NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 2,3,4,7,8-PENTACHLORODIBENZOFURAN (PeCDF)

(CAS NO. 57117-31-4)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)

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

September 2006

NTP TR 525

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

FOREWORD

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

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

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SUMMARY

Background
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) is a member of a class of chemicals containing chlorine and related in structure to dioxins. Some dioxins or dioxin-like compounds are highly toxic and cause cancer, and usually contaminated sites contain many different varieties of these dioxin-like compounds. The National Toxicology Program conducted a series of studies to try to gauge the relative toxicity of some of the more prevalent of these compounds both alone and in mixtures. This study evaluated the effects of PeCDF on female rats.

Methods
We exposed groups of 50 or 53 female rats by depositing solutions of PeCDF dissolved in corn oil through a tube directly into their stomachs five days a week for two years. Daily doses of PeCDF were 6, 20, 44, 92, or 200 nanograms (ng) of PeCDF per kilogram of body weight. Animals receiving corn oil alone served as the control group. Tissues from more than 40 sites were examined for every animal.

Results
Exposure to PeCDF caused a variety of diseases in several organs. Cancers of the liver and mouth, and to a lesser extent in the uterus, pancreas, and lung were seen in female rats exposed to PeCDF. A variety of other toxic lesions observed in exposed animals included hypertrophy, hyperplasia, and necrosis of the liver, hyperplasia of the oral mucosa, hyperplasia and metaplasia of the uterus, metaplasia of the lung, vacuolization and inflammation of the pancreas, kidney nephropathy, cystic degeneration of the adrenal cortex, atrophy of the thymus, and hyperplasia of the forestomach.

Conclusions
We conclude that PeCDF caused cancer and other toxic effects at several sites in female rats.

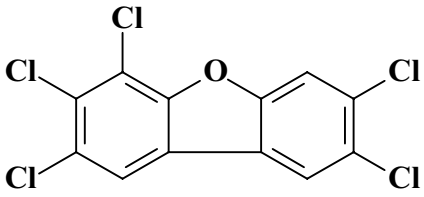
ABSTRACT

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 human tissues, mainly adipose, resulting in chronic lifetime human exposure.

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

The toxic equivalency of DLCs was nominated for evaluation because of the widespread human exposure to DLCs and the lack of data on the adequacy of the TEF methodology for predicting relative potency for cancer risk. To address this, the National Toxicology Program conducted a series of 2-year bioassays in female Harlan Sprague-Dawley rats to evaluate the chronic toxicity and carcinogenicity of DLCs and structurally related polychlorinated biphenyls (PCBs) and mixtures of these compounds.



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

CAS No. 57117-31-4

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

Synonyms: Dibenzofuran, 2,3,4,7,8-pentachloro-; 2,3,4,7,8-PeCDF; 2,3,4,7,8-PnCDF; 2,3,4,7,8-penta-CDF

2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) is not manufactured commercially other than for scientific research purposes. The main sources of PeCDF releases into the environment are from combustion and incineration sources. PeCDF was 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 PCBs. The dioxin TEF evaluation includes conducting multiple 2-year rat bioassays to evaluate the relative chronic toxicity and carcinogenicity of DLCs, structurally related PCBs, and mixtures of these compounds. While one of the aims of the dioxin TEF evaluation was a comparative analysis across studies, in this Technical Report only the results of the present PeCDF study are presented and discussed. Female Harlan Sprague-Dawley rats were administered PeCDF (at least 97% pure) in corn oil:acetone (99:1) by gavage for 14, 31, or 53 weeks or 2 years.

2-Year Study

Groups of 81 female rats were administered 6, 20, 44, 92, or 200 ng PeCDF/kg body weight in corn oil:acetone (99:1) by gavage, 5 days per week, for up to 105 weeks; a group of 81 vehicle control female rats received the corn oil/acetone vehicle alone. Up to 10 rats per group were evaluated at 14, 31, and 53 weeks. A stop-exposure group was administered 200 ng/kg PeCDF in corn oil:acetone (99:1) by gavage for 30 weeks and then the vehicle for the remainder of the study. The PeCDF in this study was at least 97% pure. Survival of dosed groups was similar to that of the vehicle control group. Mean body weights of the 200 ng/kg core and stop-exposure groups were less than those of the vehicle controls during year 2 of the study.

Thyroid Hormone Concentrations

Alterations in serum thyroid hormone levels were evaluated at the 14-, 31- and 53-week interim evaluations. There were significant decreases in total serum thyroxine (T_4) levels at the 14-week interim evaluation. There were no significant differences observed in serum free T_4, total triiodothyronine (T_3), or thyroid stimulating hormone (TSH) at 14 weeks. At both 31 and 53 weeks, there were treatment-related decreases in free and total T_4 concentrations and increases in serum T_3 levels. Serum TSH levels in dosed groups at 31 and 53 weeks were not significantly different than in the vehicle controls.

Hepatic Cell Proliferation Data

To evaluate hepatocyte replication, analysis of labeling of replicating hepatocytes with 5-bromo-2′-deoxyuridine (BrdU) was conducted at the 14-, 31-, and 53-week interim evaluations. At 14 and 53 weeks, hepatocyte BrdU-labeling indices were significantly higher in the 200 ng/kg groups compared to time-matched vehicle controls. No significant differences were observed between the dosed groups and vehicle controls at 31 weeks.

Cytochrome P450 Enzyme Activities

To evaluate the expression of known dioxin-responsive genes, CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) activity and CYP1A2-associated acetanilide-4-hydroxylase (A4H) activity were evaluated at

the 14-, 31-, and 53-week interim evaluations. Hepatic EROD and A4H activities were significantly higher in all groups administered PeCDF relative to the vehicle controls at all three interim evaluations. Pulmonary EROD was also significantly higher in all dosed groups compared to vehicle controls at 14, 31, and 53 weeks.

Determinations of PeCDF Concentrations in Tissues

The tissue disposition of PeCDF was analyzed in the liver, lung, fat, and blood of all animals at the 14-, 31-, and 53-week interim evaluations, and in 10 animals per group at the end of the 2-year study (105 weeks). In the liver of vehicle controls, PeCDF concentrations were detectable at 105 weeks. Measurable concentrations of PeCDF were not detected in fat or lung from vehicle control rats at any of the interim evaluations or at 105 weeks. Hepatic and fat concentrations were higher in groups with increasing doses of PeCDF, demonstrating a dose-related increase in tissue burden of PeCDF at each time point. No measurable concentrations of PeCDF were detected in the lungs of vehicle controls or any of the dosed groups at 14 weeks or in the lungs of the vehicle control group at 31, 53, and 105 weeks, or the 6 ng/kg group at 31 and 53 weeks. In groups with measurable levels, PeCDF concentrations were higher with respect to increasing doses. Mean levels of PeCDF in the liver, fat, lung, and blood in the 200 ng/kg group at the end of the 2-year study were 500 ng/g, 7.75 ng/g, 0.28 ng/g, and 0.04 ng/mL, respectively. Negligible PeCDF concentrations were observed in blood of the 200 ng/kg group at 53 weeks and the 92 and 200 ng/kg groups at 105 weeks. In liver and fat from the stop-exposure group, the PeCDF concentrations were between the levels observed in the 6 and 20 ng/kg groups. In the stop-exposure group, PeCDF concentration in lung was comparable to levels observed in the 6 ng/kg group. No measurable concentrations were observed in blood from the stop-exposure group.

Pathology and Statistical Analyses

There were dose-dependent increases in both absolute and relative liver weights at 14, 31, and 53 weeks, and these tended to correlate with increased incidences of hepatocellular hypertrophy. In the liver at 14 weeks, the only significant effect was an increase in the incidences of hepatocellular hypertrophy. At 53 weeks, there were significant increases in the incidences of hepatocellular hypertrophy and pigmentation.

At 2 years, there were significant dose-dependent trends for increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver. A significant dose-dependent increase in hepatic toxicity was observed and was characterized by increased incidences of numerous nonneoplastic lesions including hepatocellular hypertrophy, multinucleated hepatocytes, oval cell hyperplasia, diffuse fatty change, pigmentation, nodular hyperplasia, eosinophilic foci, hepatocellular necrosis, bile duct hyperplasia, bile duct fibrosis, cholangiofibrosis, and toxic hepatopathy.

At 2 years, three gingival squamous cell carcinomas of the oral mucosa were seen in the 200 ng/kg core and stop-exposure groups, two occurred in the 6 ng/kg group, and one occurred in each of the vehicle control, 20 ng/kg, and 92 ng/kg groups. Gingival squamous hyperplasia occurred in all groups including the vehicle controls, with increasing incidences in groups administered 44 ng/kg or greater.

The incidence of carcinoma of the uterus was marginally increased in the 92 ng/kg group at 2 years. Increased incidences of chronic active inflammation of the uterus were observed in all dosed groups, and the incidence in the 200 ng/kg stop-exposure group was greater than those in the vehicle control and 200 ng/kg core study groups. Increased incidences of squamous metaplasia of the uterus occurred in all dosed groups. In the 200 ng/kg stop-exposure group, the incidence of squamous metaplasia was significantly greater than that in the vehicle controls, but was lower than that in the 200 ng/kg core study group.

At 14-weeks, lung weights were significantly increased in the 200 ng/kg group compared to the vehicle controls. A single occurrence of a multiple cystic keratinizing epithelioma of the lung was observed in the 200 ng/kg core study group. There were increases in the incidences of bronchiolar metaplasia of the alveolar epithelium and sporadic incidences of squamous metaplasia.

One pancreatic acinar adenoma and one pancreatic acinar carcinoma were each observed in the 92 ng/kg group and in the 200 ng/kg stop-exposure group at 2 years. Significantly increased incidences of acinar cytoplasmic vacuolization and arterial chronic active inflammation and increased severity of chronic active inflammation were observed in the 200 ng/kg core study group.

Numerous nonneoplastic effects were seen in other organs including thyroid follicular cell hypertrophy, thymic atrophy, adrenal cortex cystic degeneration, nephropathy, cardiomyopathy, and squamous hyperplasia of the forestomach.

There were significantly increased incidences of mammary gland carcinoma in the 6 and 20 ng/kg groups at 2 years, with a trend to lower adjusted incidences in higher dose groups, although there was no clear dose-response pattern. Similarly, there were significantly increased incidences of pituitary gland adenoma in the 6, 20, 44, and 92 ng/kg groups. Mammary gland fibroadenoma, a spontaneous lesion in female rats, occurred at a high incidence in all groups, but was not considered treatment related.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *some evidence of carcinogenic activity** of PeCDF in female Harlan Sprague-Dawley rats, based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and gingival squamous cell carcinoma of the oral mucosa. Occurrences of cystic keratinizing epithelioma of the lung, neoplasms of the pancreatic acinus, and carcinoma of the uterus may have been related to administration of PeCDF.

PeCDF administration caused increased incidences of nonneoplastic lesions of the liver, oral mucosa, uterus, lung, pancreas, thyroid gland, thymus, adrenal cortex, kidney, heart, and forestomach in female rats.

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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 appears on page 13.

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

Concentrations in corn oil/acetone by gavage
0, 6, 20, 44, 92, or 200 ng/kg and 200 ng/kg (stop-exposure)

Body weights
200 ng/kg core study and stop-exposure groups were less than the vehicle control group

Survival rates
25/53, 22/53, 24/53, 25/53, 20/53, 23/53, 15/50

Nonneoplastic effects
Liver:
hepatocyte hypertrophy (2/53, 13/53, 17/53, 17/52, 24/53, 34/53, 14/50);
multinucleated hepatocyte (0/53, 0/53, 4/53, 13/52, 18/53, 35/53, 25/50);
oval cell hyperplasia (1/53, 4/53, 2/53, 6/52, 15/53, 35/53, 3/50);
diffuse fatty change (1/53, 4/53, 10/53, 12/52, 20/53, 26/53, 6/50);
pigmentation (13/53, 11/53, 21/53, 44/52, 42/53, 48/53, 48/50);
nodular hyperplasia (0/53, 0/53, 0/53, 3/52, 8/53, 12/53, 0/50);
eosinophilic focus (15/53, 13/53, 18/53, 18/52, 23/53, 28/53, 22/50);
necrosis (4/53, 10/53, 3/53, 3/52, 6/53, 18/53, 11/50);
bile duct hyperplasia (3/53, 2/53, 2/53, 2/52, 1/53, 13/53, 1/50);
bile duct fibrosis (1/53, 4/53, 2/53, 2/52, 3/53, 6/53, 1/50);
toxic hepatopathy (0/53, 2/53, 3/53, 8/52, 27/53, 44/53, 9/50);
cholangiofibrosis (0/53, 1/53, 0/53, 3/52, 3/53, 5/53, 3/50)
Oral Mucosa:
gingival squamous hyperplasia (15/53, 11/53, 16/53, 19/53, 22/53, 20/53, 14/50)
Uterus:
endometrial cystic hyperplasia (31/53, 29/53, 29/53, 33/52, 39/52, 37/53, 35/49);
chronic active inflammation (0/53, 5/53, 3/53, 3/52, 4/52, 3/53, 7/49);
squamous metaplasia (17/53, 25/53, 21/53, 36/52, 31/52, 35/53, 28/49)
Lung:
alveolar epithelium, metaplasia, bronchiolar (5/53, 6/53, 5/53, 9/53, 23/53, 28/52, 7/50);
squamous metaplasia (0/53, 0/53, 0/53, 2/53, 4/53, 3/52, 1/50)
Pancreas:
acinar cytoplasmic vacuolization (0/53, 0/53, 0/53, 0/52, 2/52, 23/52, 2/49);
arterial chronic active inflammation (1/53, 2/53, 1/53, 2/52, 4/52, 11/52, 1/49)
Thyroid Gland:
follicular cell hypertrophy (7/53, 13/53, 24/51, 24/53, 24/51, 22/51, 23/48)
Thymus:
atrophy (43/53, 36/49, 36/50, 44/52, 39/49, 48/51, 44/49);
severity of atrophy (2.3, 2.6, 2.7, 2.8, 3.1, 3.6, 2.9)
Adrenal Cortex:
cystic degeneration (4/53, 17/53, 14/53, 18/52, 12/53, 14/53, 12/48)
Kidney:
nephropathy (34/53, 39/53, 35/53, 42/52, 36/53, 45/53, 35/48);
severity of nephropathy (1.1, 1.2, 1.2, 1.4, 1.4, 1.5, 1.1)
Heart:
cardiomyopathy (15/53, 12/53, 19/52, 13/53, 18/53, 24/52, 13/50)
Forestomach:
squamous hyperplasia (4/53, 1/53, 5/53, 6/53, 3/52, 10/53, 5/50)

Neoplastic effects
Liver:
hepatocellular adenoma (1/53, 0/53, 1/53, 0/52, 2/53, 4/53, 1/50);
cholangiocarcinoma (0/53, 0/53, 0/53, 1/52, 1/53, 2/53, 0/50)
Oral Mucosa:
gingival squamous cell carcinoma (1/53, 2/53, 1/53, 0/53, 1/53, 3/53, 3/50)

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

Equivocal findings
Lung:
cystic keratinizing epithelioma (multiple) (0/53, 0/53, 0/53, 0/53, 0/53, 1/52, 0/50)
Pancreas:
acinar adenoma or carcinoma (0/53, 0/53, 0/53, 0/52, 2/52, 0/52, 2/49)
Uterus:
carcinoma (1/53, 1/53, 0/53, 1/53, 5/53, 2/53, 1/50)

Level of evidence of carcinogenic activity
Some 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 PeCDF on February 17, 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 February 17, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) 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 dioxin-like compounds (dioxins, PCBs, furans). Dr. J.R. Hailey, NIEHS, described the pathology review process for the TEF studies and presented examples of the characteristic spectra of neoplasms and nonneoplastic lesions of the liver and lung for these compounds.

Dr. Walker introduced the study of PeCDF by noting that PeCDF is the most potent polycholorinated dibenzofuran in the TEF scheme. He described the study design, and the reduction in body weights, the spectra of effects in the liver, lung, oral mucosa, uterus, and pancreas, and a variety of nonneoplastic lesions. The proposed conclusion was *some evidence of carcinogenic activity* of PeCDF in female Harlan Sprague-Dawley rats.

Dr. Ho, the first principal reviewer, said the study was well designed and described, and she agreed with the proposed conclusion. She emphasized that the chemical was nonmutagenic and suggested the possibility that besides interacting with the Ah receptor, it may also suppress an immune response. Dr. Ho thought the interplay of several mechanisms might explain the nonlinearity of proliferative responses.

Dr. McQueen, the second principal reviewer, also agreed with the proposed conclusion.

Dr. Birt, the third principal reviewer, agreed with the proposed conclusion and suggested expanding the description of the pathology diagnostic criteria.

Dr. Walker agreed that the chemical was a nongenotoxic carcinogen and noted that frequently the responses in laboratory animals were more skewed than linear.

Dr. Ho moved that the conclusion be accepted as written. Dr. McQueen seconded the motion. The motion was passed unanimously with 12 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 adipose tissue, resulting in chronic lifetime human exposure (Schecter *et al*., 1994a).

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, ten PCDFs, and thirteen 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 rereviewed 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 of 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 that DLCs are 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, and disruption of normal cellular processes could be a risk factor for carcinogenicity.

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

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, *in vivo* studies are weighted more than *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.

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

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.

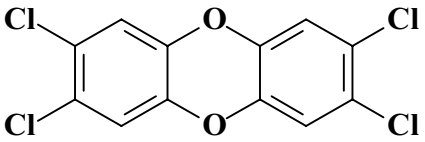
One of the implicit assumptions in the use of TEFs is that the TEQ for different compounds is dose additive. While dose additivity is supported for certain mixtures, for some biological endpoints in some models, this may not be true. As outlined by Van den Berg *et al.* (1998), the TEF methodology is 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 female Sprague-Dawley rats.
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

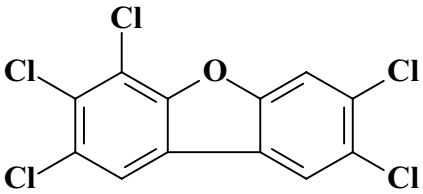


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD

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 1.0. TCDD is classified as a known human carcinogen by the NTP and the International Agency for Research on Cancer.

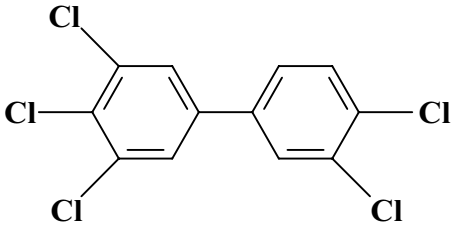


2,3,4,7,8-Pentachlordibenzofuran
PeCDF

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.

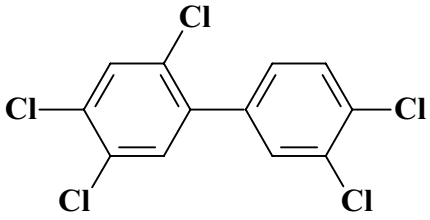


3,3′,4,4′,5-Pentachlorobiphenyl
PCB 126

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.

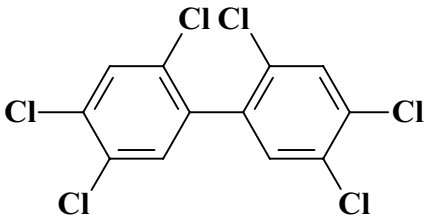


2,3′,4,4′,5-Pentachlorobiphenyl
PCB 118

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, PeCDF, and PCB 126

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, PeCDF, and PCB 126. 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 test 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 mixture (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 at the in-life stage, 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 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.

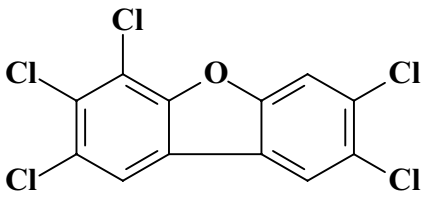
Additional "special study" animals were included at each interim necropsy. Tissues from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies. These animals were not evaluated as part of the core study.

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



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

CAS No. 57117-31-4

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

Synonyms: Dibenzofuran, 2,3,4,7,8-pentachloro-; 2,3,4,7,8-PeCDF; 2,3,4,7,8-PnCDF; 2,3,4,7,8-penta-CDF

Chemical and Physical Properties

PeCDF belongs to a family of chemicals designated polyhalogenated aromatic hydrocarbons (PHAHs). These include the polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Depending on the position and number of chlorine substitutions, the structure of these chemicals allows for 75 chlorinated dioxins, 135 chlorinated dibenzofurans, and 209 chlorinated biphenyls (USEPA, 2000b).

2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) is a solid with a melting point of 195° to 196° C (NTP Chemical Repository Information) and is stable under normal laboratory conditions.

Production, Use, and Human Exposure

PCDFs are not manufactured commercially other than for scientific research purposes. The main sources of PCDF releases into the environment are from combustion and incineration sources. Based on congener-specific profiles, combustion sources produce all 2,3,7,8-substituted PCDDs and PCDFs including PeCDF. PeCDF is the major congener emitted from cement kilns burning hazardous waste (approximately 20% of the total congener emission). Other major sources of PeCDF are metal smelting, refining, and processing; chemical manufacturing/processing (production of chlorophenols, PCBs, vinyl chloride); pulp bleaching; and existing "reservoir" sources that reflect past releases (IARC, 1997; USEPA, 2000a).

Due to high lipophilicity and low solubility in aqueous media, PCDFs accumulate in the fat tissues of animals. The highest concentrations of PCDFs are found in fish, meat, eggs, and dairy products (Schecter *et al*., 1994b; USEPA, 2000b). This results in widespread exposure of the general population to PCDFs and related dioxin-like compounds (DLCs) (Schecter *et al*., 1994a). It is estimated that 90% of human exposure to PCDFs and related dioxins occurs through ingestion of food contaminated with these compounds. A small fraction of exposure occurs via inhalation and dermal absorption.

Adult daily intake of DLCs including PCDDs, PCDFs and dioxin-like PCBs from all sources is estimated to be approximately 70 pg TCDD equivalents (TEQ)/day, where TEQ reflects the potency-adjusted mass of all DLCs covered by the World Health Organization toxic equivalency factors (TEFs). The intake from all sources of PCDDs and PCDFs is estimated at 45 pg TEQ/day and intake from dioxin-like PCBs is 25 pg TEQ/day. Approximately 90% of the daily intake is from food sources (40 pg TEQ/day for PCDDs and PCDFs and 22 pg TEQ/day for dioxin-like PCBs). Intake of PeCDF from food is approximately 6.6 pg per day and represents 16% of the total intake of PCDDs and PCDFs on a TEQ basis. This level of exposure together with the long half-life of DLCs in humans leads to persistent body burdens in humans in the range of 25 pg TEQ/g lipid (USEPA, 2000b). Depending upon dietary practices and proximity to specific sources of exposure, some populations may have higher exposure levels or body burdens. The exposure levels in the United States are similar to those seen in other industrialized countries. In contrast to the general population, several specific populations have been exposed to much higher levels of PeCDF as a result of occupational exposure.

Average levels of PCDDs and PCDFs in human tissues (average of adipose, blood, and human milk) is 34 ppt TEQ. The average level of PeCDF in human tissues in the United States is 5.4 ppt lipid TEQ, compared to 5.2 ppt TEQ for TCDD. This indicates that PeCDF represents approximately 16% of the total TEQ in human tissue contributed by PCDDs and PCDFs and occurs at similar levels, on a TEQ basis, to TCDD. In European tissue samples, PeCDF levels are on average 13 ppt TEQ (lipid-adjusted) and represent approximately 36% of the TEQ contributed by PCDDs and PCDFs. PeCDF is the highest contributor of the PCDF class of DLCs to the total TEQ based on both intake levels and tissue levels.

TOXICOKINETICS

There is an extensive body of literature on the toxicokinetics of PCDD and PCDF-related compounds (USEPA, 2000c). More than 70% of an oral dose of PeCDF was absorbed by male F344 rats; the extent of absorption was independent of dose between 0.1 and 1 µmol/kg (Brewster and Birnbaum, 1987). For comparison, Rose *et al*. (1976), reported more than 80% absorption of a 0.1 µg/kg oral dose of TCDD in male and female Sprague-Dawley rats. Once absorbed, PeCDF is readily distributed throughout the body. In rats, the whole-body elimination half-life has been estimated as 64 days. In adipose tissue in rats, PeCDF has an elimination half-life of 193 days, and the half-life in liver is 69 days. The major metabolites of PeCDF in the rat are dihydroxy-penta-chlorobiphenyl and hydroxy-penta-CDF. Other metabolites include a hydroxy-tetra-CDF, a dihydroxy-tri-CDF, a dihydroxy-tetra-CDF, and a thio-tetra-CDF (Pluess *et al*., 1987). While the toxicity of these metabolites has not been investigated, it is generally assumed that the toxicities associated with exposure to PCDDs and PCDFs are due to the parent compound, and that metabolism is a detoxification mechanism.

In humans, it has been estimated that the median whole-body half-life of PeCDF is 19.6 years (Flesch-Janys *et al*., 1996). Estimates from highly exposed individuals also indicate that elimination may be biphasic with shorter half-lives (2 to 3 years) after high-level acute exposure leading to longer half-lives at ambient levels of exposure (USEPA, 2000b).

The main sites of distribution of PeCDF in rats within the first few days after exposure are primarily to the liver and adipose tissue and lesser amounts to skin and muscle. In the Brewster and Birnbaum (1987) study, PeCDF levels in the rat liver are 30 times higher than those in adipose tissue. The ratio generally increases with time. The pattern of distribution of PeCDF in rats is due to its lipophilicity and binding to cytochrome P450 1A2 (Gillner *et al*., 1987; Diliberto *et al*., 1997). Cytochrome P450 1A2 is a known binding protein for PCDDs and PCDFs and is also inducible by PCDDs and PCDFs via the aryl hydrocarbon receptor (AhR). Since CYP1A2 is inducible in the liver, PeCDF tends to sequester in the liver at levels that would not be predicted based on its lipophilicity alone. The hepatic sequestration of PeCDF is not observed in CYP1A2 knockout mice, demonstrating the critical involvement of CYP1A2 in this process of liver sequestration (Diliberto *et al*., 1999).

TOXICITY

PeCDF is a high-potency DLC, so much of the toxicity of PeCDF is similar to that of the most potent dioxin, TCDD. Toxic effects observed with exposure to PCDDs and PCDFs include developmental and reproductive alterations, immunotoxicity, teratogenicity, carcinogenicity, and lethality (Poland and Knutson, 1982; Birnbaum, 1994b; ATSDR, 1998; Grassman *et al*., 1998; USEPA, 2000c).

Different animal species vary widely in their sensitivity to the lethal toxicity of TCDD. The oral LD_{50} of TCDD varies over 5,000-fold. Consequently the range of acute lethality for PeCDF is probably similar. The oral LD_{50} for PeCDF in guinea pigs (the most sensitive species for TCDD-induced lethality) is 10 µg/kg; by comparison the LD_{50} for TCDD is 1 µg/kg. In all species tested, the acute lethal doses of TCDD and related potent DLCs have latency periods of 1 to 2 weeks, during which animals exhibit a wasting syndrome. Other characteristic effects associated with exposure are chloracne, body weight loss, reduced body weight gain, porphyria, thymic atrophy, gastric hyperplasia/hypoplasia, hepatotoxicity, increased serum concentrations of liver enzymes, hypertriglyceridemia, increased liver weights, hepatic Vitamin A depletion, altered thyroid homeostasis, and increased expression of drug metabolizing enzymes (Poland and Knutson, 1982).

CARCINOGENICITY

Experimental Animals

PeCDF has not been tested for carcinogenicity in any animal model. Based on the similarity in mechanism of action of binding to the AhR, it is expected that the carcinogenicity of PeCDF would be similar to that 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). TCDD administered by gavage causes tumors in male and female Osborne-Mendel rats and $B6C3F_1$ mice (NTP, 1982a). In those studies, there were significant increases in the incidences of thyroid gland follicular cell adenoma in high-dose male and female rats and high-dose female mice, the incidence of neoplastic liver nodules in high-dose female mice, and the incidences of hepatocellular carcinoma in high-dose male and female mice. TCDD administered to Swiss-Webster mice by dermal application caused an increased incidence of fibrosarcoma of the integumentary system in high-dose females and yielded equivocal evidence in males (NTP, 1982b). Based on the previous NTP (1982b) studies, there is substantial evidence of carcinogenicity of TCDD in male and female rats and mice.

One of the most highly cited carcinogenicity studies for TCDD is a 2-year feed study conducted by Dow Chemical Company (Kociba *et al*., 1978). In that study, increased incidences of tumors were seen at multiple sites in Sprague-Dawley rats administered up to 100 ng TCDD/kg per day for 2 years. There were increased incidences of liver hyperplastic nodules (females), hepatocellular carcinoma (females), keratinizing squamous cell carcinoma of the lung (females), adenoma of the adrenal cortex (males), squamous cell carcinoma of nasal turbinates/hard palate (males and females), and stratified squamous cell carcinoma of the tongue (males). Significantly decreased tumor incidences were observed for pheochromocytoma of the adrenal gland (males); subcutaneous skin lipoma, fibroma, or fibroadenoma (combined) (males); benign uterine tumors, benign neoplasms of the mammary gland, mammary gland carcinoma, and pituitary gland adenoma (females); and acinar adenoma of the pancreas (males). Two evaluations of the pathology of the female liver tumor data confirmed significant increases in hepatocellular adenoma and hepatocellular carcinoma (Squire, 1980; Goodman and Sauer, 1992).

Humans

Humans have not been exposed to significant amounts of PeCDF alone. Exposure to PeCDF occurs in a mixture combined with other structurally related compounds such as PCDDs, other PCDFs, and PCBs.

Two accidental poisoning incidents in Japan and Taiwan resulted from exposures to cooking oil highly contaminated with PCDFs and PCBs. In addition to extensive reproductive and developmental effects in these populations, early follow-up studies indicated an increased mortality from liver disease and cancer, particularly liver cancer (IARC, 1997). Although recent follow-up studies do not show an increased mortality from cancer, mortality from liver disease was still elevated (Yu *et al*., 1997). Cancer mortality was also investigated in a Swedish population that consumed fatty fish from the Baltic Sea. The predominant exposure was to PeCDF and other PCDFs, and to PCBs. In this population, there was an increase in mortality from stomach cancer, squamous cell cancer of the skin, and multiple myeloma (IARC, 1997).

Other studies have examined occupational cohorts of phenoxy herbicide workers who were exposed to mixtures of PCDDs or PCDFs, and a population in Seveso, Italy that was accidentally exposed to TCDD after an explosion at a chemical plant in 1976 (USEPA, 2000c). Studies in phenoxy herbicide workers indicate an increased mortality for all cancers combined, soft tissue sarcoma, non-Hodgkins lymphoma, and lung cancer (Kogevinas *et al*., 1997; Steenland *et al.*, 1999). The most recent follow-up of the Seveso cohort showed

similar effects: an increase in all cancers combined with several specific cancers including rectal cancer, lung cancer, Hodgkins disease, non-Hodgkins lymphoma, and myeloid leukemia (Bertazzi *et al*., 2001).

Tumor Promotion Studies

Studies in Sprague-Dawley rats show that PeCDF can enhance the development of enzyme-altered hepatic foci (Waern *et al*., 1991), indicative of a tumor promotion effect. Numerous studies have also examined the promotion of altered hepatic foci by TCDD within the framework of two-stage, initiation-promotion protocols (Dragan and Schrenk, 2000). In the liver, clonal expansion of genetically altered cells leads to the formation of altered hepatocellular focal lesions identified by alterations in histomorphology or gene expression. These lesions are believed to be a precursor in the development of liver tumors (Pitot *et al*., 1991). These studies demonstrate that TCDD is a potent liver tumor promoter and this effect is dose-dependent (Pitot *et al*., 1980; Maronpot *et al*., 1993; Teeguarden *et al*., 1999), duration of exposure dependent, and reversible (Dragan *et al*., 1992; Walker *et al*., 1998, 2000). Studies also show that TCDD promotes more tumors in female rat liver than in male rat liver and that this is likely due to the enhancing effect of estrogens (Lucier *et al*., 1991; Wyde *et al*., 2001a,b, 2002). Comparison of the potency of PeCDF to induce the development of enzyme-altered hepatic foci within the rat liver in a subchronic study indicates that PeCDF is approximately five times less potent than TCDD for this endpoint (Waern *et al*., 1991).

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

Tumor promotion by PeCDF has not been evaluated in transgenic models. However, transgenic models have been used to examine the carcinogenicity of TCDD in mice (Eastin *et al*., 1998). These include the Tg.AC transgenic mouse that harbors an activated mouse v-Ha-*ras* oncogene (an intermediate in growth factor signaling). Dermal application of TCDD results in significant increases in the incidences of squamous cell papilloma in male and female Tg.AC mice, supporting the conclusions that TCDD is a tumor promoter. Subsequent studies by the NTP showed that the induction of papillomas and squamous cell carcinomas by dermal application of TCDD to hemizygous Tg.AC mice was dose-dependent (Van Birgelen *et al*., 1999; Dunson *et al*., 2000). In addition, skin papillomas are induced in this model when TCDD is administered orally.

In addition to the liver and skin, TCDD and PCDFs are tumor promoters in the lung (Anderson *et al*., 1991; Beebe *et al*., 1995). In Sprague-Dawley rats, which have a much lower spontaneous incidence of lung tumors, TCDD alone promotes the development of bronchiolar hyperplasia and alveolar-bronchiolar metaplasia (Tritscher *et al*., 2000). It was demonstrated that the induction of these lesions was reversible; incidences of these lesions returned to control levels following withdrawal of TCDD for 16 or 30 weeks.

Overall, these data demonstrate that the mode of action of PCDDs and PCDFs for carcinogenesis is probably as potent tumor promoters.

Mechanism and Biochemical Effects

PCDDs and PCDFs are generally classified as nongenotoxic and nonmutagenic. The common mechanism of action involves an initial binding to the AhR (Poland and Knutson, 1982; Safe, 1990; Whitlock, 1990; Schmidt and Bradfield, 1996). In general, the potency of effects of PCDDs and PCDFs exhibit a rank order potency similar to that seen for relative binding to the AhR. PeCDF has a binding affinity for the AhR (1.5×10^{-8} M) similar to TCDD. Therefore, much of the mechanism of action for PeCDF may be inferred based on the effects of TCDD on the AhR.

The broad spectrum of effects on hormone and growth factor systems, cytokines, and other signal transducer pathways indicates that PCDDs and PCDFs are powerful growth dysregulators (Birnbaum, 1994a). Since PCDDs and PCDFs are not directly genotoxic it is believed that the pathological responses associated with exposure are fundamentally due to binding to and activation of the AhR, subsequent alterations in the expression of AhR-regulated genes, and altered signaling of biological pathways that interact with the AhR signal transduction mechanism (Poland and Knutson, 1982).

Alterations in expression of AhR-regulated genes occur via a mechanism that involves a high-affinity interaction of ligand with an intracellular protein, the AhR, which functions as a ligand-activated transcription factor (Okey *et al*., 1994; Schmidt and Bradfield, 1996). Ligand binding initiates a signaling pathway in which the cytosolic AhR dissociates from heat shock proteins and translocates to the nucleus (Whitlock, 1993). At some point subsequent to ligand binding, the AhR associates with another protein, aromatic hydrocarbon nuclear translocator protein (ARNT) to form the nuclear DNA-binding and transcriptionally active AhR complex. Both the AhR and ARNT are members of the basic helix-loop-helix family of transcription factors (Hoffman *et al*., 1991; Burbach *et al*., 1992; Ema *et al*., 1992). The AhR-ARNT heterodimer binds with high affinity to a specific DNA sequence termed the dioxin response element (DRE). DREs have been identified in the enhancer regions of genes encoding several drug-metabolizing enzymes (Lai *et al*., 1996). The characteristic response to PCDDs and PCDFs is the transcriptional induction of the cytochome P450 1A1 gene (CYP1A1), which is mediated by binding of the AhR-ARNT complex to DREs present in the 5′flanking region of the gene. The AhR is expressed in all tissues examined (Dolwick *et al*., 1993) with a definite tissue specificity in terms of level of expression and diversity of response, indicating that PCDDs and PCDFs are likely to have some effect in every tissue. However, even with the same receptor and the same ligand, there are qualitative and quantitative differences in response, and these differences in response are likely to be involved in the tissue- and species-specificity of the response. How alterations in gene expression lead to the development of pathologies and adverse health effects associated with DLC exposure is still not known. However, it is generally accepted that most, if not all, responses require an initial step of binding to the AhR.

The most studied response to PCDDs and PCDFs is induction of the CYP1A1 class of cytochromes P450 (Whitlock, 1999). CYP1A1 is induced in most tissues including liver, lung, kidney, nasal passages, and small intestine with the highest induction in rat liver. Increased expression of CYP1A1 is a very sensitive response and serves as a useful marker for exposure to DLCs. PCDDs and PCDFs induce CYP1A1 *in vivo* and *in vitro* in human and animal models. CYP1A2 is constitutively expressed in the liver at low levels and inducible by DLCs in liver and possibly the nasal turbinates of rats (Goldstein and Linko, 1984). Induction of 7-ethoxyresorufin-*O*-deethylase activity is a marker of CYP1A1 activity. CYP1A2 is induced by DLCs and expressed primarily in the liver. Induction of acetanilide-4-hydroxylase activity is a marker of CYP1A2 activity. In addition to the well-characterized induction of CYP1A1 and CYP1A2, DLCs also induce another cytochrome P450, CYP1B1 in human cells (Sutter *et al*., 1994) and rodent tissues (Walker *et al*., 1995). CYP1B1 is active in the metabolism of numerous polycyclic aromatic hydrocarbons and arylamines and can catalyze the 4-hydroxylation of 17β-estradiol (Hayes *et al*., 1996; Murray *et al*., 2001).

PCDDs and PCDFs are believed to disrupt thyroid hormone homeostasis via the induction of the phase II enzymes, UDP-glucuronosyltransferases (UGTs). Thyroxine (T_4) production and secretion is controlled by thyroid stimulating hormone (TSH) which is under negative and positive regulation from the hypothalamus, pituitary gland, and thyroid gland by thyrotrophin releasing hormone, TSH itself, T_4, and triiodothyronine. Induction of the synthesis of UDP-glucuronosyltransferase-1 mRNA occurs by an AhR-dependent transcriptional mechanism. Consequently, a reduction in serum T_4 levels via an induction of UGT may lead to a decrease in the negative feedback inhibition on the pituitary gland. This would lead to a rise in secreted TSH, resulting in chronic hyperstimulation of the thyroid gland follicular cells.

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

Genetic Toxicology

PeCDF has not been tested in *Salmonella* reverse mutation assays. *In vivo,* PeCDF did not produce DNA adducts in rats treated with 100 µg/kg per week for 4 weeks. In humans highly exposed to mixtures of PeCDF contaminated with PCBs, sister chromatid exchange frequencies and chromosomal aberrations were similar in control and exposed populations. However, alpha-naphthoflavone-induced sister chromatid exchanges were higher in lymphocytes from individuals from the exposed population. No DNA adducts were detected in placentas from exposed individuals (IARC, 1997). By comparison, TCDD is negative in short-term tests for mutations and genotoxicity (Wassom *et al*., 1977; Whysner and Williams, 1996). Likewise, there is no consistent evidence for genotoxicity in humans exposed to TCDD. TCDD has been shown to possess only weak initiating activity in the two-stage CD-1 mouse skin assay using phorbol ester as a promoter (DiGiovanni *et al*., 1977). Although TCDD does not directly react with DNA, several studies have pointed to an increase in the formation of DNA damage (Tritscher *et al*., 1996; Wyde *et al*., 2001b), most likely through an indirect mechanism. TCDD has been shown to result in oxidative damage (Hassoun *et al*., 1998, 2000), and this is probably the reason for increased DNA strand breaks in livers of female rats exposed to lethal doses of TCDD (100 µg/kg) (Wahba *et al*., 1988). The evidence indicates that PeCDF is not directly genotoxic. Based on its similarity to TCDD, it may be inferred that PeCDF may have indirect genotoxic effects, depending upon the tissue or dose examined.

Study Rationale

The female Harlan Sprague-Dawley rat was selected as the model for the current study because this sex and species has been used frequently in chronic and subchronic studies of the action of dioxins. In addition, this was the model in which TCDD was demonstrated as a carcinogen in a gavage study conducted by Dow Chemical Company. Moreover, the incidence of liver tumors in female Harlan Sprague-Dawley rats has frequently been the primary rodent carcinogenicity dataset used by regulatory agencies worldwide for development of cancer risk guidelines for TCDD exposure. The doses chosen were based on the 1 to 100 ng/kg per day range used in the gavage study of TCDD where increased liver tumors were seen. These studies were not specifically designed to determine a no-observed-adverse-effect level or lowest-observed-adverse-effect level; rather, doses used in the present study were selected to increase dose-response data density in the 10 to 100 ng/kg range, where increases in liver and lung tumors were expected, to facilitate derivation of relative potency factors for carcinogenesis. Male rats were not studied due to the lack of induction of liver and lung tumors in the previous studies of Sprague-Dawley rats.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PeCDF

PeCDF was obtained from Cambridge Isotope Laboratories (Cambridge, MA) in two lots (CJJ-30319-43 and 29494-57), combined (labeled 080196), and used for the 2-year 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). Reports on analyses performed in support of the PeCDF study are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 080196 of the chemical, a white powder, was identified by the analytical chemistry laboratory as PeCDF by proton and carbon-13 nuclear magnetic resonance spectroscopy. The spectrum of the purity analysis sample was compared to that of a frozen reference sample of the same lot and a previously reported spectrum of the same lot. All spectra were consistent with the structure of PeCDF. The route of synthesis used to produce the test article allowed the exclusion of other isomers that are also consistent with the nuclear magnetic resonance data.

The purity of lot 080196 was determined by the analytical chemistry laboratory using gas chromatography coupled to high resolution mass spectrometry and by the study laboratory using gas chromatography with flame ionization detection.

The purity profile obtained by the analytical chemistry laboratory detected four impurities with individual relative areas greater than or equal to 0.1% and a total area of 2.4% relative to the major peak. One impurity (0.6% of peak area) was identified as a pentachlorodibenzofuran and one (0.4%) was identified as a hexachlorodibenzofuran. The other two impurities (1.4% combined) had none of the characteristics of PCBs, furans, or dioxins. The two furan impurities could not be unequivocally identified. Gas chromatography by the study laboratory indicated a purity of 101% when compared with the frozen reference sample. The overall purity of lot 080196 was determined to be 97% or greater.

To ensure stability, the bulk chemical was stored at room temperature, protected from light in amber glass bottles sealed with Teflon®-lined lids.

Formulation Materials

USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) in three lots and was used with corn oil (Spectrum Quality Products) as the vehicle in the 2-year gavage study. The identity of each lot was confirmed by the study laboratory using infrared spectroscopy. The purity of each lot was determined by GC 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 less than 3 mEq/kg.

PREPARATION OF STOCK SAMPLES

Lot 080196 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. Details concerning the preparation and use of these stock solutions are provided in Appendix C.

There were no stability studies performed on the bulk chemical, only periodic reanalysis by the study laboratory using gas chromatography. These studies indicated that PeCDF was stable as a bulk chemical for 25 months when stored protected from light at room temperature (approximately 25° C). No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving PeCDF working stocks in acetone, then diluting with corn oil (Spectrum Quality Products, Gardena, CA) to give the required concentrations (Table C2). The dose formulations were stored at room temperature in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids, for up to 35 days.

Homogeneity studies of 2.4 and 80 ng/mL dose formulations were performed by the study laboratory using gas chromatography. Stability studies of a 4.0 ng/mL dose formulation in corn oil containing 1% acetone were performed by the analytical chemistry laboratory with gas chromatography. Homogeneity was confirmed, and stability was confirmed for 35 days for dose formulations stored in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids, at –20° C, 5° C, and room temperature (approximately 25° C). Gavagability was confirmed for the 80 ng/mL dose formulation.

Periodic analyses of the dose formulations of PeCDF were conducted by the study laboratory using gas chromatography. During the 2-year study, the dose formulations were analyzed at least every 3 months (Table C3). Of the dose formulations analyzed, 42 of 53 were within 10% of the target concentrations; 15 of 21 animal room samples were within 10% of the target concentrations. Periodic analyses of the corn oil vehicle performed by the study laboratory demonstrated peroxide concentrations less than 3 mEq/kg.

2-Year Study

Study Design

Groups of 81 female rats received PeCDF in corn oil:acetone (99:1) by gavage at doses of 6, 20, 44, 92, or 200 ng/kg 5 days per week for up to 105 weeks; a group of 81 female rats received the corn oil:acetone (99:1) vehicle alone. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. For stop-exposure evaluation, a group of 50 female rats was given 200 ng/kg 5 days per week for 30 weeks then the vehicle for the remainder of the study.

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.

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 E). Sentinel rats included five males and five females at 1 month, five males at 6, 12, and 18 months, and five 200 ng/kg 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 was available *ad libitum*, and water was available *ad libitum* except during BrdU administration. 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 D.

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 of the study, 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 (except stop-exposure) and processed into serum for thyroid hormone determinations. Radioimmunoassays were performed for thyroid stimulating hormone, 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, NJ).

For cell proliferation analysis at 14, 31, and 53 weeks, up to 10 female rats per group (except stop-exposure 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 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 the vehicle control and 200 ng/kg groups by counting stained cells in the left lobe and right median lobe. Interlobular variation greater than 25% was considered significant. For the remaining rats, stained cells were counted only in the left lobe. Two thousand labeled and unlabeled hepatocyte nuclei were counted using a 20× objective and ocular grid. The labeling index was calculated as the percentage of BrdU labeled nuclei.

For determination of P450 activities, liver and lung tissue samples were collected from up to 10 rats per group (except stop-exposure) at 14, 31, and 53 weeks and stored frozen at –70° C. Microsomal suspensions were prepared using the Pearce method (Pearce *et al*., 1996). The concentration of protein in each suspension was determined using the 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 and by the acetanilide-4-hydroxylase activity assay.

Cytochrome P450 1A1 (CYP1A1)-associated 7-ethoxyresorufin-*O*-deethylase (EROD) 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) or nmol/min per mg (A4H) microsomal protein.

For analysis of tissue concentrations of PeCDF, 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. Concentrations of PeCDF in the tissue extracts were measured by capillary gas chromatography with high resolution mass spectrometry detection.

Complete necropsies and microscopic examinations were performed on all rats. At the interim evaluations, the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid gland were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 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, heart, kidney, liver, lung, oral mucosa, pancreas, stomach, thymus, thyroid gland, 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, the 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 among all the studies on dioxin-like compounds (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, four other studies (TCDD, PCB 126, the TEF mixture, and PCB 153; NTP, 2006a,b,c,d) were subjected to additional PWG review. 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 reviews 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 review 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. A different group of pathologists was also convened to provide additional guidance relative to the most appropriate classification of the hepatocellular proliferative lesions from these studies of DLCs. Participants included Drs. Jerrold Ward, Ernest McConnell, James Swenberg, Michael Elwell, Peter Bannasch, Douglas Wolf, John Cullen, and Rick Hailey. Final diagnoses for the hepatocellular proliferative lesions reflect the consensus of this complete review process.

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

Study Laboratory
Batelle Columbus Operations (Columbus, OH)

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

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

Time Held Before Study
13 days

Average Age When Study Began
8 weeks

Date of First Dose
April 7, 1999 (female rats only)

Duration of Dosing
5 days/week for 14, 31, 53 (interim evaluation), 30 (stop-exposure), or 105 weeks

Date of Last Dose
November 3, 1999 (stop-exposure)
April 2-4, 2001 (core study)

Necropsy Dates
April 3-5, 2001

Average Age at Necropsy
112 weeks

Size of Study Groups
81 (vehicle control, 6, 20, 44, 92, and 200 ng/kg), or 50 (200 ng/kg stop-exposure)

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

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

Method of Animal Identification
Tail tattoo

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

Water
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum* except during BrdU administration

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

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

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

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

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

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

Doses
0, 6, 20, 44, 92, or 200 ng/kg

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

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Necropsy was performed on all female rats. At the 14-, 31-, and 53-week interim evaluations, the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks 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 (except stop-exposure) 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 (except stop-exposure), 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 (except stop-exposure) for 7-ethoxyresorufin-*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 105 weeks, samples of blood, fat, liver, and lung were taken from up to 10 rats per group for analysis of PeCDF concentrations.

Histopathology
Complete histopathology was performed on all core study and stop-exposure rats at 2 years. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, harderian gland, heart, 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 vehicle control and 200 ng/kg rats. In the remaining dose groups, the following tissues were examined: the liver at 14, 31, and 53 weeks; the thymus at 31 and 53 weeks; and the uterus at 31 weeks.

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1a, A1b, A5a, and A5b as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3a and A3b) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the numbers of animals on which a necropsy was performed. Tables A3a and A3b also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Thyroid hormone, cell proliferation, and cytochrome P450 data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend sensitive

test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. For female Sprague-Dawley rats, the NTP historical database is limited to the seven gavage studies, conducted as part of the dioxin TEF evaluation (the current PeCDF study, TCDD, PCB 126, the TEF mixture, PCB 153, the binary mixture of PCB 126 and 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 studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year study were submitted to the NTP Archives, this study was audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

RESULTS

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for female rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 1). Survival of dosed groups was similar to that of the vehicle controls.

Body Weights and Clinical Findings

Mean body weights of 6 ng/kg rats were greater than those of the vehicle controls, and those of the 200 ng/kg core and stop-exposure groups were less than those of the vehicle controls during year 2 of the study (Figure 2 and Table 4). The mean body weights of the 44 and 92 ng/kg groups were less than those of the vehicle controls after week 73, and those of the 20 ng/kg group were generally similar to those of the vehicle controls throughout the study. No clinical findings related to chemical exposure were observed.

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

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Animals initially in study	81	81	81	81	81	81	50
14-Week interim evaluation[a]	10	10	10	10	10	10	0
31-Week interim evaluation[a]	10	10	10	10	10	10	0
53-Week interim evaluation[a]	8	8	8	8	8	8	0
Accidental deaths[a]	0	1	2	1	0	0	0
Moribund	21	20	17	19	19	19	25
Natural deaths	7	10	10	8	14	11	10
Animals surviving to study termination	25	22	24	25	20	23	15
Percent probability of survival at end of study[b]	47	42	47	48	38	43	30
Mean survival (days)[c]	655	630	606	635	603	616	605
Survival analysis[d]	P=0.089	P=0.531	P=0.765	P=0.823	P=0.272	P=0.470	P=0.057

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. The stop-exposure group is excluded from the trend test.

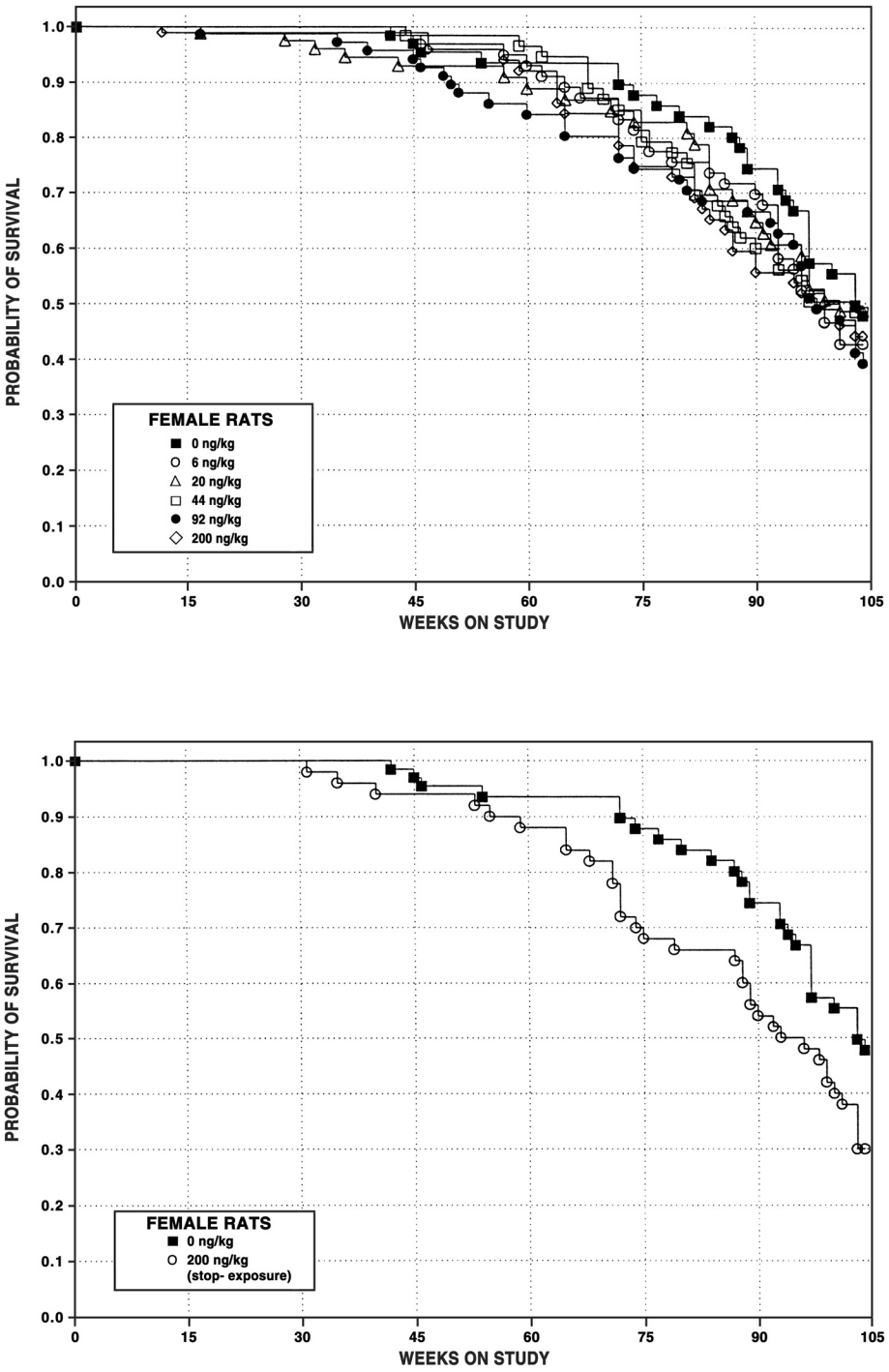


FIGURE 1
Kaplan-Meier Survival Curves for Female Rats Administered PeCDF by Gavage for 2 Years

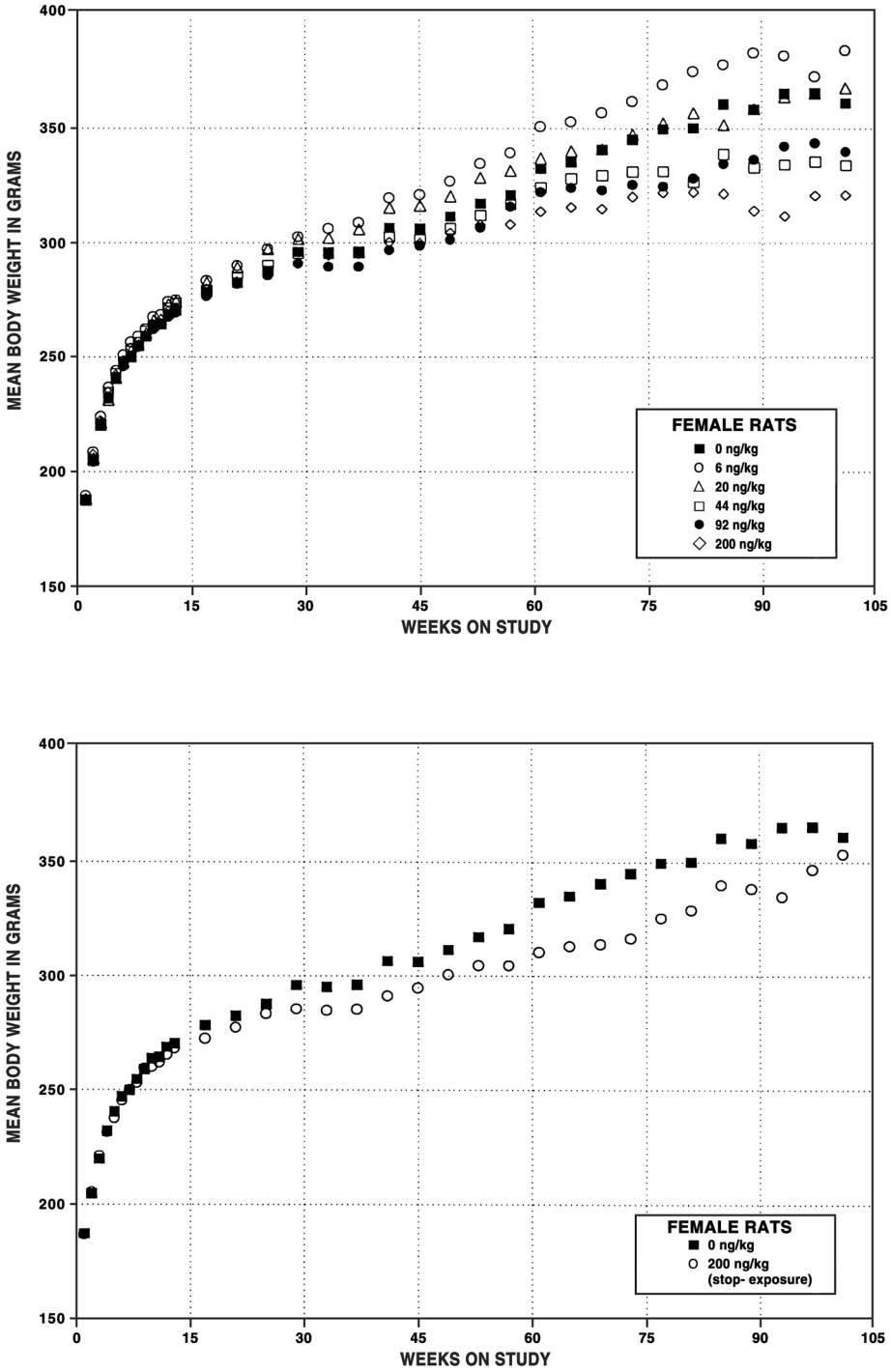


FIGURE 2
Growth Curves for Female Rats Administered PeCDF by Gavage for 2 Years

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

Weeks on Study	Vehicle Control		6 ng/kg			20 ng/kg			44 ng/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	187	98	190	101	98	188	101	98	188	100	98
2	205	98	209	102	98	206	101	98	206	100	98
3	220	98	224	102	98	221	101	98	221	100	98
4	232	98	237	102	98	231	100	98	235	101	98
5	241	98	244	102	98	241	100	98	243	101	98
6	247	98	251	102	98	248	100	98	248	100	98
7	250	98	257	103	98	253	101	98	254	101	98
8	255	98	259	102	98	255	100	98	256	101	98
9	259	98	262	101	98	260	101	98	261	101	98
10	264	98	268	101	98	266	101	98	264	100	98
11	265	98	269	102	98	267	101	98	265	100	98
12	269	98	274	102	98	273	102	98	271	101	98
13	270	98	275	102	98	274	101	98	274	101	98
17[a]	278	82	284	102	82	283	102	82	279	100	82
21	283	82	290	103	82	289	102	81	285	101	82
25	288	82	297	103	82	297	103	81	290	101	82
29	296	82	303	102	82	302	102	80	295	100	82
33[a]	295	66	306	104	66	302	102	63	296	100	66
37	296	66	309	104	66	306	103	62	296	100	66
41	307	66	320	104	66	315	103	62	303	99	66
45	306	65	321	105	64	316	103	59	302	98	65
49	312	63	327	105	63	320	103	59	306	98	65
53[a]	317	63	335	106	63	329	104	59	312	98	65
57	321	49	339	106	49	332	103	45	317	99	52
61	333	49	351	106	48	337	101	44	324	98	51
65	335	49	353	105	47	340	102	44	328	98	50
69	341	49	357	105	45	341	100	43	330	97	46
73	345	47	362	105	43	348	101	42	331	96	44
77	350	46	369	106	40	353	101	41	331	95	41
81	350	44	375	107	39	357	102	41	327	93	40
85	361	43	378	105	38	352	98	35	339	94	35
89	359	41	383	107	37	359	100	34	333	93	32
93	366	39	382	105	35	364	100	30	334	92	31
97	366	35	373	102	27	366	100	29	336	92	28
101	361	29	385	106	24	368	102	25	334	93	26
Mean for weeks											
1-13	243		248	103		245	101		246	101	
14-52	296		306	104		303	103		295	100	
53-101	347		365	105		350	101		329	95	

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

Weeks on Study	92 ng/kg			200 ng/kg			200 ng/kg (Stop-Exposure)		
	Av. Wt. (g)	Wt. (% of controls	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	187	100	98	188	100	98	187	100	50
2	204	100	98	208	101	98	206	100	50
3	221	100	98	222	101	98	221	101	50
4	233	100	98	235	101	98	232	100	50
5	241	100	98	242	101	98	238	99	50
6	246	100	98	249	101	98	246	99	50
7	251	100	98	254	102	98	250	100	50
8	254	100	98	257	101	98	253	99	50
9	259	100	98	259	100	98	260	100	50
10	262	99	98	264	100	98	260	99	50
11	264	100	98	265	100	98	262	99	50
12	268	100	98	270	100	97	266	99	50
13	269	100	98	272	101	97	268	99	50
17[a]	277	99	82	280	101	81	273	98	50
21	282	100	81	283	100	81	277	98	50
25	286	99	81	287	100	81	284	99	50
29	291	98	81	291	98	81	286	97	50
33[a]	290	98	65	295	100	65	285	97	49
37	290	98	64	295	100	65	286	96	48
41	297	97	63	300	98	65	291	95	47
45	299	98	63	300	98	65	295	96	47
49	301	97	60	304	98	63	301	97	47
53[a]	307	97	58	308	97	63	305	96	47
57	316	98	44	308	96	50	305	95	45
61	322	97	43	314	94	48	311	93	44
65	324	97	43	316	94	45	313	93	44
69	323	95	41	315	92	44	314	92	41
73	326	94	39	320	93	41	317	92	36
77	325	93	38	322	92	39	326	93	34
81	328	94	37	322	92	38	329	94	33
85	335	93	35	322	89	34	340	94	33
89	337	94	35	314	88	31	339	94	30
93	342	94	33	312	85	29	335	92	26
97	344	94	29	321	88	27	347	95	24
101	340	94	25	321	89	26	354	98	19
Mean for weeks									
1-13	243	100		245	101		242	100	
14-52	290	98		293	99		286	97	
53-101	328	95		317	91		326	94	

[a] Interim evaluations occurred during weeks 14, 31, and 53; until week 53, number of survivors includes 17 special study animals (except stop-exposure group).

Thyroid Hormone Concentrations

Assays for total thyroxine (T_4), free T_4, total triiodothyronine (T_3), and thyroid stimulating hormone (TSH) were conducted at the 14-, 31-, and 53-week interim evaluations (Table 5). A downward trend in serum total T_4 concentrations with higher PeCDF concentrations was evident at 14 weeks. Total T_4 levels in the 92 and 200 ng/kg dose groups were significantly lower than those in vehicle controls by 25.1% and 24.5%, respectively. There were no significant differences observed in serum free T_4, T_3, or TSH concentrations between PeCDF-treated groups and vehicle controls at 14 weeks.

At 31 weeks, total T_4 concentrations were significantly lower in all PeCDF-treated groups than in the vehicle controls. These decreases were more pronounced with increased doses of PeCDF. The maximal decrease was observed in the 200 ng/kg group, which was 40.0% lower than vehicle control values. Serum free T_4 concentrations were lower at all doses of PeCDF compared to vehicle controls, but only significantly lower in the 200 ng/kg group. There was an increasing trend in serum T_3 with increasing doses of PeCDF at 31 weeks. Serum T_3 was significantly higher in the 92 and 200 ng/kg groups than vehicle controls by 24.7% and 38.1%, respectively. Serum TSH levels in the 6, 20, 92, and 200 ng/kg groups were lower, but not significantly different, than vehicle control values.

At the 53-week interim evaluation, a downward trend in serum total T_4 concentrations with higher PeCDF concentrations was evident. Statistically significant depressions in mean total T_4 concentrations relative to vehicle controls were observed in the 44, 92, and 200 ng/kg groups. Serum free T_4 concentrations were significantly lower at doses equal to or greater than 44 ng/kg compared to vehicle controls. These changes were 17.4%, 21.9%, and 9.0% lower than vehicle controls for the 44, 92, and 200 ng/kg groups, respectively. An upward trend in serum T_3 with higher PeCDF concentrations was

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

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
Week 14						
n	10	10	10	10	10	10
Total T_4 (µg/dL)	5.060 ± 0.261	4.700 ± 0.302	4.720 ± 0.377	4.590 ± 0.251	3.790 ± 0.204**	3.820 ± 0.389**
Free T_4 (ng/dL)	2.246 ± 0.102	2.241 ± 0.181	2.258 ± 0.194	2.064 ± 0.238	2.000 ± 0.146	2.123 ± 0.204
Total T_3 (ng/dL)	116.553 ± 6.770	108.947 ± 6.560	120.689 ± 7.152	118.444 ± 9.143	123.614 ± 9.773	134.280 ± 7.653
TSH (ng/mL)	9.319 ± 0.904	10.744 ± 1.228[b]	10.886 ± 0.962	9.053 ± 0.637	9.071 ± 0.719	10.630 ± 0.820
Week 31						
n	10	10	10	10	10	10
Total T_4 (µg/dL)	4.170 ± 0.104	3.520 ± 0.149**	3.410 ± 0.205**	3.120 ± 0.326**	2.960 ± 0.244**	2.500 ± 0.223**
Free T_4 (ng/dL)	2.055 ± 0.141	1.910 ± 0.115	1.830 ± 0.150	1.798 ± 0.163	1.887 ± 0.100	1.590 ± 0.107*
Total T_3 (ng/dL)	137.856 ± 6.042	149.003 ± 6.785	140.961 ± 5.555	149.127 ± 8.801	171.848 ± 8.041**	190.375 ± 5.404**
TSH (ng/mL)	18.596 ± 2.295	12.403 ± 1.213	14.596 ± 1.324	18.782 ± 2.267	14.278 ± 0.963	16.174 ± 1.562
Week 53						
n	8	8	8	8	8	8
Total T_4 (µg/dL)	3.313 ± 0.136	3.238 ± 0.143	3.413 ± 0.279	2.600 ± 0.280*	2.163 ± 0.086**	2.188 ± 0.416**
Free T_4 (ng/dL)	1.796 ± 0.127	1.713 ± 0.097	1.818 ± 0.104	1.484 ± 0.082*	1.403 ± 0.072**	1.634 ± 0.218*
Total T_3 (ng/dL)	153.151 ± 4.779	151.755 ± 5.888	160.836 ± 14.004	167.289 ± 4.608[c]	177.601 ± 8.316*	181.746 ± 6.340**
TSH (ng/mL)	11.766 ± 0.906	10.656 ± 0.613	12.169 ± 1.053	12.193 ± 1.099	10.146 ± 0.897	11.654 ± 0.599

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's or 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] n=8
[c] n=7

observed at doses of 20 ng/kg or greater. These increases were statistically significant for the 92 and 200 ng/kg groups, which exceeded control values by 16.0% and 18.7%, respectively. There were no statistically significant changes in serum TSH levels for any of the PeCDF-treated groups compared to vehicle controls at 53 weeks.

Hepatic Cell Proliferation Data

Hepatocellular proliferation data at the 14-, 31-, and 53-week interim evaluations are included in Table 6. Consumption of the BrdU drinking water solutions prior to each interim evaluation was similar across groups. At 14 weeks, the hepatocellular labeling index was significantly higher (2.5-fold) in the 200 ng/kg group compared to the vehicle control group. No significant differences were observed between the other PeCDF treatment groups and the vehicle controls. The labeling index in hepatocytes was similar in all dose groups at the 31-week interim evaluation compared to the vehicle controls. At 53 weeks, the labeling index was elevated in all treatment groups compared to the vehicle controls. A statistically significant increase in labeling index at 53 weeks was observed only at 200 ng/kg, which was 2.9-fold higher than vehicle controls.

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

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Labeling index (%)						
Week 14	1.045 ± 0.187	1.070 ± 0.120	1.481 ± 0.349	1.758 ± 0.285	1.147 ± 0.205[b]	2.669 ± 0.540**
Week 31	1.746 ± 0.525	1.108 ± 0.192	1.090 ± 0.097[b]	1.398 ± 0.218	1.789 ± 0.239	1.402 ± 0.217
Week 53	1.063 ± 0.131	2.270 ± 0.426	1.936 ± 0.283	1.535 ± 0.240	1.758 ± 0.245	3.103 ± 0.408**

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

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 and acetanilide-4-hydroxylase (A4H, CYP1A2) activity. Microsomal samples from lung were analyzed for EROD activity only (Table 7).

Hepatic EROD and A4H activities were significantly higher in all groups administered PeCDF relative to the vehicle control group at the 14-, 31-, and 53-week interim evaluations. Significant induction of hepatic P450 activities occurred at the lowest dose (6 ng/kg) for all three sampling times (14, 31, and 53 weeks). Hepatic EROD activities were increased with respect to dose and were maximally induced in the 200 ng/kg dose groups; 44-, 57-, and 47-fold at 14, 31, and 53 weeks, respectively. Similarly, hepatic A4H activity was higher with increasing doses of PeCDF and was maximally induced in the 200 ng/kg group; 5.6-, 6.3-, and 4.6-fold at 14, 31, and 53 weeks, respectively.

EROD activity in the lung was significantly higher in all dose groups compared to vehicle controls at the 14-, 31-, and 53-week interim evaluations. At the lowest dose (6 ng/kg), pulmonary EROD activities were 6-fold higher than vehicle controls at 14 weeks, 41-fold higher at 31 weeks, and 18-fold higher at 53 weeks. Maximal values for pulmonary EROD activities were 24- (200 ng/kg), 86- (92 ng/kg), and 41-fold (200 ng/kg) higher than vehicle controls at 14, 31, and 53 weeks, respectively.

TABLE 7
Liver and Lung Cytochrome P450 Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Liver Microsomes						
Acetanilide-4-hydroxylase (nmol/minute per mg microsomal protein)						
Week 14	0.353 ± 0.025	0.520 ± 0.039**	0.790 ± 0.056**	0.953 ± 0.044**	1.322 ± 0.082**	1.969 ± 0.062**
Week 31	0.418 ± 0.026	0.517 ± 0.031*	0.852 ± 0.032**	1.119 ± 0.051**	1.921 ± 0.131**	2.634 ± 0.085**
Week 53	0.579 ± 0.033	0.794 ± 0.074*	1.240 ± 0.103**	1.464 ± 0.066**	1.803 ± 0.143**	2.642 ± 0.265**
7-Ethoxyresorufin-*O*-deethylase (pmol/minute per mg microsomal protein)						
Week 14	50.854 ± 4.759	243.673 ± 16.387**	689.850 ± 60.062**	1,159.81 ± 70.582**	1,713.83 ± 83.182**	2,245.94 ± 53.123**
Week 31	61.656 ± 2.775	273.042 ± 17.474**	896.659 ± 74.974**	1,546.31 ± 83.353**	2,160.98 ± 105.776**	3,506.44 ± 182.245**
Week 53	77.659 ± 3.251	444.311 ± 20.003**	1,022.05 ± 108.954**	1,656.16 ± 86.795**	2,372.65 ± 227.330**	3,674.19 ± 401.297**
Lung Microsomes						
7-Ethoxyresorufin-*O*-deethylase (pmol/minute per mg microsomal protein)						
Week 14	2.916 ± 0.325	18.608 ± 1.314**	26.149 ± 2.797**	47.181 ± 3.008**	58.637 ± 3.042**	70.244 ± 6.320**
Week 31	0.779 ± 0.054	31.655 ± 6.401**	48.026 ± 2.403**	55.041 ± 4.065**	66.941 ± 3.680**	65.055 ± 4.569**
Week 53	1.480 ± 0.121	26.481 ± 2.533**	41.529 ± 5.618**	51.420 ± 4.891**	56.790 ± 3.847**	60.949 ± 4.568**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's or Dunn's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Determinations of PeCDF Concentrations in Tissues

Concentrations of PeCDF were determined for all dose groups in liver, lung, and fat at the 14-, 31-, and 53-week interim evaluations and at the end of the 2-year study (105 weeks) (Table 8). Concentrations of PeCDF were determined in blood for the 44, 92, and 200 ng/kg groups at all time points and the 200 ng/kg stop-exposure group at 105 weeks. The highest concentrations of PeCDF were observed in the liver, followed by adipose tissue. In vehicle control liver, PeCDF concentrations were undetectable at all of the interim evaluations, but at 105 weeks, hepatic concentrations of PeCDF were 1,615 pg/g. Hepatic concentrations increased with increasing doses of PeCDF at each time point. The highest liver concentrations were observed in the 200 ng/kg group. In liver tissue from the stop-exposure group, the PeCDF concentration was 35,773 pg/g, which was between the levels observed in the 6 (17,615 pg/g) and 20 ng/kg groups (62,457 pg/g).

In the fat of vehicle controls, PeCDF concentrations were below the experimental limit of detection at all of the interim evaluations and at 105 weeks. In treated groups, adipose concentrations were higher with increasing doses of PeCDF. The highest concentrations were observed in the 200 ng/kg group. In the adipose tissue of the stop-exposure group, the PeCDF concentration was 1,467 pg/g, which was between the levels observed in the 6 (1,005 pg/g) and 20 ng/kg groups (1,943 pg/g).

No measurable lung concentrations of PeCDF were detected in vehicle controls at any time point, in any of the PeCDF-treated groups at 14 weeks, or in the 6 ng/kg group at 31 or 53 weeks. Lung concentrations of PeCDF were lower than those observed in liver and fat. In groups with measurable levels, concentrations were higher with respect to increasing doses of PeCDF. Maximal lung concentrations of 345, 241, and 282 pg/g were observed in the 200 ng/kg group at 31, 53, and 105 weeks, respectively. In the stop-exposure group, the PeCDF concentration was 89 pg/g, which was lower than the level observed in the 6 ng/kg (138 pg/g) group.

No measurable concentrations of PeCDF were observed in blood from the groups evaluated at 14 and 31 weeks. PeCDF concentrations were negligible in the 200 ng/kg group at 53 weeks and the 92 and 200 ng/kg groups at 105 weeks. No measurable concentration was observed in the blood from the stop-exposure group.

TABLE 8
Tissue Concentrations of PeCDF in Female Rats in the 2-Year Gavage Study of PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
n							
Week 14	10	10	10	10	10	10	
Week 31	10	10	10	10	10	10	
Week 53	8	8	8	8	8	8	
Week 105	10	10	10	10	10	10	10
Fat							
Week 14	BLOQ	379.5 ± 40.2	833.5 ± 169.5[b]	1,301 ± 446	1,840 ± 443	3,523 ± 570	
Week 31	BLOQ	533.1 ± 145.6	1,286 ± 327	2,089 ± 259	3,361 ± 390	5,698 ± 328	
Week 53	BLOQ	632.0 ± 128.4	1,391 ± 197	2,029 ± 254	3,307 ± 565	5,582 ± 969	
Week 105	BLOQ	1,005 ± 392	1,943 ± 535	2,761 ± 526	5,095 ± 849	7,753 ± 1,193	1,467 ± 251
Liver							
Week 14	BLOQ	4,967 ± 1,308	17,245 ± 5,576	29,781 ± 12,610	70,351 ± 23,129	132,943 ± 47,405	
Week 31	BLOQ	14,162 ± 1,900	49,271 ± 5,271	130,546 ± 14,004	264,947 ± 30,140	543,177 ± 27,228	
Week 53	BLOQ	15,206 ± 2,120	48,956 ± 5,521	108,411 ± 12,544	232,489 ± 15,080	429,922 ± 17,771	
Week 105	1,615[c]	17,615 ± 3,525	62,457 ± 9,466	125,110 ± 25,904	263,557 ± 35,999	499,565 ± 85,356	35,773 ± 6,011
Lung							
Week 14	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	
Week 31	BLOQ	BLOQ	80.72 ± 20.81[b]	126.7 ± 21.7	195.2 ± 48.4	345.1 ± 41.0	
Week 53	BLOQ	BLOQ	77.17 ± 5.86[e]	115.9 ± 17.9	161.6 ± 19.0	240.5 ± 27.7	
Week 105	BLOQ	137.6 ± 139.1[d]	165.3 ± 95.9	233.3 ± 197.9	264.4 ± 127.5	281.7 ± 105.0	88.97 ± 28.60[f]
Blood							
Week 14	NA	NA	NA	BLOQ	BLOQ	BLOQ	
Week 31	NA	NA	NA	BLOQ	BLOQ	BLOQ	
Week 53	NA	NA	NA	BLOQ	BLOQ	34.88 ± 1.40[d]	
Week 105	NA	NA	NA	BLOQ	38.41 ± 5.14[g]	42.98 ± 6.77[f]	BLOQ

[a] Data are given in pg/g tissue (fat, liver, lung) or pg/mL (blood) as the mean ± standard deviation. Mean values do not include values that were below the experimental limit of quantitation. NA=not analyzed; BLOQ=below the limit of quantitation; LOQ_{fat}=187.5 pg/g, LOQ_{liver}=375 pg/g, LOQ_{lung}=60 pg/g, LOQ_{blood}=30 pg/mL
[b] n=9
[c] n=2
[d] n=4
[e] n=6
[f] n=5
[g] n=3

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, oral mucosa, uterus, lung, pancreas, mammary gland, pituitary gland, thyroid gland, thymus, adrenal cortex, kidney, heart, forestomach, and bone marrow. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A.

Liver: The absolute liver weight of the 200 ng/kg group was significantly greater than that of the vehicle control group at 14 weeks (Table B1). Relative liver weights at 14 weeks were significantly greater than vehicle controls in all dosed groups except the 44 ng/kg group. At the 31-week interim evaluation, absolute liver weights were increased at doses of 20 ng/kg and greater, but were not significantly different from vehicle controls. Relative liver weights were significantly greater in the 20 ng/kg or greater groups compared to vehicle controls. At 53 weeks, the absolute liver weight was significantly increased in the 200 ng/kg group. Relative liver weights at 53 weeks were significantly greater than those of the vehicle controls at doses of 44 ng/kg or greater.

At 14 weeks, increased incidences of hepatocellular hypertrophy occurred in all dosed groups and tended to correlate with increased liver weight (Tables 9 and A5a). Other treatment-related lesions included diffuse fatty change and pigmentation in the 200 ng/kg group.

TABLE 9
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 PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
14-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy[a]	0	1 (1.0)[b]	3 (1.0)	3 (1.0)	4* (1.0)	7** (1.0)
Fatty Change, Diffuse	0	0	0	0	0	2 (1.0)
Pigmentation	0	0	0	0	0	2 (1.0)
31-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	1 (1.0)	3 (1.0)	6** (1.0)	8** (1.0)	8** (1.1)
Pigmentation	0	1 (1.0)	3 (1.0)	4* (1.0)	7** (1.0)	8** (1.0)
Cholangiofibrosis	0	0	0	0	0	1 (1.0)
Eosinophilic Focus	0	0	0	0	0	1
Bile Duct, Fibrosis	0	0	0	0	0	1 (1.0)
53-Week Interim Evaluation						
Number Examined Microscopically	8	8	8	8	8	8
Hepatocyte, Hypertrophy	1 (1.0)	3 (1.0)	3 (1.0)	3 (1.0)	5 (1.0)	7* (1.4)
Hepatocyte, Multinucleated	0	0	0	0	1 (1.0)	2 (1.0)
Pigmentation	0	1 (1.0)	1 (1.0)	6** (1.0)	7** (1.0)	8** (1.4)
Bile Duct, Hyperplasia	0	0	0	0	1 (1.0)	1 (1.0)
Fatty Change, Diffuse	0	0	0	0	0	3 (1.0)
Bile Duct, Cyst	0	0	0	0	0	2 (1.0)
Eosinophilic Focus	0	0	0	0	0	1
Toxic Hepatopathy	0	0	0	0	0	1 (1.0)

* 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

At 31 weeks, dose-related increased incidences of hepatocellular hypertrophy occurred in all dosed groups and tended to correlate with increased liver weight (Tables 9 and A5a). The incidences of pigmentation were significantly increased in rats administered 44 ng/kg or greater. There were single occurrences of cholangiofibrosis, eosinophilic focus, and bile duct fibrosis in the 200 ng/kg group.

At 53 weeks, dose-related increased incidences of hepatocellular hypertrophy occurred in all dosed groups and tended to correlate with increased liver weight (Tables 9 and A5a). The incidences of pigmentation were significantly increased in rats administered 44 ng/kg or greater. Sporadic occurrences of the following were observed: multinucleated hepatocytes and bile duct hyperplasia in the 92 and 200 ng/kg groups, and diffuse fatty change in the 200 ng/kg group. In the 200 ng/kg group, the occurrence of bile duct cysts, one occurrence of eosinophilic focus, and one occurrence of minimal toxic hepatopathy were observed.

At 2 years, there was a dose-dependent increase in the incidences of hepatocellular adenoma (Tables 10, A1b, and A3a). A single occurrence was observed in the vehicle control and 200 ng/kg stop-exposure groups. The incidence in the 200 ng/kg core study group exceeded the historical vehicle control range (Tables 10 and A4a). One, one, and two cholangiocarcinomas (single or multiple) were seen in the 44, 92, and 200 ng/kg core study groups, respectively. No cholangiocarcinomas have occurred in the historical vehicle controls (Tables 10 and A4a). Cholangiofibrosis was observed in all dosed groups except the 20 ng/kg group (Tables 10 and A5b), and the incidence in the 200 ng/kg core study group was significantly greater than that in the vehicle controls.

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. 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 relatively normal sized, somewhat pleomorphic hepatocytes that were arranged in abnormal lobular patterns, and the lack of proliferating biliary epithelium were important features differentiating adenoma from nodular hyperplasia.

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 (Plates 1 and 2). The epithelium forming the atypical bile ducts was often discontinuous, consisted usually of large atypical cells, and displayed degenerative changes. Mitotic figures and localized invasion of adjacent liver parenchyma were also observed. Cholangiofibrosis appeared similar to cholangiocarcinoma but was a much smaller, well demarcated lesion which did not show evidence of localized invasion.

At 2 years, the incidences of hepatocellular hypertrophy were significantly increased in all dosed groups, and increased incidences of multinucleated hepatocytes, diffuse fatty change, and pigmentation were observed in all dosed groups except the 6 ng/kg group (Tables 10 and A5b). The incidences of toxic hepatopathy and oval cell hyperplasia were significantly increased in the 44, 92, and 200 ng/kg core study groups. Nodular hyperplasia was present in groups administered 44 ng/kg or greater, and the incidences were significantly elevated in the 92 and 200 ng/kg core study groups.

Increased incidences of eosinophilic focus (single or multiple) were observed in the 92 and 200 ng/kg core and stop-exposure study groups, and increased incidences of necrosis were seen in the 200 ng/kg core study and stop-exposure groups. Increased incidences, as compared to vehicle controls, of bile duct hyperplasia and fibrosis were observed in the 200 ng/kg core study group.

In the 200 ng/kg stop-exposure group, treatment-related changes were still present at higher incidences than in vehicle controls, but at significantly lower incidences than in the 200 ng/kg core study group. These included hepatocyte hypertrophy, oval cell hyperplasia, diffuse fatty change, nodular hyperplasia, bile duct hyperplasia, and toxic hepatopathy. The incidences of multinucleated hepatocytes, necrosis, and bile duct fibrosis were also decreased in the 200 ng/kg stop-exposure group compared to the 200 ng/kg core study group, but not significantly.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Number Examined							
Microscopically	53	53	53	52	53	53	50
Hepatocyte, Hypertrophy[a]	2 (1.0)[b]	13** (1.0)	17** (1.0)	17** (1.0)	24** (1.0)	34** (1.2)	14**▲▲ (1.0)
Hepatocyte, Multinucleated	0	0	4* (1.0)	13** (1.0)	18** (1.3)	35** (1.2)	25** (1.2)
Oval Cell, Hyperplasia	1 (1.0)	4 (1.3)	2 (1.0)	6* (1.2)	15** (1.1)	35** (1.4)	3▲▲ (1.0)
Fatty Change, Diffuse	1 (1.0)	4 (1.3)	10** (1.4)	12** (1.1)	20** (1.1)	26** (1.3)	6*▲▲ (1.2)
Pigmentation	13 (1.1)	11 (1.1)	21* (1.3)	44** (1.5)	42** (1.8)	48** (1.9)	48** (1.6)
Hyperplasia, Nodular	0	0	0	3	8**	12**	0▲▲
Eosinophilic Focus (includes multiple)	15	13	18	18	23	28**	22*
Necrosis	4 (2.5)	10 (2.1)	3 (2.0)	3 (2.3)	6 (2.0)	18** (1.8)	11* (2.1)
Bile Duct, Hyperplasia	3 (1.3)	2 (1.0)	2 (1.0)	2 (1.0	1 (1.0)	13** (1.6)	1▲▲ (2.0)
Bile Duct, Fibrosis	1 (1.0)	4 (1.8)	2 (1.0)	2 (1.5)	3 (1.0)	6* (1.3)	1 (1.0)
Toxic Hepatopathy	0	2 (1.0)	3 (1.0)	8** (1.1)	27** (1.1)	44** (1.7)	9**▲▲ (1.0)
Cholangiofibrosis	0	1 (1.0)	0	3 (1.3)	3 (1.3)	5* (2.2)	3 (2.7)
Hepatocellular Adenoma[c]							
Overall rate[d]	1/53 (2%)	0/53 (0%)	1/53 (2%)	0/52 (0%)	2/53 (4%)	4/53 (8%)	1/50 (2%)
Adjusted rate[e]	2.4%	0.0%	2.7%	0.0%	5.5%	10.9%	3.0%
Terminal rate[f]	0/25 (0%)	0/22 (0%)	1/24 (4%)	0/25 (0%)	2/20 (10%)	3/23 (13%)	1/15 (7%)
First incidence (days)	678	—[i]	728 (T)	—	728 (T)	689	728 (T)
Poly-3 test[g]	P=0.006	P=0.519N	P=0.730	P=0.520N	P=0.448	P=0.140	P=0.705
Poly-3 test[h]							P=0.218N
Cholangiocarcinoma, Multiple	0	0	0	0	0	1	0
Cholangiocarcinoma (includes multiple)[j]							
Overall rate	0/53 (0%)	0/53 (0%)	0/53 (0%)	1/52 (2%)	1/53 (2%)	2/53 (4%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.6%	2.8%	5.4%	0.0%
Terminal rate	0/25 (0%)	0/22 (0%)	0/24 (0%)	1/25 (4%)	1/20 (5%)	1/23 (4%)	0/15 (0%)
First incidence (days)	—	—[k]	—	728 (T)	728 (T)	608	—
Poly-3 test	P=0.036	—	—	P=0.481	P=0.471	P=0.211	—
Poly-3 test							P=0.278N

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
▲▲ Significantly different (P≤0.01) from the 200 ng/kg core study group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 4/371 (1.1% ± 1.5%), range 0%-4%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N.**
[h] Pairwise comparison between the 200 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 0/371
[k] Value of statistic cannot be computed.

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

Oval cell hyperplasia consisted of small ovoid cells, with basophilic cytoplasm and round to ovoid nuclei, that were arranged in single or double rows and located predominantly in the portal areas. 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 hepatocytes scattered diffusely throughout the liver.

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 stained positive for iron with the Perl's stain.

Nodular hyperplasia was characterized by areas of focal hypertrophy and hyperplasia of hepatocytes that also contained proliferating bile ducts and was considered to be the result of the presence of a proliferative stimulus. Areas of nodular hyperplasia varied in size with some areas being quite large while others were smaller and were the size of larger foci. Areas of nodular hyperplasia sometimes blended with the surrounding parenchyma, although often they had distinct borders. 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 vacuolation. 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. Biliary epithelium 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. Hypertrophic, vacuolated hepatocytes with proliferating biliary epithelium were considered to be characteristic of nodular hyperplasia and were characteristics useful in differentiating nodular hyperplasia from adenoma. Nodular hyperplasia was seen most commonly in the higher dose groups in which toxic changes were more prominent. However, a lesser degree of nodular hyperplasia was sometimes seen in lower dose animals in other studies in the dioxin TEF evaluation in which toxic changes were minimal to inapparent. This suggested that nodular hyperplasia resulted from the presence of an hepatocellular proliferative stimulus that may have been independent of the toxic changes, but that the severity of the nodular hyperplasia was increased by toxicity.

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. Foci in vehicle control livers 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 (Plate 3). In contrast, foci in treated animals generally had a more definite border, the cords within the foci 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 vacuolated cytoplasm. In addition, some larger foci caused variable degrees of compression of the surrounding hepatic parenchyma. The cells were arranged in a relatively normal lobular pattern and foci sometimes contained large blood vessels and/or portal areas. The presence of proliferating bile ducts or oval cells was not considered characteristic of a focus. If proliferating bile ducts were present, they were considered indicative of nodular hyperplasia, described previously.

Necrosis consisted of scattered areas of necrotic hepatic parenchyma that were often randomly distributed, but occasionally, in more severe cases, were distributed more diffusely. Bile duct hyperplasia consisted of increased numbers of bile ducts, usually in portal areas.

Bile duct fibrosis was characterized by accumulation of fibrous connective tissue surrounding bile ducts. It was sometimes accompanied by a decrease in the height or number of epithelial cells lining the ducts and by bile duct hyperplasia.

Toxic hepatopathy included all nonneoplastic liver changes under one term. The severity of the toxic hepatopathy was graded in order to give one overall severity grade for the degree of toxicity in a liver. The purpose of this was to allow for easier comparison of the degree of toxic change among different dose groups than would be possible if the severities of all the individual nonneoplastic changes had to be compared among the different groups. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. The changes included under the diagnosis were focal cellular alteration, multinucleated hepatocytes, cystic degeneration, fatty change, inflammation, necrosis, pigmentation, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, hepatocyte degeneration, hepatocyte hypertrophy, oval cell hyperplasia, and portal fibrosis. The livers of some treated animals, especially those in lower dose groups, contained hepatocellular hypertrophy with slight inflammation and pigmentation and sometimes an occasional focus. However, these changes were also seen in interim sacrifice animals in which toxic changes were not yet considered to be present, thus the presence of these changes alone was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy. Additional changes indicative of a treatment-related effect, such as oval cell or bile duct hyperplasia or numerous foci scattered diffusely within the liver, needed to be present before toxic hepatopathy was diagnosed.

Oral Mucosa: At 2 years, three gingival squamous cell carcinomas were seen in the 200 ng/kg core and stop-exposure groups compared to one occurrence in the vehicle control group (Tables 11, A1b, A3a, and A3b). While there were no statistically significant changes in the incidences of squamous cell carcinoma in any dose group relative to the vehicle controls, the lesions were considered to be related to treatment, and the incidences in the 6 and 200 ng/kg core study and 200 ng/kg stop-exposure groups exceeded the historical control range (Tables 11 and A4b). Squamous cell carcinoma occurred within the oral mucosa of the palate and was located adjacent to the incisor 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 (Plate 4).

Gingival squamous hyperplasia occurred in all groups including the vehicle controls, with increasing incidences in the 44 ng/kg or greater groups (Tables 11 and A5b). The incidence in the 200 ng/kg stop-exposure group was similar to that in the vehicle controls. Squamous hyperplasia was a focal lesion that occurred in the stratified squamous epithelium of the gingival oral mucosa adjacent to the incisor teeth 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, at least in these cases, that the hyperplasia was secondary to the presence of the hair shafts and associated inflammation. It was unclear whether there was an association between squamous hyperplasia and squamous cell carcinoma.

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Oral Mucosa in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Number Necropsied	53	53	53	53	53	53	50
Gingival, Hyperplasia, Squamous[a]	15 (1.1)[b]	11 (1.4)	16 (1.4)	19 (1.2)	22 (1.1)	20 (1.4)	14 (1.2)
Gingival, Squamous Cell Carcinoma[c]							
Overall rate[d]	1/53 (2%)	2/53 (4%)	1/53 (2%)	0/53 (0%)	1/53 (2%)	3/53 (6%)	3/50 (6%)
Adjusted rate[e]	2.4%	5.2%	2.7%	0.0%	2.8%	8.1%	9.0%
Terminal rate[f]	1/25 (4%)	1/22 (5%)	0/24 (0%)	0/25 (0%)	0/20 (0%)	2/23 (9%)	1/15 (7%)
First incidence (days)	728 (T)	706	517	—[i]	717	668	680
Poly-3 test[g]	P=0.149	P=0.468	P=0.735	P=0.518N	P=0.728	P=0.261	P=0.249
Poly-3 test[h]							P=0.625

(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): 4/371 (1.1% ± 1.0%), range 0%-2%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group group is indicated by **N**.
[h] Pairwise comparison between the 200 ng/kg core and stop-exposure groups
[i] Not applicable; no neoplasms in animal group

Uterus: At 31 weeks, the incidence and severity of cystic endometrial hyperplasia in the 200 ng/kg group were increased (Tables 12 and A5a). At 2 years, the incidence of carcinoma in the 92 ng/kg group was marginally greater than that in the vehicle controls and exceeded the historical vehicle control range (Tables 12 and A4c). Uterine carcinomas arose from the epithelial cells of the endometrium and were characterized by endometrial glands that invaded into and through the uterine wall. Proliferations of neoplastic glands were present in the adipose tissue and mesometrium surrounding the uterus. These glands were usually lined by a single layer of cuboidal cells with large vesicular nuclei and surrounded by a moderate scirrhous reaction. There was extensive inflammation, predominantly neutrophilic, associated with the neoplasms. A single uterine carcinoma was characterized by solid sheets of cells displaying moderate to marked pleomorphism and atypia. There was a single occurrence of a squamous cell carcinoma in the uterus of a 92 ng/kg rat (Tables 12 and A1b). Squamous cell carcinoma occurred on the endometrial surface, caused dilatation of the uterus, and was characterized by irregular cords and clusters of atypical stratified squamous epithelial cells that invaded the underlying myometrium.

At 2 years, significantly increased incidences of cystic endometrial hyperplasia were observed in the 92 and 200 ng/kg core study groups (Tables 12 and A5b). Increases in the incidences of chronic active inflammation were observed in all dosed groups; the increases were not considered dose related. The incidence in the 200 ng/kg stop-exposure group was greater than those in the vehicle control and 200 ng/kg core study groups. Increased incidences of squamous metaplasia occurred in all dosed groups with comparable incidences and severities in the 44 and 200 ng/kg core study groups. In the 200 ng/kg stop-exposure group, the incidence of

TABLE 12
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
31-Week Interim Evaluation							
Number Examined Microscopically	10	10	10	10	10	10	—[c]
Endometrium, Hyperplasia, Cystic[a]	4 (1.3)[b]	2 (1.5)	4 (2.0)	6 (1.8)	7 (1.7)	8 (2.9)	
2-Year Study							
Number Necropsied	53	53	53	53	53	53	50
Endometrium, Hyperplasia, Cystic	31 (2.4)	29 (2.1)	29 (2.1)	33 (2.2)	39* (2.2)	37* (2.1)	35 (2.5)
Inflammation, Chronic Active	0	5* (2.6)	3 (2.3)	3 (3.3)	4* (3.0)	3 (2.3)	7**▲▲ (1.9)
Metaplasia, Squamous	17 (1.8)	25 (1.8)	21 (1.8)	36** (1.8)	31** (1.9)	35** (1.9)	28**▲▲ (2.1)
Adenoma	2	0	0	0	0	0	0
Carcinoma[d]							
Overall rate[e]	1/53 (2%)	1/53 (2%)	0/53 (0%)	1/53 (2%)	5/53 (9%)	2/53 (4%)	1/50 (2%)
Adjusted rate[f]	2.4%	2.6%	0.0%	2.6%	13.8%	5.4%	3.0%
Terminal rate[g]	0/25 (0%)	0/22 (0%)	0/24 (0%)	1/25 (4%)	2/20 (10%)	1/23 (4%)	1/15 (7%)
First Incidence (days)	678	706	—[j]	728 (T)	707	624	728 (T)
Poly-3 test[h]	P=0.103	P=0.740	P=0.527N	P=0.740	P=0.070	P=0.456	P=0.705
Poly-3 test[i]							P=0.555N
Squamous Cell Carcinoma[k]	0	0	0	0	1	0	0

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
▲▲ Significantly different (P≤0.01) from the 200 ng/kg core study group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Stop-exposure group not examined at 31 weeks
[d] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 3/371 (0.8% ± 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; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.
[i] Pairwise comparison between the 200 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[j] Not applicable; no neoplasms in animal group
[k] Historical incidence: 1/371 (0.3% ± 0.7%), range 0%-2%

squamous metaplasia was significantly greater than that in vehicle controls, but lower than that in the 200 ng/kg core study group.

Cystic endometrial hyperplasia had the typical appearance of a spontaneous aging change and consisted of hypercellularity of the endometrial epithelium combined with varying degrees of dilatation of the uterine glands and the uterine lumen. Chronic active inflammation consisted of accumulation of a mixture of varying numbers of neutrophils and macrophages within the uterine lumen and extending into the underlying stroma. This generally occurred in uteri with squamous metaplasia or cystic endometrial hyperplasia although it was not clear if the inflammation was related to those conditions. Thus, the significance, if any, of the chronic active inflammation is uncertain. Squamous metaplasia was generally a minimal to mild, multifocal change consisting of squamous epithelium lining endometrial glands. In severe cases, the horn of the uterus would be dilated and completely lined by keratinizing stratified squamous epithelium.

Lung: At 14 weeks, lung weights were significantly increased in the 200 ng/kg group (Table B1). At 31 weeks, no differences were observed in absolute or relative lung weights between any of the PeCDF-treated groups and the vehicle control group. At 53 weeks, no differences were observed in absolute lung weight, and the relative lung weight of the 200 ng/kg group was significantly increased.

At 2 years, a single incidence of a multiple cystic keratinizing epithelioma occurred in the 200 ng/kg core study group (Tables 13 and A1b). No cystic keratinizing epitheliomas have occurred in the historical vehicle controls (Tables 13 and A4d). The cystic keratinizing epithelioma was a large lesion that replaced a substantial amount of the normal lung parenchyma. It consisted of a cystic structure composed of an irregular wall of highly keratinized stratified squamous epithelium and a center filled with keratin. The outer portion of the lesion grew by expansion into the adjacent lung but evidence of invasion was not observed. Due to the rare occurence of this neoplasm, the presence of cystic keratinizing epithelioma may have been treatment related.

There were significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium in the 92 and 200 ng/kg core study groups; the incidence of this lesion was significantly decreased in the 200 ng/kg stop-exposure compared to the 200 ng/kg core study

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Number Examined Microscopically	53	53	53	53	53	52	50
Alveolar Epithelium, Metaplasia, Bronchiolar[a]	5 (1.0)[b]	6 (1.0)	5 (1.2)	9 (1.3)	23** (1.6)	28** (1.5)	7▲▲ (1.1)
Metaplasia, Squamous	0	0	0	2 (1.0)	4* (1.5)	3 (1.7)	1 (3.0)
Alveolar Epithelium, Hyperplasia	14 (1.1)	17 (1.1)	12 (1.2)	20 (1.2)	8 (1.1)	3** (1.0)	6 (1.0)
Cystic Keratinizing Epithelioma, Multiple[c]	0	0	0	0	0	1	0

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
▲▲ Significantly different (P≤0.01) from the 200 ng/kg core study group by the Poly-3 test
[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

group. Sporadic incidences of squamous metaplasia were noted at 44 ng/kg or greater, with only one occurrence in the 200 ng/kg stop-exposure group (Tables 13 and A5b). Incidences of alveolar epithelial hyperplasia occurred in vehicle controls and all dosed groups, with reduced incidences in the 92 and 200 ng/kg core study group and the 200 ng/kg stop-exposure group. 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, which was seen in the vehicle controls. In alveolar hyperplasia, the alveoli were lined by cuboidal epithelium and, unlike bronchiolar metaplasia, prominent mucus production was not observed. 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 minimal to mild change consisting of one or more small, irregular foci of keratinizing stratified squamous epithelium that had replaced the normal alveolar epithelium.

Pancreas: At 2 years, one acinar adenoma and one acinar carcinoma were observed in the 92 ng/kg group and in the 200 ng/kg stop-exposure group (Tables 14 and A1b). The combined incidences of acinar adenoma or carcinoma in these dosed groups exceeded the historical vehicle control range (Tables 14 and A4e). The 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. In contrast, carcinoma was a large, multinodular lesion with moderate amounts of dense fibrous stroma. Carcinoma was 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.

Increased incidences of acinar cytoplasmic vacuolization and incidences and severities of arterial chronic active inflammation occurred in the 92 and 200 ng/kg core study groups; the incidences in the 200 ng/kg stop-exposure group were significantly decreased compared to the 200 ng/kg core study group (Tables 14 and A5b). Cytoplasmic vacuolization consisted of small, clear, discrete intracytoplasmic vacuoles within pancreatic acinar cells. Sometimes these vacuoles coalesced to form larger single vacuoles. The severity of the change was determined by the degree of vacuolization per cell and the amount of tissue involved. Arterial chronic active inflammation was a focal to multifocal change characterized by a thick mantle of macrophages, lymphocytes, and plasma cells around the arteries with infiltration into the muscular layers of the artery (Plates 5 and 6). There was often fibrinoid necrosis of the vessel, and the tunica intima was frequently thickened. Endothelial cells were swollen or decreased in number. This inflammatory reaction often extended into the surrounding parenchyma. Thrombosis was sometimes seen in areas of more severe inflammation and appeared to be secondary to damage to the vessel wall caused by the inflammation.

Mammary and Pituitary Glands: At 2 years, there were significantly increased incidences of mammary gland carcinoma in the 6 and 20 ng/kg groups (vehicle control, 5/53; 6 ng/kg, 12/53; 20 ng/kg, 13/53; 44 ng/kg, 2/53; 92 ng/kg, 5/53; 200 ng/kg (core study), 3/53; 200 ng/kg (stop-exposure), 5/50; Tables A3a and A3b). These increased incidences exceeded the historical vehicle control range [42/371 (11.3% ± 2.9%), range 8%-15%]. There were also increased incidences of pituitary gland (pars distalis) adenoma in the 20 and 44 ng/kg groups (15/53, 21/53, 23/53, 23/53, 21/53, 16/53, 13/49; Tables A3a and A3b). However, these incidences of pituitary gland adenoma were within the historical vehicle control range [153/369 (41.4% ± 11.3%), range 28%-57%].

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Number Examined Microscopically	53	53	53	52	52	52	49
Acinus, Vacuolization Cytoplasmic[a]	0	0	0	0	2 (1.0)[b]	23** (1.1)	2▲▲ (1.0)
Artery, Inflammation, Chronic Active	1 (1.0)	2 (2.0)	1 (1.0)	2 (2.5)	4 (2.3)	11** (3.3)	1▲▲ (2.0)
Acinus, Adenoma	0	0	0	0	1	0	1
Acinus, Carcinoma	0	0	0	0	1	0	1
Adenoma or Carcinoma[c]							
Overall rate[d]	0/53 (0%)	0/53 (0%)	0/53 (0%)	0/52 (0%)	2/52 (4%)	0/52 (0%)	2/49 (4%)
Adjusted rate[e]	0.0%	0.0%	0.0%	0.0%	5.6%	0.0%	6.1%
Terminal rate[f]	0/25 (0%)	0/22 (0%)	0/24 (0%)	0/25 (0%)	2/20 (10%)	0/23 (0%)	2/15 (13%)
First incidence (days)	—[i]	—	—	—	728 (T)	—	728 (T)
Poly-3 test[g]	P=0.442	—[j]	—	—	P=0.202	—	P=0.215
Poly-3 test[h]							P=0.225

** Significantly different (P≤0.01) from the vehicle control group by the Poly-3 test
▲▲ Significantly different (P≤0.01) from the 200 ng/kg core study group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± 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; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Pairwise comparison between the 200 ng/kg core and stop-exposure groups
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

Thyroid Gland: At 14 weeks, dose-related increased incidences of follicular cell hypertrophy were observed in all dosed groups, and the severity was increased in the 92 and 200 ng/kg groups (Tables 15 and A5a). At 53 weeks, increased incidences were observed in the 92 and 200 ng/kg groups, with severity increased in the 200 ng/kg group. At 2 years, incidences of follicular cell hypertrophy were increased in all dosed groups (Tables 15 and A5b). The incidence in the 200 ng/kg stop-exposure group was also increased. 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 sometimes present. The affected follicles were lined by large, prominent cuboidal follicular epithelial cells that ranged from approximately one and a half to four times normal size and usually had abundant pale cytoplasm sometimes containing small, clear vacuoles. Since some degree of this change commonly occurs spontaneously, the study pathologist only diagnosed this change when at least half of the thyroid follicles in the glands were affected. A severity grade of minimal was recorded when 50% to 60% of the follicles

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

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
14-Week Interim Evaluation						
Thyroid Gland[a]	10	10	10	10	9	10
Follicular Cell, Hypertrophy[b]	0	3 (1.0)[c]	3 (1.3)	4* (1.0)	7** (1.4)	7** (2.0)
Thymus	10	9	8	9	10	10
Atrophy	0	0	3 (1.0)	0	0	2 (1.0)
31-Week Interim Evaluation						
Thymus	10	10	10	10	10	10
Atrophy	1 (1.0)	0	0	2 (1.0)	0	5 (2.4)
53-Week Interim Evaluation						
Thyroid Gland	8	8	7	8	8	8
Follicular Cell, Hypertrophy	2 (1.0)	3 (1.0)	1 (1.0)	3 (1.0)	4 (1.0)	5 (1.4)
Thymus	8	8	8	8	8	8
Atrophy	5 (1.8)	4 (2.0)	6 (1.7)	6 (2.0)	5 (2.0)	7 (2.6)

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
2-Year Study							
Thyroid Gland	53	53	51	53	51	51	48
Follicular Cell, Hypertrophy	7 (1.6)	13 (1.2)	24** (1.3)	24** (1.2)	24** (1.5)	22** (1.5)	23** (1.3)
Thymus	53	49	50	52	49	51	49
Atrophy	43 (2.3)	36 (2.6)	36 (2.7)	44 (2.8)	39 (3.1)	48 (3.6)	44 (2.9)
Adrenal Cortex	53	53	53	52	53	53	48
Degeneration, Cystic	4 (2.0)	17** (1.9)	14** (2.3)	18** (2.2)	12* (2.1)	14** (2.4)	12** (1.8)
Vacuolization Cytoplasmic	9 (1.4)	13 (1.2)	14 (1.6)	7 (1.9)	17* (1.2)	9 (1.4)	10 (1.3)
Kidney	53	53	53	52	53	53	48
Nephropathy	34 (1.1)	39 (1.2)	35 (1.2)	42* (1.4)	36 (1.4)	45** (1.5)	35 (1.1)
Heart	53	53	52	53	53	52	50
Cardiomyopathy	15 (1.1)	12 (1.0)	19 (1.1)	13 (1.0)	18 (1.0)	24* (1.1)	13 (1.1)
Stomach, Forestomach	53	53	53	53	52	53	50
Hyperplasia, Squamous	4 (1.8)	1 (1.0)	5 (1.4)	6 (2.5)	3 (2.7)	10* (2.1)	5 (2.2)
Bone Marrow	53	53	53	53	53	53	50
Hyperplasia	38 (2.8)	32 (2.9)	37 (3.1)	35 (2.9)	42 (2.9)	41 (2.8)	43 (3.0)

* 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

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. The severity was minimal to mild in nearly all affected animals in all groups, including the vehicle control and treated groups.

Thymus: At 14 weeks, there were three incidences of atrophy in the 20 ng/kg group and two incidences in the 200 ng/kg group (Tables 15 and A5a). At 31 and 53 weeks, increased incidences and severities of cortical atrophy were observed in the 200 ng/kg group. At 2 years, increased severities of atrophy were noted in all dosed groups, with the greatest increase in the 200 ng/kg core study group (Table 15). Atrophy consisted of varying degrees of loss of lymphoid cells from the cortex resulting in reduction of cortical thickness.

Adrenal Cortex: At 2 years, treatment-related, but not dose-related, changes consisted of significantly increased incidences of cystic degeneration in all dosed groups (Tables 15 and A5b). The incidence of cortical cystic degeneration was still increased in the 200 ng/kg stop-exposure group. Cortical cystic degeneration was a focal to multifocal, unilateral to bilateral lesion consisting of variably sized endothelial-lined spaces, usually containing blood and occasionally thrombi, that were located in the zonae fasciculata and reticularis. Larger lesions compressed or replaced adjacent parenchyma. Some lesions were very large, replaced much of the gland, and caused enlargement of the gland.

Increased incidences of cortical cytoplasmic vacuolization in the 6, 20, and 92 ng/kg groups were not considered dose related. Cortical cytoplasmic vacuolization was a focal to multifocal to diffuse change consisting of small, discrete, clear intracytoplasmic vacuoles. Sometimes the cytoplasm contained a large single vacuole that displaced the nucleus. The changes were morphologically consistent with the accumulation of lipid. Cytoplasmic vacuolization occurred most commonly within foci of hypertrophy.

Kidney: At 2 years, increased incidences and severities of nephropathy (Tables 15 and A5b) were observed in all treated groups, with the highest incidence in the 200 ng/kg core study group. The incidence in the 200 ng/kg stop-exposure group was less than in the 200 ng/kg core study group. Nephropathy was generally a minimal to mild change, although sometimes moderate to marked nephropathy was seen. It had the typical appearance of this lesion as seen in aging rats, and was similar to that observed in Fischer F344/N 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.

Heart: At 2 years, a significantly increased incidence of minimal to mild cardiomyopathy (Tables 15 and A5b) was observed in the 200 ng/kg core study group. Cardiomyopathy had the typical microscopic appearance of this lesion as seen in aging rats, and appeared similar to cardiomyopathy seen in aging Fischer F344/N rats (MacKenzie and Alison, 1990). It was a multifocal, generally minimal to mild lesion consisting of hypereosinophilic myofibers that lacked cross striations, infiltrates of mononuclear cells, separation of myofibers by myxomatous material (bluish material on H&E stain), and eventually replacement of myofibers by fibrous connective tissue. The severity was graded based upon the number and extent of foci of myocardial degeneration. Minimal cardiomyopathy consisted of a few scattered foci while mild cardiomyopathy consisted of a greater number of lesions more diffusely scattered within the myocardium.

Forestomach: At 2 years, the incidence of squamous cell hyperplasia in the 200 ng/kg core study group was significantly greater than that in the vehicle control group (Tables 15 and A5b); severities were increased in the 44 ng/kg or greater groups. Squamous hyperplasia of the forestomach epithelium was generally a minimal to moderate, focal or occasionally diffuse change characterized by varying degrees of thickening of the stratified squamous epithelium up to approximately five times normal thickness in more severe cases. Sometimes the hyperplasia occurred around a focal ulcer, although most cases occurred without the presence of an apparent ulcer.

Bone Marrow: At 2 years, the incidences of hyperplasia were slightly increased in the 92 and 200 ng/kg core study groups and in the 200 ng/kg stop-exposure group (Tables 15 and A5b).

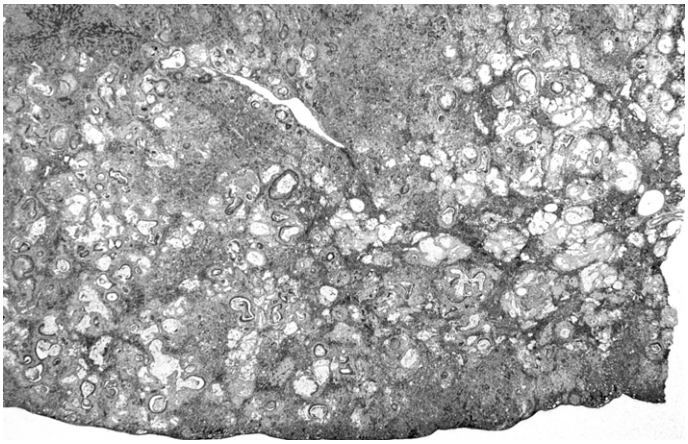


PLATE 1
Cholangiocarcinoma in the liver of a female rat administered 200 ng/kg PeCDF by gavage for 2 years. In contrast to cholangiofibrosis, cholangiocarcinoma is larger in size and widely involves the hepatic parenchyma. H&E; 4x

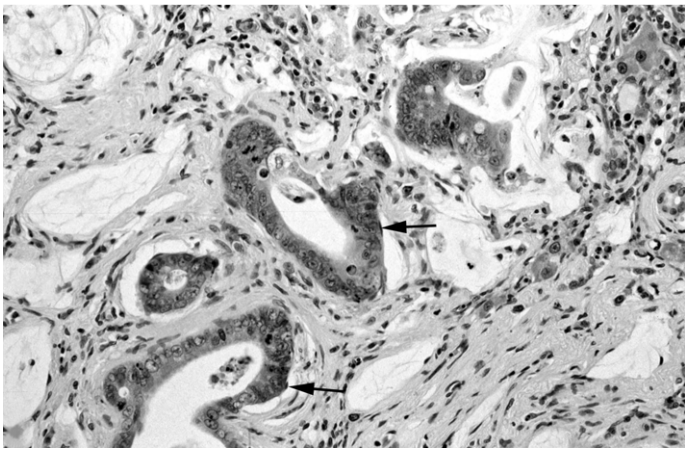


PLATE 2
Higher magnification of Plate 1. Note the lesion consisting of fibrous connective tissue stroma containing numerous atypical bile ducts (arrows). H&E; 66x

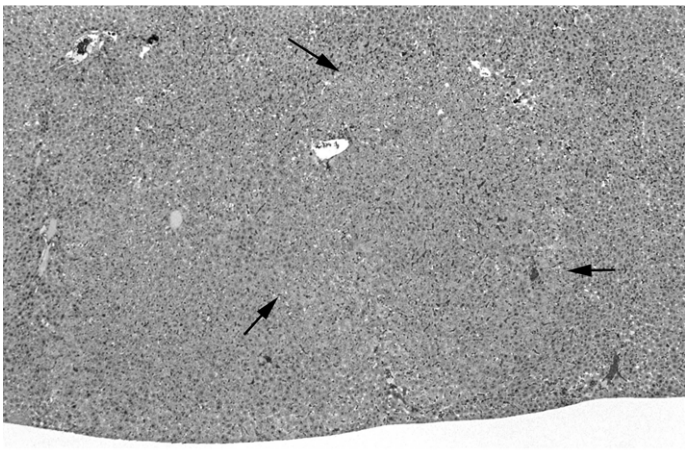


PLATE 3
Eosinophilic focus in the liver of a female rat administered 200 ng/kg PeCDF by gavage for 2 years. Note the margins of the focus are distinct, with a minimal degree of compression of the surrounding hepatic parenchyma (arrows). H&E; 10x

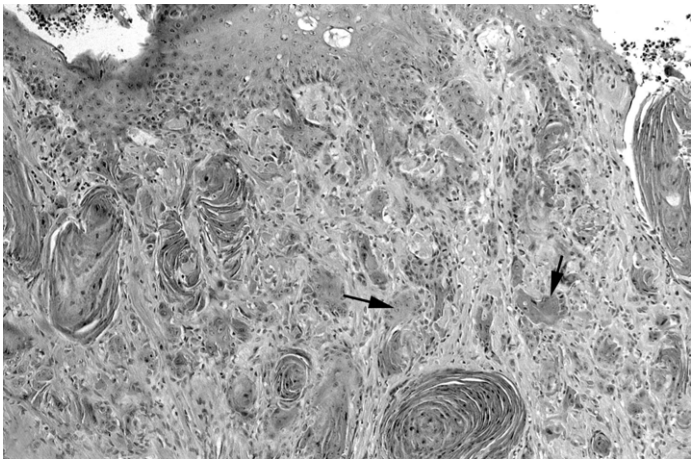


PLATE 4
Squamous cell carcinoma in the gingival epithelium of a female rat administered 200 ng/kg PeCDF by gavage for 2 years. There is invasion of squamous cells (arrows) into the underlying stroma. H&E; 33x

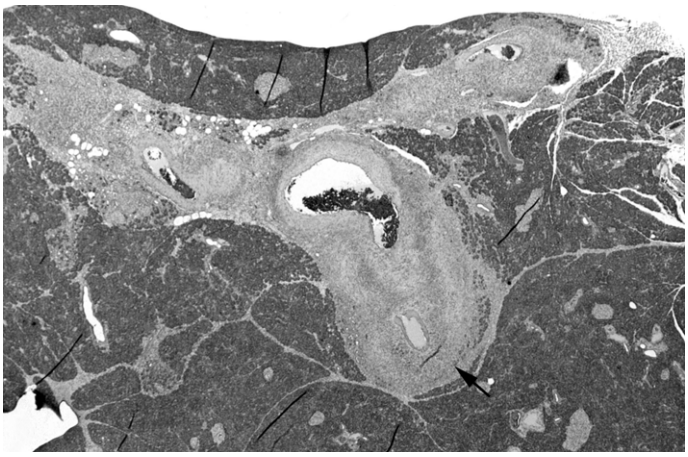


PLATE 5
Arterial inflammation (arrow) in the pancreas of a female rat administered 200 ng/kg PeCDF by gavage for 2 years. H&E; 5x

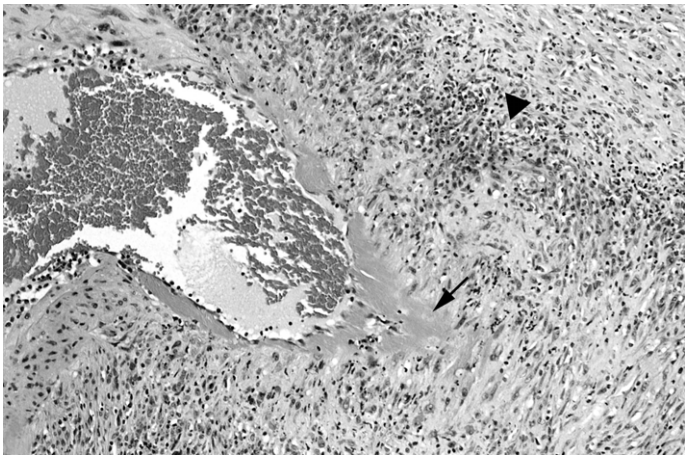


PLATE 6
Arterial inflammation in the pancreas of a female rat administered 200 ng/kg PeCDF by gavage for 2 years. Note the fibrinoid necrosis in the intima and media (arrow) and chronic inflammation in the media and adventitia (arrow head). H&E; 33x

DISCUSSION AND CONCLUSIONS

This 2-year study of the chronic toxicity and carcinogenicity of PeCDF in female Harlan Sprague-Dawley rats is one in a series of studies carried out as part of a multistudy NTP initiative examining the relative chronic toxicity and carcinogenicity of dioxin-like compounds (DLCs) and structurally related polychlorinated biphenyls (PCBs) (see Overview section). While one of the primary aims of this dioxin toxic equivalency factor (TEF) evaluation was an analysis of the comparative carcinogenicity of TCDD, PeCDF, and PCB 126, only the results of the PeCDF study are described in this Technical Report. Where appropriate, a qualitative comparison to neoplastic responses seen in the gavage study of TCDD conducted as part of the dioxin TEF evaluation was made (NTP, 2006a). A quantitative analysis of the effects observed in this study to responses observed with other compounds studied as part of the dioxin TEF evaluation is presented elsewhere (Toyoshiba *et al*., 2004; Walker *et al*., 2005).

PeCDF is the most potent of the polychlorinated dibenzofurans for the induction of TCDD-like activities. Dose selection for this study of PeCDF was based on prior observations made in a 2-year carcinogenicity study of TCDD by Dow Chemical Company (Kociba *et al.*, 1978). In that study, Spartan Sprague-Dawley rats were exposed to doses of 1, 10, or 100 ng TCDD/kg body weight. Increases in liver adenomas were observed at doses of 10 and 100 ng/kg. Given the World Health Organization's (WHO) TEF for PeCDF of 0.5, the dose range for the present study was selected as 6 to 200 ng PeCDF/kg body weight per day. For the carcinogenicity study, five doses of 6, 20, 44, 92, and 200 ng/kg per day were used to provide more information on the shape of the dose-response curve.

In the present study, there was no significant effect on survival in any dosed group. The survival of the stop-exposure group was lower than the other continuous treatment groups but was not significantly different from the vehicle control group. Body weight gain especially in the latter half of the study tended to be lower in the higher dose groups. Specifically, the terminal body weight in the 200 ng/kg group was significantly lower (88% relative to controls). In general, there was no significant effect on body weight at the interim evaluations; there was a significant effect on liver weight in the 92 and 200 ng/kg groups at 14, 31 and 53 weeks.

The principal findings of this 2-year study were increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and gingival squamous cell carcinoma of the oral mucosa following chronic administration of PeCDF in corn oil:acetone (99:1) by gavage at doses up to 200 ng/kg, five days per week. Based on increased incidences of these neoplasms in the TCDD and PCB 126 studies conducted as part of the TEF evaluation, these increases were attributed to PeCDF administration. In addition, cystic keratinizing lung epithelioma in dosed animals may have been related to PeCDF treatment because there were also increased incidences in the TCDD and PCB 126 studies, and this neoplasm is rare. In addition, the increased incidences of pancreatic acinar neoplasms in the 92 ng/kg group and uterine carcinomas in the 92 and 200 ng/kg groups may have been related to PeCDF administration.

The principal nonneoplastic finding in this study was significant hepatotoxicity. In addition, increases in the incidences of nonneoplastic lesions occurred in the lung, oral mucosa, uterus, pancreas, thyroid gland, thymus, adrenal cortex, kidney, heart, and forestomach.

Chronic exposure led to significant accumulation of PeCDF in liver, fat, and lung and detectable levels in blood. The significant accumulation in fat is consistent with the lipophilic nature of this compound. Previous studies of DLCs indicate that the liver and fat are the main depots for DLCs in rodents and together contribute 70% to 80% of the total body burden within the animal (DeVito *et al*., 1995).

As expected, the levels in liver were higher than those in fat on a wet weight basis. This is likely due to the sequestration of DLCs in the liver as a result of binding to CYP1A2, which is inducible by DLCs (Diliberto *et al.*, 1997). In this study, 2-year administration at doses of 6, 20, 44, 92, and 200 ng/kg per day resulted in

mean levels of 18, 63, 125, 264, and 500 ng PeCDF/g liver, respectively. The relationship between intake and tissue liver levels was linear. In the TCDD feed study by Kociba *et al.* (1978), terminal liver TCDD levels were 5 ng/g at the 10 ng/kg dose and 24 ng/g at the 100 ng/kg dose. By comparison, terminal liver levels in the TCDD study conducted as part of the dioxin TEF evaluation were 2.2 and 9.3 ng/g in the 10 and 100 ng/kg groups, respectively (NTP, 2006a). On a toxic equivalents (TEQ) basis (using the WHO TEF factor of 0.5 for PeCDF), the liver burden of PeCDF at the 10 ng TEQ/kg and 100 ng TEQ/kg doses (31.5 ng TEQ/g and 250 ng TEQ/g) are approximately 14- to 27-fold higher than those seen in the dioxin TEF evaluation TCDD study (NTP, 2006a), and approximately 6- to 10-fold higher than that observed in the Kociba *et al.* (1978) dosed feed study.

Cessation of daily treatment with PeCDF in the stop-exposure group led to a decline in levels of PeCDF in all tissues examined. At the end of the study, the mean level of PeCDF in the liver of the stop-exposure group was 36 ng/g compared to 500 ng/g in animals exposed for the full 2 years at the same dose. This level of 36 ng/g was twofold higher than that observed at the end of the study in animals exposed for 2 years to 6 ng/kg per day (mean liver level of 18 ng/g). Therefore, interpretation of the pathology results in the stop-exposure animals must consider that exposure *per se* does not end on cessation of daily administration of compounds. While PeCDF levels in the stop-exposure group declined significantly over the remainder of the study due to the CYP1A2 binding and lipophilic nature of the compound, animals were still continually exposed throughout the course of the study. Exposure to DLCs also can occur as a result of low levels of these compounds present in rodent feed.

There was measurable PeCDF in the liver of control animals at the longer durations of exposure. These concentrations can be attributed to the ingestion of very low levels of PeCDF that are normally present in rodent chow (Feeley and Jordan, 1998; Jordan and Feeley, 1999). Accumulation of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) has been observed in control animals in other studies (Vanden Heuvel *et al.*, 1994). Therefore, all experimental treatments in the TEF studies were made in addition to a background of exposure to DLCs normally present in feed, and the vehicle control group exposure is not zero. Levels of PCDDs and PCDFs were analyzed in NTP-2000 feed (Table D5). The mean level of PeCDF in NTP-2000 feed was 0.041 ± 0.082 pg/g feed.

Increased expression of CYP1A1 and CYP1A2 is a characteristic response to DLCs in the liver and is directly linked to binding and activation of the aryl hydrocarbon receptor (AhR) by DLCs (Whitlock, 1993). In many cases the relative potency for induction of CYP1A1 *in vivo* is an appropriate surrogate for the dioxin-like activity of a given compound and provides the basis for many TEFs (Van den Berg *et al.*, 1998). In this study, increased CYP1A1 and CYP1A2 activities as a result of PeCDF administration were observed at all time points and at all doses used. The maximum observed levels of EROD activity were higher than those seen in the TCDD dioxin TEF evaluation study (NTP, 2006a). The finding that the liver was a target following exposure to PeCDF was expected. It was also expected that exposure to this compound would lead to increases in these specific dioxin-like responses. While not discussed in this report, data on altered expression of CP1A1 and CYP1A2 together with data from the other studies of DLCs conducted as part of the dioxin TEF evaluation have been used for an evaluation of the additivity of relative potency of DLCs for these endpoints (Toyoshiba *et al*., 2004).

Numerous studies have examined the toxicity of DLCs and PCBs and have demonstrated that the liver is a principal target organ for the action of these compounds. In the present study of PeCDF, the principal hepatic neoplastic response observed was an increased trend in the incidence of cholangiocarcinoma and hepatocellular adenoma. The liver effects seen in this study are consistent with those seen in the dioxin TEF evaluation study of TCDD (NTP, 2006a). In that study, there were significant increases in the incidences of cholangiocarcinoma and hepatocellular adenoma in the 100 ng/kg group. These data indicate that the pattern of responses of the liver to PeCDF was qualitatively similar to that of TCDD.

The principal nonneoplastic finding in this study was the extensive hepatotoxicity induced by PeCDF treatment. The incidence and pattern of hepatic toxicity exhibited a clear dose and duration dependence and preceded neoplastic effects in the liver. There were significant increases in the incidence and severity of hepatic toxicity, with increases in severity occurring at higher doses and longer durations of exposure. Hepatic toxicity was

characterized by foci of cellular alterations, multinucleated hepatocytes, cystic degeneration, fatty change, inflammation, necrosis, pigmentation, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, hepatocyte degeneration, hepatocyte hypertrophy, oval cell hyperplasia, and portal fibrosis. A comprehensive term of toxic hepatopathy was also used, reflecting the overall severity grade of the nonneoplastic effects. The purpose of this term was to allow for easier comparison of the degree of toxic change among different dose groups than would be possible if the severities of all the individual nonneoplastic changes had to be compared among the different groups. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. When treated animals occasionally had just a few of these changes present it was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy.

The only treatment-related effect observed in the liver at the 14-week interim evaluation was hepatocyte hypertrophy. At 31 weeks, both hepatocyte hypertrophy and pigmentation were observed. These changes continued to be observed at the 53-week time point together with fatty change and multinucleated hepatocytes. At the end of the 2-year study, there were additional changes indicative of increased toxic hepatopathy including altered hepatic foci, bile duct hyperplasia, bile duct fibrosis, necrosis, oval cell hyperplasia, and nodular hyperplasia.

The trend for increased incidences of hepatocellular adenoma is consistent with observations made in the TCDD study conducted as part of the dioxin TEF evaluation (NTP, 2006a) and previously observed effects of TCDD and hexachlorodioxins on the liver (Kociba *et al*., 1978; NCI, 1980; NTP, 1982a). However, in this study the incidence of hepatocellular adenoma was lower than that seen previously. In a reevaluation of the Kociba *et al*. (1978) TCDD dosed feed study of Spartan Sprague-Dawley rats, the incidences of hepatocellular adenomas were 2/86, 9/50, and 14/45 in the 0 ng/kg, 10 ng/kg, and 100 ng/kg dose groups, respectively. No hepatocellular carcinomas were observed in the present study compared to 4/45 in the 100 ng/kg group in the TCDD dosed feed study (Goodman and Sauer, 1992). In the NTP (1982a) gavage study of Osborne-Mendel rats, the incidence of liver "neoplastic nodules" in female rats was 12/49 (24%) at a weekly dose of 500 ng TCDD/kg per day; the incidence of neoplastic nodules or hepatocellular carcinoma was 14/49 (29%). There was no significant effect in male rats. In the NCI (1980) study of a mixture of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin in Osborne-Mendel rats, the incidences of neoplastic nodules or hepatocellular carcinoma (combined) were 5/75 (7%), 10/50 (20%), 12/50 (24%), 30/50 (60%), at doses of 0, 1,250, 2,500, or 5,000 ng/kg per week, respectively. Given the TEF of 0.1 for hexachlorodioxins (Van den Berg *et al*., 1998), these weekly doses are over a similar range of TEQs as those used in the present gavage study of PeCDF.

The incidences of neoplasms were lower than expected given the disposition of PeCDF and the increased incidence of hepatocellular adenoma observed in the TCDD study conducted as part of the dioxin TEF evaluation (NTP, 2006a). Terminal liver levels of PeCDF, when expressed as TEQs using the WHO TEF for PeCDF of 0.5, were 27-fold higher than the TCDD levels seen in animals dosed with 100 ng TCDD/kg where significantly increased incidences of cholangiocarcinoma and hepatocellular adenoma were observed (NTP, 2006a). Based on the higher mean liver levels of PeCDF, higher incidences of neoplasms would have been expected in the present study. Based on the lower magnitude of body weight reduction and lower severity of hepatotoxicity at the highest dose in the present study compared to the highest dose used in the TCDD study conducted in the dioxin TEF evaluation, it is likely that higher doses of PeCDF could have been used.

Pathology nomenclature practices have changed since the Kociba *et al*. (1978) study. In the initial Kociba *et al*. (1978) evaluation, there was an increased incidence of hepatocellular carcinoma, but proliferative lesions that did not meet the diagnostic criteria for carcinoma were generally classified as hyperplastic (neoplastic) nodules, and these were also increased in treated groups. The original Kociba *et al*. (1978) study indicated a 47% incidence of "hepatocellular hyperplastic nodules" in the 100 ng TCDD/kg group compared to a 9% incidence in control animals. A subsequent reevaluation of the proliferative lesions from that study (Squire, 1980) changed the categories to neoplastic nodule and hepatocellular carcinoma, and while a treatment-related effect remained, more lesions were diagnosed in the control and 10 ng/kg groups. Subsequent to the Kociba *et al*. (1978) study there was an evolution of nomenclature for hepatocellular proliferative lesions, and a reevaluation of the slides from the study. In that evaluation, neoplasms were classified as adenoma or carcinoma. Using the newer nomenclature, the incidence of hepatocellular adenoma was 31% at 100 ng TCDD/kg, the highest dose

(Goodman and Sauer, 1992). Significant hepatotoxicity was noted in the Kociba *et al*. (1978) TCDD study and the NCI (1980) and the NTP (1982a) TCDD studies. A summary of the pathology reevaluation is provided in NTP Technical Report 521 on TCDD (NTP, 2006a).

The spectrum of lesions observed in the current study is not common in NTP studies, and there is a lack of biological information relative to the progression and behavior of these lesions. These lesions generally occurred on a background of toxic hepatopathy, the components of which are listed previously and described in the results section. It is generally accepted that, in the rat, hepatocellular adenoma and hepatocellular carcinoma represent a morphological and biological continuum (Narama *et al.* 2003; Hailey *et al.*, 2005). Foci of cellular alteration are often part of that continuum, but not always. In high dose animals, proper categorization of the lesions was further complicated by the presence of the toxic hepatopathy. While the biological behavior of hepatocellular lesions within this study and other studies conducted as part of the dioxin TEF evaluation (NTP, 2006a,b,c) is uncertain, the morphology suggests that, in this study, eosinophilic foci and mixed cell foci, nodular hyperplasia, and potentially adenoma were a continuum. Carcinomas were not observed in the present study.

The foci of cellular alterations seen in treated animals generally differed from the typical foci seen in vehicle control animals. Foci seen in vehicle control animals were usually smaller, lacked discrete borders and blended with the surrounding parenchyma, produced little or no compression, and consisted of cells that were similar in size to normal cells. In contrast, foci in treated livers generally had discrete borders, produced some compression of the adjacent parenchyma, and consisted of large, hypertrophic, often vacuolated cells. The significant hypertrophy resulted in a greater degree of compression of adjacent hepatic parenchyma than is often seen with foci of hepatocellular alteration. At 2 years, focal lesions were observed that resembled foci of hepatocellular alteration, but were larger and often nodular, with greater compression of surrounding hepatic parenchyma and more disorganization of hepatic cords. As with foci, these lesions generally contained a somewhat normal hepatic structure including portal triads with biliary tracts. Additionally, these focal lesions contained variable numbers of randomly scattered biliary epithelium that often formed profiles of small glands/ductules. The large size of the lesions and presence of scattered biliary epithelium suggested a proliferative response of both cell types, and therefore were considered to have progressed beyond a simple focus of cellular alteration. However, because of the somewhat normal hepatic structure and the dual cellular composition, the lesions were considered to be hyperplastic rather than neoplastic and were diagnosed as nodular hyperplasia.

In the higher dose animals with severe toxic hepatopathy, there was evidence of hepatocyte degeneration and loss, and a regenerative response by the damaged liver. The term of "hyperplasia, nodular" was selected as the inclusive term, and was characterized by areas of focal hypertrophy and hyperplasia of hepatocytes that also contained proliferating biliary epithelium. This lesion was considered to be the result of the presence of a proliferative stimulus. Nodular hyperplasia varied in size, but generally appeared morphologically similar whether in a high dose animal with severe toxic hepatopathy or in a lower dose animal where the toxic hepatopathy was minimal to nonexistent. Nodular hyperplasia was seen most commonly in the higher dose groups in which prominent toxic changes were present. However, a lesser degree of nodular hyperplasia was sometimes seen in lower dose animals in which the only evidence of liver pathology may have been hepatocellular 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 significant toxic hepatopathy. This does not explain, however, these responses in animals that lack significant toxic hepatopathy. This indicates that some type of other stimulus, rather than regeneration secondary to degeneration and necrosis of the hepatic parenchyma, may have contributed to the proliferative lesions observed in this study.

Regarding the potential pathogenesis for the foci and nodular hyperplasia, the earliest treatment-related hepatocellular change seen in these studies, noted in interim sacrifice animals, was a diffuse hepatocellular hypertrophy. With continued dosing, poorly demarcated foci of prominent hypertrophic, often vacuolated, hepatocytes resembling those seen in foci and nodular hyperplasia were seen superimposed on the background of diffuse hypertrophy. It appeared that, with continued dosing,

the poorly demarcated foci of hypertrophic cells grew, giving rise to lesions diagnosed as foci, 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 usually was larger than a focus, had a distinct border, and produced more compression of surrounding normal hepatic parenchyma. Adenomas were composed of mildly to moderately pleomorphic hepatocytes with a subjectively increased nucleus to cytoplasmic ratio. Cells lacked the normal architectural arrangements of hepatic lobules, and while a few bile ducts may have been present within an adenoma, they were usually found at the periphery of the lesion and were considered entrapped. Proliferating biliary epithelium or oval cells were usually absent in hepatocellular adenoma.

The increased incidence of cholangiocarcinoma was an unexpected finding but consistent with observations made in the other TEF evaluation studies (NTP, 2006a,b,c,d,e,f). Spontaneous cholangioma and cholangiocarcinoma are apparently rare in the Harlan Sprague-Dawley rat and were not observed in vehicle control animals from this group of 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 in dosed groups differed morphologically from spontaneous cholangiocarcinoma, and 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 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 cholangiocarcinoma 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 nonneoplastic diagnosis, and there were lesions at the other end of the spectrum that appeared aggressive. While there were specific diagnostic criteria for cholangiofibrosis versus cholangiocarcinoma, some of the lesions did not readily fit the criteria and posed a diagnostic challenge.

Other chemicals, including furan, have increased 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 in the furan study were positive for growth and metastases. Transplantation studies using lesions from the present study were not conducted.

The mechanism underlying the increase in cholangiocarcinoma may be multifactorial. PeCDF clearly had an effect on bile duct proliferation in this study. This may be an indirect response to the toxicity observed as a result of the action of the DLC on the hepatocytes or due to a direct effect on the biliary cells themselves. Tritscher *et al.* (1995) showed a high degree of staining for TGF alpha in bile duct cells after exposure to TCDD in female rats. The 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 observed can produce oxidative stress that may also result in promotion of DNA damage. Consequently, the oxidative stress may be only a secondary phenomenon due to the ongoing response to the toxic hepatopathy. In addition, there may be a direct stimulatory effect on the oval cells themselves. This is supported by the increased incidences of oval cell hyperplasia in the present study. Oval cells may differentiate into hepatocytes and/or biliary epithelium; this may explain why both hepatocellular proliferative and biliary lesions were observed.

Previous studies of DLCs and PCBs have rarely seen biliary lesions despite data showing that bile ducts are targets for DLCs. In this study of PeCDF and prior studies of TCDD, there were increases in bile duct hyperplasia. In an initiation-promotion study, cholangiocarcinoma was seen in 1/14 DEN–initiated animals exposed to 100 ng TCDD/kg per day for 60 weeks (Walker *et al*., 2000). In the bioassays of Aroclor 1254, no cholangiocarcinomas were observed (Mayes *et al.*, 1998). In addition, there was no increased incidence of cholangioarcinoma in the TCDD feed study of Kociba *et al.* (1978).

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 like PeCDF require initial binding to the AhR. Recent data indicates 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).

Studies have shown that, in initiation-promotion models of hepatocarcinogenesis, PeCDF is a potent promoter of altered hepatic foci (Waern *et al*., 1991). Given that TCDD and related compounds such as PeCDF are not direct acting genotoxic agents and are potent growth dysregulators, it is believed that their predominant mode of action is as tumor promoters. Within a conceptual multistage model of carcinogenesis, promotion mediated by these compounds via the AhR may be due to an increase in the net growth rate of initiated cells due to selective growth advantage or decreased rate of cell death via suppression of apoptosis. In studies with TCDD, there are significant increases in hepatocyte replication as judged by BrdU labeling studies (Maronpot *et al*., 1993; Walker *et al*., 1998; Wyde *et al*., 2001a). Studies by Stinchcombe *et al*. (1995), Worner and Schrenk (1996), and Bohnenbuerger *et al.* (2001) have also shown a suppression of apoptosis by TCDD and PCBs. In addition, altered growth regulation may be due to alterations in intercellular communication, which have also been observed in the livers of rats exposed to DLCs (Baker *et al*., 1995; Wärngård *et al*., 1996; Bager *et al*., 1997). While DLCs are not direct acting genotoxic agents, there are data indicating that persistent AhR active compounds may be indirectly genotoxic. This may contribute to an increase in the number of cells within the liver capable of undergoing promotion (Moolgavkar *et al*., 1996; Portier *et al*., 1996). It is hypothesized that the indirect genotoxicity may be via an AhR dependent induction of CYP1 family cytochromes P450 that leads to an induction of oxidative stress because of inefficient electron transfer during P450 metabolism (Park *et al*., 1996) or the production of redox active estradiol metabolites as a result of CYP1 mediated estrogen metabolism (Lucier *et al*., 1991; Kohn *et al*., 1993). Studies have shown an induction of oxidative stress and DNA damage by high dose acute exposure to TCDD (Stohs *et al*., 1990). The induction of lipid peroxidation and single stranded DNA breaks was also observed in tissues from the present PeCDF study (Hassoun *et al*., 2000, 2002). Other studies on the female specific tumor promotion response in rats have shown an induction of oxidative DNA damage and hepatocyte replication by TCDD that is female specific and estrogen dependent (Lucier *et al*., 1991; Tritscher *et al*., 1996; Wyde *et al*., 2001a,b).

In the present study of PeCDF, there was only a single occurrence of lung cystic keratinizing epithelioma (CKE) in the 200 ng/kg group. By comparison, significant increases in the incidences of CKE were observed in the TCDD and PCB 126 studies conducted as part of the dioxin TEF evaluation (NTP, 2006a,b). Given this, and that this neoplasm is rare and has not been observed in any of the vehicle control groups in the dioxin TEF evaluation studies, the observed CKE in the present study may have been related to treatment.

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. While no direct comparison has been made between CKE and the keratinizing squamous cell carcinoma observed in the Kociba *et al.* (1978) study, given the keratinizing nature of the lesion, it is possible that these may represent the same lesion. It should be noted that CKE was not a diagnostic term consistently used at the time of the Kociba *et al.* (1978) study. 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).

Assuming that the observed CKE may have been related to treatment, the low incidence of CKE in this PeCDF study at the 200 ng/kg dose (a dose equivalent to 100 ng TCDD/kg assuming a TEF of 0.5) suggests that higher doses could have been used resulting in a higher incidence of CKE. In the TCDD study conducted as part of the dioxin TEF evaluation (NTP, 2006a), the incidence of CKE was 0/53 in the 46 ng TCDD/kg group suggesting that at least for TCDD, this neoplasm has a steep dose response over this dose range.

In contrast to the present study, a recent study of the high TEQ PCB mixture Aroclor 1254 in Charles River Sprague-Dawley rats demonstrated no increases in the incidence of any type of lung tumor (Mayes *et al*., 1998) despite a high incidence of hepatocellular neoplasms. While Aroclor 1254 contains a significant TEQ contribution by PCB 126, this mixture also contains mono-*ortho* and di-*ortho* PCBs.

In the current study, the incidences of bronchiolar metaplasia of the alveolar epithelium were significantly increased in the 92 and 200 ng/kg groups at 2 years and exhibited a significantly increased trend across the dose range. In addition, there was an increase in the incidence of alveolar squamous metaplasia in the 92 ng/kg group. These findings are consistent with those made in the TCDD study conducted as part of the dioxin TEF evaluation (NTP, 2006a) and in a two stage initiation-promotion model of TCDD 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 was a significantly decreased incidence of alveolar epithelial hyperplasia in the 200 ng PeCDF/kg dose group.

PeCDF 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 in alveoli or alveolar ducts. Histochemical analyses of mucin and GSTPi in lung tissue from the dioxin TEF evaluation studies indicates that this does appear to be similar to bronchiolar epithelium and is distinct from alveolar epithelial hyperplasia (Brix *et al*., 2004). It is not clear though 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 lung. CYP1A1 is 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 alveolar epithelial 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 of PeCDF 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 PeCDF on the lung may be an indirect effect due to the disruption of retinoid homeostasis in the liver. It is known that in rodents, mobilization of retinoid stores by TCDD and DLCs leads to a disruption in retinoid homeostasis and vitamin A deficiency (Van Birgelen *et al*., 1994, 1995a; Fiorella *et al*., 1995; Fattore *et al*., 2000; Schmidt *et al*., 2003). A characteristic of retinoid deficiency is abnormal epithelial differentiation to a keratinized squamous

phenotype (Lancillotti *et al*., 1992; Lotan, 1994). The action of DLC may therefore be a disruption of retinoid action leading to altered growth and differentiation of the lung epithelium, resulting in squamous metaplasia and CKE.

In the gavage study of TCDD conducted as part of the dioxin TEF evaluation (NTP, 2006a), there was a significant increase in the incidence of gingival squamous cell carcinoma of the oral mucosa. In the present study, there was an elevated incidence of gingival squamous cell carcinoma in the 200 ng/kg core study and stop-exposure groups. While these incidences were not statistically elevated over concurrent vehicle controls, they exceeded the historical control range in Harlan Sprague-Dawley rats used in the dioxin TEF evaluation. Given the site concordance and presence in both core and stop-exposure groups, the observed neoplasms were attributed to PeCDF administration. In the present study, there was also an increased trend in incidence of gingival hyperplasia of the oral mucosa. Cases of gingival squamous cell carcinoma were seen in vehicle controls (1/53) and in several dosed groups (6, 20, 92, and 200 ng/kg). The incidence was elevated in the 200 ng/kg group (3/53), although this increase was not statistically significant.

In the TCDD feed study by Kociba *et al*. (1978), there was an increase in the incidence of stratified squamous cell carcinoma of the hard palate/nasal turbinates in both male and female rats. The gingival squamous cell carcinomas in the dioxin TEF evaluation TCDD study (NTP, 2006a) were adjacent to the molars and invaded into the hard palate/nasal turbinate areas. This suggests that the lesions seen in the present study and in the Kociba *et al*. (1978) study are similar.

In the present study, there was an increase in the incidence of squamous metaplasia of the uterus in the 44, 92, and 200 ng/kg core study groups and increased incidences of cystic endometrial hyperplasia in the 92 and 200 ng/kg groups. In addition, the incidence of carcinoma of the uterus was marginally increased in the 92 ng/kg group and two carcinomas occurred in the 200 ng/kg group. The increased incidence of uterine carcinoma may have been related to treatment with PeCDF.

In the present study, there was an increase in the incidence of adrenal cystic degeneration at all doses tested. There was also an increase in the incidence of cytoplasmic vacuolization in the 92 ng/kg group. There were no significant increases in the incidences of any adrenal cortical neoplasm.

In the Kociba *et al*. (1978) dosed feed study of TCDD in Spartan Sprague-Dawley rats, there were significant increases in the incidences of adrenal cortical adenomas observed in male, but not female rats, at 100 ng/kg. A significant reduction in the incidence of benign pheochromocytoma was also observed in male rats only in the 2-year TCDD feed study by Kociba *et al*. (1978) (33% and 14% in controls and 100 ng/kg, respectively). There was no significant effect on the incidence of benign pheochromocytoma in the present PeCDF study or in the dioxin TEF evaluation TCDD gavage study (NTP, 2006a).

In the present study, there were increases in the incidences of adenoma or carcinoma (combined) of the acinus of the pancreas in the 92 ng/kg group and the 200 ng/kg stop-exposure group. While the increases were not statistically elevated over concurrent vehicle controls, it may have been related to PeCDF treatment. The increase in the incidence of pancreatic acinar adenoma observed in the TCDD study (NTP, 2006a) may also have been related to TCDD exposure.

In the present study, there was an increase in the incidence of acinar cytoplasmic vacuolization and chronic active arterial inflammation following exposure to 200 ng/kg. There was also an increased trend in incidence of islet pancreatic hyperplasia. By comparison, in the dioxin TEF evaluation TCDD study (NTP, 2006a), a significant effect on the incidence of cytoplasmic vacuolization was seen at a lower dose (46 ng/kg) than for acinar atrophy (100 ng/kg only). Cytoplasmic vacuolization may be a precursor to the acinar atrophy that was seen with TCDD (NTP, 2006a) and with PCB 126 (NTP, 2006b). Atrophy of the pancreatic acinus may be related to the down regulation of cholecystokinin (CCK). As shown by Lee *et al.* (2000), intestinal CCK is reduced by exposure to DLCs. 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 DLCs. 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 Otsuka Long-Evans Tokushima Fatty 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).

Administration of PeCDF in this study significantly increased the incidence of cardiomyopathy in the 200 ng/kg group. Similarly, an increase in the incidence of cardiomyopathy was observed in the dioxin TEF

evaluation TCDD study (Jokinen *et al*., 2003; NTP, 2006a). However, the average severity of cardiomyopathy was unaffected. Cardiomyopathy is a common, spontaneously occurring degenerative change of myocardial fibers that is seen in rats. Its cause in the rat is unknown, but age of onset and severity are affected by diet, environment, and stress. Significant increases in the incidence of cardiomyopathy were observed at doses of 300 ng/kg per day and above. The microscopic appearance of cardiomyopathy is the same in both the vehicle controls and treated animals and was typical of that described for spontaneous lesions. This finding may suggest that exposure to the chemicals increased the occurrence of the spontaneous change.

The heart is a target for TCDD and related DLCs in both rodents and humans (Peterson *et al*., 1993; Flesch-Janys *et al*., 1995; Walker and Catron, 2000; Heid *et al*., 2001). In the 2-year study in which Sprague-Dawley rats were administered up to 0.1 µg/kg per day of TCDD in the feed, Kociba *et al*. (1978) reported an increase in myocardial degenerative change above background levels in female rats.

In this study, the incidence of thymic atrophy was elevated after 31 weeks in the 200 ng/kg group only. There was no significant effect on the incidence of thymic atrophy at 14 or 53 weeks. In addition, there was a significantly increased trend in the incidences of thymic atrophy at the end of the 2-year study. Thymic atrophy is one of the characteristic 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).

By comparison, the incidences of thymic atrophy were significantly elevated at 46 ng/kg or greater at 14 weeks and at 22 ng/kg or greater at the end of the TCDD study, indicative of a much more robust response. Thymic atrophy may be related to the reduction in body weight gain observed in these animals as seen in short term feed restriction studies (Levin *et al*., 1993). Significant reductions in body weight gain were observed in the TCDD study (NTP, 2006a) conducted as part of the dioxin TEF evaluation. The reductions in body weight gain in the present study of PeCDF were not as pronounced. That the thymic atrophy response to PeCDF was much lower than that seen in the TCDD study is consistent with other comparative observations, suggesting that higher doses of PeCDF could have been used and may have been more comparable to the TCDD doses.

In this 2-year study, there was no significant effect on the incidence of thyroid gland neoplasia. There was a trend toward lower incidence of C-cell adenoma in PeCDF-treated animals. Similarly, there were lower incidences of C-cell adenomas in female rats in the NTP (2006a) TCDD study and in exposed male rats in the (Kociba *et al*., 1978) TCDD feed study.

In the present study, there were increases in the incidences of follicular cell hypertrophy at all doses at 14-weeks, no increases at 31 weeks, and an increased trend in incidence at 53 weeks. At 2 years, there were increased incidences in all groups administered 20 ng/kg or greater including the stop-exposure group. At the end of the 2-year gavage study of TCDD in Osborne-Mendel rats (NTP, 1982a), there were significant increases in the incidences of thyroid gland follicular cell adenomas in male rats and a nonsignificant increase in females.

Alterations in thyroid hormone homeostasis by TCDD and related DLCs are well established (Van Birgelen *et al*., 1994,1995b; Schmidt *et al*., 2003). Analyses of thyroid hormones in the present study confirmed this effect. A significant reduction in total thyroxine (T_4) was seen at all interim time points at 92 and 200 ng/kg. Total triiodothyronine (T_3) levels were elevated at these doses at 31 and 53 weeks. However, there was no effect on thyroid stimulating hormone (TSH) levels. UDP-glucuronosyltransferase (UDPGT) is an enzyme in the liver that is inducible by DLCs. The disruption of thyroid hormone homeostasis by DLCs is believed to be due to the increase in T_4 glucuronidation as a result of increased hepatic expression of UDPGT. This leads to decreased negative feedback inhibition of the thyroid gland and over-expression of TSH (Curran and DeGroot, 1991). Kohn *et al*. (1996) developed a mathematical model of the effects of TCDD on UDPGT expression and thyroid hormone homeostasis that is consistent with this mechanism. 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). Despite alterations in T_4 and T_3 levels at the early time points in the present study, there was no effect on TSH levels at any time point. Therefore, it is possible that the lack of follicular cell neoplasia in this study reflects the lack of a sustained long-term increase on TSH levels.

In this study, there were significantly increased incidences of carcinoma of the mammary gland in the 6 and 20 ng/kg groups and increased incidences of pituitary gland adenoma in the 6, 20, and 44 ng/kg groups.

Overall, there was a trend to lower adjusted incidence of mammary gland carcinoma following PeCDF exposure, although the incidence of these neoplasms showed no clear dose-response pattern. Mammary gland carcinoma was seen in vehicle control animals at low incidence (9%). Fibroadenoma is a spontaneous lesion in female rats and occurred at high incidence (64%). There was no effect on the incidence of fibroadenoma in the mammary gland.

In contrast, significantly lower incidences of mammary gland and pituitary gland neoplasms were observed in animals exposed to 100 ng TCDD/kg body weight in the 2-year feed study by Kociba *et al.* (1978). Similarly, there were significantly lower incidences of spontaneous mammary gland fibroadenoma, adenoma, or carcinoma (combined) and pituitary gland adenoma in the NTP (2006a) TCDD study. It is believed that the lower incidence of mammary gland and pituitary gland neoplasms in exposed rats is related to a general endocrine effect as a result of reductions in body weight gain associated with exposure. An association between reduced body weight gain and lower incidence of mammary gland and pituitary gland neoplasms has been observed in many NTP studies of F344/N rats (Seilkop, 1995). In this study, mean terminal body weights of the 6, 20, 44, 92 and 200 ng/kg groups at 2 years were 9% higher, 0% lower, 7% lower, 3% lower, and 12% lower than the vehicle control weight, respectively. Consequently it is unlikely that increased neoplasm incidences in the mid-dose groups are due to body weight changes.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *some evidence of carcinogenic activity** of PeCDF in female Harlan Sprague-Dawley rats, based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and gingival squamous cell carcinoma of the oral mucosa. Occurrences of cystic keratinizing epithelioma of the lung, neoplasms of the pancreatic acinus, and carcinoma of the uterus may have been related to administration of PeCDF.

PeCDF administration caused increased incidences of nonneoplastic lesions of the liver, oral mucosa, uterus, lung, pancreas, thyroid gland, thymus, adrenal cortex, kidney, heart, and forestomach in female rats.

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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 appears on page 13.

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APPENDIX A
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF PeCDF

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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 PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
Disposition Summary						
Animals initially in study	28	28	28	28	28	28
14-Week interim evaluation	10	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8	8
Animals examined microscopically	28	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
Integumentary System

Mammary gland	(10)	(2)	(1)	(1)	(10)
Carcinoma			1 (100%)		
Fibroadenoma	1 (10%)	2 (100%)			

Systems Examined with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE A1a
Summary of the Incidence of Neoplasms in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
53-Week Interim Evaluation						
Endocrine System						
Thyroid gland	(8)	(8)	(7)	(8)	(8)	(8)
C-cell, adenoma	1 (13%)	1 (13%)				
Integumentary System						
Mammary gland	(8)	(1)	(1)		(2)	(8)
Fibroadenoma		1 (100%)			1 (50%)	
Systems Examined 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		2	1		
53-Week interim evaluation	1	2			1	
Total primary neoplasms						
31-Week interim evaluation	1		2	1		
53-Week interim evaluation	1	2			1	
Total animals with benign neoplasms						
31-Week interim evaluation	1		2			
53-Week interim evaluation	1	2			1	
Total benign neoplasms						
31-Week interim evaluation	1		2			
53-Week interim evaluation	1	2			1	
Total animals with malignant neoplasms						
31-Week interim evaluation				1		
Total malignant neoplasms						
31-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 PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Disposition Summary							
Animals initially in study	53	53	53	53	53	53	50
Early deaths							
Accidental deaths		1	2	1			
Moribund	21	20	17	19	19	19	25
Natural deaths	7	10	10	8	14	11	10
Survivors							
Terminal sacrifice	25	22	24	25	20	23	15
Animals examined microscopically	53	53	53	53	53	53	50
Alimentary System							
Intestine large, colon	(53)	(53)	(53)	(53)	(51)	(53)	(49)
Carcinoma, metastatic, uterus				1 (2%)			
Intestine large, rectum	(53)	(53)	(53)	(53)	(52)	(53)	(50)
Carcinoma, metastatic, uterus						1 (2%)	
Squamous cell carcinoma, metastatic, uterus							1 (2%)
Intestine large, cecum	(53)	(53)	(53)	(52)	(51)	(53)	(48)
Intestine small, duodenum	(53)	(53)	(53)	(53)	(52)	(53)	(49)
Carcinoma, metastatic, uterus						1 (2%)	
Intestine small, jejunum	(53)	(53)	(53)	(52)	(52)	(53)	(48)
Leiomyoma						1 (2%)	
Liver	(53)	(53)	(53)	(52)	(53)	(53)	(50)
Carcinoma, metastatic, uterus					1 (2%)	1 (2%)	
Cholangiocarcinoma				1 (2%)	1 (2%)	1 (2%)	
Cholangiocarcinoma, multiple						1 (2%)	
Hepatocellular adenoma	1 (2%)		1 (2%)		2 (4%)	4 (8%)	1 (2%)
Oral mucosa	(52)	(53)	(52)	(53)	(53)	(53)	(50)
Gingival, squamous cell carcinoma	1 (2%)	2 (4%)	1 (2%)		1 (2%)	3 (6%)	3 (6%)
Pancreas	(53)	(53)	(53)	(52)	(52)	(52)	(49)
Carcinoma, metastatic, uterus					1 (2%)	1 (2%)	
Acinus, adenoma					1 (2%)		1 (2%)
Acinus, carcinoma					1 (2%)		1 (2%)
Stomach, forestomach	(53)	(53)	(53)	(53)	(52)	(53)	(50)
Fibrosarcoma						1 (2%)	
Squamous cell papilloma							1 (2%)
Stomach, glandular	(53)	(53)	(53)	(53)	(52)	(53)	(50)
Carcinoma, metastatic, uterus						1 (2%)	
Cardiovascular System							
Heart	(53)	(53)	(52)	(53)	(53)	(52)	(50)
Schwannoma malignant	1 (2%)	2 (4%)		2 (4%)			

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Endocrine System							
Adrenal cortex	(53)	(53)	(53)	(52)	(53)	(53)	(48)
Adenoma	1 (2%)	1 (2%)			2 (4%)		1 (2%)
Carcinoma	1 (2%)				1 (2%)		
Adrenal medulla	(53)	(53)	(53)	(52)	(53)	(53)	(48)
Pheochromocytoma malignant			1 (2%)				
Pheochromocytoma complex	1 (2%)						
Pheochromocytoma benign	4 (8%)	5 (9%)	2 (4%)	5 (10%)	1 (2%)	2 (4%)	1 (2%)
Bilateral, pheochromocytoma benign				1 (2%)			
Pancreatic islets	(53)	(53)	(53)	(52)	(52)	(52)	(49)
Adenoma			1 (2%)	1 (2%)	1 (2%)	2 (4%)	
Carcinoma			1 (2%)				
Parathyroid gland	(49)	(51)	(44)	(51)	(49)	(49)	(47)
Adenoma		2 (4%)					
Pituitary gland	(53)	(53)	(53)	(53)	(53)	(53)	(49)
Carcinoma		1 (2%)					
Pars distalis, adenoma	15 (28%)	21 (40%)	23 (43%)	23 (43%)	21 (40%)	16 (30%)	13 (27%)
Thyroid gland	(53)	(53)	(51)	(53)	(51)	(51)	(48)
Bilateral, C-cell, adenoma		1 (2%)	1 (2%)	1 (2%)			
C-cell, adenoma	13 (25%)	9 (17%)	8 (16%)	8 (15%)	6 (12%)	7 (14%)	5 (10%)
C-cell, carcinoma	2 (4%)	2 (4%)	2 (4%)	2 (4%)		1 (2%)	
Follicular cell, adenoma		1 (2%)					
General Body System							
None							
Genital System							
Clitoral gland	(53)	(51)	(53)	(53)	(50)	(53)	(50)
Adenoma			1 (2%)				
Carcinoma, metastatic, mammary gland		1 (2%)					
Ovary	(53)	(53)	(53)	(52)	(52)	(53)	(47)
Cystadenoma		1 (2%)					
Granulosa cell tumor malignant		1 (2%)					
Granulosa cell tumor benign		1 (2%)	1 (2%)				
Luteoma	1 (2%)						1 (2%)
Sertoli cell tumor malignant				1 (2%)			
Uterus	(53)	(53)	(53)	(52)	(52)	(53)	(49)
Adenoma	2 (4%)						
Carcinoma	1 (2%)	1 (2%)		1 (2%)	5 (10%)	2 (4%)	1 (2%)
Fibroma	1 (2%)						
Hemangioma							1 (2%)
Leiomyoma				1 (2%)			
Polyp stromal	8 (15%)	3 (6%)	5 (9%)	5 (10%)	6 (12%)	3 (6%)	4 (8%)
Polyp stromal, multiple	1 (2%)				1 (2%)	1 (2%)	
Schwannoma malignant				1 (2%)		1 (2%)	
Squamous cell carcinoma					1 (2%)		

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Genital System (continued)							
Uterus (continued)	(53)	(53)	(53)	(52)	(52)	(53)	(49)
Cervix, polyp stromal			1 (2%)				
Cervix, sarcoma stromal		1 (2%)					
Cervix, schwannoma malignant		1 (2%)					
Cervix, squamous cell carcinoma			1 (2%)				1 (2%)
Vagina	(1)	(1)	(1)				
Polyp			1 (100%)				
Sarcoma stromal		1 (100%)					
Squamous cell papilloma	1 (100%)						
Hematopoietic System							
Bone marrow	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Lymph node	(5)	(4)	(4)	(4)	(3)	(7)	(3)
Deep cervical, carcinoma, metastatic, thyroid gland			1 (25%)				
Inguinal, carcinoma, metastatic, mammary gland						1 (14%)	
Mediastinal, carcinoma, metastatic, thyroid gland			1 (25%)				
Lymph node, mandibular	(53)	(53)	(51)	(53)	(51)	(51)	(49)
Lymph node, mesenteric	(53)	(53)	(53)	(52)	(52)	(53)	(49)
Carcinoma, metastatic, uterus					1 (2%)		
Spleen	(53)	(53)	(53)	(52)	(52)	(51)	(49)
Carcinoma, metastatic, uterus						1 (2%)	
Thymus	(53)	(49)	(50)	(52)	(49)	(51)	(49)
Osteosarcoma, metastatic, skin						1 (2%)	
Thymoma malignant, metastatic, mammary gland							1 (2%)
Integumentary System							
Mammary gland	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Adenoma	1 (2%)	1 (2%)	2 (4%)		1 (2%)		2 (4%)
Carcinoma	5 (9%)	9 (17%)	11 (21%)	2 (4%)	5 (9%)	3 (6%)	5 (10%)
Carcinoma, multiple		3 (6%)	2 (4%)				
Fibroadenoma	24 (45%)	23 (43%)	23 (43%)	21 (40%)	22 (42%)	17 (32%)	25 (50%)
Fibroadenoma, multiple	10 (19%)	12 (23%)	7 (13%)	8 (15%)	12 (23%)	13 (25%)	9 (18%)
Osteosarcoma, metastatic, skin						1 (2%)	
Skin	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Basal cell carcinoma					1 (2%)		1 (2%)
Fibroma		1 (2%)	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Fibrosarcoma			1 (2%)				1 (2%)
Fibrous histiocytoma						1 (2%)	
Keratoacanthoma						1 (2%)	
Neural crest tumor						1 (2%)	
Neurofibroma		1 (2%)					
Osteosarcoma						1 (2%)	
Sarcoma							1 (2%)
Schwannoma malignant		1 (2%)					
Subcutaneous tissue, hemangioma				1 (2%)			

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Musculoskeletal System							
Bone	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Fibrosarcoma, metastatic, skin							1 (2%)
Osteosarcoma		1 (2%)					
Skeletal muscle				(2)			
Rhabdomyosarcoma				1 (50%)			
Nervous System							
Brain	(53)	(53)	(53)	(53)	(53)	(53)	(49)
Astrocytoma malignant			1 (2%)		1 (2%)	1 (2%)	
Carcinoma, metastatic, pituitary gland		1 (2%)					
Granular cell tumor malignant				1 (2%)			
Cranial nerve, squamous cell carcinoma, metastatic, oral mucosa					1 (2%)		
Respiratory System							
Lung	(53)	(53)	(53)	(53)	(53)	(52)	(50)
Alveolar/bronchiolar adenoma						1 (2%)	
Carcinoma, metastatic, mammary gland	1 (2%)				1 (2%)	2 (4%)	
Carcinoma, metastatic, thyroid gland	1 (2%)		1 (2%)				
Carcinoma, metastatic, uterus					1 (2%)	1 (2%)	
Cystic keratinizing epithelioma, multiple						1 (2%)	
Fibrous histiocytoma, metastatic, skin						1 (2%)	
Histiocytic sarcoma						1 (2%)	
Osteosarcoma, metastatic, skin						1 (2%)	
Squamous cell carcinoma, metastatic, uterus							1 (2%)
Nose	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Sarcoma			1 (2%)				
Special Senses System							
Eye	(53)	(53)	(52)	(53)	(52)	(52)	(49)
Sarcoma						1 (2%)	
Harderian gland	(53)	(53)	(53)	(53)	(52)	(52)	(49)
Carcinoma, metastatic, oral mucosa		1 (2%)					
Zymbal's gland					(1)		
Adenoma					1 (100%)		

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Urinary System							
Kidney	(53)	(53)	(53)	(52)	(53)	(53)	(48)
Lipoma	1 (2%)						
Nephroblastoma			1 (2%)		1 (2%)		
Renal tubule, adenoma			1 (2%)		1 (2%)		
Urinary bladder	(53)	(52)	(53)	(53)	(51)	(53)	(50)
Carcinoma, metastatic, uterus					1 (2%)		
Systemic Lesions							
Multiple organs[b]	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Histiocytic sarcoma						1 (2%)	
Leukemia mononuclear	1 (2%)	1 (2%)				1 (2%)	
Lymphoma malignant				1 (2%)			
Neoplasm Summary							
Total animals with primary neoplasms[c]	48	49	49	44	48	46	43
Total primary neoplasms	97	110	102	89	96	90	80
Total animals with benign neoplasms	45	45	43	42	45	41	40
Total benign neoplasms	84	83	79	76	78	70	66
Total animals with malignant neoplasms	12	20	21	13	16	16	12
Total malignant neoplasms	13	27	23	13	18	19	14
Total animals with metastatic neoplasms	2	3	1	1	3	5	3
Total metastatic neoplasms	2	3	3	1	7	14	4
Total animals with uncertain neoplasms-benign or malignant						1	
Total uncertain neoplasms						1	

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: Vehicle Control

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

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: Vehicle Control

Number of Days on Study	7 1 5	7 2 0	7 2 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 4 2	0 6 0	0 3 5	0 1 1	0 1 9	0 2 1	0 2 3	0 2 4	0 2 6	0 3 4	0 6 5	0 8 9	0 9 8	0 1 5	0 2 0	0 3 1	0 3 3	0 4 6	0 4 7	0 4 9	0 6 4	0 8 6	0 9 5	0 9 7	0 5 4	0 9 2	0 9 3	0 9 6	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
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hepatocellular adenoma																													1
Mesentery														+															1
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	52
Gingival, squamous cell carcinoma																								X					1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth	+	+		+		+	+	+		+	+	+		+	+	+	+	+	+	+				+	+	+	+	+	33
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant											X																		1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma														X															1
Carcinoma																													1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma complex																						X							1
Pheochromocytoma benign												X		X												X			4
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma		X		X			X			X		X			X					X	X					X			15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
C-cell, adenoma	X			X			X	X							X	X							X			X	X		13
C-cell, carcinoma												X								X									2
General Body System																													
None																													

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: Vehicle Control

Number of Days on Study	2 8 9	3 1 0	3 2 1	3 7 7	5 0 0	5 0 0	5 1 3	5 3 6	5 5 4	5 8 8	6 0 3	6 1 0	6 1 9	6 1 9	6 5 1	6 5 1	6 5 2	6 5 9	6 7 5	6 7 8	6 7 8	6 7 8	6 7 8	6 9 9	7 1 5
Carcass ID Number	0 4 1	0 0 1	0 5 2	0 0 2	0 0 5	0 1 2	0 5 7	0 1 4	0 1 8	0 6 1	0 9 1	0 9 4	0 4 4	0 6 2	0 2 5	0 5 3	0 2 8	0 8 8	0 1 3	0 2 7	0 3 2	0 5 0	0 5 9	0 3 0	0 2 9
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																									
Oviduct																+									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma or carcinoma																					X	X			
Fibroma																						X			
Polyp stromal														X						X	X				X
Polyp stromal, multiple										X															
Vagina																									
Squamous cell papilloma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node													+							+					
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma														X			X								
Fibroadenoma	X		X		X		X	X	X	X	X				X						X	X			
Fibroadenoma, multiple						X						X				X			X	X			X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland														X											
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: Vehicle Control

Number of Days on Study	7 1 5	7 2 0	7 2 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 4 2	0 6 0	0 3 5	0 1 1	0 1 9	0 2 1	0 2 3	0 2 4	0 2 6	0 3 4	0 6 5	0 8 9	0 9 8	1 5	2 0	2 9	3 3	3 6	4 7	4 9	6 4	6 8	6 5	9 7	5 4	5 9	9 3	9 6	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Luteoma	X																												1
Oviduct																													1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma or carcinoma								X																					3
Fibroma																													1
Polyp stromal				X									X								X				X				8
Polyp stromal, multiple																													1
Vagina				+																									1
Squamous cell papilloma				X																									1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node					+								+												+				5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																X													1
Carcinoma							X			X		X																	5
Fibroadenoma		X	X		X		X						X		X		X		X			X	X	X	X			X	24
Fibroadenoma, multiple	X									X																X	X		10
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, mammary gland																													1
Carcinoma, metastatic, thyroid gland												X																	1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: Vehicle Control

Number of Days on Study	2	3	3	3	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7
	8	1	2	7	0	0	1	3	5	8	0	1	1	1	5	5	5	5	7	7	7	7	7	9	1
	9	0	1	7	0	0	3	6	4	8	3	0	9	9	1	1	2	9	5	8	8	8	8	9	5
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	5	0	0	1	5	1	1	6	9	9	4	6	2	5	2	8	1	2	3	5	5	3	2
	1	1	2	2	5	2	7	4	8	1	1	4	4	2	5	3	8	8	3	7	2	0	9	0	9
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X																					

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: Vehicle Control

Number of Days on Study	7 1 5	7 2 0	7 2 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 4 2	0 6 0	0 3 5	0 1 1	0 1 9	0 2 1	0 2 3	0 2 4	0 2 6	0 3 4	0 6 5	0 8 9	0 9 8	1 5	2 0	3 1	3 3	4 6	4 7	4 9	6 4	8 6	9 5	9 7	5 4	9 2	9 3	9 6	Total Tissues/ Tumors
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lipoma																											X	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leukemia mononuclear																												1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 6 ng/kg

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 6 ng/kg

Number of Days on Study	6 6 8	6 8 7	6 8 9	6 9 3	7 0 6	7 0 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 0 4	1 5 7	1 5 8	1 9 3	1 0 7	1 8 7	1 0 3	1 0 8	1 2 9	1 5 3	1 6 5	1 6 8	1 7 2	1 7 5	1 7 7	1 7 8	1 5 4	1 6 2	1 7 0	1 9 1	1 9 7	1 1 0	1 0 2	1 1 3	1 1 5	1 6 1	1 8 0	1 9 6	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
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Mesentery																					+								2
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Gingival, squamous cell carcinoma					X			X																					2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth	+				+	+		+			+		+	+		+	+	+		+			+		+		+		25
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant			X													X													2
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma										X																			1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign						X										X							X						5
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	51
Adenoma																												X	2
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																													1
Pars distalis, adenoma	X	X	X			X					X		X	X	X	X	X			X	X	X	X		X			X	21
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma																													1
C-cell, adenoma	X					X			X	X		X			X	X													9
C-cell, carcinoma																											X		2
Follicular cell, adenoma																										X			1
General Body System																													
None																													

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 6 ng/kg

Number of Days on Study	2 8 9	2 9 6	3 2 1	3 9 3	4 2 0	4 3 2	4 5 1	4 6 7	4 9 8	5 0 3	5 1 6	5 2 6	5 3 0	5 4 7	5 8 7	6 0 0	6 2 4	6 3 5	6 4 5	6 4 7	6 5 1	6 5 1	6 5 1	6 6 5	6 6 7
Carcass ID Number	1 6 4	1 4 2	1 8 9	1 3 0	1 1 2	1 0 9	1 8 8	1 7 9	1 6 7	1 6 3	1 7 3	1 7 6	1 2 7	1 9 5	1 1 1	1 5 5	1 4 1	1 5 2	1 0 6	1 6 9	1 0 5	1 5 6	1 5 9	1 9 2	1 9 0
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma														X											
Granulosa cell tumor malignant																							X		
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Polyp stromal														X											
Cervix, sarcoma stromal										X															
Cervix, schwannoma malignant								X																	
Vagina																									
Sarcoma stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+			+	+											+						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X									X					X						
Carcinoma, multiple																								X	
Fibroadenoma	X		X	X		X			X		X				X		X			X	X		X	X	
Fibroadenoma, multiple																X			X			X			X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																				X					
Neurofibroma																									
Schwannoma malignant																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																								X	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 6 ng/kg

Number of Days on Study	6 6 8	6 8 7	6 8 9	6 9 3	7 0 6	7 0 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 0 4	1 5 7	1 5 8	1 9 3	1 0 7	1 8 7	1 0 3	1 0 8	1 2 9	1 5 3	1 6 5	1 6 8	1 7 2	1 7 5	1 7 7	1 7 8	1 5 4	1 6 2	1 7 0	1 9 1	1 9 7	1 0 1	1 0 2	1 1 3	1 1 5	1 6 1	1 8 0	1 9 6	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, mammary gland				X																									1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cystadenoma																													1
Granulosa cell tumor malignant																													1
Granulosa cell tumor benign									X																				1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma					X																								1
Polyp stromal						X									X														3
Cervix, sarcoma stromal																													1
Cervix, schwannoma malignant																													1
Vagina																						+							1
Sarcoma stromal																						X							1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																													4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma										X																			1
Carcinoma				X		X													X		X			X		X			9
Carcinoma, multiple											X												X						3
Fibroadenoma	X	X	X		X				X					X			X	X	X					X		X			23
Fibroadenoma, multiple				X		X					X	X			X					X			X					X	12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma																													1
Neurofibroma			X																										1
Schwannoma malignant																										X			1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Osteosarcoma						X																							1
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, pituitary gland																													1
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 6 ng/kg

Number of Days on Study	2 8 9	2 9 6	3 2 1	3 9 3	4 2 0	4 3 2	4 5 1	4 6 7	4 9 8	5 0 3	5 1 6	5 2 6	5 3 0	5 4 7	5 8 7	6 0 0	6 2 4	6 3 5	6 4 5	6 4 7	6 5 1	6 5 1	6 5 1	6 6 5	6 6 7
Carcass ID Number	1 6 4	1 4 2	1 8 9	1 3 0	1 1 2	1 0 9	1 8 8	1 7 9	1 6 7	1 6 3	1 7 3	1 7 6	1 2 7	1 9 5	1 1 5	1 5 5	1 4 1	1 5 2	1 0 6	1 6 9	1 0 5	1 5 6	1 5 9	1 9 2	1 9 0
Special Senses System																									
Ear		+																							
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, oral mucosa																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																		X							

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 6 ng/kg

Number of Days on Study	6 6 8	6 8 7	6 8 9	6 9 3	7 0 6	7 0 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 0 4	1 5 7	1 5 8	1 9 3	1 0 7	1 8 7	1 0 3	1 0 8	1 2 9	1 5 3	1 6 5	1 6 8	1 7 2	1 7 5	1 7 7	1 7 8	1 5 4	1 6 2	1 7 0	1 9 1	1 9 7	1 0 1	1 0 2	1 1 3	1 1 5	1 6 1	1 8 0	1 9 6	Total Tissues/ Tumors
Special Senses System																													
Ear																													1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, oral mucosa					X																								1
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leukemia mononuclear																													1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 20 ng/kg

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 20 ng/kg

Number of Days on Study	6 7 5	6 7 7	6 8 9	7 0 1	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 5 7	2 5 9	2 3 9	2 4 3	2 0 3	2 1 0	2 1 2	2 1 3	2 2 7	2 3 7	2 4 0	2 5 5	2 6 6	2 9 7	2 0 1	2 2 5	2 2 8	2 8 6	2 0 5	2 0 8	2 2 1	2 3 6	2 4 1	2 4 6	2 4 7	2 5 2	2 8 8	2 9 0	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hepatocellular adenoma																										X		1
Mesentery												+																1
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	52
Gingival, squamous cell carcinoma																												1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth			+		+	+	+	+	+					+		+	+				+					+		22
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma malignant																									X			1
Pheochromocytoma benign				X									X															2
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma														X														1
Carcinoma																							X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma	X	X	X		X	X	X	X	X			X		X			X	X	X		X			X	X			23
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Bilateral, C-cell, adenoma											X																	1
C-cell, adenoma						X				X							X		X							X		8
C-cell, carcinoma																								X				2
General Body System																												
None																												

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 20 ng/kg

Number of Days on Study	1 1 6	1 9 6	2 1 9	2 4 6	2 9 7	3 0 0	3 0 1	3 9 3	4 2 0	4 5 1	4 9 7	5 1 7	5 6 1	5 7 0	5 8 4	5 8 6	5 8 7	5 8 7	6 0 5	6 1 9	6 2 9	6 3 6	6 3 8	6 6 8	6 7 5
Carcass ID Number	2 6 9	2 7 7	2 0 6	2 0 7	2 7 5	2 6 0	2 5 8	2 3 0	2 2 3	2 6 7	2 2 2	2 4 4	2 0 9	2 9 6	2 0 4	2 2 9	2 7 1	2 8 9	2 1 5	2 3 8	2 4 2	2 5 6	2 7 3	2 8 7	2 2 6
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																									
Cervix, polyp stromal																									
Cervix, squamous cell carcinoma																									
Vagina																					+				
Polyp																					X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+											+									+	+		
Deep cervical, carcinoma, metastatic, thyroid gland																						X			
Mediastinal, carcinoma, metastatic, thyroid gland																						X			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma			X	X	X									X										X	
Carcinoma, multiple																									
Fibroadenoma								X				X	X		X		X	X		X		X		X	X
Fibroadenoma, multiple										X													X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																X									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 20 ng/kg

Number of Days on Study	6 7 5	6 7 7	6 8 9	7 0 1	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 5 5 7	2 5 5 9	2 3 3 9	2 4 4 3	2 0 3 3	2 1 0	2 1 1 2	2 1 1 3	2 2 3 7	2 3 7 7	2 4 4 0	2 5 5 5	2 6 6 6	2 9 6 7	2 0 1 1	2 2 2 5	2 2 8 8	2 8 6 6	2 0 5 5	2 0 0 8	2 2 1	2 3 6	2 4 1	2 4 4 6	2 4 7 7	2 5 2	2 8 8	2 9 0	Total Tissues/ Tumors
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma				X																								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granulosa cell tumor benign											X																	1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp stromal	X					X											X									X	X	5
Cervix, polyp stromal										X																		1
Cervix, squamous cell carcinoma									X																			1
Vagina																												1
Polyp																												1
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																												4
Deep cervical, carcinoma, metastatic, thyroid gland																												1
Mediastinal, carcinoma, metastatic, thyroid gland																												1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	50
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma							X											X										2
Carcinoma						X	X							X					X		X				X			11
Carcinoma, multiple			X																				X					2
Fibroadenoma	X		X				X	X	X		X	X		X	X	X		X		X					X			23
Fibroadenoma, multiple				X	X					X									X			X						7
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma										X																		1
Fibrosarcoma	X																											1
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Astrocytoma malignant								X																				1
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, thyroid gland																												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Sarcoma																												1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 20 ng/kg

Number of Days on Study	1	1	2	2	2	3	3	3	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	
	1	9	1	4	9	0	0	9	2	5	9	1	6	7	8	8	8	0	1	2	3	3	6	7	
	6	6	9	6	7	0	1	3	0	1	7	7	1	0	4	6	7	5	9	9	6	8	8	5	
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	6	7	0	0	7	6	5	3	2	6	2	4	0	9	0	2	7	8	1	3	4	5	7	8	2
	9	7	6	7	5	0	8	0	3	7	2	4	9	6	4	9	1	9	5	8	2	6	3	7	6
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma		X																							
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 20 ng/kg

Number of Days on Study	6 7 5	6 7 7	6 8 9	7 0 1	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 5 7	2 5 9	2 3 9	2 4 3	2 0 3	2 1 0	2 1 2	2 1 3	2 2 7	2 3 7	2 4 0	2 5 5	2 6 6	2 9 7	2 0 1	2 2 5	2 2 8	2 8 6	2 0 5	2 0 8	2 2 1	2 3 6	2 4 1	2 4 6	2 4 7	2 5 2	2 8 8	2 9 0	Total Tissues/ Tumors
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nephroblastoma																													1
Renal tubule, adenoma																										X			1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 44 ng/kg

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 44 ng/kg

Number of Days on Study	6 7 5	6 7 5	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	3 0 2	3 0 6	3 0 7	3 1 1	3 2 1	3 2 2	3 3 8	3 4 0	3 4 1	3 4 6	3 8 1	3 8 8	3 9 6	3 0 1	3 0 4	3 0 8	3 3 7	3 7 4	3 8 4	3 3 5	3 4 4	3 4 4	3 5 9	3 6 0	3 6 7	3 8 3	3 8 5	3 8 6	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus																		X										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Cholangiocarcinoma																							X					1
Mesentery													+					+										3
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth		+		+	+	+	+		+		+		+	+	+			+	+				+			+	+	30
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant																								X				2
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pheochromocytoma benign								X			X					X	X							X				5
Bilateral, pheochromocytoma benign																		X										1
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma																								X				1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma		X		X		X		X	X	X		X	X	X	X		X	X	X				X	X	X		X		23
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma						X																						1
C-cell, adenoma	X									X	X				X							X	X	X	X			8
C-cell, carcinoma									X						X													2
General Body System																												
None																												

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 44 ng/kg

Number of Days on Study	3 0 2	4 1 0	4 2 9	4 7 0	4 7 0	4 7 0	4 7 2	4 8 9	4 9 9	5 2 5	5 2 5	5 2 5	5 4 8	5 6 1	5 7 0	5 7 0	5 7 3	5 8 9	5 9 9	6 0 3	6 1 6	6 2 4	6 4 5	6 4 5	6 6 8
Carcass ID Number	3 3 2	3 4 8	3 3 1	3 1 5	3 2 4	3 5 7	3 4 3	3 7 2	3 3 9	3 0 3	3 0 7	3 4 2	3 5 0	3 3 3	3 0 9	3 1 3	3 9 8	3 3 6	3 7 0	3 7 1	3 0 5	3 4 5	3 0 6	3 5 8	3 1 0
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sertoli cell tumor malignant																									
Uterus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Leiomyoma																									
Polyp stromal																									
Schwannoma malignant			X																						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node							+											+	+					+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma	X	X			X	X					X		X				X			X		X	X		X
Fibroadenoma, multiple												X						X	X					X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Subcutaneous tissue, hemangioma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle															+										
Rhabdomyosarcoma															X										
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor malignant																						X			
Peripheral nerve																		+							
Spinal cord																		+							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 44 ng/kg

Number of Days on Study	6 7 5	6 7 5	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	3 0 2	3 0 6	3 0 7	3 1 1	3 1 2	3 2 2	3 3 8	3 4 0	3 4 1	3 4 6	3 8 1	3 8 8	3 9 6	3 0 1	3 0 4	3 0 8	3 3 7	3 7 4	3 8 4	3 3 5	3 4 4	3 4 7	3 5 9	3 6 0	3 6 7	3 8 3	3 8 5	3 8 6	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Sertoli cell tumor malignant												X																	1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma																		X											1
Leiomyoma					X																								1
Polyp stromal					X														X			X		X				X	5
Schwannoma malignant																													1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																													4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																	X								X				2
Fibroadenoma	X		X				X	X			X		X	X					X					X				X	21
Fibroadenoma, multiple		X								X		X											X						8
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma											X																		1
Subcutaneous tissue, hemangioma															X														1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle															+														2
Rhabdomyosarcoma																													1
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granular cell tumor malignant																													1
Peripheral nerve																													1
Spinal cord																													1
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 44 ng/kg

Number of Days on Study	3	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	
	0	1	2	7	7	7	7	8	9	2	2	2	4	6	7	7	7	8	9	0	1	2	4	4	6
	2	0	9	0	0	0	2	9	9	5	5	5	8	1	0	0	3	9	9	3	6	4	5	5	8
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	3	4	3	1	2	5	4	7	3	0	0	4	5	3	0	1	9	3	7	7	0	4	0	5	1
	2	8	1	5	4	7	3	2	9	3	7	2	0	3	9	3	8	6	0	1	5	5	6	8	0
Urinary System																									
Kidney	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																		X							

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 44 ng/kg

Number of Days on Study	6 7 5	6 7 5	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	3 0 2	3 6 6	3 7 3	3 1 1	3 2 1	3 2 2	3 3 8	3 4 0	3 4 1	3 4 6	3 8 1	3 8 8	3 9 6	3 0 1	3 0 4	3 0 8	3 3 7	3 7 4	3 8 4	3 3 5	3 4 4	3 4 7	3 5 9	3 6 0	3 6 7	3 8 3	3 8 5	3 8 6	Total Tissues/ Tumors
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymphoma malignant																												1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 92 ng/kg

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 92 ng/kg

Number of Days on Study	6 7 5	6 7 5	6 8 1	7 0 7	7 1 7	7 2 1	7 2 1	7 2 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 6 1	4 8 6	4 5 6	4 2 4	4 7 0	4 6 9	4 7 8	4 3 5	4 0 1	4 0 4	4 0 5	4 3 1	4 5 0	4 5 7	4 6 4	4 6 5	4 9 3	4 9 6	4 3 3	4 5 8	4 6 7	4 8 9	4 9 7	4 3 2	4 8 1	4 8 4	4 9 4	4 9 5	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus							X																						1
Cholangiocarcinoma											X																		1
Hepatocellular adenoma										X							X												2
Mesentery												+									+								2
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Gingival, squamous cell carcinoma					X																								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, uterus							X																						1
Acinus, adenoma													X																1
Acinus, carcinoma																		X											1
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Tooth	+	+		+		+		+	+	+	+		+		+				+	+	+		+	+	+		+	+	29
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma			X																X										2
Carcinoma			X																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign																				X									1
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma							X																						1
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma				X			X	X	X				X		X			X		X		X			X			X	21
Thyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
C-cell, adenoma						X					X	X								X								X	6
General Body System																													
None																													

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 92 ng/kg

Number of Days on Study	1 1 3	2 4 1	2 6 7	3 1 5	3 2 1	3 3 7	3 4 8	3 5 3	3 8 2	4 2 0	4 5 1	4 5 4	5 0 0	5 0 0	5 1 3	5 5 5	5 6 1	5 8 0	6 1 9	6 4 2	6 5 1	6 6 2	6 6 7	6 6 8	6 7 5
Carcass ID Number	4 9 2	4 6 0	4 4 6	4 5 5	4 0 3	4 4 9	4 6 6	4 8 5	4 6 3	4 4 8	4 5 2	4 9 1	4 7 9	4 8 8	4 1 2	4 5 9	4 5 1	4 5 4	4 0 2	4 6 8	4 2 5	4 7 6	4 9 8	4 1 1	4 2 3
Genital System																									
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Carcinoma																									
Polyp stromal																							X	X	
Polyp stromal, multiple																									
Squamous cell carcinoma																								X	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																									
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Carcinoma, metastatic, uterus																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma							X																		
Fibroadenoma					X	X		X		X	X	X	X			X			X					X	X
Fibroadenoma, multiple															X						X	X	X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant												X													
Cranial nerve, squamous cell carcinoma, metastatic, oral mucosa																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland							X																		
Carcinoma, metastatic, uterus																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 92 ng/kg

Number of Days on Study	6 7 5	6 7 5	6 8 1	7 0 7	7 1 7	7 2 1	7 2 1	7 2 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 6 1	4 8 6	4 5 6	4 2 4	4 7 0	4 6 9	4 7 8	4 3 5	4 0 1	4 0 4	4 0 5	4 3 1	4 5 0	4 5 7	4 6 4	4 6 5	4 9 3	4 9 6	4 3 3	4 5 8	4 6 7	4 8 9	4 9 7	4 3 2	4 8 1	4 8 4	4 9 4	4 9 5	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Oviduct	+																												1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma				X		X	X					X								X									5
Polyp stromal					X		X							X						X									6
Polyp stromal, multiple																		X											1
Squamous cell carcinoma																													1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node	+						+															+							3
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, uterus							X																						1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma													X																1
Carcinoma										X	X	X													X				5
Fibroadenoma	X		X			X		X	X		X					X	X				X	X		X					22
Fibroadenoma, multiple		X		X						X				X	X			X					X					X	12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Basal cell carcinoma																													1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Astrocytoma malignant																													1
Cranial nerve, squamous cell carcinoma, metastatic, oral mucosa					X																								1
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, mammary gland																													1
Carcinoma, metastatic, uterus							X																						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 92 ng/kg

Number of Days on Study	1	2	2	3	3	3	3	3	3	4	4	4	5	5	5	5	5	6	6	6	6	6	6	6	
	1	4	6	1	2	3	4	5	8	2	5	5	0	0	1	5	6	8	1	4	5	6	6	6	7
	3	1	7	5	1	7	8	3	2	0	1	4	0	0	3	5	1	0	9	2	1	2	7	8	5
Carcass ID Number	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	9	6	4	5	0	4	6	8	6	4	5	9	7	8	1	5	5	5	0	6	2	7	9	1	2
	2	0	6	5	3	9	6	5	3	8	2	1	9	8	2	9	1	4	2	8	5	6	8	1	3
Special Senses System																									
Eye	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma	X																								
Renal tubule, adenoma																									
Urinary bladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Carcinoma, metastatic, uterus																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 92 ng/kg

Number of Days on Study	6 7 5	6 7 5	6 8 1	7 0 7	7 1 7	7 2 1	7 2 1	7 2 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	4 4 6 1	4 8 1 6	4 5 6 6	4 2 4 4	4 7 0 0	4 6 9 9	4 7 8 8	4 3 5 5	4 0 1 1	4 0 0 4	4 0 5 5	4 3 1 1	4 5 0 0	4 5 5 7	4 6 4 4	4 6 5 5	4 9 3 3	4 9 6 6	4 3 3 3	4 5 8 8	4 6 7 7	4 8 9 9	4 9 7 9	4 3 2 2	4 8 1 1	4 8 4 4	4 9 4 4	4 9 5 5	Total Tissues/ Tumors
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Zymbal's gland																										+			1
Adenoma																										X			1
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nephroblastoma																													1
Renal tubule, adenoma																						X							1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, uterus							X																						1
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg

Number of Days on Study	0 7 8	3 2 6	3 2 9	3 9 8	4 1 2	4 4 2	4 4 2	4 4 3	4 5 1	4 9 8	4 9 8	4 9 8	5 1 3	5 1 3	5 4 9	5 7 0	5 7 0	5 7 7	5 8 6	5 9 7	6 0 3	6 0 8	6 2 4	6 4 4	6 5 9
Carcass ID Number	5 5 2	5 0 7	5 5 5	5 6 3	5 7 3	5 3 7	5 6 8	5 8 4	5 7 2	5 6 7	5 7 4	5 9 4	5 1 7	5 5 3	5 8 7	5 7 1	5 7 7	5 6 4	5 6 6	5 4 3	5 4 2	5 4 6	5 0 6	5 7 9	5 4 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																								X	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																								X	
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																								X	
Cholangiocarcinoma																									
Cholangiocarcinoma, multiple																						X			
Hepatocellular adenoma																									
Mesentery																							+		+
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gingival, squamous cell carcinoma																									
Pancreas	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																								X	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																								X	
Tooth								+	+	+	+			+		+	+	+			+			+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																								X	
Pancreatic islets	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma															X			X	X			X	X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
C-cell, adenoma																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 PeCDF: 200 ng/kg

Number of Days on Study	6 6 8	6 8 9	7 0 6	7 0 6	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	5 5 7 6	5 8 1	5 3 6	5 9 8	5 6 2	5 1 9	5 3 8	5 3 9	5 4 8	5 4 9	5 6 1	5 7 8	5 8 2	5 8 3	5 9 7	5 1 0	5 1 8	5 4 5	5 4 7	5 5 0	5 8 0	5 8 6	5 9 5	5 1 6	5 5 1	5 5 4	5 9 2	5 9 3	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus																												1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus																												1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leiomyoma																				X								1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus																												1
Cholangiocarcinoma																			X									1
Cholangiocarcinoma, multiple																												1
Hepatocellular adenoma		X				X														X				X				4
Mesentery																			+						+			4
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Gingival, squamous cell carcinoma	X								X																X			3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, uterus																												1
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibrosarcoma																											X	1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus																												1
Tooth	+				+	+	+	+	+	+		+	+					+	+	+	+	+	+	+	+		+	29
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign															X													2
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma										X		X																2
Parathyroid gland	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma	X			X	X							X	X			X			X				X	X	X	X		16
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
C-cell, adenoma							X			X					X				X		X							7
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 PeCDF: 200 ng/kg

Number of Days on Study	0 7 8	3 2 6	3 2 9	3 9 8	4 1 2	4 4 2	4 4 2	4 4 3	4 5 1	4 9 8	4 9 8	4 9 8	5 1 3	5 1 3	5 4 9	5 7 0	5 7 0	5 7 7	5 8 6	5 9 7	6 0 3	6 0 8	6 2 4	6 2 4	6 5 9
Carcass ID Number	5 5 2	5 0 7	5 5 5	5 6 3	5 7 3	5 3 7	5 6 8	5 8 4	5 7 2	5 6 7	5 7 4	5 9 4	5 1 7	5 5 3	5 8 7	5 7 1	5 7 7	5 6 4	5 6 6	4 4 3	4 4 2	4 4 6	5 0 6	5 7 9	5 4 1
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								X	
Polyp stromal																									X
Polyp stromal, multiple										X															
Schwannoma malignant				X																					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+					+															+		
Inguinal, carcinoma, metastatic, mammary gland								X																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																								X	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M
Osteosarcoma, metastatic, skin																	X								
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma								X		X															
Fibroadenoma		X				X				X		X	X		X			X				X			
Fibroadenoma, multiple																	X				X			X	
Osteosarcoma, metastatic, skin																	X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrous histiocytoma							X																		
Keratoacanthoma																									
Neural crest tumor																									
Osteosarcoma																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant														X											
Peripheral nerve											+														
Spinal cord											+														

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg

Number of Days on Study	6 6 8	6 8 9	7 0 6	7 0 6	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	5 7 6	5 8 1	5 3 6	5 9 8	5 6 2	5 1 9	5 3 8	5 3 9	5 4 8	5 4 9	5 6 1	5 7 8	5 8 2	5 8 3	5 9 7	5 1 0	5 1 8	5 4 5	5 4 7	5 5 0	5 8 0	5 8 6	5 9 5	5 1 6	5 5 1	5 5 4	5 9 2	5 9 3	Total Tissues/ Tumors
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma						X																							2
Polyp stromal									X													X							3
Polyp stromal, multiple																													1
Schwannoma malignant																													1
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node	+				+																					+		+	7
Inguinal, carcinoma, metastatic, mammary gland																													1
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma, metastatic, uterus																													1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Osteosarcoma, metastatic, skin																													1
Integumentary System																													
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma						X																							3
Fibroadenoma		X	X						X		X	X		X											X		X	X	17
Fibroadenoma, multiple				X	X	X				X					X				X	X	X		X	X					13
Osteosarcoma, metastatic, skin																													1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma											X																		1
Fibrous histiocytoma																													1
Keratoacanthoma									X																				1
Neural crest tumor																		X											1
Osteosarcoma																													1
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Astrocytoma malignant																													1
Peripheral nerve																													1
Spinal cord																													1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg

Number of Days on Study	0 7 8	3 2 6	3 2 9	3 9 8	4 1 2	4 4 2	4 4 2	4 4 3	4 5 1	4 9 8	4 9 8	4 9 8	5 1 3	5 1 3	5 4 9	5 7 0	5 7 0	5 7 7	5 8 6	5 9 7	6 0 3	6 0 8	6 2 4	6 2 4	6 5 9
Carcass ID Number	5 5 2	5 0 7	5 5 5	5 6 3	5 7 3	5 3 7	5 6 8	5 8 4	5 7 2	5 6 7	5 7 4	5 9 4	5 1 7	5 5 3	5 8 7	5 7 1	5 7 7	5 6 4	5 6 6	5 4 3	5 4 2	5 4 6	5 0 6	5 7 9	5 4 1
Respiratory System																									
Lung	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X																						
Carcinoma, metastatic, mammary gland								X		X															
Carcinoma, metastatic, uterus																								X	
Cystic keratinizing epithelioma, multiple																									
Fibrous histiocytoma, metastatic, skin							X																		
Histiocytic sarcoma			X																						
Osteosarcoma, metastatic, skin																	X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Sarcoma																X									
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Lacrimal gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Leukemia mononuclear																						X			

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg

Number of Days on Study	6 6 8	6 8 9	7 0 6	7 0 6	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	5 7 6	5 8 1	5 3 6	5 9 8	5 6 2	5 1 9	5 3 8	5 3 9	5 4 8	5 4 9	5 6 1	5 7 8	5 8 2	5 8 3	5 9 7	5 1 0	5 1 8	5 4 5	5 4 7	5 5 0	5 8 0	5 8 6	5 9 5	5 1 6	5 5 1	5 5 4	5 9 2	5 9 3	Total Tissues/ Tumors
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Alveolar/bronchiolar adenoma																													1
Carcinoma, metastatic, mammary gland																													2
Carcinoma, metastatic, uterus																													1
Cystic keratinizing epithelioma, multiple									X																				1
Fibrous histiocytoma, metastatic, skin																													1
Histiocytic sarcoma																													1
Osteosarcoma, metastatic, skin																													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Sarcoma																													1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lacrimal gland																			+										1
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Histiocytic sarcoma																													1
Leukemia mononuclear																													1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg (Stop-Exposure)

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg (Stop-Exposure)

Number of Days on Study	6 6 7	6 8 0	6 8 9	6 9 2	6 9 9	7 0 1	7 1 5	7 1 5	7 1 6	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	
Carcass ID Number	6 3 2	6 3 0	6 3 3	6 4 0	6 1 3	6 2 5	6 2 4	6 5 0	6 0 3	6 1 1	6 0 5	6 0 6	6 1 0	6 1 5	6 1 7	6 1 9	6 2 0	6 3 4	6 4 6	6 4 9	6 2 7	6 4 5	6 4 8	6 0 8	6 2 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, uterus							X																			1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																				X						1
Mesentery																		+								2
Oral mucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gingival, squamous cell carcinoma		X			X															X						3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																				X						1
Carcinoma																	X									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth				+		+			+		+				+	+	+		+	+	+		+		+	24
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																				X						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign																					X					1
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X			X	X		X	X				X				X				X	X	X			13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma						X									X				X						X	5
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg (Stop-Exposure)

Number of Days on Study	2 1 5	2 4 0	2 7 6	3 7 0	3 8 4	4 1 3	4 5 1	4 5 1	4 7 6	4 9 1	4 9 5	5 0 0	5 0 0	5 0 3	5 1 2	5 2 5	5 4 7	6 0 5	6 1 0	6 1 0	6 1 9	6 1 9	6 2 5	6 3 8	6 5 1
Carcass ID Number	6 2 1	6 3 9	6 0 7	6 0 1	6 0 2	6 3 1	6 4 1	6 4 2	6 4 7	6 3 6	6 4 3	6 1 8	6 2 2	6 0 4	6 3 7	6 3 5	6 4 4	6 0 9	6 1 2	6 1 4	6 2 6	6 2 8	6 2 3	6 1 6	6 3 8
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Carcinoma																									
Hemangioma																									
Polyp stromal							X						X								X				
Cervix, squamous cell carcinoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Thymoma malignant, metastatic, mammary gland																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Carcinoma													X												
Fibroadenoma			X	X			X	X			X	X	X		X		X		X		X			X	
Fibroadenoma, multiple																				X		X	X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																X									
Fibroma																									
Fibrosarcoma						X																			
Sarcoma		X																							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin						X																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, uterus																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg (Stop-Exposure)

Number of Days on Study	6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	6 8 8 9 9 0 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 3 3
	7 0 9 2 9 1 5 5 6 6 8 8 8 8 8 8 8 8 8 8 9 9 9 0 0
Carcass ID Number	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
	3 3 3 4 1 2 2 5 0 3 1 0 0 1 1 1 1 2 3 4 4 2 4 4 0
	2 0 3 0 3 5 4 0 3 1 5 6 0 5 7 9 0 4 6 9 7 5 8 8 9
Genital System	
Clitoral gland	+ +
Ovary	+ +
Luteoma	X
Uterus	+ +
Carcinoma	X
Hemangioma	X
Polyp stromal	X
Cervix, squamous cell carcinoma	X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ + +
Lymph node, mandibular	+ +
Lymph node, mesenteric	+ +
Spleen	+ +
Thymus	+ +
Thymoma malignant, metastatic, mammary gland	X
Integumentary System	
Mammary gland	+ +
Adenoma	X
Carcinoma	X X X X
Fibroadenoma	X X X X X X X X X X X X X X
Fibroadenoma, multiple	X X X X X
Skin	+ +
Basal cell carcinoma	
Fibroma	X
Fibrosarcoma	
Sarcoma	
Musculoskeletal System	
Bone	+ +
Fibrosarcoma, metastatic, skin	
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Squamous cell carcinoma, metastatic, uterus	X
Nose	+ +
Trachea	+ +

Carcass ID Number	Total Tissues/ Tumors
Genital System	
Clitoral gland	50
Ovary	47
Luteoma	1
Uterus	49
Carcinoma	1
Hemangioma	1
Polyp stromal	4
Cervix, squamous cell carcinoma	1
Hematopoietic System	
Bone marrow	50
Lymph node	3
Lymph node, mandibular	49
Lymph node, mesenteric	49
Spleen	49
Thymus	49
Thymoma malignant, metastatic, mammary gland	1
Integumentary System	
Mammary gland	50
Adenoma	2
Carcinoma	5
Fibroadenoma	25
Fibroadenoma, multiple	9
Skin	50
Basal cell carcinoma	1
Fibroma	1
Fibrosarcoma	1
Sarcoma	1
Musculoskeletal System	
Bone	50
Fibrosarcoma, metastatic, skin	1
Nervous System	
Brain	49
Respiratory System	
Lung	50
Squamous cell carcinoma, metastatic, uterus	1
Nose	50
Trachea	50

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg (Stop-Exposure)

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of PeCDF: 200 ng/kg (Stop-Exposure)

Number of Days on Study	6 6 7	6 8 0	6 8 9	6 9 2	6 9 9	7 0 1	7 1 5	7 1 5	7 1 6	7 1 6	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	
Carcass ID Number	6 3 2	6 3 0	6 3 3	6 4 0	6 1 3	6 2 5	6 2 4	6 5 0	6 0 3	6 1 1	6 0 5	6 0 6	6 1 0	6 1 5	6 1 7	6 1 9	6 2 0	6 3 4	6 4 6	6 4 9	6 2 7	6 4 5	6 4 8	6 0 8	6 2 9	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
Adrenal Medulla: Pheochromocytoma Benign						
Overall rate[a]	4/53 (8%)	5/53 (9%)	2/53 (4%)	6/52 (12%)	1/53 (2%)	2/53 (4%)
Adjusted rate[b]	9.5%	12.9%	5.4%	15.8%	2.8%	5.4%
Terminal rate[c]	3/25 (12%)	2/22 (9%)	1/24 (4%)	6/25 (24%)	1/20 (5%)	1/23 (4%)
First incidence (days)	659	651	701	728 (T)	728 (T)	624
Poly-3 test[d]	P=0.164N	P=0.447	P=0.400N	P=0.304	P=0.227N	P=0.397N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma						
Overall rate	5/53 (9%)	5/53 (9%)	3/53 (6%)	6/52 (12%)	1/53 (2%)	2/53 (4%)
Adjusted rate	11.9%	12.9%	8.2%	15.8%	2.8%	5.4%
Terminal rate	4/25 (16%)	2/22 (9%)	2/24 (8%)	6/25 (24%)	1/20 (5%)	1/23 (4%)
First incidence (days)	659	651	701	728 (T)	728 (T)	624
Poly-3 test	P=0.105N	P=0.578	P=0.431N	P=0.427	P=0.139N	P=0.270N
Liver: Hepatocellular Adenoma						
Overall rate	1/53 (2%)	0/53 (0%)	1/53 (2%)	0/52 (0%)	2/53 (4%)	4/53 (8%)
Adjusted rate	2.4%	0.0%	2.7%	0.0%	5.5%	10.9%
Terminal rate	0/25 (0%)	0/22 (0%)	1/24 (4%)	0/25 (0%)	2/20 (10%)	3/23 (13%)
First incidence (days)	678	—[e]	728 (T)	—	728 (T)	689
Poly-3 test	P=0.006	P=0.519N	P=0.730	P=0.520N	P=0.448	P=0.140
Liver: Cholangiocarcinoma						
Overall rate	0/53 (0%)	0/53 (0%)	0/53 (0%)	1/52 (2%)	1/53 (2%)	2/53 (4%)
Adjusted rate	0.0%	0.0%	0.0%	2.6%	2.8%	5.4%
Terminal rate	0/25 (0%)	0/22 (0%)	0/24 (0%)	1/25 (4%)	1/20 (5%)	1/23 (4%)
First incidence (days)	—	—[f]	—	728 (T)	728 (T)	608
Poly-3 test	P=0.036	—	—	P=0.481	P=0.471	P=0.211
Mammary Gland: Fibroadenoma						
Overall rate	34/53 (64%)	35/53 (66%)	30/53 (57%)	29/53 (55%)	34/53 (64%)	30/53 (57%)
Adjusted rate	68.4%	74.6%	70.0%	62.0%	75.0%	69.0%
Terminal rate	14/25 (56%)	13/22 (59%)	15/24 (63%)	11/25 (44%)	13/20 (65%)	15/23 (65%)
First incidence (days)	289	289	393	302	321	326
Poly-3 test	P=0.536	P=0.321	P=0.521	P=0.325N	P=0.307	P=0.565
Mammary Gland: Fibroadenoma or Adenoma						
Overall rate	35/53 (66%)	36/53 (68%)	30/53 (57%)	29/53 (55%)	35/53 (66%)	30/53 (57%)
Adjusted rate	70.4%	76.7%	70.0%	62.0%	77.2%	69.0%
Terminal rate	15/25 (60%)	14/22 (64%)	15/24 (63%)	11/25 (44%)	14/20 (70%)	15/23 (65%)
First incidence (days)	289	289	393	302	321	326
Poly-3 test	P=0.482N	P=0.312	P=0.579N	P=0.251N	P=0.294	P=0.533N
Mammary Gland: Carcinoma						
Overall rate	5/53 (9%)	12/53 (23%)	13/53 (25%)	2/53 (4%)	5/53 (9%)	3/53 (6%)
Adjusted rate	11.8%	29.8%	32.2%	5.2%	13.5%	7.9%
Terminal rate	3/25 (12%)	6/22 (27%)	7/24 (29%)	2/25 (8%)	4/20 (20%)	1/23 (4%)
First incidence (days)	619	420	219	728 (T)	348	443
Poly-3 test	P=0.021N	P=0.036	P=0.021	P=0.259N	P=0.543	P=0.417N
Mammary Gland: Adenoma or Carcinoma						
Overall rate	6/53 (11%)	13/53 (25%)	14/53 (26%)	2/53 (4%)	6/53 (11%)	3/53 (6%)
Adjusted rate	14.1%	32.3%	34.6%	5.2%	16.2%	7.9%
Terminal rate	4/25 (16%)	7/22 (32%)	8/24 (33%)	2/25 (8%)	5/20 (25%)	1/23 (4%)
First incidence (days)	619	420	219	728 (T)	348	443
Poly-3 test	P=0.012N	P=0.040	P=0.024	P=0.167N	P=0.522	P=0.297N

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma						
Overall rate	38/53 (72%)	40/53 (75%)	37/53 (70%)	31/53 (58%)	38/53 (72%)	31/53 (58%)
Adjusted rate	75.4%	82.8%	80.1%	66.3%	82.2%	70.0%
Terminal rate	16/25 (64%)	16/22 (73%)	18/24 (75%)	13/25 (52%)	16/20 (80%)	15/23 (65%)
First incidence (days)	289	289	219	302	321	326
Poly-3 test	P=0.209N	P=0.249	P=0.376	P=0.215N	P=0.278	P=0.357N
Oral Mucosa: Squamous Cell Carcinoma						
Overall rate	1/53 (2%)	2/53 (4%)	1/53 (2%)	0/53 (0%)	1/53 (2%)	3/53 (6%)
Adjusted rate	2.4%	5.2%	2.7%	0.0%	2.8%	8.1%
Terminal rate	1/25 (4%)	1/22 (5%)	0/24 (0%)	0/25 (0%)	0/20 (0%)	2/23 (9%)
First incidence (days)	728 (T)	706	517	—	717	668
Poly-3 test	P=0.149	P=0.468	P=0.735	P=0.518N	P=0.728	P=0.261
Pancreas: Adenoma or Carcinoma						
Overall rate	0/53 (0%)	0/53 (0%)	0/53 (0%)	0/52 (0%)	2/52 (4%)	0/52 (0%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	5.6%	0.0%
Terminal rate	0/25 (0%)	0/22 (0%)	0/24 (0%)	0/25 (0%)	2/20 (10%)	0/23 (0%)
First incidence (days)	—	—	—	—	728 (T)	—
Poly-3 test	P=0.442	—	—	—	P=0.202	—
Pituitary Gland (Pars Distalis): Adenoma						
Overall rate	15/53 (28%)	21/53 (40%)	23/53 (43%)	23/53 (43%)	21/53 (40%)	16/53 (30%)
Adjusted rate	34.7%	52.1%	57.2%	55.9%	51.8%	40.7%
Terminal rate	8/25 (32%)	12/22 (55%)	13/24 (54%)	16/25 (64%)	8/20 (40%)	8/23 (35%)
First incidence (days)	603	600	420	470	420	549
Poly-3 test	P=0.344N	P=0.075	P=0.027	P=0.034	P=0.081	P=0.367
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma or Carcinoma						
Overall rate	15/53 (28%)	22/53 (42%)	23/53 (43%)	23/53 (43%)	21/53 (40%)	16/53 (30%)
Adjusted rate	34.7%	54.3%	57.2%	55.9%	51.8%	40.7%
Terminal rate	8/25 (32%)	12/22 (55%)	13/24 (54%)	16/25 (64%)	8/20 (40%)	8/23 (35%)
First incidence (days)	603	600	420	470	420	549
Poly-3 test	P=0.311N	P=0.050	P=0.027	P=0.034	P=0.081	P=0.367
Thyroid Gland (C-Cell): Adenoma						
Overall rate	13/53 (25%)	10/53 (19%)	9/51 (18%)	9/53 (17%)	6/51 (12%)	7/51 (14%)
Adjusted rate	29.9%	25.1%	24.5%	23.4%	16.9%	19.4%
Terminal rate	8/25 (32%)	5/22 (23%)	6/24 (25%)	8/25 (32%)	4/20 (20%)	5/23 (22%)
First incidence (days)	500	530	570	675	642	570
Poly-3 test	P=0.149N	P=0.404N	P=0.386N	P=0.340N	P=0.138N	P=0.207N
Thyroid Gland (C-Cell): Adenoma or Carcinoma						
Overall rate	15/53 (28%)	12/53 (23%)	10/51 (20%)	10/53 (19%)	6/51 (12%)	7/51 (14%)
Adjusted rate	34.5%	29.9%	27.2%	26.0%	16.9%	19.4%
Terminal rate	10/25 (40%)	6/22 (27%)	7/24 (29%)	9/25 (36%)	4/20 (20%)	5/23 (22%)
First incidence (days)	500	530	570	675	642	570
Poly-3 test	P=0.055N	P=0.415N	P=0.322N	P=0.275N	P=0.063N	P=0.104N
Uterus: Stromal Polyp						
Overall rate	9/53 (17%)	3/53 (6%)	6/53 (11%)	5/53 (9%)	7/53 (13%)	4/53 (8%)
Adjusted rate	20.9%	7.8%	16.3%	13.1%	19.1%	10.7%
Terminal rate	4/25 (16%)	1/22 (5%)	5/24 (21%)	5/25 (20%)	3/20 (15%)	2/23 (9%)
First incidence (days)	588	547	675	728 (T)	667	498
Poly-3 test	P=0.343N	P=0.084N	P=0.405N	P=0.261N	P=0.532N	P=0.172N

TABLE A3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
Uterus: Stromal Polyp or Stromal Sarcoma						
Overall rate	9/53 (17%)	4/53 (8%)	6/53 (11%)	5/53 (9%)	7/53 (13%)	4/53 (8%)
Adjusted rate	20.9%	10.2%	16.3%	13.1%	19.1%	10.7%
Terminal rate	4/25 (16%)	1/22 (5%)	5/24 (21%)	5/25 (20%)	3/20 (15%)	2/23 (9%)
First incidence (days)	588	503	675	728 (T)	667	498
Poly-3 test	P=0.303N	P=0.149N	P=0.405N	P=0.261N	P=0.532N	P=0.172N
Uterus: Carcinoma						
Overall rate	1/53 (2%)	1/53 (2%)	0/53 (0%)	1/53 (2%)	5/53 (9%)	2/53 (4%)
Adjusted rate	2.4%	2.6%	0.0%	2.6%	13.8%	5.4%
Terminal rate	0/25 (0%)	0/22 (0%)	0/24 (0%)	1/25 (4%)	2/20 (10%)	1/23 (4%)
First incidence (days)	678	706	—	728 (T)	707	624
Poly-3 test	P=0.103	P=0.740	P=0.527N	P=0.740	P=0.070	P=0.456
Uterus: Adenoma or Carcinoma						
Overall rate	3/53 (6%)	1/53 (2%)	0/53 (0%)	1/53 (2%)	5/53 (9%)	2/53 (4%)
Adjusted rate	7.1%	2.6%	0.0%	2.6%	13.8%	5.4%
Terminal rate	1/25 (4%)	0/22 (0%)	0/24 (0%)	1/25 (4%)	2/20 (10%)	1/23 (4%)
First incidence (days)	678	706	—	728 (T)	707	624
Poly-3 test	P=0.269	P=0.341N	P=0.145N	P=0.341N	P=0.275	P=0.560N
All Organs: Benign Neoplasms						
Overall rate	45/53 (85%)	45/53 (85%)	43/53 (81%)	42/53 (79%)	45/53 (85%)	41/53 (77%)
Adjusted rate	89.0%	92.2%	94.3%	88.0%	96.3%	88.6%
Terminal rate	21/25 (84%)	20/22 (91%)	22/24 (92%)	22/25 (88%)	19/20 (95%)	20/23 (87%)
First incidence (days)	289	289	393	302	321	326
Poly-3 test	P=0.472N	P=0.414	P=0.278	P=0.571N	P=0.146	P=0.613N
All Organs: Malignant Neoplasms						
Overall rate	12/53 (23%)	20/53 (38%)	21/53 (40%)	13/53 (25%)	16/53 (30%)	16/53 (30%)
Adjusted rate	27.6%	47.1%	48.8%	31.5%	39.7%	37.0%
Terminal rate	7/25 (28%)	8/22 (36%)	10/24 (42%)	8/25 (32%)	6/20 (30%)	5/23 (22%)
First incidence (days)	377	420	196	302	113	329
Poly-3 test	P=0.454N	P=0.044	P=0.030	P=0.437	P=0.168	P=0.236
All Organs: Benign or Malignant Neoplasms						
Overall rate	48/53 (91%)	49/53 (92%)	49/53 (92%)	44/53 (83%)	48/53 (91%)	46/53 (87%)
Adjusted rate	92.9%	96.7%	99.1%	89.8%	96.8%	93.3%
Terminal rate	22/25 (88%)	21/22 (96%)	24/24 (100%)	22/25 (88%)	19/20 (95%)	21/23 (91%)
First incidence (days)	289	289	196	302	113	326
Poly-3 test	P=0.415N	P=0.325	P=0.121	P=0.414N	P=0.317	P=0.626

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for the 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 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 A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of PeCDF

	Vehicle Control	200 ng/kg	200 ng/kg (Stop-Exposure)
Adrenal Medulla: Pheochromocytoma Benign			
Overall rate[a]	4/53 (8%)	2/53 (4%)	1/48 (2%)
Adjusted rate[b]	9.5%	5.4%	3.1%
Terminal rate[c]	3/25 (12%)	1/23 (4%)	1/15 (7%)
First incidence (days)	659	624	728 (T)
Poly-3 test[d]		P=0.397N	P=0.286N
Poly-3 test[e]			P=0.559N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma			
Overall rate	5/53 (9%)	2/53 (4%)	1/48 (2%)
Adjusted rate	11.9%	5.4%	3.1%
Terminal rate	4/25 (16%)	1/23 (4%)	1/15 (7%)
First incidence (days)	659	624	728 (T)
Poly-3 test		P=0.270N	P=0.186N
Poly-3 test			P=0.559N
Liver: Hepatocellular Adenoma			
Overall rate	1/53 (2%)	4/53 (8%)	1/50 (2%)
Adjusted rate	2.4%	10.9%	3.0%
Terminal rate	0/25 (0%)	3/23 (13%)	1/15 (7%)
First incidence (days)	678	689	728 (T)
Poly-3 test		P=0.140	P=0.705
Poly-3 test			P=0.218N
Mammary Gland: Fibroadenoma			
Overall rate	34/53 (64%)	30/53 (57%)	34/50 (68%)
Adjusted rate	68.4%	69.0%	79.1%
Terminal rate	14/25 (56%)	15/23 (65%)	9/15 (60%)
First incidence (days)	289	326	276
Poly-3 test		P=0.565	P=0.171
Poly-3 test			P=0.186
Mammary Gland: Fibroadenoma or Adenoma			
Overall rate	35/53 (66%)	30/53 (57%)	34/50 (68%)
Adjusted rate	70.4%	69.0%	79.1%
Terminal rate	15/25 (60%)	15/23 (65%)	9/15 (60%)
First incidence (days)	289	326	276
Poly-3 test		P=0.533N	P=0.232
Poly-3 test			P=0.186
Mammary Gland: Carcinoma			
Overall rate	5/53 (9%)	3/53 (6%)	5/50 (10%)
Adjusted rate	11.8%	7.9%	14.9%
Terminal rate	3/25 (12%)	1/23 (4%)	4/15 (27%)
First incidence (days)	619	443	500
Poly-3 test		P=0.417N	P=0.497
Poly-3 test			P=0.295
Mammary Gland: Adenoma or Carcinoma			
Overall rate	6/53 (11%)	3/53 (6%)	7/50 (14%)
Adjusted rate	14.1%	7.9%	20.6%
Terminal rate	4/25 (16%)	1/23 (4%)	5/15 (33%)
First incidence (days)	619	443	500
Poly-3 test		P=0.297N	P=0.346
Poly-3 test			P=0.113

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of PeCDF

	Vehicle Control	200 ng/kg	200 ng/kg (Stop-Exposure)
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma			
Overall rate	38/53 (72%)	31/53 (58%)	36/50 (72%)
Adjusted rate	75.4%	70.0%	83.8%
Terminal rate	16/25 (64%)	15/23 (65%)	11/15 (73%)
First incidence (days)	289	326	276
Poly-3 test		P=0.357N	P=0.227
Poly-3 test			P=0.083
Oral Mucosa: Squamous Cell Carcinoma			
Overall rate	1/53 (2%)	3/53 (6%)	3/50 (6%)
Adjusted rate	2.4%	8.1%	9.0%
Terminal rate	1/25 (4%)	2/23 (9%)	1/15 (7%)
First incidence (days)	728 (T)	668	680
Poly-3 test		P=0.261	P=0.249
Poly-3 test			P=0.625
Pancreas: Adenoma or Carcinoma			
Overall rate	0/53 (0%)	0/52 (0%)	2/49 (4%)
Adjusted rate	0.0%	0.0%	6.1%
Terminal rate	0/25 (0%)	0/23 (0%)	2/15 (13%)
First incidence (days)	—[f]	—[g]	728 (T)
Poly-3 test			P=0.215
Poly-3 test			P=0.225
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	15/53 (28%)	16/53 (30%)	13/49 (27%)
Adjusted rate	34.7%	40.7%	38.0%
Terminal rate	8/25 (32%)	8/23 (35%)	5/15 (33%)
First incidence (days)	603	549	610
Poly-3 test		P=0.367	P=0.488
Poly-3 test			P=0.509N
Skin: Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma			
Overall rate	0/53 (0%)	2/53 (4%)	3/50 (6%)
Adjusted rate	0.0%	5.4%	8.6%
Terminal rate	0/25 (0%)	1/23 (4%)	0/15 (0%)
First incidence (days)	—	442	240
Poly-3 test		P=0.213	P=0.099
Poly-3 test			P=0.475
Thyroid Gland (C-Cell): Adenoma			
Overall rate	13/53 (25%)	7/51 (14%)	5/48 (10%)
Adjusted rate	29.9%	19.4%	15.0%
Terminal rate	8/25 (32%)	5/23 (22%)	3/15 (20%)
First incidence (days)	500	570	525
Poly-3 test		P=0.207N	P=0.111N
Poly-3 test			P=0.437N
Thyroid Gland (C-Cell): Adenoma or Carcinoma			
Overall rate	15/53 (28%)	7/51 (14%)	5/48 (10%)
Adjusted rate	34.5%	19.4%	15.0%
Terminal rate	10/25 (40%)	5/23 (22%)	3/15 (20%)
First incidence (days)	500	570	525
Poly-3 test		P=0.104N	P=0.049N
Poly-3 test			P=0.437N

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of PeCDF

	Vehicle Control	200 ng/kg	200 ng/kg (Stop-Exposure)
Uterus: Stromal Polyp			
Overall rate	9/53 (17%)	4/53 (8%)	4/50 (8%)
Adjusted rate	20.9%	10.7%	11.5%
Terminal rate	4/25 (16%)	2/23 (9%)	1/15 (7%)
First incidence (days)	588	498	451
Poly-3 test		P=0.172N	P=0.224N
Poly-3 test			P=0.608
Uterus: Carcinoma			
Overall rate	1/53 (2%)	2/53 (4%)	1/50 (2%)
Adjusted rate	2.4%	5.4%	3.0%
Terminal rate	0/25 (0%)	1/23 (4%)	1/15 (7%)
First incidence (days)	678	624	728 (T)
Poly-3 test		P=0.456	P=0.427N
Poly-3 test			P=0.555N
All Organs: Benign Neoplasms			
Overall rate	45/53 (85%)	41/53 (77%)	40/50 (80%)
Adjusted rate	89.0%	88.6%	91.2%
Terminal rate	21/25 (84%)	20/23 (87%)	13/15 (87%)
First incidence (days)	289	326	276
Poly-3 test		P=0.613N	P=0.511
Poly-3 test			P=0.483
All Organs: Malignant Neoplasms			
Overall rate	12/53 (23%)	16/53 (30%)	13/50 (26%)
Adjusted rate	27.6%	37.0%	35.7%
Terminal rate	7/25 (28%)	5/23 (22%)	5/15 (33%)
First incidence (days)	377	329	240
Poly-3 test		P=0.236	P=0.301
Poly-3 test			P=0.556N
All Organs: Benign or Malignant Neoplasms			
Overall rate	48/53 (91%)	46/53 (87%)	43/50 (86%)
Adjusted rate	92.9%	93.3%	94.2%
Terminal rate	22/25 (88%)	21/23 (91%)	14/15 (93%)
First incidence (days)	289	326	240
Poly-3 test		P=0.626	P=0.577
Poly-3 test			P=0.624

(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 dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.
[e] Pairwise comparison between the 200 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

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

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

[a] Data as of February 24, 2005

TABLE A4b
Historical Incidence of Squamous Cell Carcinoma in the Oral Mucosa of Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls
Historical Incidence	
PCB 126	0/53
TCDD	1/53
PeCDF	1/53
TEF Mixture	1/53
PCB 153	0/53
Binary Mixture of PCB 126/PCB 153	0/53
Binary Mixture of 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 24, 2005

TABLE A4c
Historical Incidence of Uterus Neoplasms in Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Squamous Cell Carcinoma
Historical Incidence			
PCB 126	0/53	0/53	0/53
TCDD	0/53	0/53	0/53
PeCDF	2/53	1/53	0/53
TEF Mixture	0/53	1/53	0/53
PCB 153	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 153	0/53	0/53	1/53
Binary Mixture of PCB 126/PCB 118	0/53	1/53	0/53
Overall Historical Incidence			
Total (%)	2/371 (0.5%)	3/371 (0.8%)	1/371 (0.3%)
Mean ± standard deviation	0.5% ± 1.4%	0.8% ± 1.0%	0.3% ± 0.7%
Range	0%-4%	0%-2%	0%-2%

[a] Data as of February 24, 2005

TABLE A4d
Historical Incidence of Cystic Keratinizing Epithelioma in the Lung of Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls
Historical Incidence	
PCB 126	0/53
TCDD	0/53
PeCDF	0/53
TEF Mixture	0/53
PCB 153	0/52
Binary Mixture of PCB 126/PCB 153	0/53
Binary Mixture of PCB 126/PCB 118	0/53
Overall Historical Incidence	
Total	0/370

[a] Data as of February 24, 2005

TABLE A4e
Historical Incidence of Pancreas Neoplasms in Vehicle Control Female Sprague-Dawley Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence			
PCB 126	1/51	0/51	1/51
TCDD	0/51	0/51	0/51
PeCDF	0/53	0/53	0/53
TEF Mixture	0/52	0/52	0/52
PCB 153	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 153	0/53	0/53	0/53
Binary Mixture of PCB 126/PCB 118	0/53	0/53	0/53
Overall Historical Incidence			
Total (%)	1/366 (0.3%)	0/366	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 24, 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 PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
Disposition Summary						
Animals initially in study	28	28	28	28	28	28
14-Week interim evaluation	10	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8	8
Animals examined microscopically	28	28	28	28	28	28
14-Week Interim Evaluation						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fatty change, diffuse						2 (20%)
Inflammation	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Pigmentation						2 (20%)
Hepatocyte, hypertrophy		1 (10%)	3 (30%)	3 (30%)	4 (40%)	7 (70%)
Pancreas	(10)					(10)
Acinus, atrophy	1 (10%)					
Endocrine System						
Thyroid gland	(10)	(10)	(10)	(10)	(9)	(10)
Follicular cell, hypertrophy		3 (30%)	3 (30%)	4 (40%)	7 (78%)	7 (70%)
Genital System						
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy	1 (10%)	1 (10%)	1 (10%)	1 (10%)	3 (30%)	
Uterus	(10)					(10)
Metaplasia, squamous	1 (10%)					
Endometrium, hyperplasia, cystic	4 (40%)					3 (30%)
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	10 (100%)					10 (100%)
Thymus	(10)	(9)	(8)	(9)	(10)	(10)
Atrophy			3 (38%)			2 (20%)
Necrosis				1 (11%)		
Respiratory System						
Lung	(10)					(10)
Inflammation						1 (10%)
Inflammation, chronic active	2 (20%)					4 (40%)

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

TABLE A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
14-Week Interim Evaluation (continued)						
Systems Examined with No 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)	(10)
Basophilic focus		2 (20%)		1 (10%)		
Cholangiofibrosis						1 (10%)
Eosinophilic focus						1 (10%)
Fatty change, diffuse					1 (10%)	
Inflammation	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus	1 (10%)	3 (30%)	2 (20%)	1 (10%)	2 (20%)	1 (10%)
Mixed cell focus, multiple	4 (40%)	1 (10%)	3 (30%)	2 (20%)		4 (40%)
Pigmentation		1 (10%)	3 (30%)	4 (40%)	7 (70%)	8 (80%)
Bile duct, fibrosis						1 (10%)
Bile duct, hyperplasia					1 (10%)	
Hepatocyte, hypertrophy		1 (10%)	3 (30%)	6 (60%)	8 (80%)	8 (80%)
Endocrine System						
Adrenal cortex	(10)					(10)
Hypertrophy	1 (10%)					2 (20%)
Pituitary gland	(10)					(10)
Hyperplasia	1 (10%)					
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy	2 (20%)	1 (10%)	5 (50%)	3 (30%)	2 (20%)	3 (30%)
Genital System						
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy	5 (50%)	7 (70%)	7 (70%)	6 (60%)	8 (80%)	9 (90%)
Cyst	1 (10%)			1 (10%)		2 (20%)
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative				1 (10%)	1 (10%)	1 (10%)
Metaplasia, squamous	5 (50%)	6 (60%)	3 (30%)	5 (50%)	6 (60%)	3 (30%)
Endometrium, hyperplasia, cystic	4 (40%)	2 (20%)	4 (40%)	6 (60%)	7 (70%)	8 (80%)
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	10 (100%)					10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			2 (20%)		5 (50%)

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 PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
31-Week Interim Evaluation (continued)						
Integumentary System						
Mammary gland	(10)		(2)	(1)	(1)	(10)
Cyst	1 (10%)				1 (100%)	
Hyperplasia	1 (10%)					
Systems Examined with No Lesions Observed						
Cardiovascular System						
General Body System						
Musculoskeletal System						
Nervous System						
Respiratory System						
Special Senses System						
Urinary System						
53-Week Interim Evaluation						
Alimentary System						
Liver	(8)	(8)	(8)	(8)	(8)	(8)
Angiectasis	1 (13%)					
Basophilic focus	1 (13%)	1 (13%)		3 (38%)		1 (13%)
Clear cell focus, multiple						1 (13%)
Eosinophilic focus						1 (13%)
Fatty change, diffuse						3 (38%)
Hepatodiaphragmatic nodule		1 (13%)				
Inflammation	8 (100%)	8 (100%)	8 (100%)	7 (88%)	8 (100%)	8 (100%)
Mixed cell focus	2 (25%)	1 (13%)	3 (38%)	1 (13%)	3 (38%)	
Mixed cell focus, multiple	5 (63%)	5 (63%)	3 (38%)	7 (88%)	3 (38%)	6 (75%)
Necrosis	1 (13%)	1 (13%)	2 (25%)			
Pigmentation		1 (13%)	1 (13%)	6 (75%)	7 (88%)	8 (100%)
Toxic hepatopathy						1 (13%)
Bile duct, cyst						2 (25%)
Bile duct, fibrosis	1 (13%)		1 (13%)	1 (13%)		
Bile duct, hyperplasia					1 (13%)	1 (13%)
Hepatocyte, hypertrophy	1 (13%)	3 (38%)	3 (38%)	3 (38%)	5 (63%)	7 (88%)
Hepatocyte, multinucleated					1 (13%)	2 (25%)
Pancreas	(8)					(8)
Hyperplasia	1 (13%)					
Inflammation, chronic active	1 (13%)					
Endocrine System						
Adrenal cortex	(8)					(8)
Degeneration, cystic						1 (13%)
Hyperplasia	1 (13%)					2 (25%)
Hypertrophy	4 (50%)					6 (75%)
Vacuolization cytoplasmic						2 (25%)
Pituitary gland	(8)					(8)
Hyperplasia						2 (25%)
Pars distalis, hyperplasia	1 (13%)					

TABLE A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
53-Week Interim Evaluation (continued)						
Endocrine System (continued)						
Thyroid gland	(8)	(8)	(7)	(8)	(8)	(8)
C-cell, hyperplasia						2 (25%)
Follicular cell, hypertrophy	2 (25%)	3 (38%)	1 (14%)	3 (38%)	4 (50%)	5 (63%)
Genital System						
Ovary	(8)	(1)	(2)	(1)		(8)
Atrophy	8 (100%)					7 (88%)
Cyst	1 (13%)		2 (100%)			
Oviduct		(1)				
Cyst		1 (100%)				
Uterus	(8)	(1)	(2)	(1)	(1)	(8)
Inflammation, chronic active			2 (100%)		1 (100%)	
Inflammation, suppurative	2 (25%)					1 (13%)
Metaplasia, squamous	8 (100%)	1 (100%)	1 (50%)			7 (88%)
Endometrium, hyperplasia, cystic	6 (75%)			1 (100%)	1 (100%)	8 (100%)
Epithelium, hyperplasia			2 (100%)			
Vagina	(8)					(8)
Vacuolization cytoplasmic						1 (13%)
Hematopoietic System						
Spleen	(8)					(8)
Pigmentation	8 (100%)					8 (100%)
Thymus	(8)	(8)	(8)	(8)	(8)	(8)
Atrophy	5 (63%)	4 (50%)	6 (75%)	6 (75%)	5 (63%)	7 (88%)
Integumentary System						
Mammary gland	(8)	(1)	(1)		(2)	(8)
Cyst			1 (100%)		1 (50%)	1 (13%)
Hyperplasia						3 (38%)
Inflammation, chronic			1 (100%)		1 (50%)	
Respiratory System						
Lung	(8)	(1)				(8)
Infiltration cellular, histiocyte	4 (50%)	1 (100%)				2 (25%)
Urinary System						
Kidney	(1)					
Inflammation, chronic	1 (100%)					

TABLE A5a
Summary of the Incidence of Nonneoplastic Lesions in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
53-Week Interim Evaluation (continued)						
Systems Examined with No Lesions Observed						
Cardiovascular System						
General Body System						
Musculoskeletal System						
Nervous System						
Special Senses System						

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Disposition Summary							
Animals initially in study	53	53	53	53	53	53	50
Early deaths							
Accidental deaths		1	2	1			
Moribund	21	20	17	19	19	19	25
Natural deaths	7	10	10	8	14	11	10
Survivors							
Terminal sacrifice	25	22	24	25	20	23	15
Animals examined microscopically	53	53	53	53	53	53	50
Alimentary System							
Esophagus	(53)	(53)	(52)	(53)	(53)	(53)	(50)
Cyst				2 (4%)			
Perforation		1 (2%)	2 (4%)	1 (2%)			
Muscularis, inflammation		2 (4%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Periesophageal tissue, inflammation		1 (2%)	2 (4%)	2 (4%)	1 (2%)		
Intestine large, colon	(53)	(53)	(53)	(53)	(51)	(53)	(49)
Edema					1 (2%)		
Inflammation					1 (2%)	1 (2%)	
Parasite metazoan	2 (4%)						
Intestine large, rectum	(53)	(53)	(53)	(53)	(52)	(53)	(50)
Parasite metazoan	4 (8%)	5 (9%)	5 (9%)	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Artery, inflammation, chronic active		1 (2%)		1 (2%)			
Intestine large, cecum	(53)	(53)	(53)	(52)	(51)	(53)	(48)
Inflammation					1 (2%)		
Artery, inflammation, chronic active		1 (2%)				2 (4%)	
Artery, mineralization					1 (2%)		
Intestine small, ileum	(53)	(53)	(53)	(52)	(52)	(53)	(48)
Parasite metazoan	1 (2%)						
Liver	(53)	(53)	(53)	(52)	(53)	(53)	(50)
Angiectasis	6 (11%)	3 (6%)	4 (8%)	1 (2%)		1 (2%)	1 (2%)
Basophilic focus	10 (19%)	11 (21%)	9 (17%)	10 (19%)	12 (23%)	6 (11%)	9 (18%)
Basophilic focus, multiple	12 (23%)	12 (23%)	11 (21%)	6 (12%)	3 (6%)	1 (2%)	4 (8%)
Cholangiofibrosis		1 (2%)		3 (6%)	3 (6%)	5 (9%)	3 (6%)
Clear cell focus	11 (21%)	1 (2%)	7 (13%)	2 (4%)		3 (6%)	4 (8%)
Clear cell focus, multiple	2 (4%)	5 (9%)	3 (6%)	1 (2%)	6 (11%)	8 (15%)	2 (4%)
Degeneration, cystic						1 (2%)	1 (2%)
Eosinophilic focus	7 (13%)	11 (21%)	9 (17%)	9 (17%)	11 (21%)	10 (19%)	8 (16%)
Eosinophilic focus, multiple	8 (15%)	2 (4%)	9 (17%)	9 (17%)	12 (23%)	18 (34%)	14 (28%)
Fatty change, diffuse	1 (2%)	4 (8%)	10 (19%)	12 (23%)	20 (38%)	26 (49%)	6 (12%)
Fatty change, focal	5 (9%)	6 (11%)	5 (9%)	6 (12%)	6 (11%)	4 (8%)	7 (14%)
Hematopoietic cell proliferation	24 (45%)	28 (53%)	27 (51%)	24 (46%)	39 (74%)	31 (58%)	31 (62%)
Hemorrhage	1 (2%)						
Hepatodiaphragmatic nodule	3 (6%)	3 (6%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, nodular				3 (6%)	8 (15%)	12 (23%)	
Inflammation	47 (89%)	44 (83%)	47 (89%)	50 (96%)	48 (91%)	50 (94%)	48 (96%)
Mixed cell focus	5 (9%)		1 (2%)	2 (4%)	2 (4%)	1 (2%)	5 (10%)
Mixed cell focus, multiple	28 (53%)	24 (45%)	23 (43%)	27 (52%)	25 (47%)	25 (47%)	19 (38%)
Necrosis	4 (8%)	10 (19%)	3 (6%)	3 (6%)	6 (11%)	18 (34%)	11 (22%)
Pigmentation	13 (25%)	11 (21%)	21 (40%)	44 (85%)	42 (79%)	48 (91%)	48 (96%)

[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 PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Alimentary System (continued)							
Liver (continued)	(53)	(53)	(53)	(52)	(53)	(53)	(50)
Toxic hepatopathy	2 (4%)	3 (6%)	8 (15%)	27 (51%)	44 (83%)	9 (18%)	
Artery, inflammation, chronic active					1 (2%)		
Bile duct, cyst	7 (13%)	4 (8%)	2 (4%)	3 (6%)	2 (4%)	7 (13%)	2 (4%)
Bile duct, fibrosis	1 (2%)	4 (8%)	2 (4%)	2 (4%)	3 (6%)	6 (11%)	1 (2%)
Bile duct, hyperplasia	3 (6%)	2 (4%)	2 (4%)	2 (4%)	1 (2%)	13 (25%)	1 (2%)
Centrilobular, degeneration	4 (8%)	2 (4%)	3 (6%)	4 (8%)	4 (8%)	5 (9%)	7 (14%)
Hepatocyte, hypertrophy	2 (4%)	13 (25%)	17 (32%)	17 (33%)	24 (45%)	34 (64%)	14 (28%)
Hepatocyte, multinucleated			4 (8%)	13 (25%)	18 (34%)	35 (66%)	25 (50%)
Oval cell, hyperplasia	1 (2%)	4 (8%)	2 (4%)	6 (12%)	15 (28%)	35 (66%)	3 (6%)
Portal, fibrosis						1 (2%)	
Mesentery	(1)	(2)	(1)	(3)	(2)	(4)	(2)
Inflammation		1 (50%)					
Inflammation, chronic active							1 (50%)
Necrosis			1 (100%)				1 (50%)
Artery, inflammation, chronic active		1 (50%)		2 (67%)	1 (50%)	4 (100%)	
Fat, necrosis	1 (100%)			1 (33%)	1 (50%)		
Oral mucosa	(52)	(53)	(52)	(53)	(53)	(53)	(50)
Gingival, cyst				1 (2%)			
Gingival, hyperplasia				1 (2%)			
Gingival, hyperplasia, squamous	15 (29%)	11 (21%)	16 (31%)	19 (36%)	22 (42%)	20 (38%)	14 (28%)
Pancreas	(53)	(53)	(53)	(52)	(52)	(52)	(49)
Cyst					1 (2%)		
Cytoplasmic alteration						3 (6%)	
Inflammation, chronic active	2 (4%)	1 (2%)	1 (2%)	1 (2%)		4 (8%)	
Lipomatosis				1 (2%)			
Acinus, atrophy	3 (6%)	1 (2%)	1 (2%)	1 (2%)		3 (6%)	
Acinus, hyperplasia		1 (2%)					
Acinus, vacuolization cytoplasmic					2 (4%)	23 (44%)	2 (4%)
Artery, inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	2 (4%)	4 (8%)	11 (21%)	1 (2%)
Duct, dilatation						1 (2%)	
Salivary glands	(53)	(53)	(51)	(53)	(51)	(51)	(49)
Atrophy	1 (2%)					2 (4%)	
Duct, inflammation		1 (2%)					
Stomach, forestomach	(53)	(53)	(53)	(53)	(52)	(53)	(50)
Cyst						1 (2%)	
Edema		1 (2%)					
Erosion							1 (2%)
Hyperkeratosis			1 (2%)				1 (2%)
Hyperplasia, squamous	4 (8%)	1 (2%)	5 (9%)	6 (11%)	3 (6%)	10 (19%)	5 (10%)
Inflammation	1 (2%)	1 (2%)		2 (4%)	2 (4%)	2 (4%)	1 (2%)
Mineralization				1 (2%)			
Ulcer		1 (2%)	2 (4%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Artery, inflammation, chronic active					1 (2%)	3 (6%)	
Stomach, glandular	(53)	(53)	(53)	(53)	(52)	(53)	(50)
Erosion			1 (2%)	2 (4%)	1 (2%)		
Hyperplasia				1 (2%)			
Inflammation	1 (2%)			1 (2%)			
Mineralization	2 (4%)	9 (17%)	1 (2%)	3 (6%)	2 (4%)	4 (8%)	3 (6%)
Ulcer	1 (2%)						
Artery, inflammation, chronic active					1 (2%)	1 (2%)	
Serosa, inflammation		1 (2%)					
Tooth	(33)	(25)	(22)	(30)	(29)	(29)	(24)
Peridontal tissue, inflammation	33 (100%)	25 (100%)	22 (100%)	30 (100%)	29 (100%)	29 (100%)	24 (100%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Cardiovascular System							
Blood vessel	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Aorta, mineralization		1 (2%)		1 (2%)	2 (4%)		
Aorta, adventitia, inflammation, chronic, active					1 (2%)		
Heart	(53)	(53)	(52)	(53)	(53)	(52)	(50)
Cardiomyopathy	15 (28%)	12 (23%)	19 (37%)	13 (25%)	18 (34%)	24 (46%)	13 (26%)
Hemorrhage	2 (4%)						
Infiltration cellular, mononuclear cell						1 (2%)	
Inflammation	1 (2%)		1 (2%)	1 (2%)			
Mineralization				1 (2%)	1 (2%)	1 (2%)	
Artery, inflammation, chronic active					1 (2%)	2 (4%)	
Epicardium, inflammation		1 (2%)				1 (2%)	
Endocrine System							
Adrenal cortex	(53)	(53)	(53)	(52)	(53)	(53)	(48)
Angiectasis	31 (58%)	37 (70%)	30 (57%)	33 (63%)	27 (51%)	35 (66%)	29 (60%)
Atrophy	3 (6%)			1 (2%)		4 (8%)	
Degeneration, cystic	4 (8%)	17 (32%)	14 (26%)	18 (35%)	12 (23%)	14 (26%)	12 (25%)
Hematopoietic cell proliferation			1 (2%)		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)			1 (2%)			
Hyperplasia	8 (15%)	10 (19%)	10 (19%)	7 (13%)	10 (19%)	11 (21%)	7 (15%)
Hypertrophy	42 (79%)	43 (81%)	43 (81%)	46 (88%)	44 (83%)	45 (85%)	36 (75%)
Necrosis	4 (8%)		2 (4%)		2 (4%)		3 (6%)
Vacuolization cytoplasmic	9 (17%)	13 (25%)	14 (26%)	7 (13%)	17 (32%)	9 (17%)	10 (21%)
Bilateral, inflammation, chronic active							1 (2%)
Adrenal medulla	(53)	(53)	(53)	(52)	(53)	(53)	(48)
Hyperplasia	11 (21%)	12 (23%)	14 (26%)	14 (27%)	7 (13%)	10 (19%)	7 (15%)
Pancreatic islets	(53)	(53)	(53)	(52)	(52)	(52)	(49)
Hyperplasia						2 (4%)	
Parathyroid gland	(49)	(51)	(44)	(51)	(49)	(49)	(47)
Fibrosis							1 (2%)
Hyperplasia		1 (2%)	1 (2%)		1 (2%)	1 (2%)	
Hypertrophy		1 (2%)					
Pituitary gland	(53)	(53)	(53)	(53)	(53)	(53)	(49)
Angiectasis	20 (38%)	21 (40%)	20 (38%)	13 (25%)	12 (23%)	14 (26%)	13 (27%)
Atrophy	1 (2%)						
Cyst	2 (4%)			1 (2%)	1 (2%)	1 (2%)	1 (2%)
Cytoplasmic alteration	1 (2%)				1 (2%)		2 (4%)
Vacuolization cytoplasmic	2 (4%)	2 (4%)	1 (2%)				2 (4%)
Pars distalis, hyperplasia	22 (42%)	16 (30%)	14 (26%)	11 (21%)	15 (28%)	19 (36%)	19 (39%)
Pars intermedia, hyperplasia	1 (2%)						
Rathke's cleft, pars intermedia, hyperplasia					1 (2%)		
Thyroid gland	(53)	(53)	(51)	(53)	(51)	(51)	(48)
Inflammation			1 (2%)				
C-cell, hyperplasia	15 (28%)	16 (30%)	9 (18%)	13 (25%)	9 (18%)	11 (22%)	15 (31%)
Follicle, cyst	1 (2%)			1 (2%)	1 (2%)		
Follicular cell, hypertrophy	7 (13%)	13 (25%)	24 (47%)	24 (45%)	24 (47%)	22 (43%)	23 (48%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
General Body System							
None							
Genital System							
Clitoral gland	(53)	(51)	(53)	(53)	(50)	(53)	(50)
Hyperplasia, squamous			1 (2%)				
Inflammation	52 (98%)	47 (92%)	46 (87%)	51 (96%)	47 (94%)	47 (89%)	48 (96%)
Duct, cyst	40 (75%)	37 (73%)	34 (64%)	41 (77%)	44 (88%)	40 (75%)	39 (78%)
Ovary	(53)	(53)	(53)	(52)	(52)	(53)	(47)
Atrophy	43 (81%)	40 (75%)	38 (72%)	43 (83%)	45 (87%)	45 (85%)	39 (83%)
Cyst	11 (21%)	18 (34%)	20 (38%)	17 (33%)	18 (35%)	13 (25%)	12 (26%)
Fibrosis				1 (2%)			
Inflammation, chronic active	1 (2%)				1 (2%)	1 (2%)	
Oviduct	(1)				(1)		
Cyst	1 (100%)						
Inflammation, chronic active					1 (100%)		
Uterus	(53)	(53)	(53)	(52)	(52)	(53)	(49)
Adenomyosis	1 (2%)			1 (2%)	1 (2%)		
Congestion							1 (2%)
Cyst		1 (2%)			1 (2%)		
Hemorrhage					1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic active		5 (9%)	3 (6%)	3 (6%)	4 (8%)	3 (6%)	7 (14%)
Inflammation, suppurative	3 (6%)	6 (11%)	1 (2%)	7 (13%)	3 (6%)	4 (8%)	3 (6%)
Metaplasia, squamous	17 (32%)	25 (47%)	21 (40%)	36 (69%)	31 (60%)	35 (66%)	28 (57%)
Ulcer				1 (2%)	3 (6%)	1 (2%)	
Cervix, cyst						1 (2%)	
Endometrium, hyperplasia, cystic	31 (58%)	29 (55%)	29 (55%)	33 (63%)	39 (75%)	37 (70%)	35 (71%)
Hematopoietic System							
Bone marrow	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Atrophy			2 (4%)				
Hyperplasia	38 (72%)	32 (60%)	37 (70%)	35 (66%)	42 (79%)	41 (77%)	43 (86%)
Hyperplasia, reticulum cell				1 (2%)			
Lymph node	(5)	(4)	(4)	(4)	(3)	(7)	(3)
Hyperplasia, plasma cell						1 (14%)	
Iliac, ectasia		1 (25%)					1 (33%)
Iliac, hyperplasia, plasma cell							1 (33%)
Lumbar, ectasia	2 (40%)	1 (25%)			1 (33%)		1 (33%)
Lumbar, hemorrhage	2 (40%)	1 (25%)					
Lumbar, hyperplasia, histiocytic				1 (25%)	1 (33%)		
Lumbar, hyperplasia, plasma cell		1 (25%)	1 (25%)	1 (25%)		1 (14%)	1 (33%)
Mediastinal, ectasia					2 (67%)	2 (29%)	
Mediastinal, hemorrhage			1 (25%)			1 (14%)	
Mediastinal, hyperplasia, lymphoid			1 (25%)				
Mediastinal, hyperplasia, plasma cell		1 (25%)		1 (25%)	1 (33%)	2 (29%)	
Popliteal, ectasia	1 (20%)						
Renal, ectasia							1 (33%)
Renal, hyperplasia, plasma cell							1 (33%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Hematopoietic System (continued)							
Lymph node, mandibular	(53)	(53)	(51)	(53)	(51)	(51)	(49)
Congestion			1 (2%)				
Ectasia		1 (2%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, histiocytic		1 (2%)					
Hyperplasia, lymphoid				1 (2%)		1 (2%)	1 (2%)
Hyperplasia, plasma cell	38 (72%)	40 (75%)	38 (75%)	31 (58%)	30 (59%)	29 (57%)	29 (59%)
Lymph node, mesenteric	(53)	(53)	(53)	(52)	(52)	(53)	(49)
Atrophy				1 (2%)			
Hemorrhage	1 (2%)		1 (2%)			1 (2%)	
Hyperplasia, histiocytic						1 (2%)	
Hyperplasia, lymphoid		1 (2%)	1 (2%)			1 (2%)	
Hyperplasia, plasma cell		1 (2%)				1 (2%)	
Spleen	(53)	(53)	(53)	(52)	(52)	(51)	(49)
Accessory spleen	1 (2%)		1 (2%)				
Congestion		1 (2%)			1 (2%)		
Hematopoietic cell proliferation	49 (92%)	49 (92%)	48 (91%)	51 (98%)	46 (88%)	50 (98%)	49 (100%)
Hemorrhage		1 (2%)					
Infarct			1 (2%)				
Necrosis		1 (2%)					
Pigmentation	52 (98%)	45 (85%)	50 (94%)	48 (92%)	49 (94%)	51 (100%)	48 (98%)
Lymphatic, angiectasis			1 (2%)				
Lymphoid follicle, atrophy	7 (13%)	8 (15%)	7 (13%)	6 (12%)	12 (23%)	4 (8%)	2 (4%)
Red pulp, atrophy	2 (4%)						
Red pulp, hyperplasia		1 (2%)					
Thymus	(53)	(49)	(50)	(52)	(49)	(51)	(49)
Atrophy	43 (81%)	36 (73%)	36 (72%)	44 (85%)	39 (80%)	48 (94%)	44 (90%)
Hemorrhage	1 (2%)		1 (2%)		1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)				
Inflammation		1 (2%)					
Integumentary System							
Mammary gland	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Cyst	1 (2%)	2 (4%)		5 (9%)	4 (8%)	3 (6%)	3 (6%)
Hyperplasia	33 (62%)	29 (55%)	26 (49%)	20 (38%)	20 (38%)	17 (32%)	19 (38%)
Inflammation							1 (2%)
Inflammation, granulomatous	2 (4%)	4 (8%)	3 (6%)	4 (8%)	4 (8%)	3 (6%)	1 (2%)
Skin	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Hyperkeratosis							1 (2%)
Inflammation	2 (4%)	1 (2%)					
Lip, inflammation, chronic active							1 (2%)
Ulcer	2 (4%)	1 (2%)					
Conjunctiva, inflammation						1 (2%)	
Musculoskeletal System							
Bone	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Osteopetrosis							1 (2%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Nervous System							
Brain	(53)	(53)	(53)	(53)	(53)	(53)	(49)
Edema				1 (2%)			
Gliosis				1 (2%)			
Hemorrhage	1 (2%)					1 (2%)	
Hydrocephalus	1 (2%)			1 (2%)		1 (2%)	
Mineralization							1 (2%)
Peripheral nerve				(1)		(1)	
Radicular neuropathy						1 (100%)	
Spinal cord				(1)		(1)	
Degeneration						1 (100%)	
Hemorrhage				1 (100%)			
Necrosis				1 (100%)			
Respiratory System							
Lung	(53)	(53)	(53)	(53)	(53)	(52)	(50)
Congestion							1 (2%)
Edema					1 (2%)		
Hemorrhage					1 (2%)		2 (4%)
Infiltration cellular, histiocyte	44 (83%)	44 (83%)	40 (75%)	40 (75%)	41 (77%)	36 (69%)	30 (60%)
Inflammation	5 (9%)	8 (15%)	7 (13%)	6 (11%)	7 (13%)	8 (15%)	5 (10%)
Metaplasia, squamous				2 (4%)	4 (8%)	3 (6%)	1 (2%)
Mineralization				1 (2%)	1 (2%)		1 (2%)
Alveolar epithelium, hyperplasia	14 (26%)	17 (32%)	12 (23%)	20 (38%)	8 (15%)	3 (6%)	6 (12%)
Alveolar epithelium, metaplasia, bronchiolar	5 (9%)	6 (11%)	5 (9%)	9 (17%)	23 (43%)	28 (54%)	7 (14%)
Alveolus, metaplasia, squamous			1 (2%)				
Nose	(53)	(53)	(53)	(53)	(53)	(53)	(50)
Inflammation	8 (15%)	3 (6%)	8 (15%)	11 (21%)	7 (13%)	6 (11%)	7 (14%)
Mineralization	1 (2%)						
Nasolacrimal duct, inflammation							1 (2%)
Olfactory epithelium, metaplasia			1 (2%)				
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	1 (2%)	6 (11%)	3 (6%)	2 (4%)
Respiratory epithelium, metaplasia						1 (2%)	
Septum, inflammation	1 (2%)	2 (4%)	3 (6%)	2 (4%)	5 (9%)	3 (6%)	
Squamous epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)				
Trachea	(53)	(53)	(52)	(53)	(53)	(53)	(50)
Inflammation					1 (2%)	1 (2%)	1 (2%)
Artery, peritracheal tissue, inflammation, chronic active						1 (2%)	
Peritracheal tissue, inflammation		1 (2%)	2 (4%)	1 (2%)			
Special Senses System							
Eye	(53)	(53)	(52)	(53)	(52)	(52)	(49)
Hemorrhage		1 (2%)					
Inflammation		1 (2%)			1 (2%)		
Cornea, inflammation						1 (2%)	
Lens, degeneration		1 (2%)					
Retina, atrophy			1 (2%)				1 (2%)
Retina, degeneration		1 (2%)					
Harderian gland	(53)	(53)	(53)	(53)	(52)	(52)	(49)
Inflammation	16 (30%)	9 (17%)	10 (19%)	13 (25%)	8 (15%)	6 (12%)	6 (12%)

TABLE A5b
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of PeCDF

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop-Exposure)
Urinary System							
Kidney	(53)	(53)	(53)	(52)	(53)	(53)	(48)
Accumulation, hyaline droplet		1 (2%)					3 (6%)
Calculus microscopic observation only	5 (9%)	2 (4%)	4 (8%)	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Cyst				1 (2%)	2 (4%)	1 (2%)	
Fibrosis						1 (2%)	
Hemorrhage					1 (2%)		
Infarct							1 (2%)
Inflammation							1 (2%)
Inflammation, chronic active					1 (2%)	1 (2%)	
Inflammation, suppurative	2 (4%)	1 (2%)	1 (2%)	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Mineralization	37 (70%)	45 (85%)	42 (79%)	43 (83%)	43 (81%)	31 (58%)	41 (85%)
Necrosis					2 (4%)		
Nephropathy	34 (64%)	39 (74%)	35 (66%)	42 (81%)	36 (68%)	45 (85%)	35 (73%)
Pigmentation	2 (4%)						
Artery, inflammation, chronic active				1 (2%)	1 (2%)		
Capsule, hemorrhage	1 (2%)						
Pelvis, dilatation		1 (2%)			2 (4%)	1 (2%)	1 (2%)
Pelvis, inflammation	1 (2%)	1 (2%)			5 (9%)	1 (2%)	1 (2%)
Renal tubule, dilatation			1 (2%)				
Renal tubule, hyperplasia			1 (2%)				
Renal tubule, necrosis		1 (2%)					
Renal tubule, nephropathy						1 (2%)	
Transitional epithelium, hyperplasia	2 (4%)	2 (4%)	1 (2%)	2 (4%)	6 (11%)	5 (9%)	3 (6%)
Urinary bladder	(53)	(52)	(53)	(53)	(51)	(53)	(50)
Infiltration cellular, lymphoid						1 (2%)	
Inflammation	2 (4%)	3 (6%)	1 (2%)	1 (2%)	6 (12%)	1 (2%)	2 (4%)
Transitional epithelium, hyperplasia		1 (2%)			4 (8%)	4 (8%)	3 (6%)
Transitional epithelium, metaplasia, squamous					1 (2%)		

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

TABLE B1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF 152

TABLE B1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of PeCDF[a]

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Necropsy body wt						
Week 14	271 ± 4	276 ± 6	264 ± 6	275 ± 8	265 ± 5	280 ± 6
Week 31	318 ± 7	295 ± 9	306 ± 12	308 ± 11	300 ± 10	277 ± 3**
Week 53	315 ± 9	329 ± 11	338 ± 10	310 ± 12	299 ± 9	307 ± 13
L. Kidney						
Week 14						
Absolute	0.805 ± 0.017	0.865 ± 0.019	0.831 ± 0.018	0.783 ± 0.017	0.774 ± 0.014	0.837 ± 0.032
Relative	2.967 ± 0.036	3.144 ± 0.067	3.163 ± 0.074	2.863 ± 0.071	2.927 ± 0.058	2.991 ± 0.079
Week 31						
Absolute	0.866 ± 0.019	0.867 ± 0.016	0.875 ± 0.031	0.877 ± 0.029	0.887 ± 0.029	0.809 ± 0.023
Relative	2.731 ± 0.052	2.955 ± 0.079	2.874 ± 0.081	2.856 ± 0.062	2.964 ± 0.070	2.918 ± 0.061
Week 53						
Absolute	1.002 ± 0.018	1.003 ± 0.023	1.041 ± 0.020	1.017 ± 0.032	0.989 ± 0.039	0.986 ± 0.032
Relative	3.201 ± 0.097	3.060 ± 0.070	3.093 ± 0.076	3.296 ± 0.080	3.317 ± 0.137	3.232 ± 0.123
Liver						
Week 14						
Absolute	8.448 ± 0.290	9.440 ± 0.314	9.250 ± 0.284	9.163 ± 0.400	9.045 ± 0.252	10.622 ± 0.197**
Relative	31.070 ± 0.631	34.261 ± 0.919**	35.085 ± 0.644**	33.250 ± 0.671	34.151 ± 0.700**	38.118 ± 0.849**
Week 31						
Absolute	9.655 ± 0.253	9.481 ± 0.324	10.693 ± 0.699	10.465 ± 0.519	10.751 ± 0.490	10.188 ± 0.146
Relative	30.443 ± 0.636	32.169 ± 0.691	34.711 ± 0.954**	33.918 ± 0.845**	35.838 ± 1.041**	36.806 ± 0.445**
Week 53						
Absolute	10.424 ± 0.324	10.589 ± 0.617	11.911 ± 0.421	11.338 ± 0.504	11.026 ± 0.322	12.587 ± 0.616**
Relative	33.255 ± 1.099	32.051 ± 1.014	35.289 ± 0.908	36.565 ± 0.342**	36.867 ± 0.711**	40.905 ± 0.765**
Lung						
Week 14						
Absolute	1.584 ± 0.088	1.649 ± 0.069	1.702 ± 0.091	1.599 ± 0.088	1.575 ± 0.068	1.950 ± 0.146*
Relative	5.831 ± 0.290	6.019 ± 0.313	6.447 ± 0.273	5.805 ± 0.225	5.940 ± 0.224	6.944 ± 0.421*
Week 31						
Absolute	2.215 ± 0.083	1.943 ± 0.105	1.957 ± 0.076	2.136 ± 0.083	2.198 ± 0.069	2.021 ± 0.098
Relative	7.015 ± 0.315	6.575 ± 0.243	6.457 ± 0.280	6.969 ± 0.269	7.366 ± 0.239	7.294 ± 0.332
Week 53						
Absolute	1.814 ± 0.070	1.820 ± 0.062	1.807 ± 0.035	1.758 ± 0.080	1.814 ± 0.079	2.027 ± 0.074
Relative	5.789 ± 0.251	5.539 ± 0.138	5.379 ± 0.177	5.680 ± 0.171	6.088 ± 0.304	6.633 ± 0.254*
L. Ovary						
Week 14						
Absolute	0.062 ± 0.005	0.072 ± 0.004	0.065 ± 0.006	0.065 ± 0.005	0.065 ± 0.003	0.070 ± 0.005
Relative	0.225 ± 0015	0.262 ± 0.010	0.244 ± 0.018	0.235 ± 0.014	0.244 ± 0.011	0.251 ± 0.018
Week 31						
Absolute	0.071 ± 0.004	0.054 ± 0.002*[b]	0.061 ± 0.006	0.063 ± 0.006	0.060 ± 0.004	0.054 ± 0.003*
Relative	0.222 ± 0.012	0.186 ± 0.009[b]	0.197 ± 0.015	0.201 ± 0.015	0.200 ± 0.008	0.195 ± 0.008
Week 53						
Absolute	0.054 ± 0.001	0.053 ± 0.003	0.077 ± 0.008*	0.058 ± 0.003	0.054 ± 0.003	0.064 ± 0.009
Relative	0.172 ± 0.007	0.162 ± 0.011	0.226 ± 0.024	0.187 ± 0.007	0.182 ± 0.013	0.211 ± 0.031

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

	Vehicle Control	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Necropsy body wt						
Week 14	271 ± 4	276 ± 6	264 ± 6	275 ± 8	265 ± 5	280 ± 6
Week 31	318 ± 7	295 ± 9	306 ± 12	308 ± 11	300 ± 10	277 ± 3**
Week 53	315 ± 9	329 ± 11	338 ± 10	310 ± 12	299 ± 9	307 ± 13
Spleen						
Week 14						
Absolute	0.595 ± 0.023	0.598 ± 0.020	0.559 ± 0.023	0.610 ± 0.033	0.597 ± 0.023	0.622 ± 0.022
Relative	2.195 ± 0.075	2.176 ± 0.078	2.116 ± 0.058	2.216 ± 0.097	2.257 ± 0.082	2.228 ± 0.076
Week 31						
Absolute	0.550 ± 0.021	0.526 ± 0.024	0.577 ± 0.025	0.589 ± 0.025	0.548 ± 0.019	0.465 ± 0.016*
Relative	1.734 ± 0.057	1.788 ± 0.069	1.890 ± 0.053	1.923 ± 0.083	1.835 ± 0.059	1.679 ± 0.057
Week 53						
Absolute	0.504 ± 0.014	0.549 ± 0.024	0.621 ± 0.028*	0.512 ± 0.030	0.475 ± 0.017	0.524 ± 0.039
Relative	1.609 ± 0.056	1.674 ± 0.075	1.838 ± 0.064	1.656 ± 0.082	1.590 ± 0.051	1.708 ± 0.122
Thymus						
Week 14						
Absolute	0.329 ± 0.020	0.333 ± 0.030	0.293 ± 0.010	0.278 ± 0.014	0.299 ± 0.025	0.307 ± 0.022
Relative	1.214 ± 0.072	1.206 ± 0.102	1.115 ± 0.039	1.017 ± 0.057	1.135 ± 0.094	1.107 ± 0.087
Thyroid gland						
Week 14						
Absolute	0.026 ± 0.002	0.026 ± 0.002	0..025 ± 0.002	0.024 ± 0.002	0.023 ± 0.001	0.024 ± 0.002
Relative	0.095 ± 0.008	0.096 ± 0.006	0.094 ± 0.005	0.087 ± 0.006	0.089 ± 0.005	0.087 ± 0.006
Week 31						
Absolute	0.023 ± 0.002	0.024 ± 0.002	0.021 ± 0.001	0.026 ± 0.002	0.024 ± 0.002	0.019 ± 0.001[b]
Relative	0.072 ± 0.004	0.080 ± 0.004	0.069 ± 0.006	0.084 ± 0.006	0.078 ± 0.006	0.068 ± 0.003[b]
Week 53						
Absolute	0.025 ± 0.002	0.028 ± 0.001	0.028 ± 0.001[c]	0.026 ± 0.002	0.027 ± 0.002	0.026 ± 0.002
Relative	0.077 ± 0.005	0.084 ± 0.003	0.086 ± 0.005[c]	0.084 ± 0.004	0.089 ± 0.006	0.084 ± 0.005

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=7

APPENDIX C
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

PeCDF

PeCDF was obtained from Cambridge Isotope Laboratories (Cambridge, MA) in two lots (CJJ-30319-43 and 29494-57), combined (labeled 080196), and used for the 2-year 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, Batelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the PeCDF study are on file at the National Institute of Environmental Health Sciences.

Lot 080196 of the chemical, a white powder, was identified by the analytical chemistry laboratory as PeCDF by proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. The spectrum of the purity analysis sample was compared to that of the frozen reference sample of the same lot and a previously reported spectrum of the same lot. All spectra were consistent with the structure of PeCDF. The route of synthesis used to produce the test article allows the exclusion of other isomers that are also consistent with the nuclear magnetic resonance data. The NMR spectra are presented in Figures C1 and C2.

The purity of lot 080196 was determined by the analytical chemistry laboratory using gas chromatography by system A (Table C1) and by the study laboratory using gas chromatography by system B (Table C1).

The purity profile obtained by system A detected four impurities with individual relative areas greater than or equal to 0.1%, and a total area of 2.4% relative to the major peak. One impurity (0.6% of peak area) was identified as a pentachlorodibenzofuran and one (0.4%) was identified as a hexachlorodibenzofuran. The other two impurities (1.4% combined) had none of the characteristics of PCBs, furans, or dioxins. The two furan impurities could not be unequivocally identified. Gas chromatography by system B indicated a purity of 101% when compared with the frozen reference sample. The overall purity of lot 080196 was determined to be 97% or greater.

To ensure stability, the bulk chemical was stored at room temperature, protected from light in amber glass bottles sealed with Teflon®-lined lids.

Formulation Materials

Acetone was obtained from Spectrum Quality Products (Gardena, CA) in three lots, and was used for the 2-year study. The identity of acetone was confirmed by the study laboratory using infrared spectroscopy. The spectrum agreed with that in the literature (*Aldrich*, 1985). The purity of acetone was determined by gas chromatography by system C (Table C1); there were no impurities greater than or equal to 0.1%.

Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

PREPARATION OF STOCK SAMPLES

Lot 080196 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 PeCDF in 100 mL of acetone. A formulation stock solution was prepared at a target concentration of 80 µg/mL by dissolving 40 mg of PeCDF in 500 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, chemical reference stocks of 100 µg for periodic purity determinations, and dose formulation working stocks. Dose formulation working stocks were prepared by transferring the required volumes of respective solutions into 15 mL amber glass containers, evaporating the solvent, and sealing with Teflon®-lined lids. Analysis of representative samples of dose formulation concentrations were performed using system B to confirm PeCDF concentrations. Stock solutions were stored at room temperature.

No stability studies of the bulk chemical were performed, only periodic reanalysis of frozen reference stocks by the study laboratory using gas chromatography by system B. These studies indicated that PeCDF was stable as a bulk chemical for 25 months when stored protected from light at room temperature (approximately 25° C). No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by dissolving PeCDF working stocks in acetone and diluting with corn oil (Spectrum Quality Products) to give the required concentrations (Table C2). The dose formulations were stored at room temperature in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids, for up to 35 days.

Homogeneity studies of 2.4 and 80 ng/mL dose formulations were performed by the study laboratory using gas chromatography by system D (Table C1). Stability studies of a 4.0 ng/mL dose formulation in corn oil containing 1% acetone were performed by the analytical chemistry laboratory with gas chromatography by system E (Table C1). Homogeneity was confirmed, and stability was confirmed for 35 days for dose formulations stored in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids, at –20° C, 5° C, and room temperature (approximately 25° C). Gavagability was confirmed for the 80 ng/mL dose formulation.

Periodic analyses of the dose formulations of PeCDF were conducted by the study laboratory using gas chromatography by system E. During the 2-year study, the dose formulations were analyzed at least every 3 months (Table C3). Of the dose formulations analyzed, 42 of 53 were within 10% of the target concentrations and all were within 14% of target; 71% 15 of 21 of the animal room samples were within 10% of the target concentrations and all were within 19% of target.

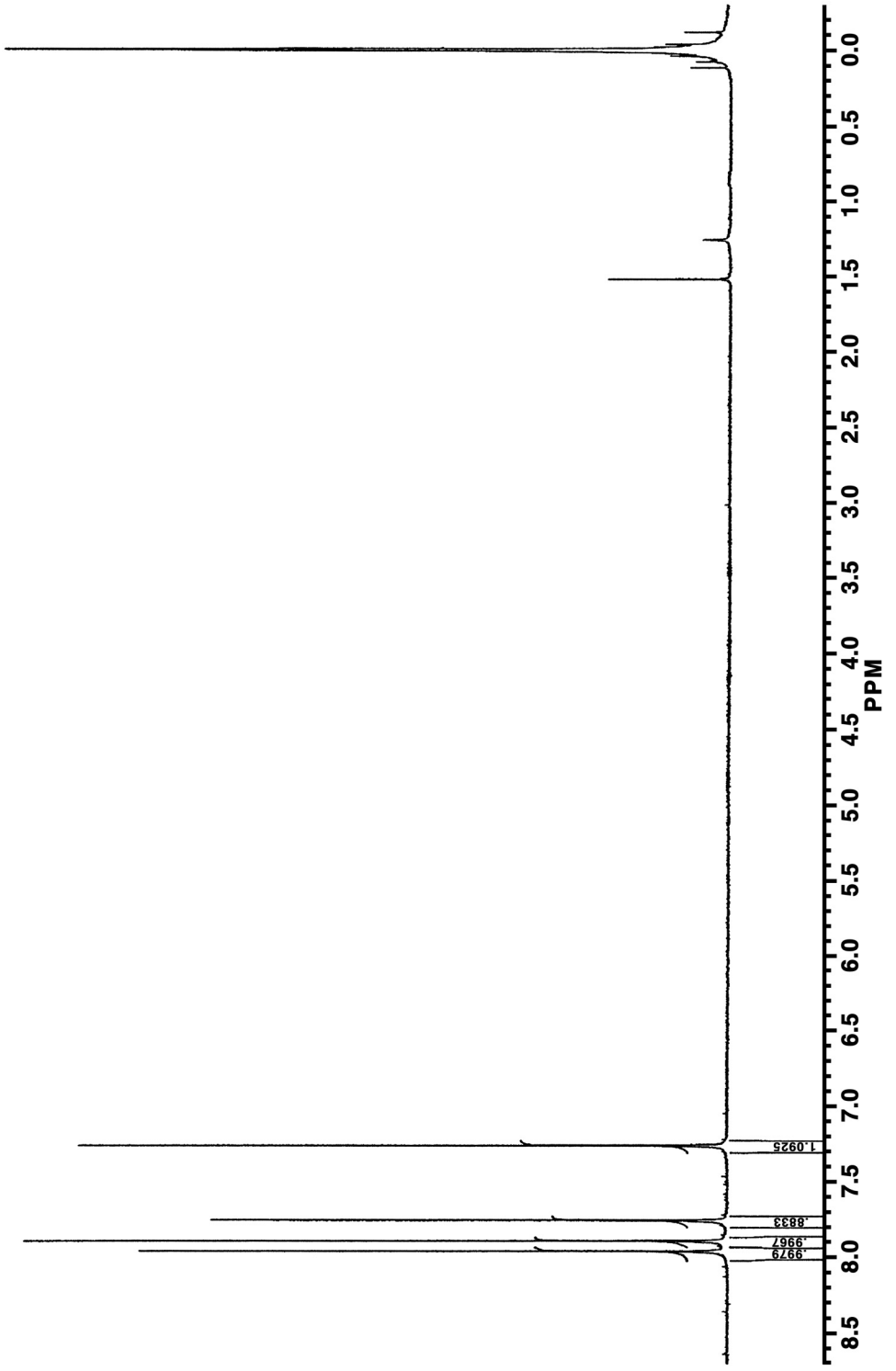

FIGURE C1
Proton Nuclear Magnetic Resonance Spectrum of PeCDF

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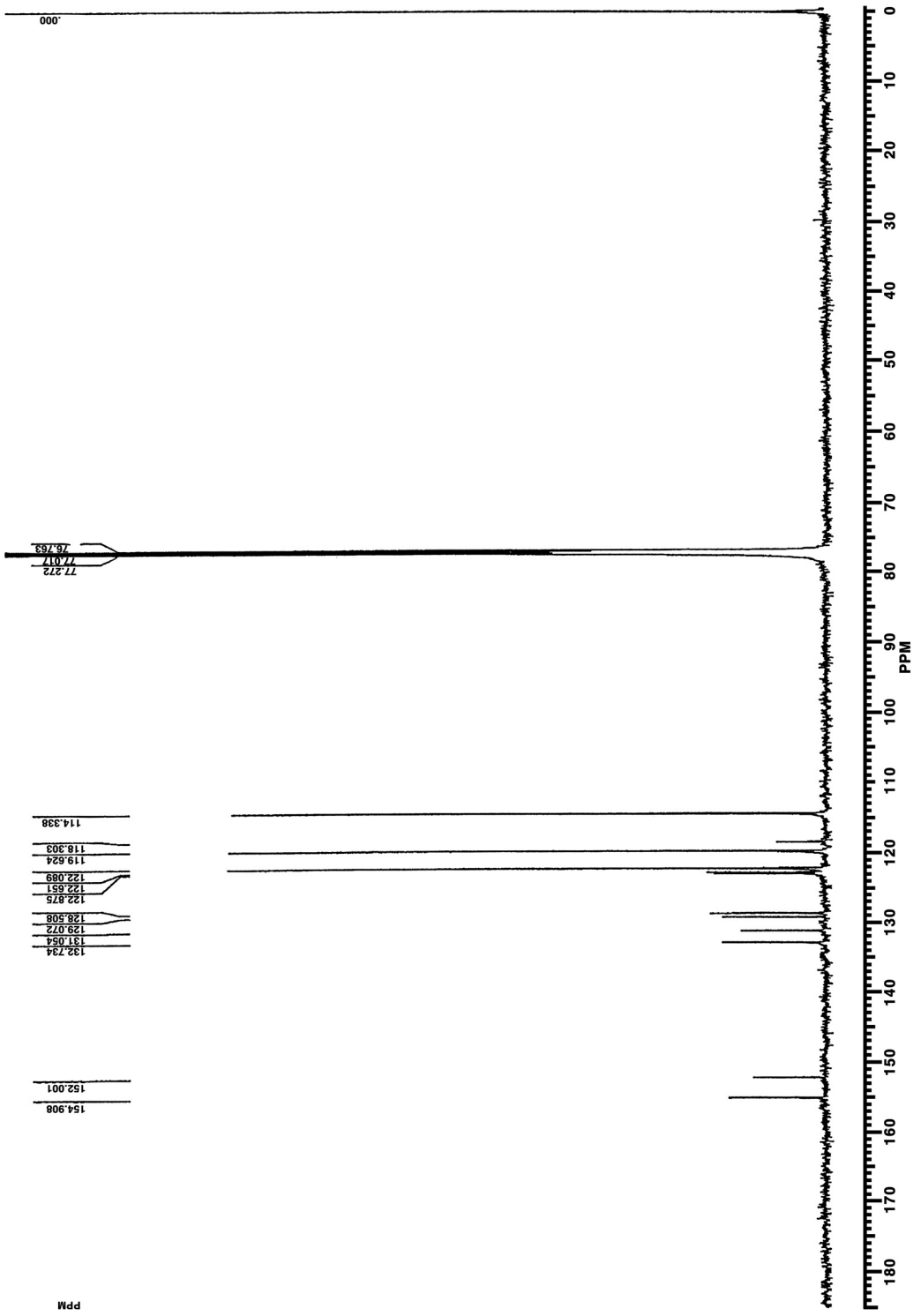

FIGURE C2
Carbon-13 Nuclear Magnetic Resonance Spectrum of PeCDF

TABLE C1
Gas Chromatography Systems Used in the 2-Year Gavage Study of PeCDF[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometer, high resolution	DB-5 (MS), 15 m × 0.25 mm, fused silica, 0.25-µm film thickness	Helium at 4 psi	50° C for 1 minute, then 8° C/minute to 300° C, held for 10 minutes
System B			
Flame ionization	PTE-5 (QTM), 15 m × 0.53 mm, 0.5-µm film thickness	Helium at 5 mL/minute	45° C for 5 minutes; then 15° C/minute to 300° C; held for 5 minutes
System C			
Flame ionization	Supelco 20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcoport, 2.4 m × 2 mm	Nitrogen at 30 mL/minute	40° C for 4 minutes, then 10° C/minute to 170° C
System D			
Mass spectrometer, selected ion recording	DB-5 (MS), 15 m × 0.25 mm, fused silica, 0.25-µm film thickness	Helium (ultra pure) at 1 mL/minute	100° C for 1 minute, then 15° C/minute to 285° C, held for 2 minutes
System E			
Mass spectrometer, selected ion recording	DB-5 (MS), 15 m × 0.25 mm, fused silica, 0.25-µm film thickness	Helium at 4 mL/minute	80° C, then 25° C/minute to 225° C, then 35°C/minute to 310° C, held for 4 minutes

[a] Gas chromatographs were manufactured by Carlo Erba/Fisons, Ltd. (Valencia, CA) (system A, D, and E) and Hewlett Packard (Palo Alto, CA) (systems B and C), mass spectrometers were manufactured by VG (Cheshire, UK) (Systems A, D, and E)

TABLE C2
Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of PeCDF

Preparation
Dose formulation working stocks were prepared by transferring the appropriate volumes from the 2.4 ng/mL (low concentration) and 80 ng/mL (high concentration) stock solutions into 15 mL amber glass bottles, evaporating the acetone, and sealing the vials with Teflon®-lined lids. The dose formulation working stocks were made to produce 2-L formulations of the desired target concentrations.

Dose formulations were prepared by filling a 2-L volumetric flask approximately half full with corn oil. Twenty mL of acetone was measured in a graduated cylinder and added in four 5 mL aliquots to the appropriate stock standard vial, capped, shaken, and sonicated for 30 seconds, and transferred by pipet to the volumetric flask after each rinse. The contents of the volumetric flask were diluted to volume with corn oil, capped, and stirred on a stirplate for 3 hours, with periodic inverting and shaking.

Chemical Lot Number
080196

Maximum Storage Time
35 days

Storage Conditions
Working stocks of PeCDF were stored in 15-mL amber glass vials, sealed with Teflon®-lined lids at room temperature.
Dose formulations were stored in 2-L amber glass screw-cap bottles with Teflon®-lined lids at room temperature (approximately 25° C).

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

TABLE C3
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of PeCDF

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration[a] (ng/mL)	Difference from Target (%)
March 23, 1999	March 26-27, 1999	2.4	2.085[b]	–13
		8.0	6.847[b]	–14
		17.6	15.66[b]	–11
		36.8	32.67[b]	–11
		80.0	68.93[b]	–14
		80.0	75.02	–6
	May 19-20, 1999[c]	2.4	2.393	0
		8.0	7.923	–1
		17.6	17.00	–3
		36.8	35.09	–5
		80.0	72.87	–9
		80.0	77.79	–3
June 15, 1999	June 28-29, 1999	2.4	2.153	–10
		8.0	7.546	–6
		17.6	15.84	–10
		36.8	33.13	–10
		80.0	71.08[b]	–11
		80.0	71.84	–10
September 3, 1999	September 9-10, 1999	2.4	2.109[b]	–12
		8.0	7.219	–10
		17.6	15.89	–10
		36.8	32.87[b]	–11
		80.0	70.18[b]	–12
		80.0	70.91[b]	–11
November 23, 1999	December 1-2, 1999	2.4	2.206	–8
		8.0	7.479	–7
		17.6	16.16	–8
		36.8	33.83	–8
		80.0	70.40[b]	–12
	January 11-12, 2000[c]	2.4	1.950	–19
		8.0	7.130	–11
		17.6	15.50	–12
		36.8	32.74	–11
		80.0	68.48	–14
February 15, 2000	February 22-23, 2000	2.4	2.234 ± 0.019	–7
		8.0	7.488 ± 0.296	–6
		17.6	16.14 ± 0.08	–8
		36.8	34.16 ± 1.58	–7
		80.0	72.82 ± 3.13	–9
May 9, 2000	May 11-12, 2000	2.4	2.314 ± 0.025	–4
		8.0	7.530 ± 0.046	–6
		17.6	16.37 ± 0.030	–7
		36.8	34.45 ± 0.77	–6
		80.0	72.61 ± 1.64	–9

TABLE C3
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of PeCDF

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration (ng/mL)	Difference from Target (%)
July 5, 2000	July 10-11, 2000	2.4	2.210 ± 0.009	–8
		8.0	7.587 ± 0.200	–5
		17.6	16.71 ± 0.75	–5
		36.8	35.58 ± 0.42	–3
		80.0	76.74 ± 1.57	–4
	August 21-22, 2000[c]	2.4	2.285 ± 0.075	–5
		8.0	7.651 ± 0.106	–4
		17.6	16.33 ± 0.43	–7
		36.8	34.76 ± 0.74	–6
		80.0	73.57 ± 0.96	–8
August 31, 2000	September 8-9, 2000	2.4	2.267 ± 0.033	–6
		8.0	7.585 ± 0.154	–5
		17.6	16.28 ± 0.31	–8
		36.8	34.24 ± 1.30	–7
		80.0	72.99 ± 0.72	–9
November 21, 2000	November 28-29, 2000	2.4	2.311 ± 0.200	–4
		8.0	7.213 ± 0.285	–10
		17.6	15.92 ± 1.21	–10
		36.8	34.79 ± 1.11	–5
		80.0	74.91 ± 1.99	–6
March 2, 2001	March 7-8, 2001	2.4	2.330 ± 0.187	–3
		8.0	8.529 ± 0.464	+7
		17.6	17.79 ± 1.40	+1
		36.8	36.96 ± 2.33	0
		80.0	85.98 ± 3.01	+7
	April 11-12, 2001[c]	2.4	2.278 ± 0.104	–5
		8.0	7.048 ± 0.543	–12
		17.6	17.70 ± 0.61	+1
		36.8	33.05 ± 1.95	–10
		80.0	72.22 ± 2.50	–10

[a] Reported value is the average of duplicate analyses, or of the average of quadruplicate analyses ± standard deviation. Dosing volume = 2.5 mL/kg; 2.4 ng/mL= 6 ng/kg, 8.0 ng/mL=20 ng/kg, 17.6 ng/mL = 44 ng/kg, 36.8 ng/mL = 92 ng/kg, and 80.0 ng/mL=200 ng/kg.
[b] Concentration was not within ± 10% of target; NTP approved use of formulation.
[c] Animal room samples

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

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TABLE D1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE D2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
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 D3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.7 ± 0.41	13.1 – 14.5	26
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.6	26
Crude fiber (% by weight)	9.0 ± 0.63	7.9 – 10.5	26
Ash (% by weight)	5.0 ± 0.21	4.7 – 5.4	26
Amino Acids (% of total diet)			
Arginine	0.731 ± 0.050	0.670 – 0.800	8
Cystine	0.224 ± 0.012	0.210 – 0.240	8
Glycine	0.684 ± 0.041	0.620 – 0.740	8
Histidine	0.333 ± 0.018	0.310 – 0.350	8
Isoleucine	0.524 ± 0.046	0.430 – 0.590	8
Leucine	1.061 ± 0.061	0.960 – 1.130	8
Lysine	0.708 ± 0.056	0.620 – 0.790	8
Methionine	0.401 ± 0.035	0.350 – 0.460	8
Phenylalanine	0.598 ± 0.036	0.540 – 0.640	8
Threonine	0.501 ± 0.051	0.430 – 0.590	8
Tryptophan	0.126 ± 0.014	0.110 – 0.150	8
Tyrosine	0.390 ± 0.056	0.280 – 0.460	8
Valine	0.640 ± 0.049	0.550 – 0.690	8
Essential Fatty Acids (% of total diet)			
Linoleic	3.97 ± 0.284	3.59 – 4.54	8
Linolenic	0.30 ± 0.042	0.21 – 0.35	8
Vitamins			
Vitamin A (IU/kg)	5,160 ± 999	3,460 – 7,790	26
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)[b]	7.7 ± 0.81	6.3 – 9.2	26
Riboflavin (ppm)	5.6 ± 1.12	4.20 – 7.70	8
Niacin (ppm)	74.3 ± 5.94	66.4 – 85.8	8
Pantothenic acid (ppm)	22.5 ± 3.96	17.4 – 29.1	8
Pyridoxine (ppm)[b]	9.04 ± 2.37	6.4 – 12.4	8
Folic acid (ppm)	1.64 ± 0.38	1.26 – 2.32	8
Biotin (ppm)	0.333 ± 0.15	0.225 – 0.704	8
Vitamin B_{12} (ppb)	68.7 ± 63.0	18.3 – 174.0	8
Choline (ppm)[b]	3,155 ± 325	2,700 – 3,790	8
Minerals			
Calcium (%)	1.012 ± 0.047	0.903 – 1.100	26
Phosphorus (%)	0.576 ± 0.027	0.517 – 0.628	26
Potassium (%)	0.659 ± 0.022	0.627 – 0.691	8
Chloride (%)	0.357 ± 0.027	0.300 – 0.392	8
Sodium (%)	0.189 ± 0.019	0.160 – 0.212	8
Magnesium (%)	0.199 ± 0.009	0.185 – 0.213	8
Sulfur (%)	0.178 ± 0.021	0.153 – 0.209	8
Iron (ppm)	160 ± 14.7	135 – 177	8
Manganese (ppm)	50.3 ± 4.82	42.1 – 56.0	8
Zinc (ppm)	50.7 ± 6.59	43.3 – 61.1	8
Copper (ppm)	6.29 ± 0.828	5.08 – 7.59	8
Iodine (ppm)	0.461 ± 0.187	0.233 – 0.843	8
Chromium (ppm)	0.542 ± 0.128	0.330 – 0.707	7
Cobalt (ppm)	0.23 ± 0.049	0.20 – 0.30	7

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.18 ± 0.068	0.10 – 0.37	26
Cadmium (ppm)	0.04 ± 0.007	0.04 – 0.07	26
Lead (ppm)	0.11 ± 0.102	0.05 – 0.54	26
Mercury (ppm)	<0.02		26
Selenium (ppm)	0.20 ± 0.040	0.14 – 0.28	26
Aflatoxins (ppb)	<5.00		26
Nitrate nitrogen (ppm)[c]	10.9 ± 3.07	9.04 – 21.1	26
Nitrite nitrogen (ppm)[c]	<0.61		26
BHA (ppm)[d]	<1.0		26
BHT (ppm)[d]	<1.0		26
Aerobic plate count (CFU/g)	12 ± 6	10 – 40	26
Coliform (MPN/g)	1.2 ± 1.7	0.0 – 3.6	26
Escherichia coli (MPN/g)	<10		26
Salmonella (MPN/g)	Negative		26
Total nitrosoamines (ppb)[e]	4.4 ± 1.27	2.1 – 7.5	26
N-Nitrosodimethylamine (ppb)[e]	1.7 ± 0.51	1.0 – 3.0	26
N-Nitrosopyrrolidine (ppb)[e]	2.7 ± 1.00	1.0 – 5.1	26
Pesticides (ppm)			
α-BHC	<0.01		26
β-BHC	<0.02		26
γ-BHC	<0.01		26
δ-BHC	<0.01		26
Heptachlor	<0.01		26
Aldrin	<0.01		26
Heptachlor epoxide	<0.01		26
DDE	<0.01		26
DDD	<0.01		26
DDT	<0.01		26
HCB	<0.01		26
Mirex	<0.01		26
Methoxychlor	<0.05		26
Dieldrin	<0.01		26
Endrin	<0.01		26
Telodrin	<0.01		26
Chlordane	<0.05		26
Toxaphene	<0.10		26
Estimated PCBs	<0.20		26
Ronnel	<0.01		26
Ethion	<0.02		26
Trithion	<0.05		26
Diazinon	<0.10		26
Methyl chlorpyrifos	0.158 ± 0.112	0.023 – 0.499	26
Methyl parathion	<0.02		26
Ethyl parathion	<0.02		26
Malathion	0.187 ± 0.129	0.020 – 0.401	26
Endosulfan I	<0.01		26
Endosulfan II	<0.01		26
Endosulfan sulfate	<0.03		26

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

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

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

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

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

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

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

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

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

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

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

APPENDIX E
SENTINEL ANIMAL PROGRAM

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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 randomly selected male and female rats during 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
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
M. arthritidis	Study termination

RESULTS

All serology tests were negative.

APPENDIX F
SINGLE-DOSE TOXICOKINETIC STUDY IN FEMALE SPRAGUE-DAWLEY RATS

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SINGLE-DOSE TOXICOKINETIC STUDY IN FEMALE SPRAGUE-DAWLEY RATS

INTRODUCTION

A single dose of PeCDF was administered by gavage to female Harlan Sprague-Dawley rats at 6 or 200 ng/kg. PeCDF levels were determined in postdose blood, lung, liver, and fat tissue samples at early time points (up to 24 hours) and late time points (5 to 365 days), and the results were analyzed to establish basic toxicokinetic parameters. Bioavailability was not determined from this study because no intravenous administration was conducted.

MATERIALS AND METHODS

PeCDF was procured in two lots (CJJ-30319-43 and 29494-57) from Cambridge Isotope Laboratories. The two lots were combined, renamed lot number 080196, and characterized as described in Appendix C. Formulations for the study were prepared in corn oil with 1% acetone, also as described in Appendix C.

Female rats (20 to 22 weeks of age at the start of the study) were used for the study. They were administered 6 or 200 ng/kg of formulation in a volume of 2.5 mL/kg. Groups of five rats per time point were bled and then tissues were harvested at 0.5, 1, 1.5, 2, 3, 8, 16, and 24 hours and at 5, 12, 32, 61, 92, 120, 166, 212, 250, 281, 309, 341, and 365 days post dosing. The animals were anesthetized with a CO_2/O_2 mixture and as much blood as possible was collected by cardiac puncture from each rat. Blood samples were collected in EDTA tubes and stored at 5° C for analysis. Following blood collection, the rats were euthanized with CO_2 and their lungs, livers, and mesenteric fat were collected. Tissue weights were recorded and the samples were frozen at –20° C for analysis.

For analysis, 100 µL of 12,500 pg/mL ^{13}C-PeCDF was added to each 1.0 mL or gram of blood or tissue sample as an internal standard and the sample was saponified with 40% potassium hydroxide in ethanol. The samples were extracted with hexane and the extract was further cleaned up on silica gel and florasil solid phase extraction columns. The eluate from the second extraction was evaporated to dryness and reconstituted in nonane. The nonane solutions were analyzed using gas chromatography with high resolution mass spectrometry on an RTX-5 MS column 15 m × 0.25 mm (ID), 0.25 µm film thickness (Restek, Bellefonte, PA), using an oven program (100° C for 1 minute, to 285° C at 15° C/minute, then held for 2 minutes). Spectra were collected at 50 eV at 339.8783/0.3 ms for PeCDF and 351.9186/0.3 ms for the internal standard. Responses for PeCDF were quantitated using least-squares linear regression of a calibration curve generated from matched tissues from untreated Sprague-Dawley rats spiked with PeCDF. Table F1 provides figures of merit from the validation of the methods used.

Noncompartmental modeling with WinNonlin® version 4.0 (Pharsight Corp., Mountain View, CA) was used to derive toxicokinetic parameters in fat (from concentration versus time data from the 200 ng/kg group), and in liver (from concentration versus time data from the 6 and 200 ng/kg groups).

RESULTS AND DISCUSSION

The absence of measurable concentrations of PeCDF in blood following administration of 6 or 200 ng/kg precluded characterizing these data by compartmental analysis. Low concentrations in fat (6 ng/kg group) and lung (6 and 200 ng/kg groups) prevented noncompartmental analysis of these data as well (Table F2).

Measured blood PeCDF concentrations for the 6 and 200 ng/kg groups were generally indistinguishable from background levels, suggesting rapid absorption and systemic distribution. Lung tissue from both groups and fat tissue from the low dose group also exhibited concentrations of PeCDF less than the limits of quantitation (LOQ) for the method. The data from tissues having PeCDF levels above the limits of quantitation are presented in Figures F1 to F3.

Toxicokinetic modeling using the data generated in this study was limited to simple noncompartmental analyses that included calculations of areas under the concentration versus time curve and estimates of terminal elimination rate constants where appropriate. Since PeCDF concentrations in blood were generally indistinguishable from background levels, absorption and elimination kinetics from blood were not obtained. Therefore, volume of distribution, absorption and elimination rates, and clearance were not calculated. However, several observations are possible from the data. First, the absence of measurable concentrations in blood together with measurable concentrations in tissues does indicate that absorption occurred. Measurement of PeCDF concentrations in liver, fat, and lungs within approximately one hour after dosing indicates that absorption occurred within a relatively short time period. Based on a comparison of tissue concentration versus time profiles for the 200 ng/kg group, distribution to tissues occurred within approximately one hour after dosing and overall T_{max} values occurred within the first 24 hours for liver and lung tissues, whereas the overall T_{max} for fat was not observed until day 32.

C_{max} was not distinguishable in fat samples from the 6 ng/kg group, but increased in the following order: blood<lung<fat<liver for the 200 ng/kg group. Approximately ten-fold greater concentrations of PeCDF were measured in liver as compared to fat in the 200 ng/kg group, and were approximately 70 to 80 times that observed in lung. A comparison of elimination half-lives revealed that PeCDF is equally persistent in fat and liver.

The dose proportionality exhibited in liver concentrations from the high dose group compared to those from the low dose group suggests that the dosages tested were within a dose-proportional range, and no saturation of absorption or elimination occurred.

TABLE F1
Figures of Merit for Assays of PeCDF in the Single Gavage Dose Toxicokinetic Study in Female Sprague-Dawley Rats

Tissue	Linearity[a]	Limit of Quantitation	Precision[b]	Accuracy[c]
Blood	>0.99	30 pg/mL	Within 15%	15% or less
Lung	>0.99	60 pg/g	Within 15%	15% or less
Liver	>0.99	50 pg/g	Within 15%	15% or less
Fat	>0.99	37.5 pg/g	Within 15%	15% or less

[a] Correlation coefficient
[b] Standard deviation of quality control samples
[c] Relative error in determined versus prepared concentration of calibration standards

TABLE F2
Toxicokinetic Parameter Estimates in Female Sprague-Dawley Rats after a Single Gavage Dose of PeCDF

Parameter	Dose (ng/kg)	Tissue Parameter Estimate[a]		
		Lung	Liver	Fat
C_{max} (observed) (pg/g)	6	—	182 ± 37	—
	200	77[b]	5,960 ± 1,570	412 ± 99
T_{max} (observed) (days)	6	—	5	—
	200	0.083	1	32
k_{elim} (day^{-1})	6	—	0.0046	—
	200	—	0.0059	0.0046
$t_{1/2\ elim}$ (days)	6	—	151	—
	200	—	118	152
AUC_T[c]	6	—	22,900	—
(days •pg/g)	200	—	674,000	68,500
AUC_∞[d]	6	—	38,800	—
(days • pg/g)	200	—	746,000	88,500
AUC_T[c]/Dose	6	—	3,820	—
(days • pg/g)/(ng/kg)	200	—	3,370	342
AUC_∞[d]/Dose	6	—	6,470	—
(days • pg/g)/(ng/kg)	200	—	3,730	442

[a] Values are reported as the absolute value or mean ± standard deviation
[b] n=2
[c] AUC_T values were calculated by the trapezoidal method, assuming that the PeCDF concentration at t=0 was 0 pg/g tissue, and using the observed tissue PeCDF concentration-time data from time 0 to the last time point (T) where T is greater than LOQ.
[d] AUC_∞ values were calculated by the trapezoidal method assuming that the PeCDF concentration at t=0 was 0 pg/g tissue, using the observed tissue PeCDF concentration-time data, and extrapolating to infinity using k_{elim}

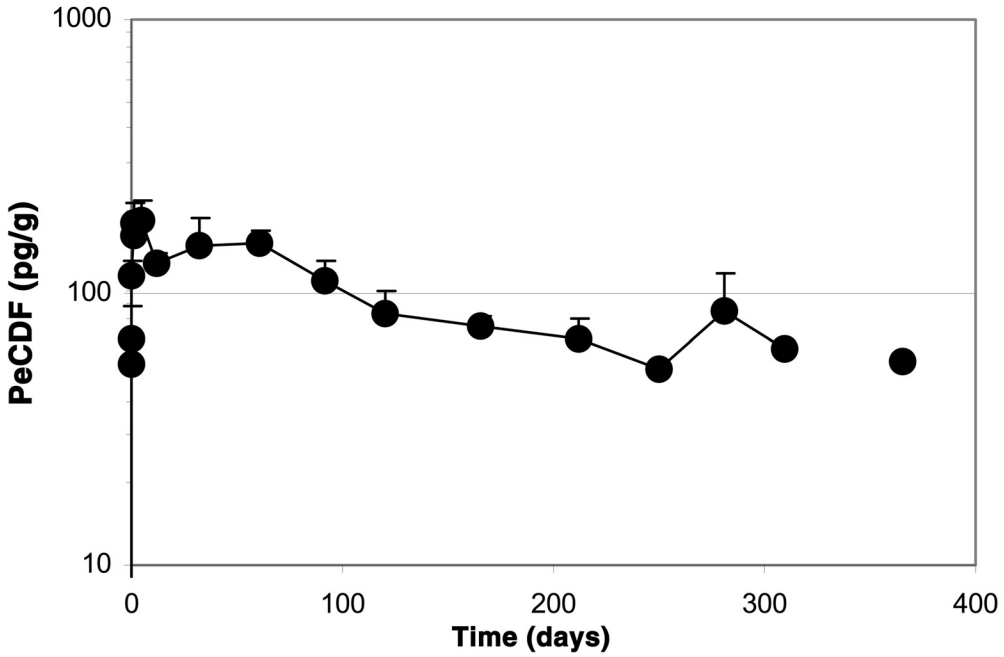

FIGURE F1
Liver Concentrations of PeCDF in Female Sprague-Dawley Rats after a Single Gavage Dose of 6 ng/kg PeCDF

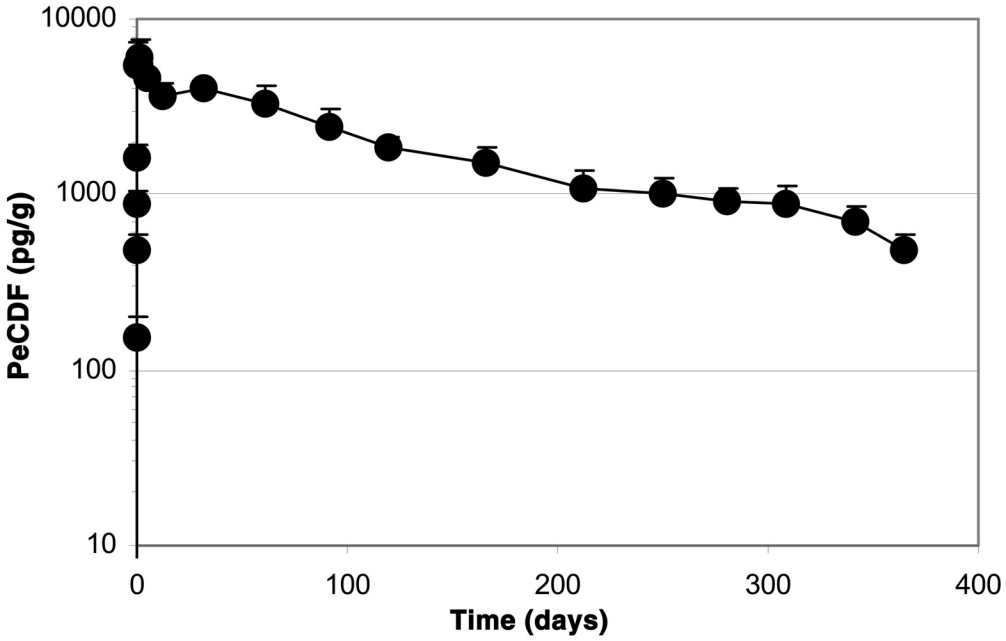

FIGURE F2
Liver Concentrations of PeCDF in Female Sprague-Dawley Rats after a Single Gavage Dose of 200 ng/kg PeCDF

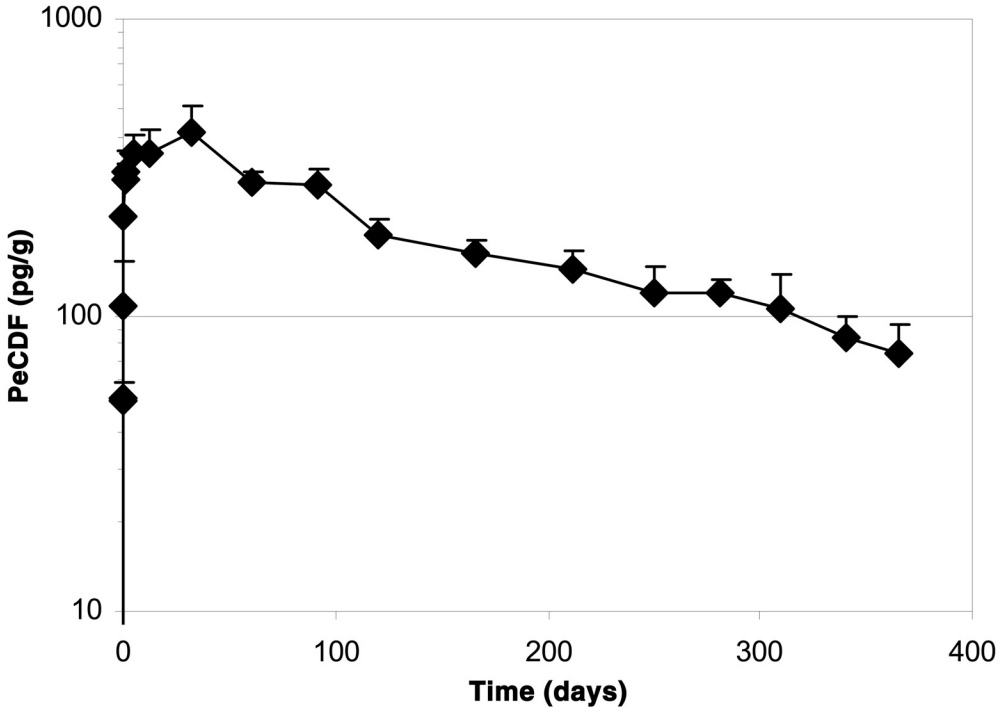

FIGURE F3
Fat Concentrations of PeCDF in Female Sprague-Dawley Rats after a Single Gavage Dose of 200 ng/kg PeCDF

APPENDIX G
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A goal for the physiologically based pharmacokinetic (PBPK) modeling of the disposition data from the dioxin toxic equivalency factor (TEF) evaluation 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 model are the same or can be deduced for related compounds.

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 and 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.

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

MODEL DEVELOPMENT

For the current dioxin TEF evaluation model the same basic model structure was used for all compounds studied, with some of the model parameters, such as metabolism or binding to the AhR, unique to each model. The model 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 and the evolution of these and other PBPK models of TCDD can be found elsewhere (USEPA, 2000c).

Kohn's model for TCDD includes compartments for fat, liver, kidney, gastrointestinal tract, muscle, and viscera. Blood is distributed among arterial, venous, and tissue capillary spaces. The model also includes equations for the liver amounts of AhR, CYP1A1, CYP1A2, and CYP1B1, as well as equations describing basal expression, induction by TCDD, and degradation of the mRNA for each of these. The amount of each enzyme depends on the time-lagged concentration of the corresponding mRNA. TCDD in the liver may bind to CYP1A2 and AhR. A key to the model is that the induction rates for all four represented mRNAs depend on the time-lagged concentration of AhR bound to TCDD. Induction increases from zero to a maximum rate as the concentration of AhR-TCDD increases. Since transthyretin (also known as prealbumin) can bind hydroxylated polychlorinated dibenzodioxins and single doses of TCDD can cause a prolonged decrease in this protein, a dose-dependent decrease was included in the model. This bound TCDD cannot enter the tissues in the model and may become free in the blood by dissociation or proteolysis. To allow the model 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 three main steps to building a PBPK model for the dioxin TEF evaluation studies; conversion of Kohn's model, addition of a lung compartment, and addition of the study-specific body weights. A copy of Kohn's model coded in the ScoP simulation was converted to both a Matlab model and a Simulink model. Simulations from the three models were compared to each other to confirm that the conversion to Matlab/Simulink was accurate. Next, a lung compartment was added to the Simulink model because the NTP data for the TEF studies includes lung tissue concentrations. The lung compartment is diffusion-limited and includes the same equations as those used in the liver for AhR, CYP1A1, and CYP1B1. The lung and liver compartments use the same gene expression parameters on a per liter basis. The final step was to include the rat body weights from each study rather than the body weight function from Kohn's model. Body weights were available weekly for the first 12 weeks of the studies and then monthly for the remainder of the studies. Interpolation of the mean body weights was used to estimate the body weight as a function of time.

Most of the parameters from Kohn's model (Kohn *et. al.*, 2001) were used in the new model. The only new parameters were the lung partition coefficient and the lung permeability factor. Values of the lung partition coefficient and the lung permeability factor parameters were estimated by optimization, fitting the model predictions to the tissue data (liver, lung, fat, blood).

Many of the parameters in Kohn's model are specific for TCDD. The parameters to change for a PeCDF-specific model relate to tissue partition coefficients, diffusion of PeCDF, binding, metabolism, and CYP1A2 induction. Kohn estimated the partition coefficients and diffusion parameters by fitting data, so all of these parameters were estimated for PeCDF. Two metabolism parameters in the Hill equation ($V_{metabolism}$ and $K_{metabolism}$) were estimated. The model has a single parameter, $k_{binding}$, for binding of PeCDF to the AhR, CYP1A2, or protein in the blood. There are different parameters for the dissociation from the AhR, CYP1A2, or protein in the blood, K_{AhR}, $K_{CYP1A2\ PeCDF}$, and $Kd_{protein}$, respectively. All four of these binding parameters were estimated from the PeCDF data. The last group of parameters that were estimated for PeCDF relate to the induction of the AhR and CYP1A2. The values of 21 parameters were estimated by optimization, fitting the model predictions to the liver, lung, fat, and blood data. All other model parameters are from Kohn's model (Kohn *et al*., 2001).

Results and Discussion

The model fits the data across the dose range and in all tissues. Diffusion parameters are in the same range for PeCDF as TCDD with values between 0.05 and 1.6. One difference is the fat permeability with a value of 0.02 for TCDD and 0.8 for PeCDF (data not shown). The partition coefficients are not very different between PeCDF and TCDD. The major differences between PeCDF and TCDD are in the binding parameters. The first difference is for the binding of PeCDF to the AhR, CYP1A2, or protein in the blood ($k_{binding}$). The estimate of $k_{binding}$ is 1,000 nmole per day for TCDD and 76.6 nmole per day for PeCDF, so there is much less binding for PeCDF. A couple of the dissociation parameters are also very different for TCDD and PeCDF, but one is nearly the same. The estimates of dissociation from blood protein ($Kd_{protein}$) are 10.97 nM for PeCDF and 10.0 nM for TCDD. Parameters for dissociation of PeCDF from the AhR and CYP1A2 are 0.001 nM and 0.95 nM, respectively. These parameters are 0.27 nM and 30.0 nM in the TCDD model. The much smaller values for PeCDF imply that PeCDF stays bound to the AhR and CYP1A2. The parameter estimate for CYP1A2 induction is almost four times higher for PeCDF than for TCDD, 105.9 and 28.4 nmole/L per day, respectively. The maximum metabolism rate is higher for PeCDF (23.5 nmole/L per day) than for TCDD (9.12 nmole/L per day) and the metabolism saturates at a higher level for PeCDF (6.0 nM) than for TCDD (0.97 nM). Identifying unique and optimal parameters may be an issue when fitting a model with 21 parameters. The parameters for PeCDF are in feasible ranges and the model fits across the dose levels. Additional study, particularly binding studies, would aid the verification of the parameters.

TABLE G1
Parameter Estimates for the PeCDF Model

Parameter	Model Value	Unit
$k_{binding}$	76.6	/nmole per day
$Kd_{protein}$	10.97	nM
K_{AhR}	0.0010	nM
$K_{CYP1A2\ PeCDF}$	0.95	nM
$V_{CYP1A2\ induction}$	105.9	nmole/L per day
Background	0.09	ng/kg per day
Fat diffusion	0.8	—
Muscle diffusion	0.05	—
Viscera diffusion	0.66	—
Liver diffusion	1.62	—
Kidney diffusion	0.48	—
Gastrointestinal tract diffusion	0.62	—
Lung diffusion	0.6	—
$V_{metabolism}$	23.5	nmole/L per day
$K_{metabolism}$	6.0	nM
Fat partition	162.5	—
Muscle partition	9.0	—
Viscera partition	8.0	—
Liver partition	5.2	—
Kidney partition	5.2	—
Gastrointestinal tract partition	8.7	—

TABLE G2
Fixed Parameters from the TCDD Model

Parameter	Model Value	Unit
lt (lag time for induced expression)	0.2	day
Cardiac output	14.7	L/hr per $kg^{0.7}$
$V_{protein}$ (blood binding protein)	300	nmole/L per day
$Ki_{protein}$ (inhibition of blood protein production)	0.0006	nM
$n_{metabolism}$	1.12	–
$k_{subchronic\ absorption}$	0.65	$kg^{0.75}$/day
$k_{absorption}$	4.8	$kg^{0.75}$/day
$k_{binding}$	1,000	/nmole per day
$k_{Ah\ degradation}$	2.16	/day
$k_{Ah\ TCDD\ degradation}$	5.15	/day
$k_{proteolysis}$	0.2727	/day
$k_{deadenylation}$	576	nt/day
$k_{mRNA\ degradation}$	13.4	/day
k_{urine}	5.36	/day
k_{bile}	3.81	/day
k_{feces}	1.152	/day
k_{lysis}	200	/day
$critical_{accumulation}$	0.6	nmole
$k_{recovery}$	0.01	/day
$critical_{concentration}$	2	nM

TABLE G3
Gene Expression Parameters Given in the Kohn *et al.* (2001) Model

	Aryl Hydrocarbon Receptor	CYP1A1	CYP1A2	CYP1B1
Expression nmole/L per day	0.0177	0.023	0.42	0.00001
Induction *(V)* nmole/L per day	0.08[a]	13.5	55.0[a]	3.92
Induction *(K)* nM	8.3[a]	2.04	4.41	13
Induction *(N)* nt	128	156	196	74
Induction *(Ka)* nM	NA	1.16	NA	10.0
Synthesis *(V)* nmole/L per day	3,000	3,000	2,400	520
$K_{ribosome}$ nM	2.68	NA	NA	NA

[a] From optimization

TABLE G4
Physiological Parameters

	Fraction of Body Weight	Fraction Capillary	Fraction of Cardiac Output
Liver	0.0373	0.138	0.039
Fat	0.07	0.02	0.065
Muscle	0.542	0.02	0.334
Viscera	0.163	0.075	0.248
Kidney	0.0148	0.16	0.133
Gastrointestinal tract	0.075	0.0265	0.181
Lung	0.005	0.36	–
Arterial	0.0044	–	–
Venous	0.0132	–	–
Liver (hepatic artery only)			

TABLE G5
Initial Conditions for Protein in Blood[a]

Arterial	0.539
Venous	1.616
Gastrointestinal tract	0.0755
Fat	0.283
Muscle	0.741
Viscera	0.413
Liver	0.328
Kidney	0.0778

[a] Conditions are measured in nmoles.

TABLE G6
Initial Conditions for Cytochrome P450s and the Aryl Hydrocarbon Receptor (AhR)[a]

AhRmRNApA	1.8×10^{-5}
AhRmRNA	1.9×10^{-5}
AhR	0.0241
CYP1A1mRNApA	7.59×10^{-5}
CYP1A1mRNA	1.1×10^{-5}
CYP1A1	1.696
CYP1A2mRNApA	1.69×10^{-3}
CYP1A2mRNA	4.72×10^{-4}
CYP1A2	4.527
CYP1B1mRNApA	2.026×10^{-7}
CYP1B1mRNA	1.59×10^{-10}
CYP1B1	0.00242

[a] Conditions are measured in nmoles.

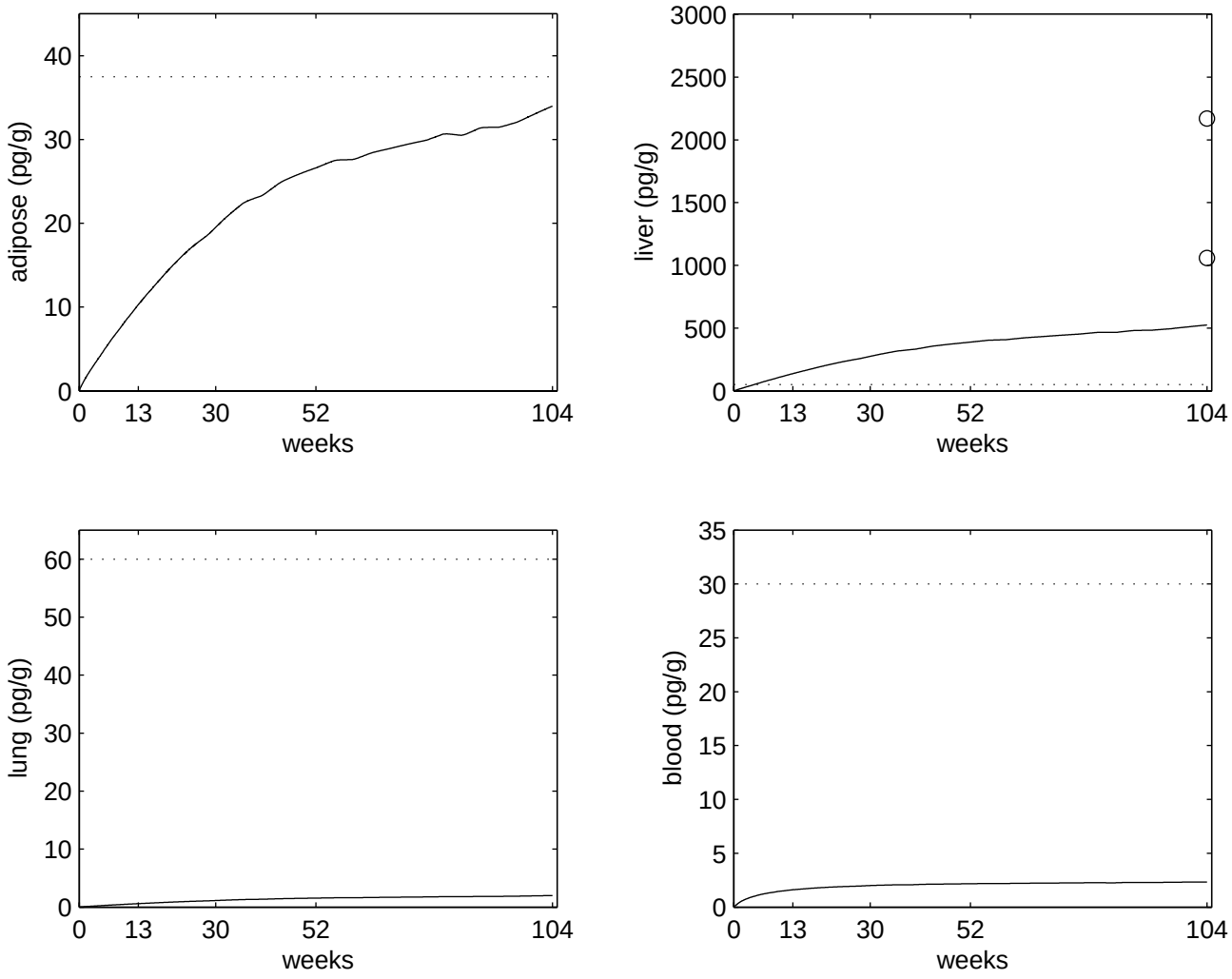

FIGURE G1
Model Predictions (–) and Individual Tissue Data (○) for the Vehicle Control Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

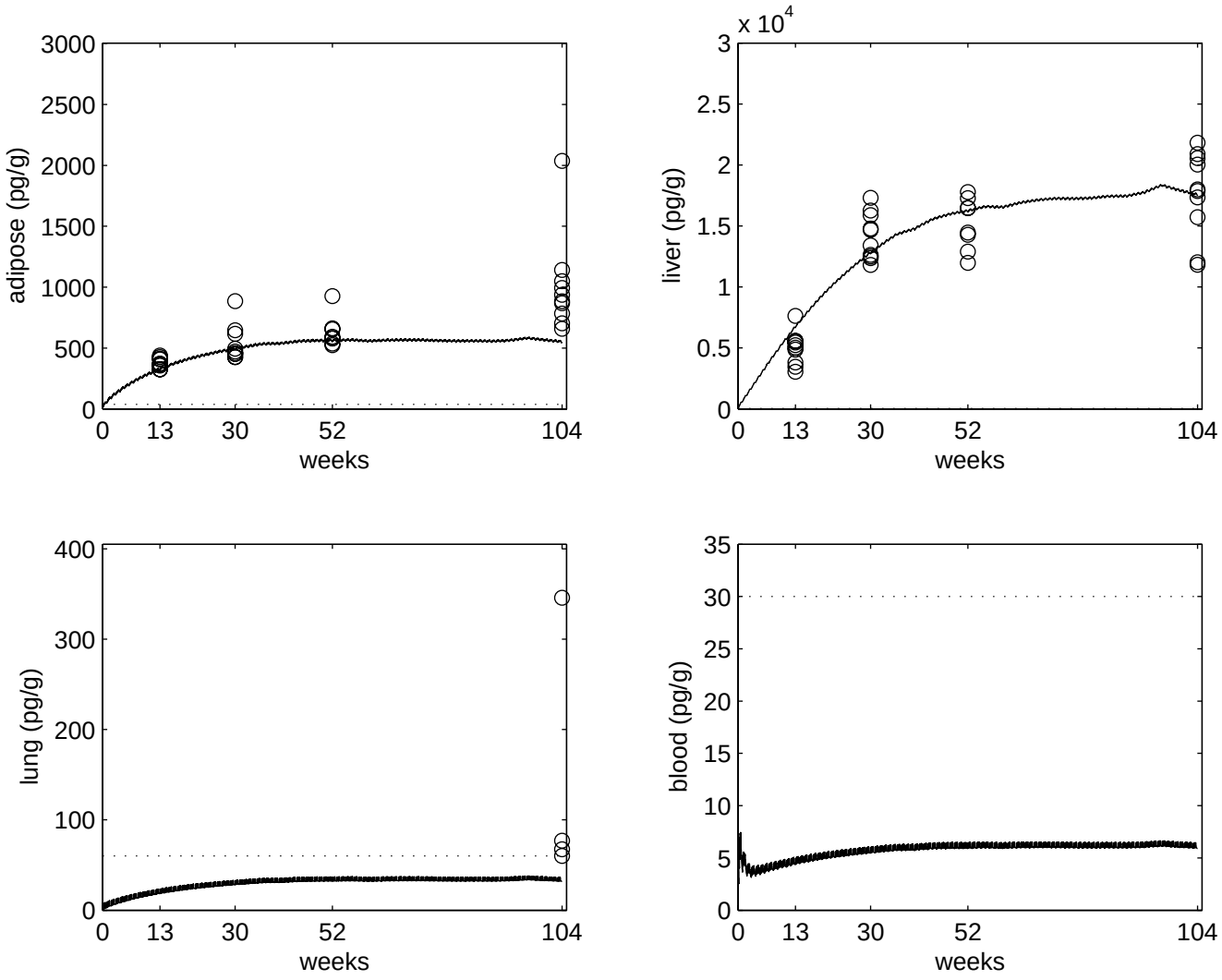

FIGURE G2
Model Predictions (–) and Individual Tissue Data (○) for the 6 ng/kg Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

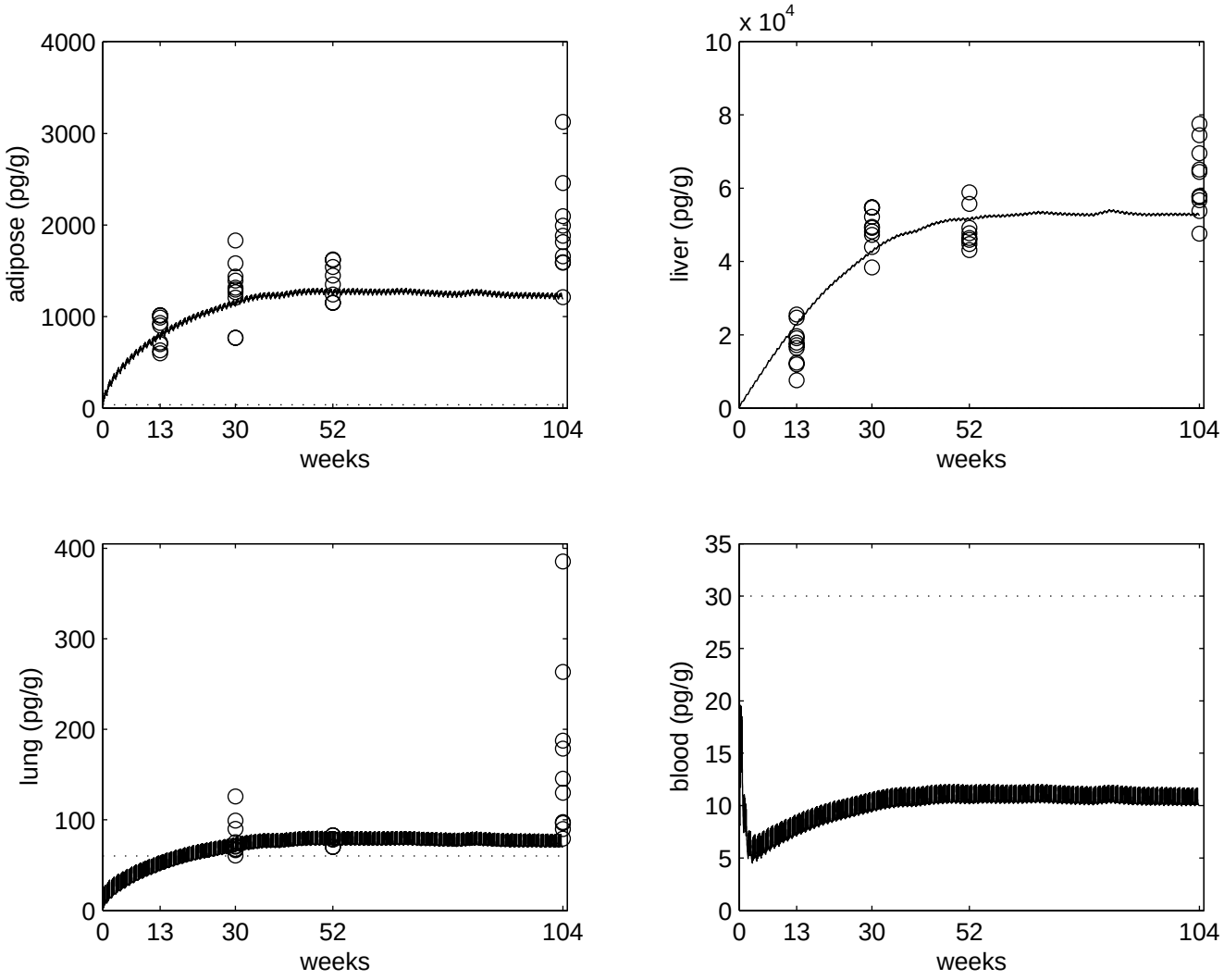

FIGURE G3
Model Predictions (–) and Individual Tissue Data (○) for the 20 ng/kg Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

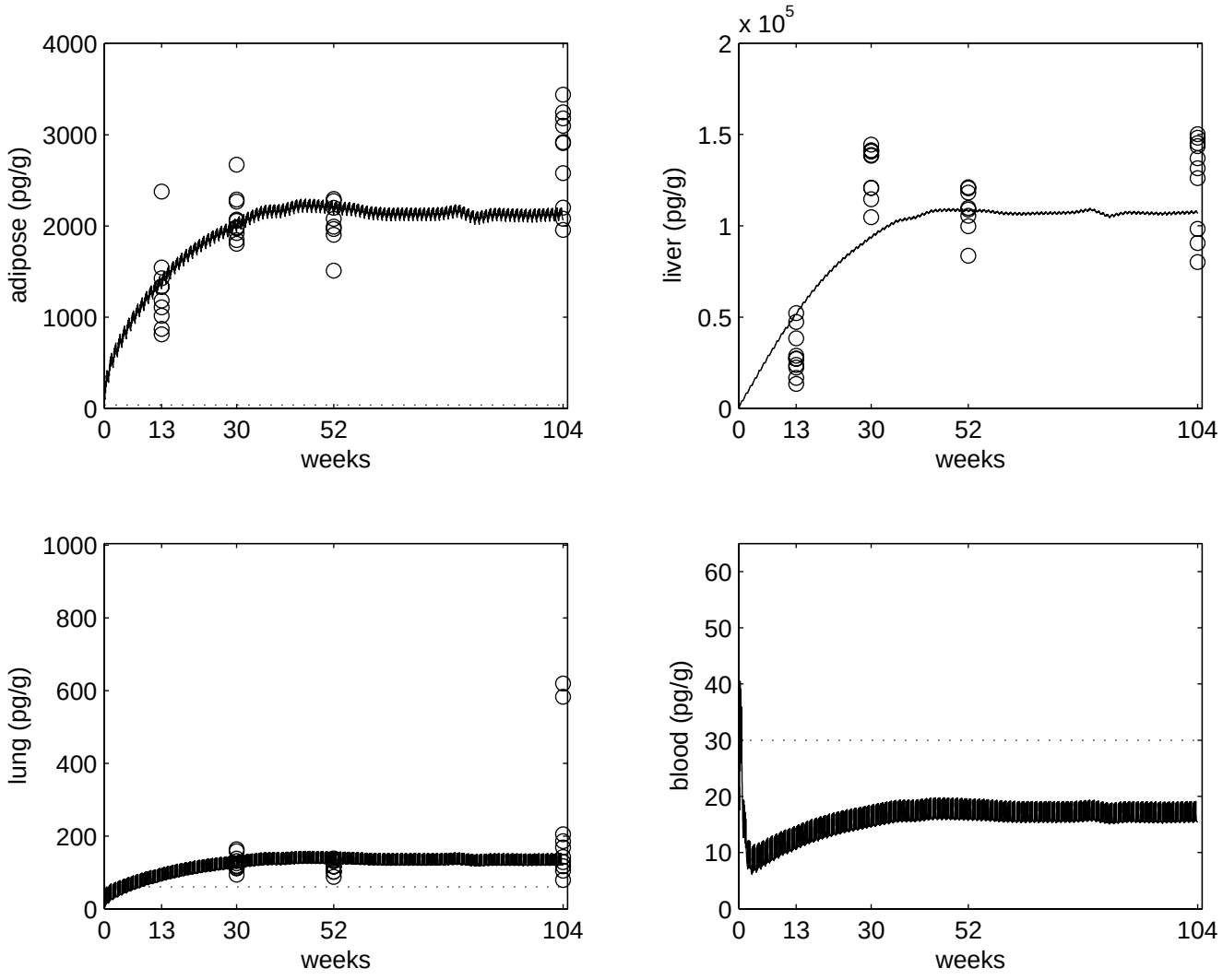

FIGURE G4
Model Predictions (–) and Individual Tissue Data (○) for the 44 ng/kg Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

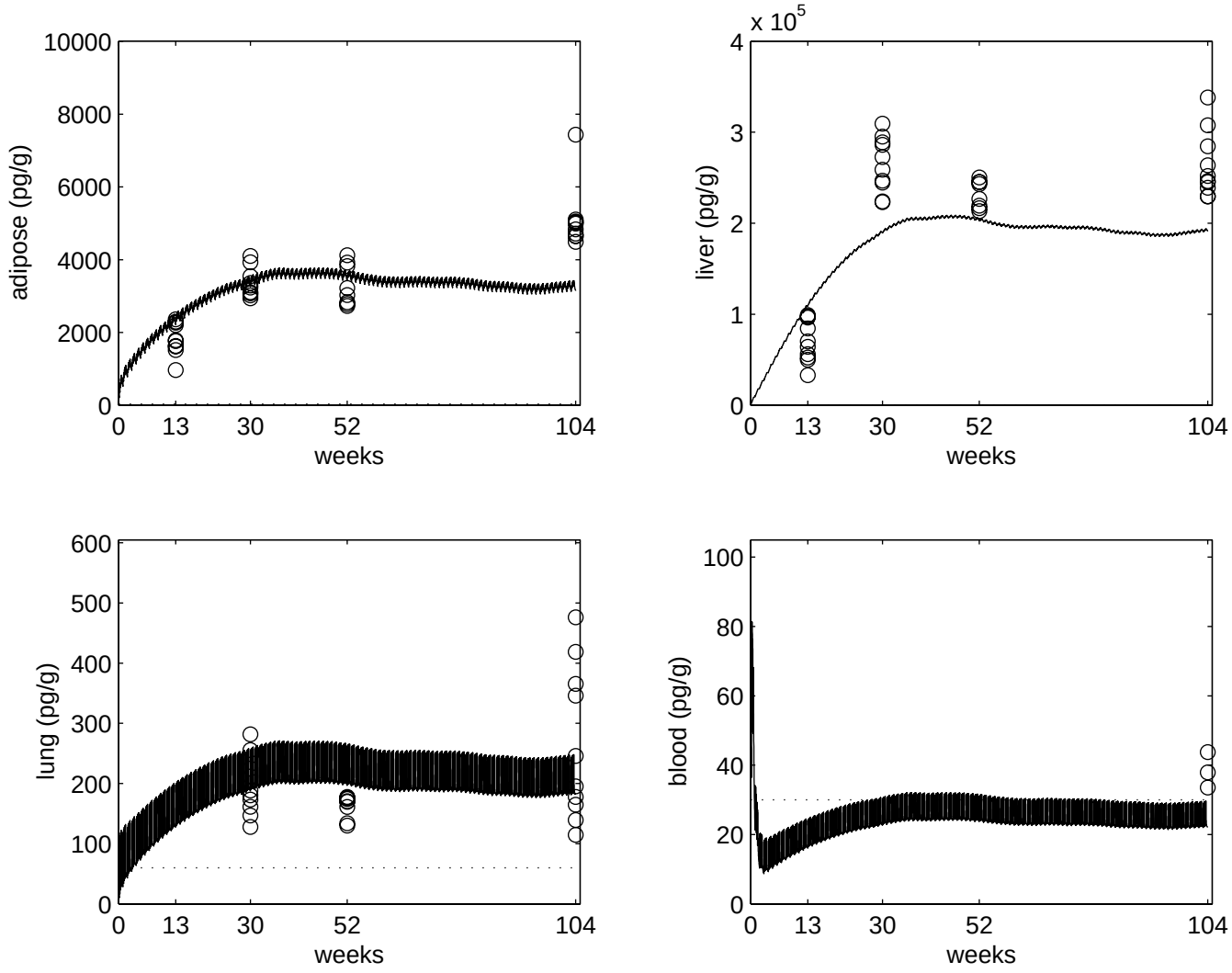

FIGURE G5
Model Predictions (–) and Individual Tissue Data (○) for the 92 ng/kg Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

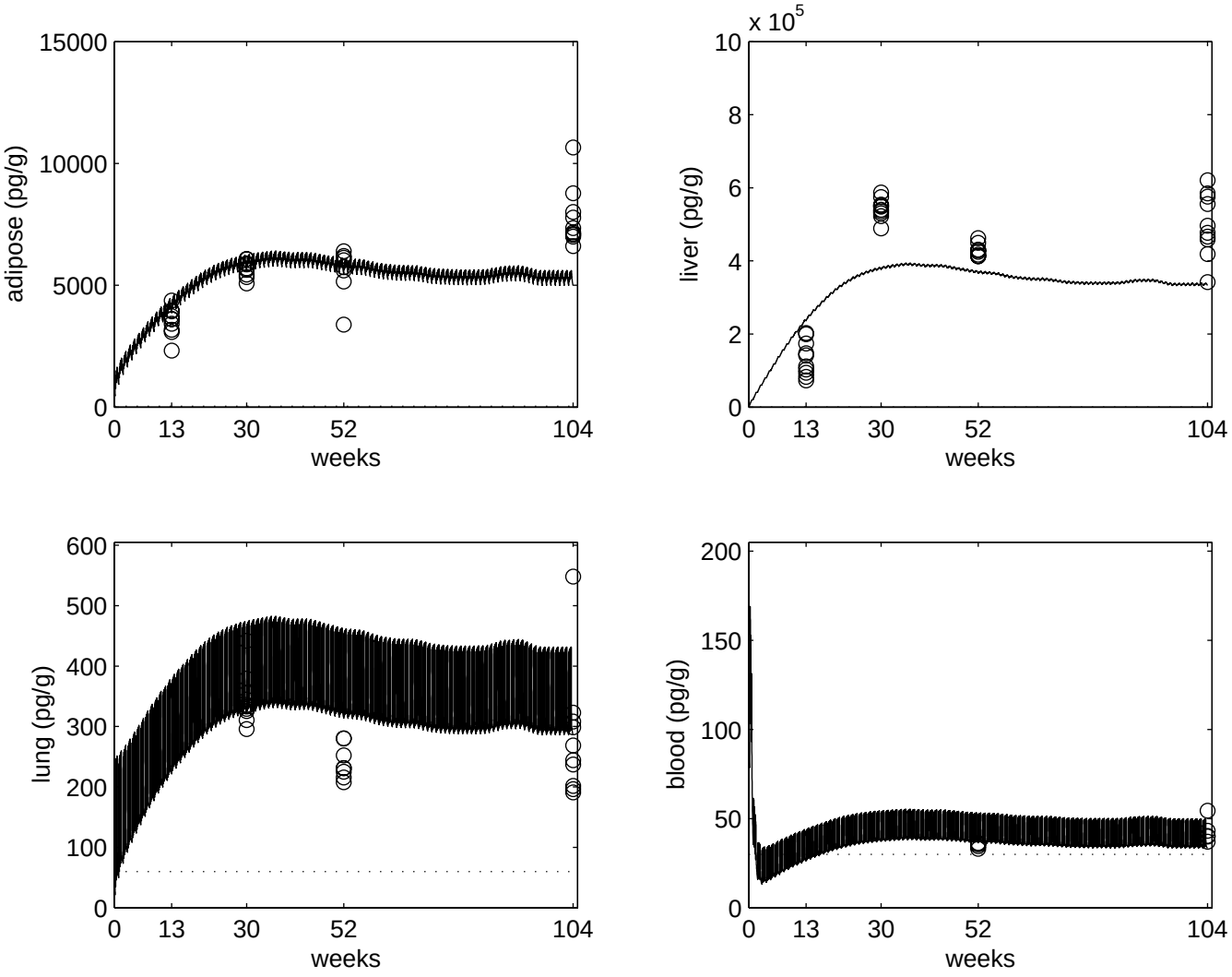

FIGURE G6
Model Predictions (–) and Individual Tissue Data (○) for the 200 ng/kg Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

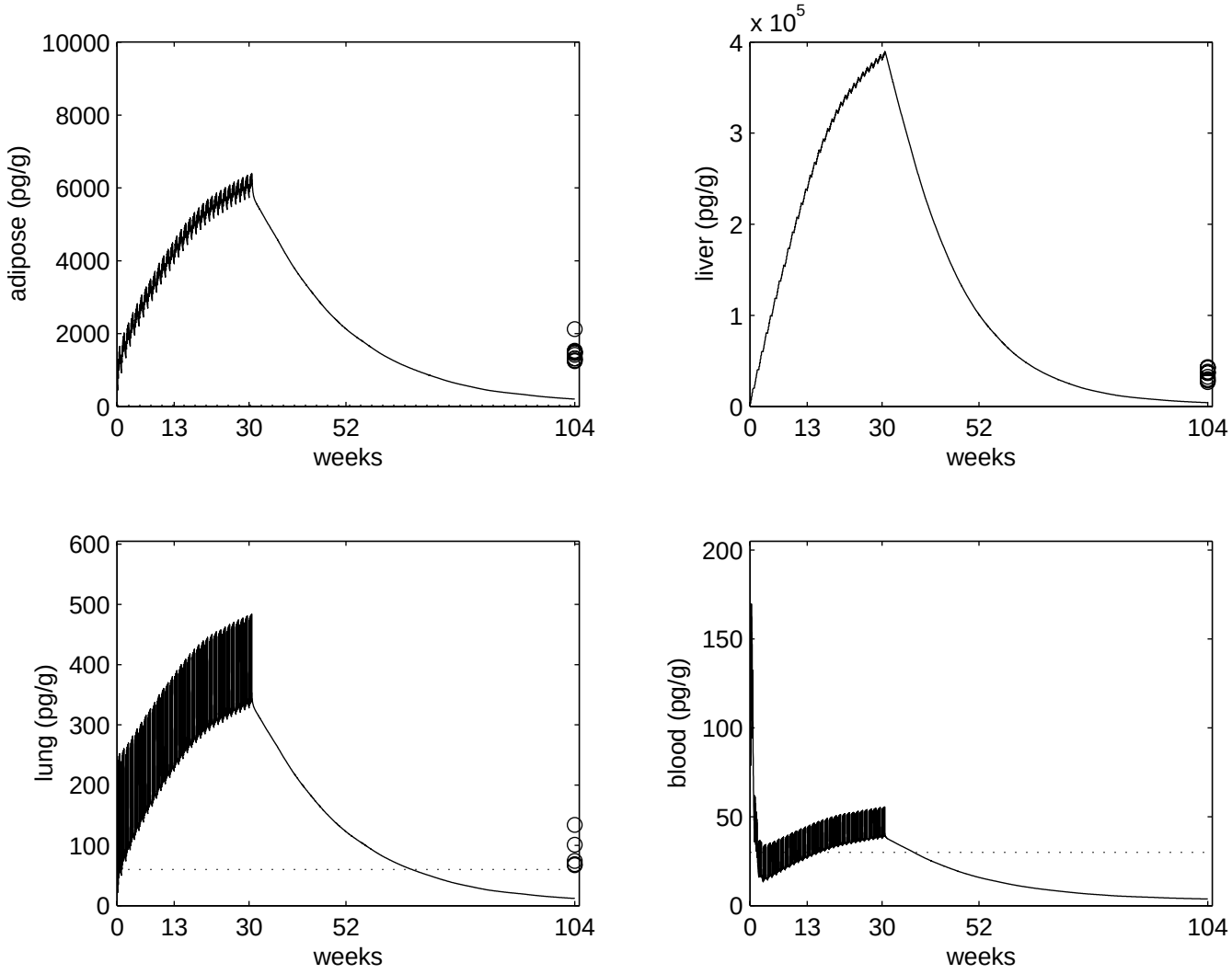

FIGURE G7
Model Predictions (–) and Individual Tissue Data (○) for the 200 ng/kg Stop-Exposure Group in the 2-Year Study
The limit of detection is labeled with a dotted line.

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.