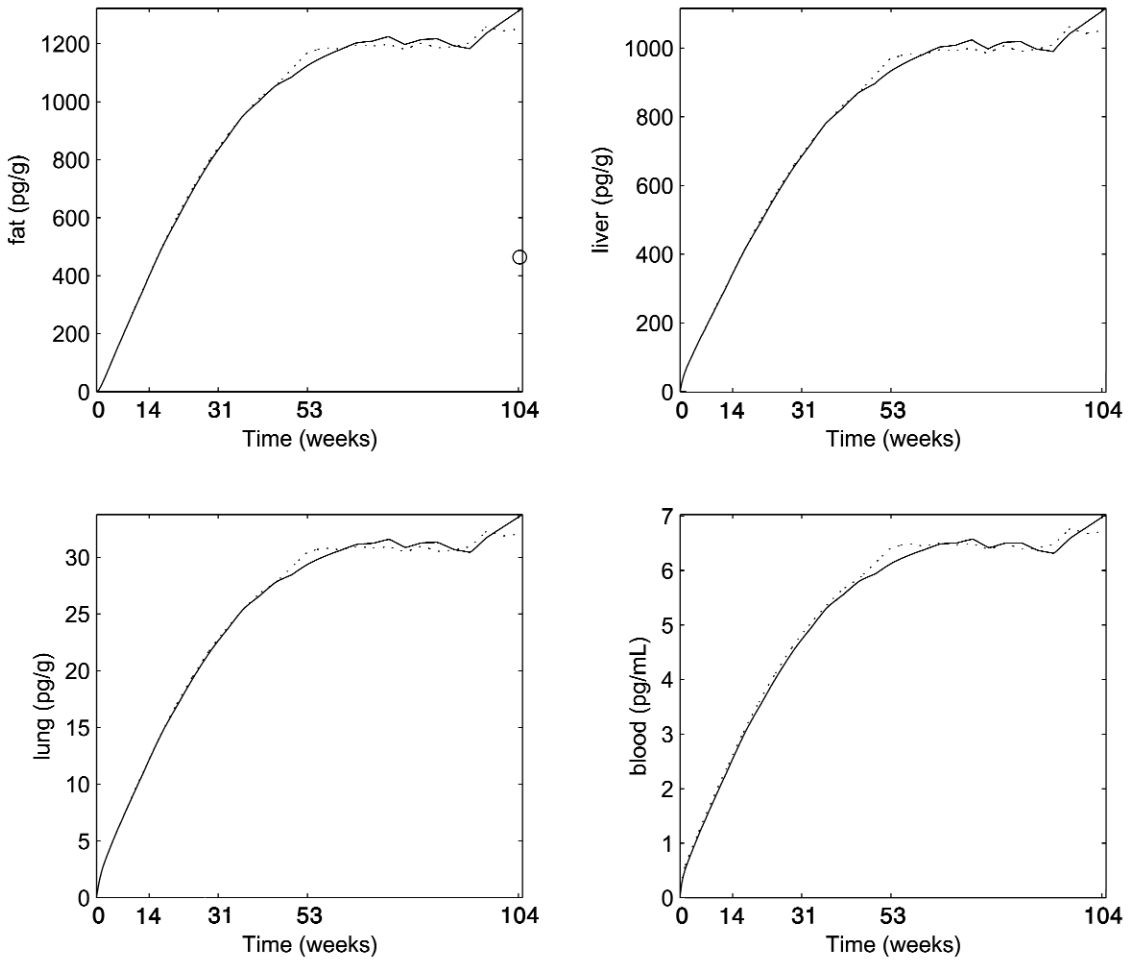

FIGURE G1
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 1 Female Rats (Vehicle Control) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

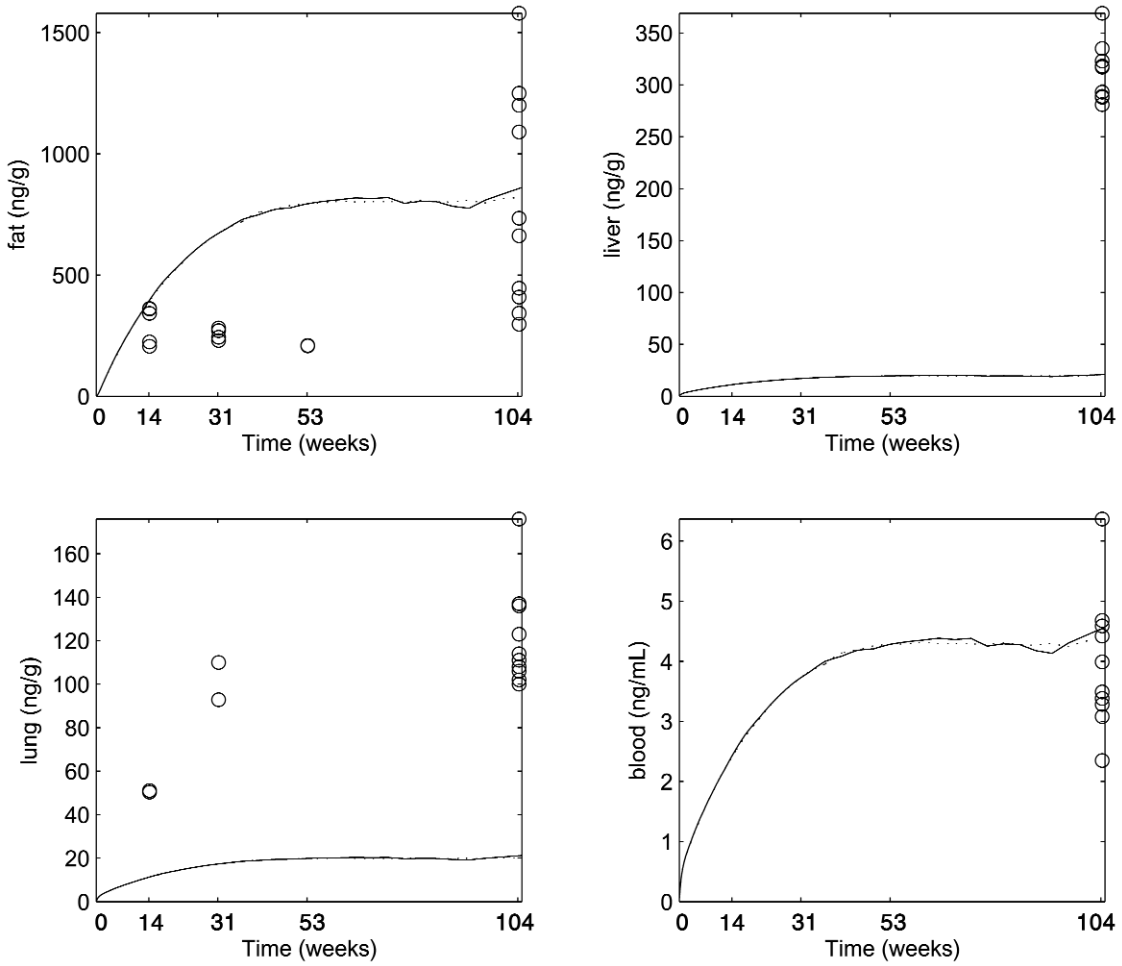

FIGURE G2
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 1 Female Rats (Vehicle Control) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

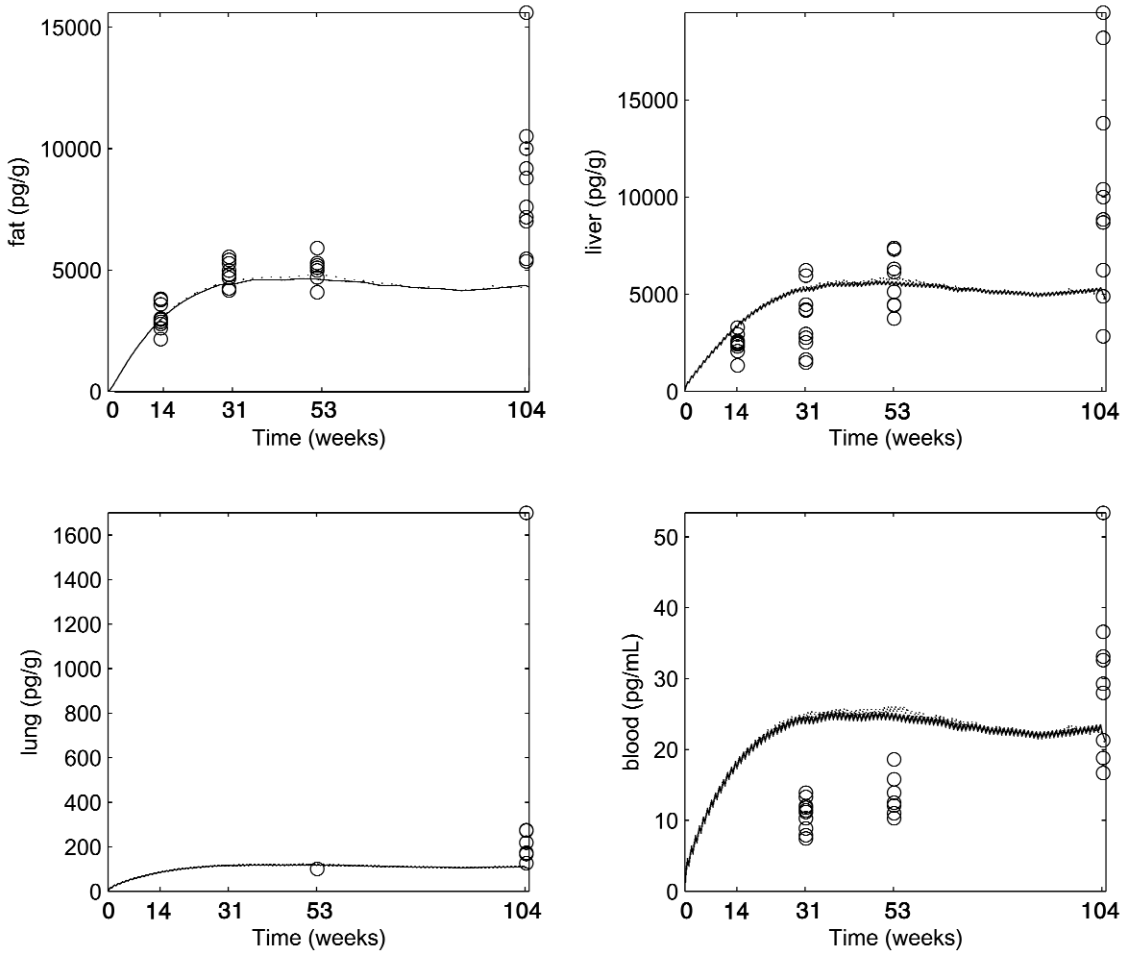

FIGURE G3
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 2 Female Rats (10 ng/kg:10 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

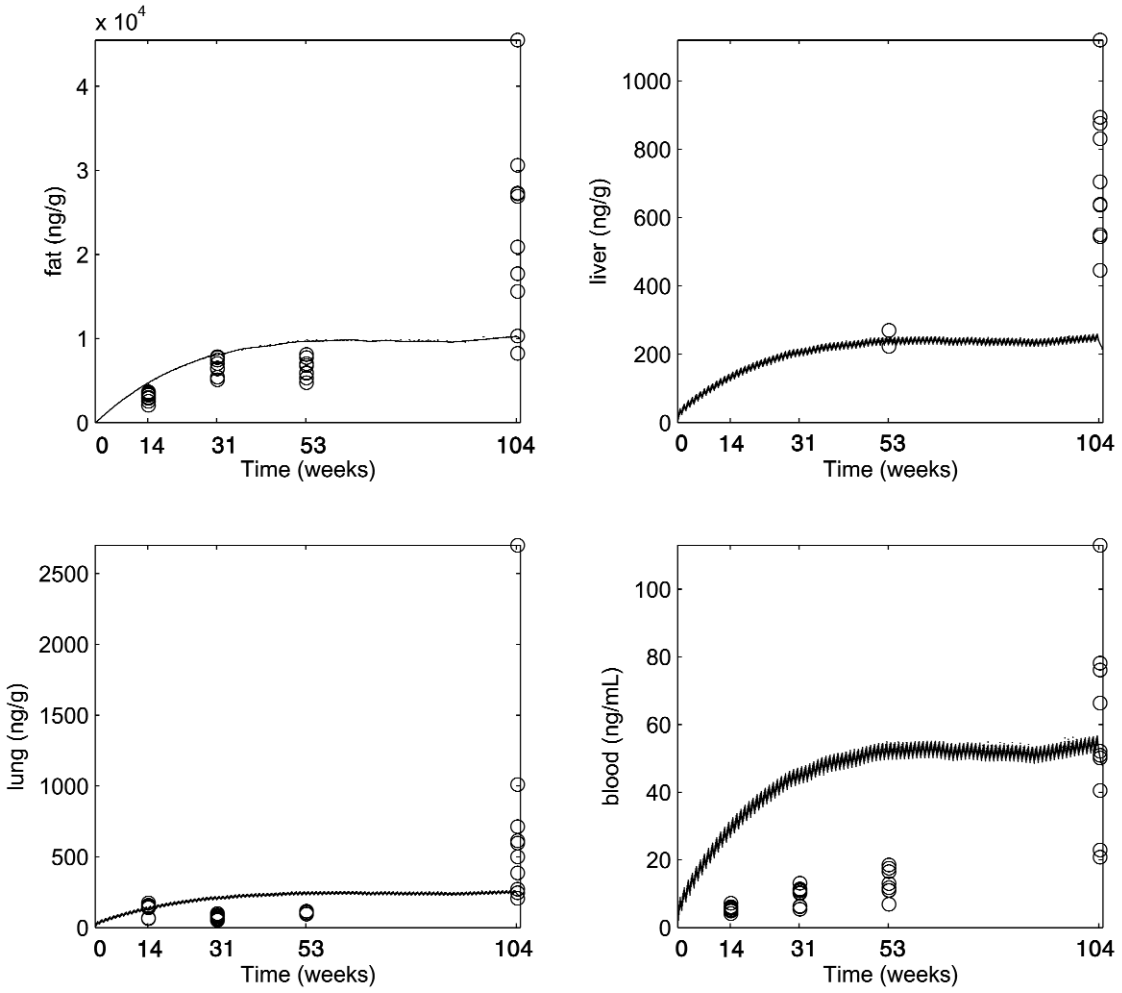

FIGURE G4
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 2 Female Rats (10 ng/kg:10 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

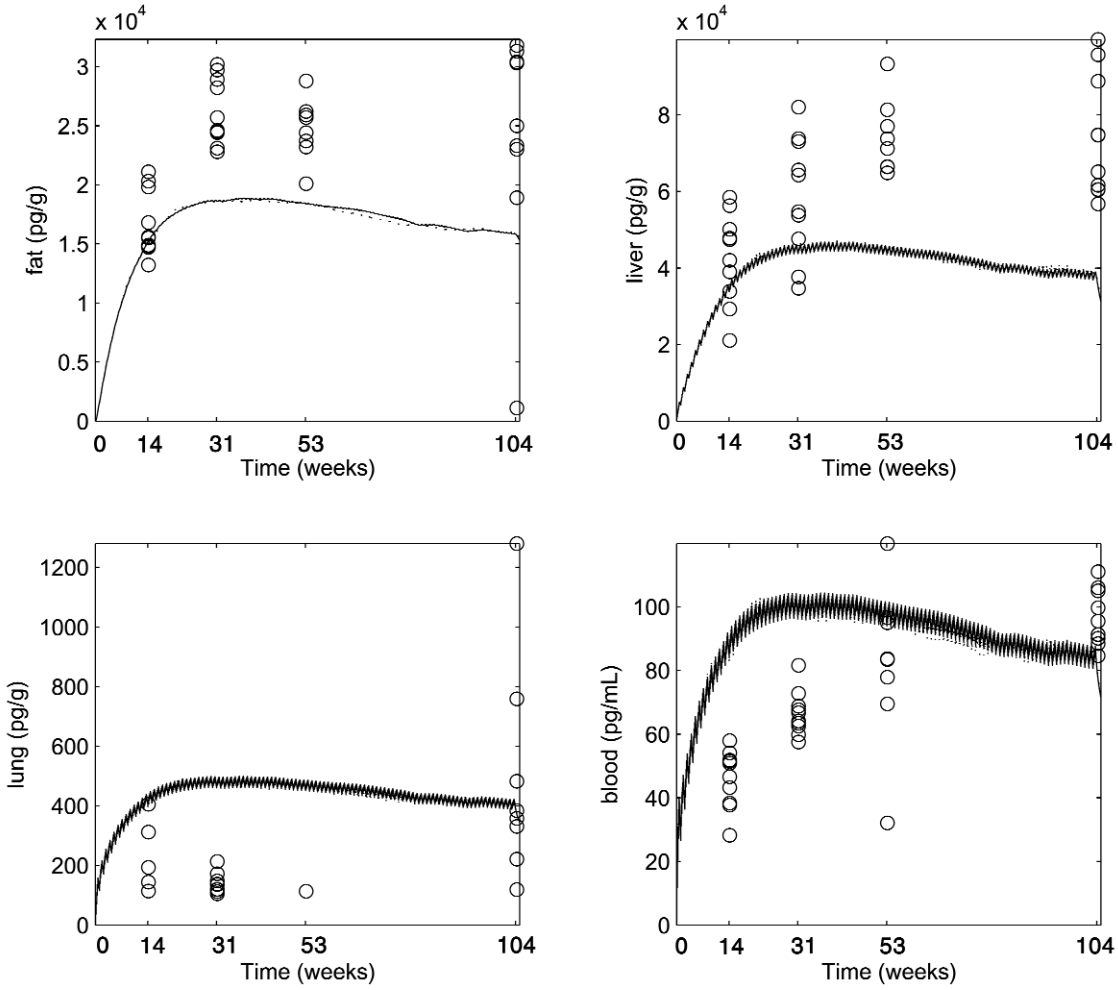

FIGURE G5
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 3 Female Rats (100 ng/kg:100 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

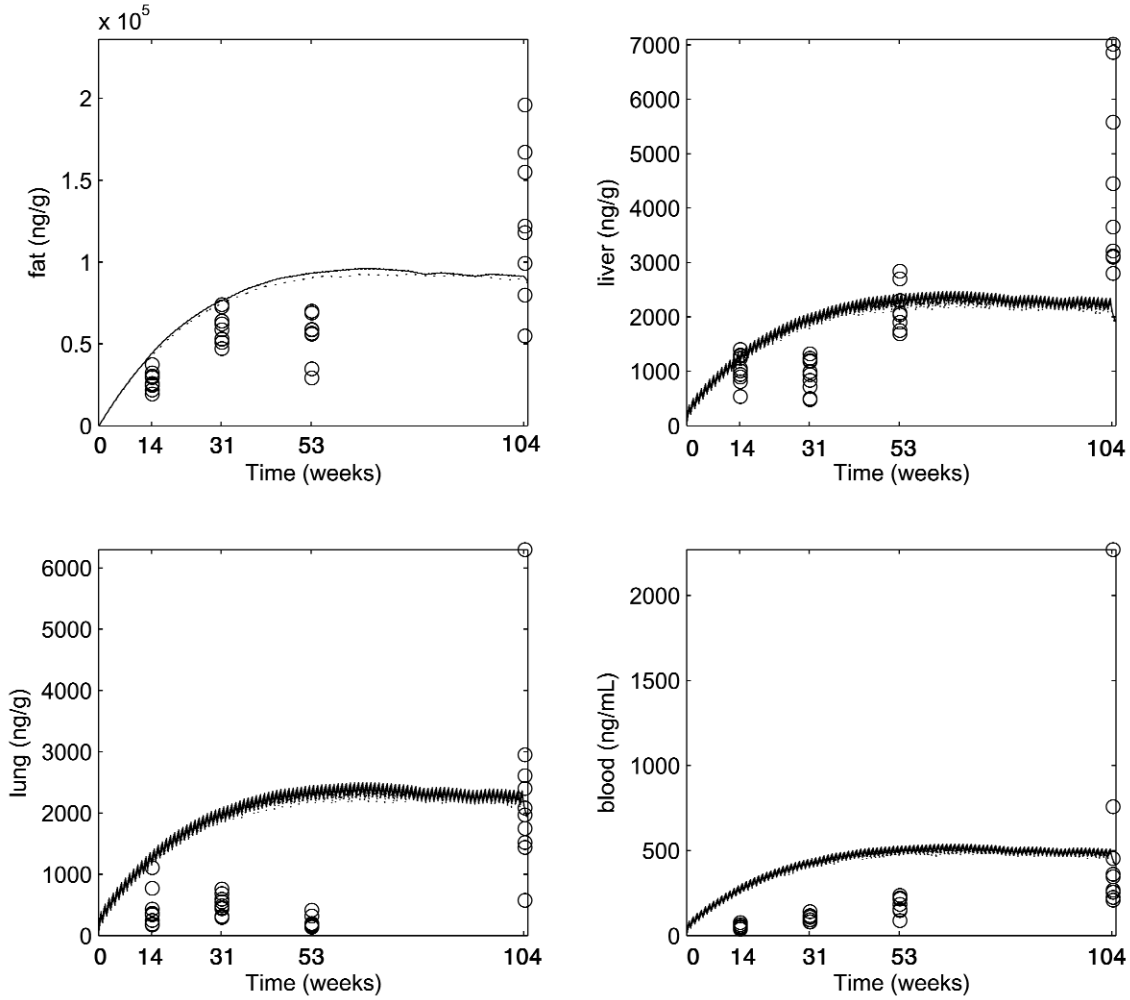

FIGURE G6
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 3 Female Rats (100 ng/kg:100 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

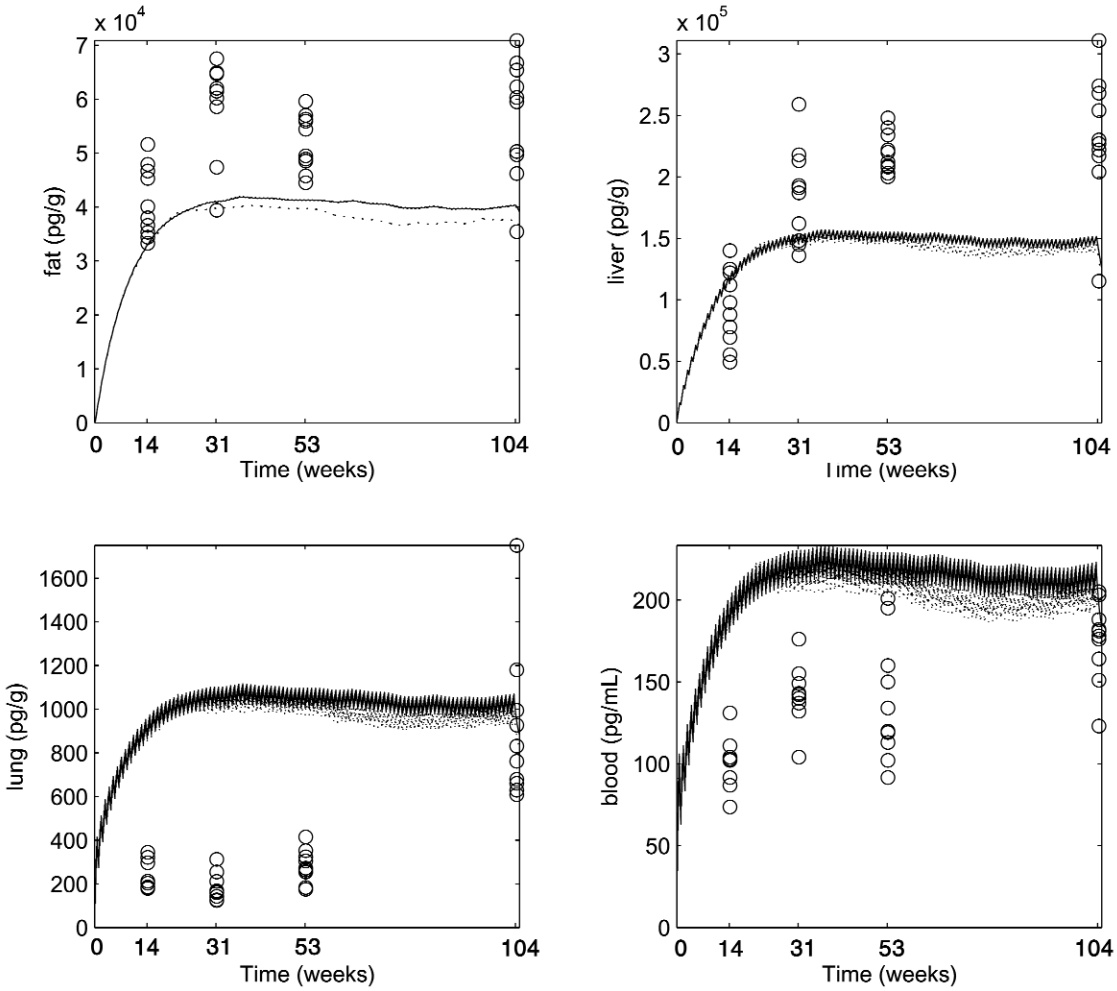

FIGURE G7
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 4 Female Rats (300 ng/kg:100 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

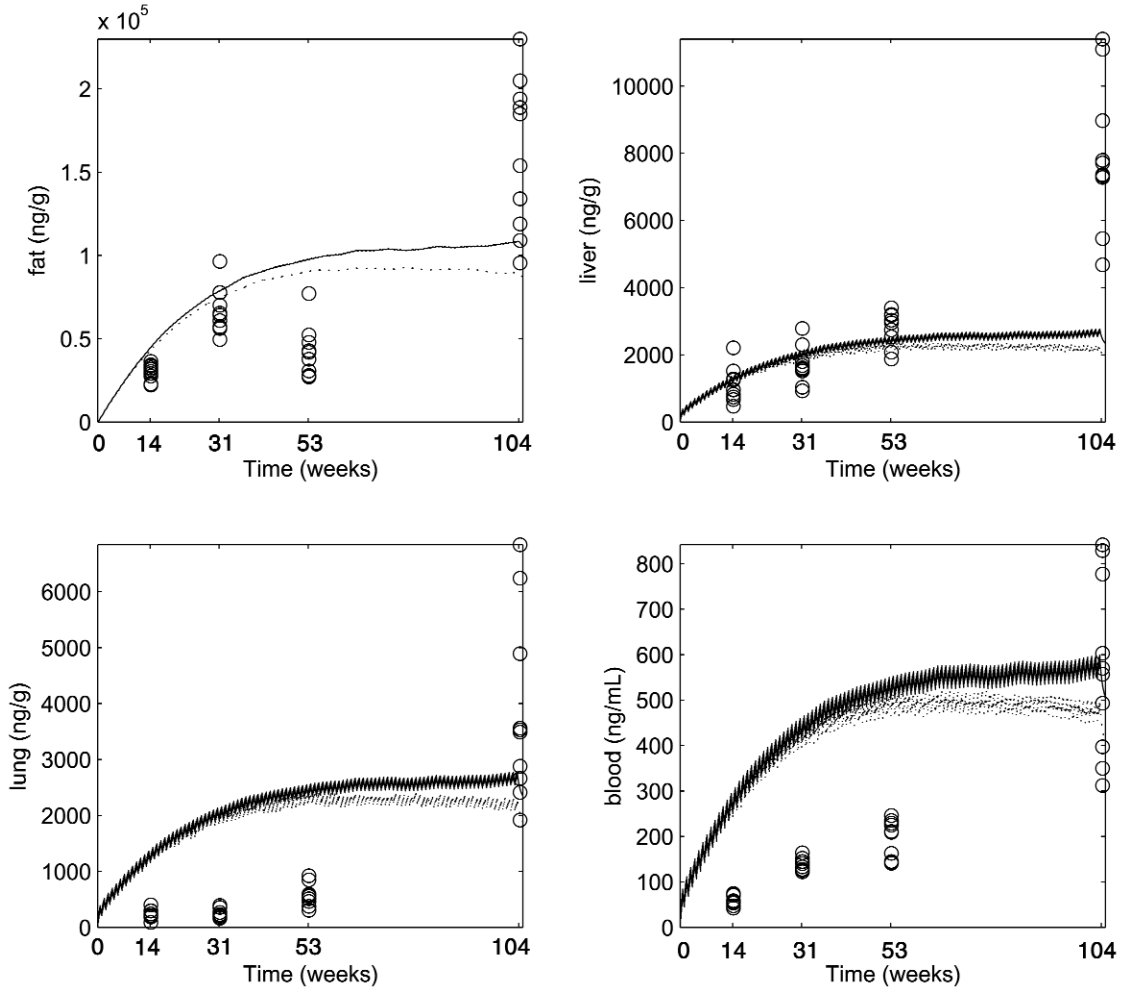

FIGURE G8
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 4 Female Rats (300 ng/kg:100 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

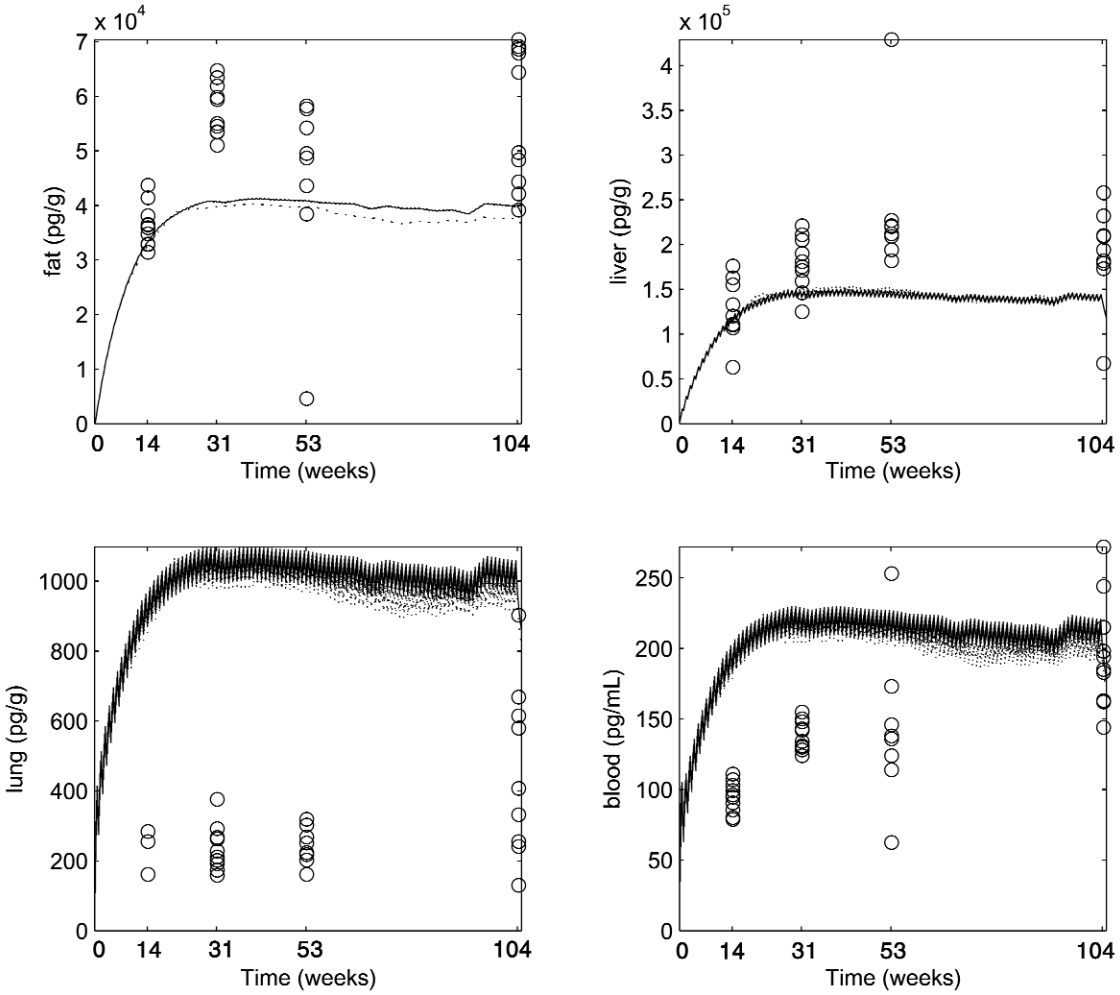

FIGURE G9
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 5 Female Rats (300 ng/kg:300 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

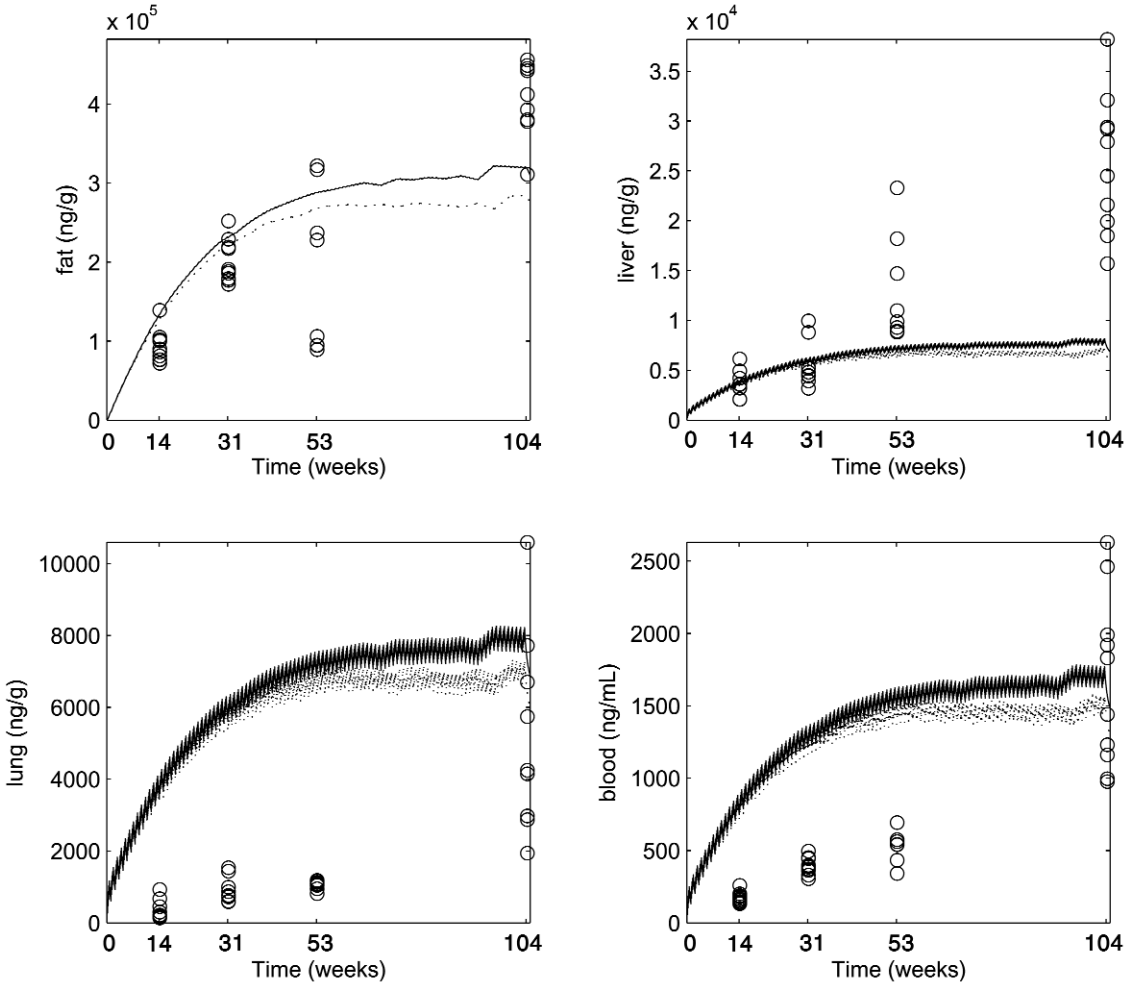

FIGURE G10
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 5 Female Rats (300 ng/kg:300 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

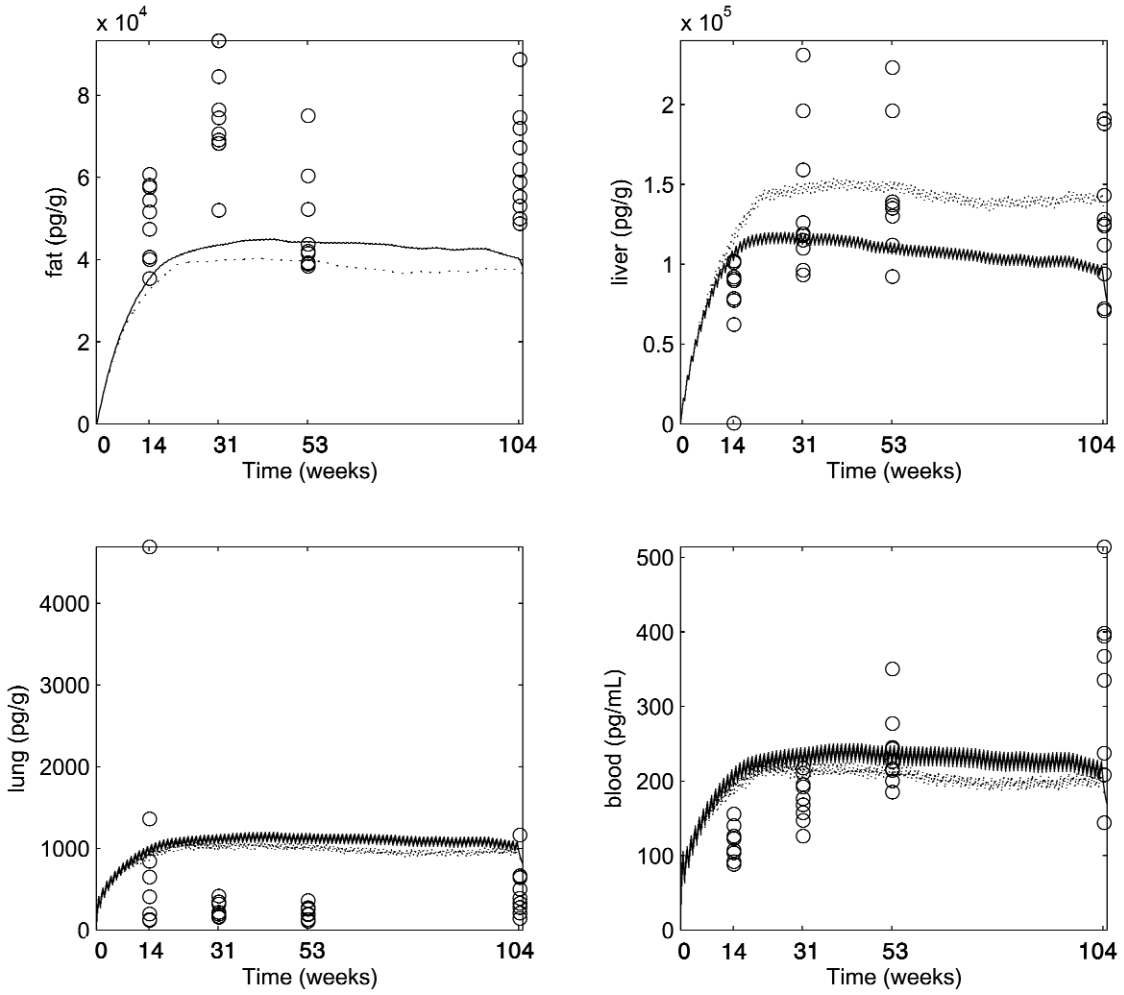

FIGURE G11
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 6 Female Rats (300 ng/kg:3,000 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

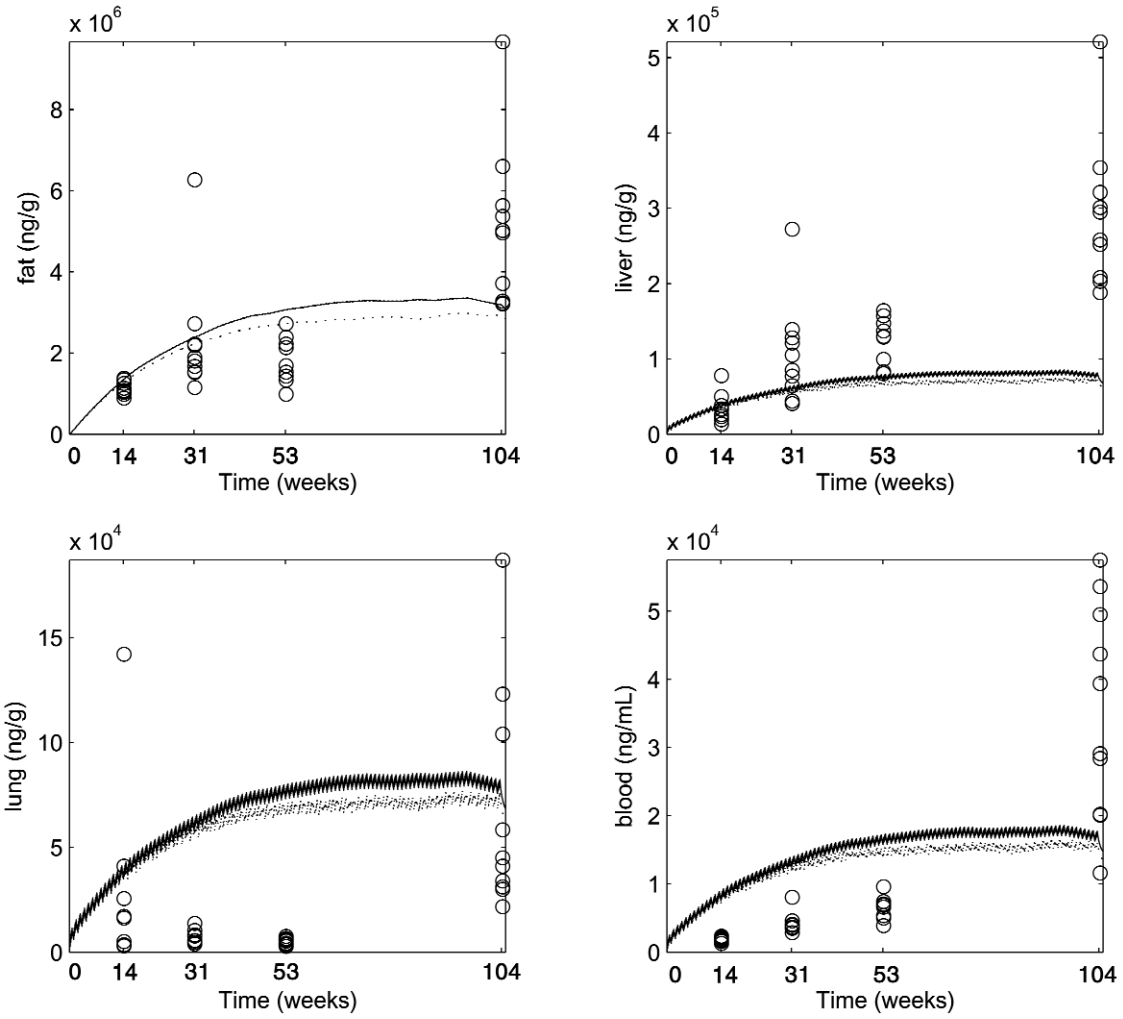

FIGURE G12
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 6 Female Rats (300 ng/kg:3,000 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

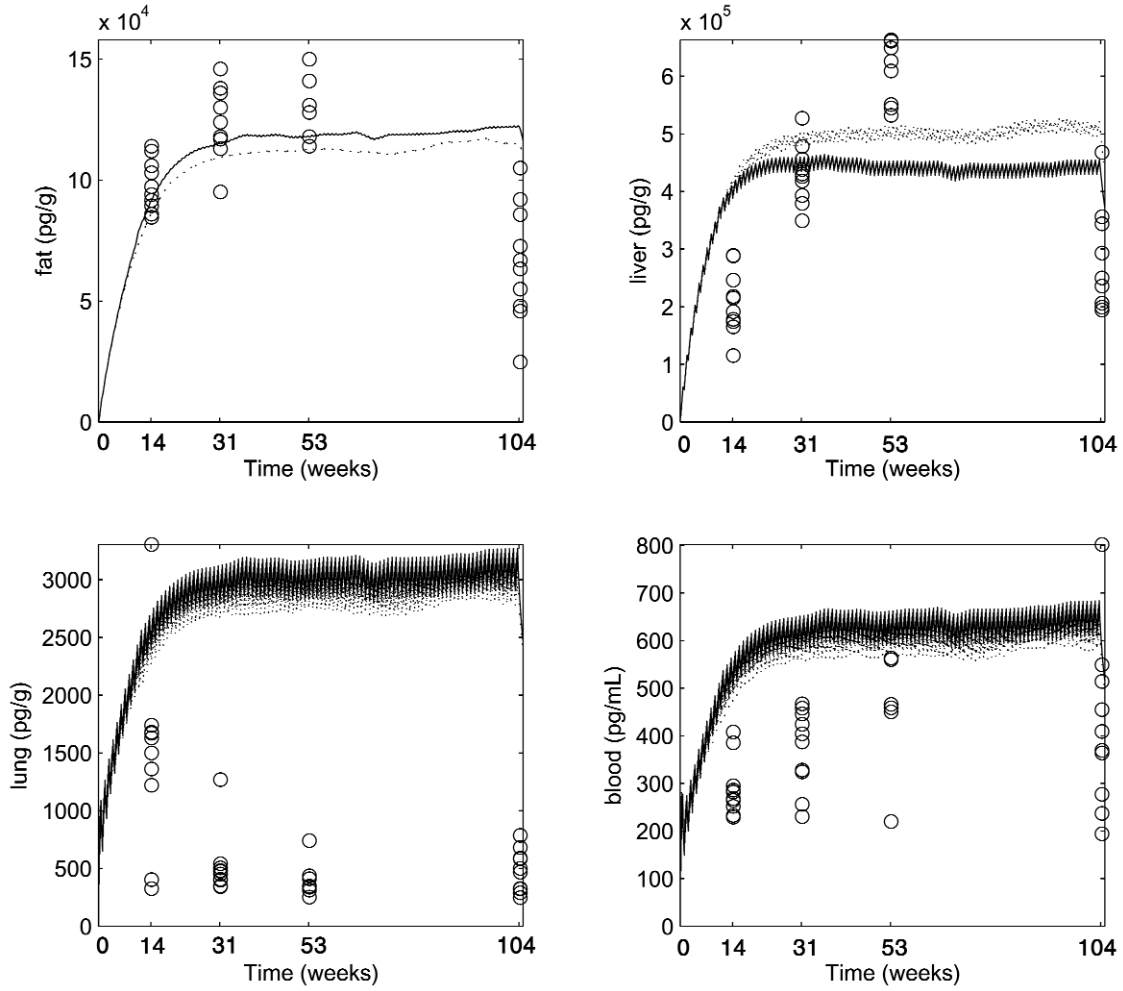

FIGURE G13
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 126 for Group 7 Female Rats (1,000 ng/kg:1,000 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 126 concentrations when PCB 126 was administered alone.

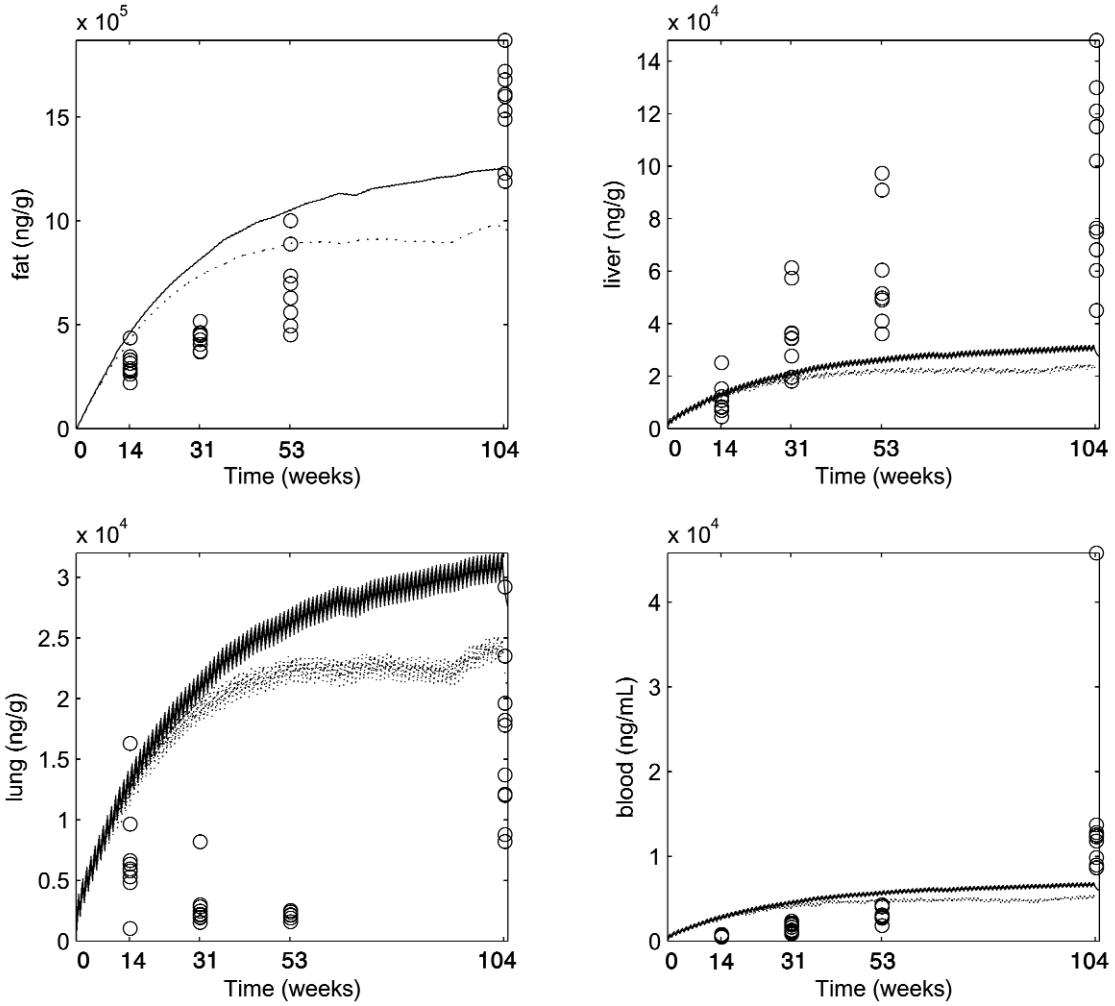

FIGURE G14
Model Predicted (–) and Measured (○) Tissue Concentrations of PCB 153 for Group 7 Female Rats (1,000 ng/kg:1,000 µg/kg) in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted line shows model predicted PCB 153 concentrations when PCB 153 was administered alone.

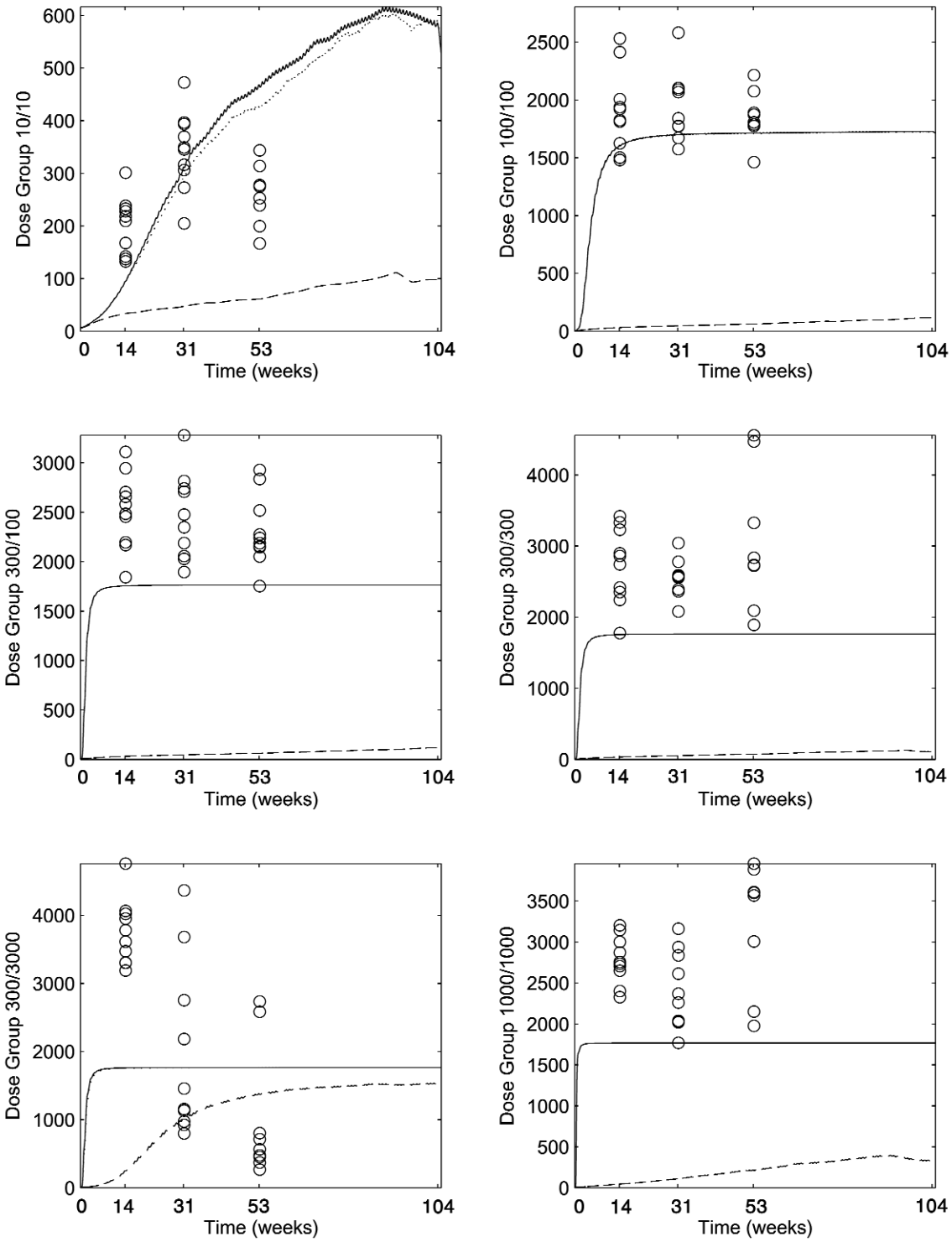

FIGURE G15
Model Predicted (–) and Measured (○) Activities of 7-Ethoxyresorufin-*O*-deethylase (pmole/minute per mg microsomal protein) in the Liver of Dosed Groups of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted and dashed lines show model predicted enzyme activities when PCB 126 or PCB 153, respectively, was administered alone.

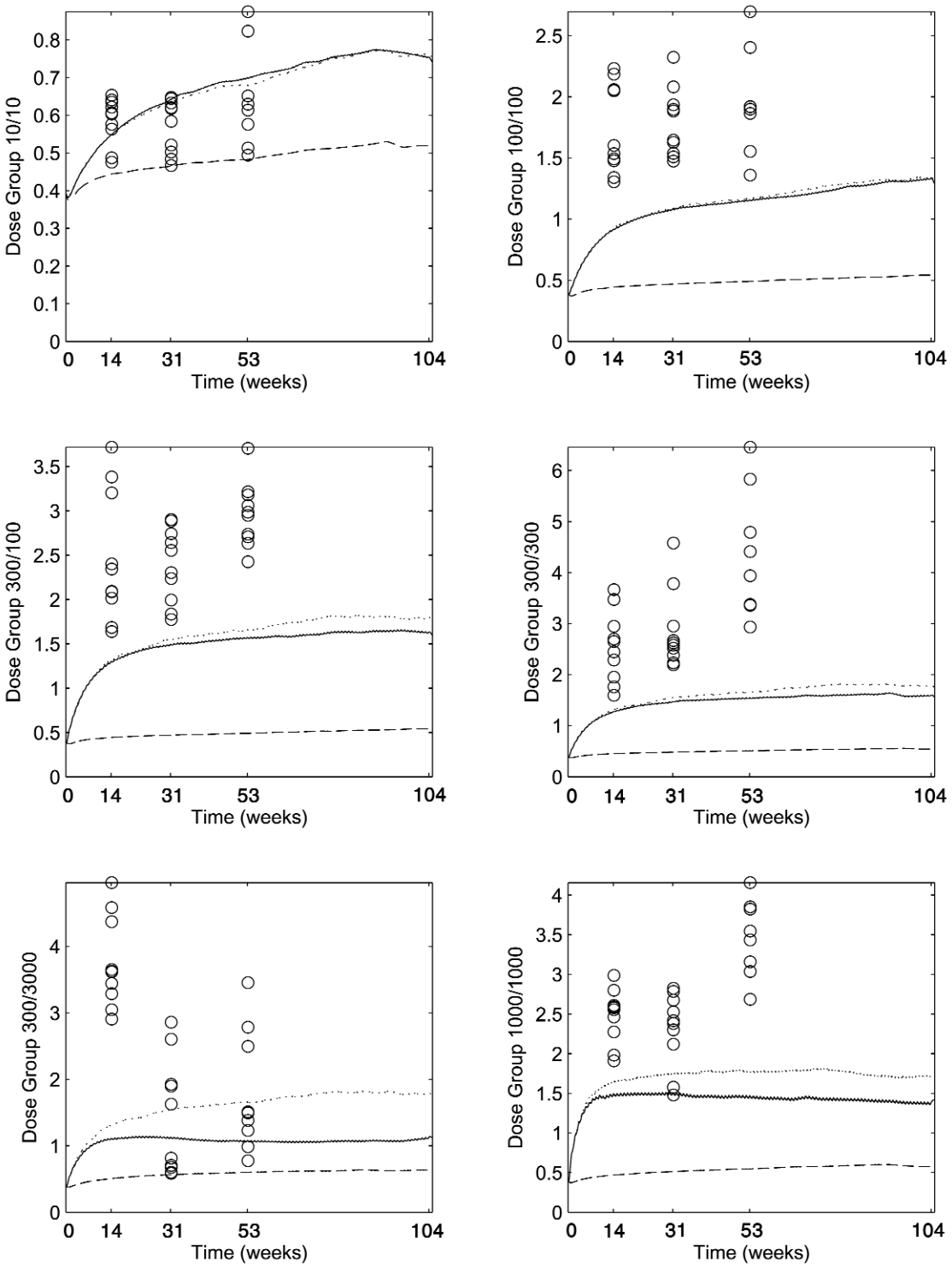

FIGURE G16
Model Predicted (–) and Measured (○) Activities of Acetanilide-4-hydroxylase (nmole/minute per mg microsomal protein) in the Liver of Dosed Groups of Female Rats in the 2-Year Gavage Study of a Binary Mixture of PCB 126 and PCB 153
The dotted and dashed lines show model predicted enzyme activities when PCB 126 or PCB 153, respectively, was administered alone.

APPENDIX H
ASSOCIATED PUBLICATIONS

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

Brix, A.E., Jokinen, M.P., Walker, N.J., Sells, D.M., and Nyska, A. (2004). Characterization of bronchiolar metaplasia of the alveolar epithelium in female Sprague-Dawley rats exposed to 3,3′,4,4′,5-pentachlorobiphenyl (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.

Hassoun, E.A., Li, F., Abushaban, A., and Stohs, S.J. (2000). The relative abilities of TCDD and its congeners to induce oxidative stress in the hepatic and brain tissues of rats after subchronic exposure. *Toxicology* **145**, 103-113.

Hassoun, E.A., Li, F., Abushaban, A., and Stohs, S.J. (2001). Production of superoxide anion, lipid peroxidation and DNA damage in the hepatic and brain tissues of rats after subchronic exposure to mixtures of TCDD and its congeners. *J. Appl. Toxicol.* **21**, 211-219.

Hassoun, E.A., Wang, H., Abushaban, A., and Stohs, S.J. (2002). Induction of oxidative stress in the tissues of rats after chronic exposure to TCDD, 2,3,4,7,8-pentachlorodibenzofuran, and 3,3′,4,4′,5-pentachlorobiphenyl. *J. Toxicol. Environ. Health A.* **65**, 825-842.

Jokinen, M.P., Walker, N.J., Brix, A.E., Sells, D.M., Haseman, J.K., and Nyska, A. (2003). Increase in cardiovascular pathology in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3,3′,4,4′,5-pentachlorobiphenyl. *Cardiovasc. Toxicol.* **3**, 299-310.

Lee, H.M., He, Q., Englander, E.W., and Greeley, G.H., Jr. (2000). Endocrine disruptive effects of polychlorinated aromatic hydrocarbons on intestinal cholecystokinin in rats. *Endocrinology* **141**, 2938-2944.

Nyska, A., Jokinen, M.P., Brix, A.E., Sells, D.M., Wyde, M.E., Orzech, D., Haseman, J.K., Flake, G., and Walker, N.J. (2004). Exocrine pancreatic pathology in female Harlan Sprague-Dawley rats after chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and dioxin-like compounds. *Environ. Health Perspect.* **112**, 903-909.

Nyska, A., Yoshizawa, K., Jokinen, M.P., Brix, A.E., Sells, D.M., Wyde, M.E., Orzech, D.P., Kissling, G.E., and Walker, N.J. (2005). Olfactory epithelial metaplasia and hyperplasia in female Harlan Sprague-Dawley rats following chronic treatment with polychlorinated biphenyls. *Toxicol. Pathol.* **33**, 371-377.

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

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

Vezina, C.M., Walker, N.J., and Olson, J.R. (2004). Subchronic exposure to TCDD, PeCDF, PCB 126, and PCB 153: Effect on hepatic gene expression. *Environ. Health Perspect.* **112**, 1636-1644.

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

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

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