NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF A MIXTURE OF

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) (CAS NO. 1746-01-6), 2,3,4,7,8-PENTACHLORODIBENZOFURAN (PeCDF) (CAS NO. 57117-31-4), AND 3,3′,4,4′,5-PENTACHLOROBIPHENYL (PCB 126) (CAS NO. 57465-28-8)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)

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

September 2006

NTP TR 526

NIH Publication No. 06-4462

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

FOREWORD

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

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

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

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SUMMARY

Background
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) are members of a class of chemicals containing chlorine and related in structure to dioxins. Some dioxins or dioxin-like compounds are highly toxic and cause cancer, 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 a mixture of TCDD, PeCDF, and PCB 126 on female rats.

Methods
Mixtures of the three chemicals were prepared in the ratio of one part TCDD, two parts PeCDF, and ten parts PCB 126. These were estimated to give approximately equal toxic contributions from each chemical. We exposed groups of 53 female rats by depositing the mixtures dissolved in corn oil through a tube directly into their stomachs five days a week for two years. Animals receiving corn oil alone served as the control group. Tissues from more than 40 sites were examined for every animal.

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

Conclusions
We conclude that a mixture of TCDD, PeCDF, and PCB 126 caused cancer and other toxic effects at several sites in female rats.

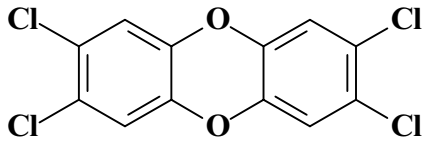
ABSTRACT

Dioxin Toxic Equivalency Factor Evaluation Overview

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

Since human exposure to DLCs always 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.

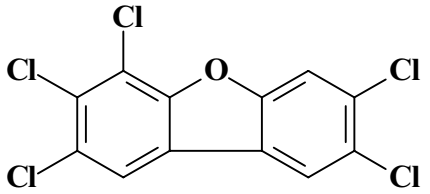


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

CAS No. 1746-01-6

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

Synonyms: Dioxin; dioxine; TCDBD; 2,3,7,8-TCDD; 2,3,7,8-tetrachlorodibenzo(b,e)(1,4)dioxin; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2,3,6,7-tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-tetrachlorodibenzo-1,4-dioxin; tetrachlorodibenzodioxin; 2,3,6,7-tetrachlorodibenzoidioxin; tetradioxin

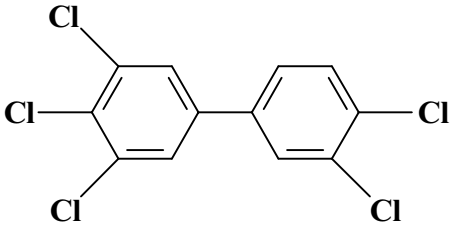


2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)

CAS No. 57117-31-4

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

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



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

CAS No. 57465-28-8

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

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

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) are not manufactured commercially other than for scientific research purposes. The main sources of TCDD and PeCDF releases into the environment are from metal smelting, refining, and processing; combustion and incineration sources; chemical manufacturing and processing; biological and photochemical processes; and existing reservoir sources that reflect past releases. 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. TCDD, PeCDF, and PCB 126 were selected for study by the National Toxicology Program as 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. Female Harlan Sprague-Dawley rats were administered a mixture of TCDD, PeCDF, and PCB 126 (henceforth referred to as the TEF mixture) in corn oil:acetone (99:1) by gavage for 14, 31, or 53 weeks or 2 years. 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 study of the mixture of TCDD, PeCDF, and PCB 126 are presented and discussed.

2-Year Study

Groups of 81 female rats were administered 10, 22, 46, or 100 ng toxic equivalents (TEQ)/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. Actual doses used for each compound in the mixture were: for 10 ng TEQ/kg: 3.3 ng/kg TCDD, 6.6 ng/kg PeCDF, and 33.3 ng/kg PCB 126; for 22 ng TEQ/kg: 7.3 ng/kg TCDD, 14.5 ng/kg PeCDF, and 73.3 ng/kg PCB 126; for 46 ng TEQ/kg: 15.2 ng/kg TCDD, 30.4 ng/kg PeCDF, and 153 ng/kg PCB 126; and for 100 ng TEQ/kg: 33 ng/kg TCDD, 66 ng/kg PeCDF, and 333 ng/kg PCB 126. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. Survival of all dosed groups of rats was similar to that of the vehicle control group. Mean body weights of the 22 and 46 ng TEQ/kg groups were less than those of the vehicle control groups after week 69 of the study. Mean body weights of the 100 ng TEQ/kg group were less than those of the vehicle control group after week 37 of the study.

Thyroid Hormone Concentrations

Alterations in serum thyroid hormone concentrations were evaluated at the 14-, 31-, and 53-week interim evaluations. At 14, 31, and 53 weeks, there were dose-dependent reductions in total serum and free thyroxine concentrations. There were dose-dependent increases in serum triiodothyronine concentrations at 14 and 31 weeks. No changes in serum thyroid stimulating hormone concentrations were observed at any time point.

Hepatic Cell Proliferation Data

To evaluate hepatocyte replication, analysis of labeling of replicating hepatocytes with 5-bromo-2′-deoxyuridine was conducted at the interim evaluations. At 14 weeks, no effects on the hepatocellular labeling index were observed in the dosed groups compared to the vehicle controls. At 31 and 53 weeks, the hepatocellular labeling index was significantly higher in the 46 and 100 ng TEQ/kg groups compared to the vehicle controls.

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 interim time points. Liver and lung EROD (CYP1A1) activities and hepatic A4H (CYP1A2) activities were significantly greater in all dosed groups than in the vehicle controls at all interim evaluations (14, 31, and 53 weeks).

Determinations of TCDD, PeCDF, and PCB 126 Concentrations in Tissues

Tissue concentrations of TCDD, PeCDF, and PCB 126 were analyzed in the fat, liver, lung, and blood at each interim evaluation and at the end of the 2-year study (105 weeks). The highest concentrations of TCDD, PeCDF, and PCB 126 were observed in the liver followed by fat. Liver and fat concentrations of TCDD, PeCDF, and PCB 126 at each interim evaluation and at 105 weeks were higher in groups with increasing doses of the mixture and generally increased with duration of dosing. In the lung, PeCDF was present at detectable concentrations in the 46 and 100 ng TEQ/kg groups at

14 and 31 weeks. Measurable concentrations of TCDD and PCB 126 were observed at 14 and 31 weeks in the lung of rats in all dosed groups with the highest concentrations observed in the 100 ng TEQ/kg group. At 53 weeks, concentrations of TCDD, PeCDF, and PCB 126 in the lung generally increased with increasing dose. At 105 weeks, detectable concentrations of TCDD, PeCDF, and PCB 126 in the lung were observed in all dosed groups. In blood, TCDD and PCB 126 concentrations at 14 and 31 weeks generally increased with increasing dose. Blood concentrations of PeCDF were detectable in the 46 and 100 ng TEQ/kg groups at 14 weeks and at 22 ng TEQ/kg or greater at 31 weeks. At 53 and 105 weeks, concentrations of TCDD, PeCDF, and PCB 126 in blood generally increased with increasing dose and duration of dosing.

Pathology and Statistical Analyses

Relative liver weights were significantly increased in all dosed groups at 14, 31, and 53 weeks and correlated with increased incidences of hepatocellular hypertrophy. Increasing duration of exposure led to an increase in the spectrum, incidence, and severity of nonneoplastic effects. The only significant effect at 14 weeks was increased incidences of hepatocellular hypertrophy. At 53 weeks, there was a significant effect on the incidences of hepatocellular hypertrophy, multinucleated hepatocytes, pigmentation, focal fatty change, bile duct hyperplasia, and toxic hepatopathy.

At 2 years, there were significant increases in the incidences of hepatocellular adenoma and cholangiocarcinoma of the liver. There was an increase in hepatic toxicity characterized by increases in the incidences of numerous nonneoplastic lesions including hepatocyte hypertrophy, multinucleated hepatocytes, pigmentation, inflammation, diffuse fatty change, bile duct hyperplasia, oval cell hyperplasia, nodular hyperplasia, eosinophilic focus, cholangiofibrosis, bile duct cysts, necrosis, portal fibrosis, mixed cell focus, and toxic hepatopathy.

In the lung, there were dose-dependent increases in the incidences of bronchiolar metaplasia of the alveolar epithelium at 53 weeks and at 2 years and squamous metaplasia at 2 years. At 2 years, there was a dose-dependent increase in the incidences of cystic keratinizing epithelioma.

In the pancreas, there were increases in the incidences of numerous nonneoplastic lesions including arterial chronic active inflammation, acinar cytoplasmic vacuolization, acinar atrophy, chronic active inflammation, and duct dilatation. At 2 years, incidences of acinar adenoma or acinar carcinoma that exceeded the historical control ranges were seen in all dosed groups except the 100 ng TEQ/kg group.

Treatment-related increases in the incidences of nonneoplastic lesions were seen in other organs including hyperplasia, cystic degeneration, atrophy, and cytoplasmic vacuolization of the adrenal cortex; gingival squamous hyperplasia of the oral mucosa; squamous metaplasia of the uterus; atrophy of the thymus (incidence and severity); chronic active inflammation of the ovary; nephropathy of the kidney (incidence and severity); cardiomyopathy; bone marrow hyperplasia; transitional epithelium of the urinary bladder; chronic active inflammation of the mesenteric artery; and follicular cell hypertrophy of the thyroid gland.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of the mixture of TCDD, PeCDF, and PCB 126 in female Harlan Sprague-Dawley rats based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and cystic keratinizing epithelioma of the lung. Neoplasms of the pancreatic acinus may have been related to administration of the mixture of TCDD, PeCDF, and PCB 126.

Administration of the mixture of TCDD, PeCDF, and PCB 126 caused increased incidences of nonneoplastic lesions of the liver, lung, pancreas, adrenal cortex, oral mucosa, uterus, thymus, ovary, kidney, heart, bone marrow, urinary bladder, mesenteric artery, and thyroid gland 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 the TEF Mixture in Female Sprague-Dawley Rats

Doses in corn oil/acetone by gavage
0 ng TEQ/kg
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)

Body weights
22, 46, and 100 ng TEQ/kg groups less than the vehicle control group

Survival rates
16/53, 23/53, 24/53, 23/53, 8/53

Nonneoplastic effects
Liver:
hepatocyte hypertrophy (1/53, 27/53, 34/53, 46/53, 50/51)
multinucleated hepatocyte (0/53, 12/53, 10/53, 39/53, 51/51)
pigmentation (4/53, 35/53, 41/53, 48/53, 51/51)
inflammation (36/53, 50/53, 45/53, 50/53, 50/51)
diffuse fatty change (3/53, 5/53, 14/53, 34/53, 36/51)
bile duct hyperplasia (2/53, 3/53, 5/53, 25/53, 42/51)
oval cell hyperplasia (0/53, 1/53, 1/53, 26/53, 42/51)
nodular hyperplasia (0/53, 1/53, 3/53, 11/53, 38/51)
eosinophilic focus (5/53, 9/53, 11/53, 20/53, 19/51)
cholangiofibrosis (0/53, 2/53, 3/53, 4/53, 17/51)
bile duct cyst (1/53, 3/53, 3/53, 4/53, 9/51)
necrosis (3/53, 1/53, 9/53, 3/53, 15/51)
portal fibrosis (0/53, 0/53, 0/53, 0/53, 11/51)
mixed cell focus (21/53, 32/53, 35/53, 36/53, 17/51)
toxic hepatopathy (0/53, 5/53, 14/53, 38/53, 47/51)
Lung:
alveolar epithelium, metaplasia, bronchiolar (0/53, 20/53, 33/53, 41/53, 40/53)
squamous metaplasia (2/53, 0/53, 2/53, 8/53, 11/53)
Pancreas:
arterial chronic active inflammation (0/52, 6/53, 3/53, 8/53, 14/51)
acinar cytoplasmic vacuolization (1/52, 0/53, 3/53, 15/53, 30/51)
acinar atrophy (3/52, 2/53, 7/53, 7/53, 20/51)
chronic active inflammation (3/52, 1/53, 6/53, 7/53, 16/51)
duct dilatation (0/52, 0/53, 0/53, 0/53, 5/51)
Adrenal Cortex:
hyperplasia (12/52, 26/53, 23/53, 25/53, 21/51)
cystic degeneration (9/52, 15/53, 19/53, 25/53, 16/51)
atrophy (0/52, 3/53, 0/53, 0/53, 18/51)
cytoplasmic vacuolization (6/52, 13/53, 11/53, 7/53, 15/51)
Oral Mucosa:
gingival squamous hyperplasia (8/53, 17/53, 18/53, 26/53, 30/53)
Uterus:
squamous metaplasia (21/52, 32/53, 32/53, 35/53, 30/51)
Thymus:
atrophy (32/52, 43/48, 45/50, 50/53, 48/50)
severity of atrophy (2.3, 2.9, 3.3, 3.7, 3.9)
Ovary:
chronic active inflammation (0/52, 2/52, 1/53, 2/53, 5/51)
Kidney:
nephropathy (26/52, 41/53, 40/53, 47/53, 49/51)
severity of nephropathy (1.1, 1.2, 1.3, 1.3, 2.1)
Heart:
cardiomyopathy (11/53, 26/53, 31/53, 30/52, 32/53)

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

Nonneoplastic effects (continued)
Bone Marrow:
hyperplasia (36/53, 36/53, 34/53, 41/53, 48/53)
Urinary Bladder:
transitional epithelial hyperplasia (0/52, 0/52, 1/53, 3/53, 4/50)
Mesentery:
arterial chronic active inflammation (0/52, 1/53, 0/53, 3/53, 6/51)
Thyroid Gland:
follicular cell hypertrophy (4/53, 13/53, 12/51, 18/52, 23/51)

Neoplastic effects
Liver:
hepatocellular adenoma (0/53, 1/53, 1/53, 1/53, 11/51)
cholangiocarcinoma (0/53, 0/53, 2/53, 7/53, 9/51)
Lung:
cystic keratinizing epithelioma (0/53, 0/53, 0/53, 2/53, 20/53)

Equivocal findings
Pancreas:
acinus adenoma or carcinoma (0/52, 1/53, 2/53, 2/53, 0/51)

Level of evidence of carcinogenic activity
Clear evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on a mixture of TCDD, PeCDF, and PCB 126 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 study of a mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. N.J. Walker, NIEHS, presented the background, design, and goals of the NTP study series on the toxic equivalency factor (TEF) evaluations of mixtures of dioxin-like compounds (dioxins, PCBs, furans). 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 the mixture of TCDD, PeCDF, and PCB 126 by noting that the primary purpose of the study was to test dose additivity in toxic response. He also noted that these three chemicals together contribute 40% of the total dioxin toxic equivalence to which humans are exposed. Dr. Walker described the study design and the responses in the liver, lung, adrenal cortex, and pancreas and a variety of nonneoplastic effects. The proposed conclusion was *clear evidence of carcinogenic activity* of a mixture of TCDD, PeCDF, and PCB 126 in female Harlan Sprague-Dawley rats.

Dr. Roberts, the first principal reviewer, felt the study was rationally designed and well conducted. Given the large number of sites affected, he suggested adding subheadings to the discussion section.

Dr. Vore, the second principal reviewer, also felt the study was well conducted and agreed with the proposed conclusion. She asked if dose additivity was expected.

Dr. Andrews, the third principal reviewer, also agreed with the proposed conclusion. He sought some discussion about the issue of using a rodent bioassay designed to test for full carcinogens to assess the promotional effects of dioxins. He also suggested some additional discussion on the timing of thyroid function in the rat, to help clarify the relative increases and decreases in thyroid hormone levels at the interim evaluations.

Dr. Walker said the fuller question of dose additivity would be explored once the entire set of TEF studies had been completed. He explained that the chemicals were tested in full cancer studies because of criticisms that earlier promotional studies were not complete. While promotion may be the major effect of the dioxins, it may not be the only mechanism operating.

Dr. C.J. Portier, NIEHS, emphasized that the program was careful to attribute promotion effects just to results of properly controlled initiation-promotion studies; otherwise, "nongenotoxic mechanism" would be a better characterization of the effect. Drs. Klaunig and Andrews agreed.

Dr. Roberts moved that the conclusion be accepted as written. Dr. Vore 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 is 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 leads 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 DLCs.

Polyhalogenated Aromatic Hydrocarbon Mixtures and Toxic Equivalency Factors

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

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

potency factors. The TEF determination is a subjective assessment because the relative potency factors are derived from the literature and there is considerable variability in the types of studies, endpoints analyzed, and quality of procedures. Types of procedures for calculation of relative potency factors vary from a comparative dose response assessment (e.g., ratio of ED_{50} or EC_{50}) to a simple administered dose ratio calculation. In evaluating different studies and endpoints, *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.

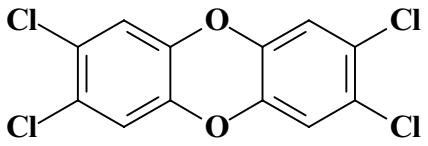
One of the implicit assumptions in the use of TEFs is that the TEQ for different compounds is dose additive. While dose additivity is supported for certain mixtures, this may not be true for some biological endpoints in some models. As outlined by Van den Berg *et al.* (1998), the TEF methodology is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

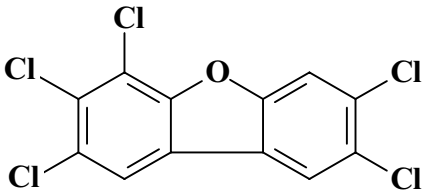


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD

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.

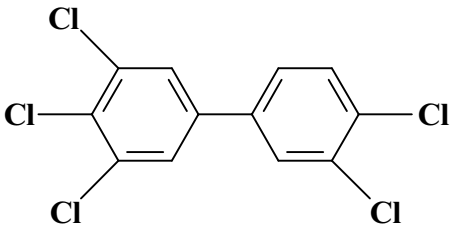


2,3,4,7,8-Pentachlorodibenzofuran
PeCDF

CAS No. 57117-31-4

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

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

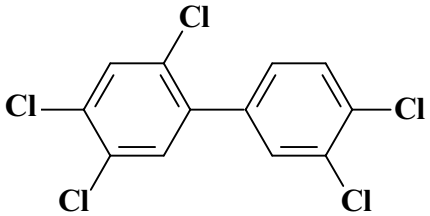


3,3′,4,4′,5-Pentachlorobiphenyl
PCB 126

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.

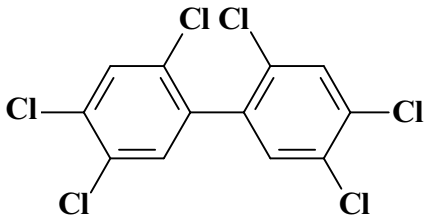


2,3′,4,4′,5-Pentachlorobiphenyl
PCB 118

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 comprised 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, to 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 100 ng/kg is at or near the predicted maximum tolerated dose.

Interim necropsies at 14, 31, and 53 weeks were incorporated into the studies for the examination of mechanistically based biomarkers of AhR- or PCB-mediated effects. These endpoints included alterations in cyto-chromes 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.

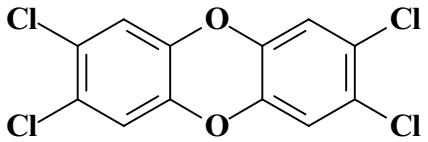
Additional "special study" animals were included at each interim necropsy. Tissues from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies. These animals were not evaluated as part of the core study.

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

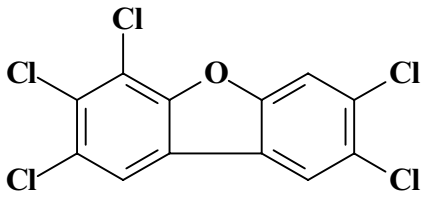


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

CAS No. 1746-01-6

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

Synonyms: Dioxin; dioxine; TCDBD; 2,3,7,8-TCDD; 2,3,7,8-tetrachlorodibenzo(b,e)(1,4)dioxin; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2,3,6,7-tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-tetrachlorodibenzo-1,4-dioxin; tetrachlorodibenzodioxin; 2,3,6,7-tetrachlorodibenzoidioxin; tetradioxin

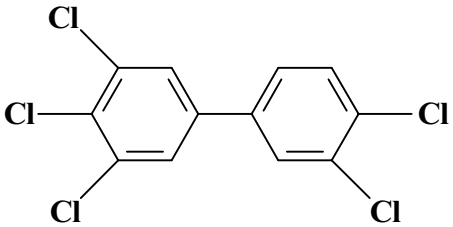


2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)

CAS No. 57117-31-4

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

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



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

CAS No. 57465-28-8

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

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

Chemical and Physical Properties

TCDD, PeCDF, and PCB 126 belong 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). NTP technical reports for TCDD, PeCDF, and PCB 126 discuss the specific chemical and physical properties of these chemicals (NTP, 2006a,b,c).

Production, Use, and Human Exposure

TCDD and PeCDF are not manufactured commercially other than for scientific research purposes. The main sources of TCDD and PeCDF releases into the environment are from combustion and incineration sources; metal smelting, refining and processing; chemical manufacturing and processing; biological and photochemical processes; and existing reservoir sources that reflect past releases (USEPA, 2000a). Based on congener-specific profiles, combustion sources produce all 2,3,7,8-substituted PCDDs and PCDFs including TCDD and PeCDF. PeCDF is the major congener emitted from cement kilns burning hazardous waste (approximately 20% of the total congener emission). PCB mixtures, including PCB 126, were commercially produced between 1929 and 1977 for the electric industry as dielectric insulating fluids for transformers and capacitors. PCBs were produced for use in hydraulic fluids, plastics, and paints. The manufacture and use of PCBs in the United States was stopped in 1977 after the increasing detection of PCB residues in the environment in the 1960s and 1970s. However, they continue to be released into the environment through the use and disposal of products containing PCBs, as by-products during the manufacture of certain organic chemicals, and during combustion of some waste materials (USEPA, 2000a).

Due to high lipophilicity and the low solubility of these compounds in aqueous media, PCDDs, PCDFs, and PCBs accumulate in the fat tissues of animals. The highest concentrations of PCDDs, PCDFs, and PCBs 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 PCDDs, PCDFs, and PCBs (Schecter *et al*., 1994a). It is estimated that 90% of human exposure to PCDDs, PCDFs, and PCBs 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 dioxin-like compounds (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 TCDD, PeCDF, and PCB 126 from food is estimated to be 5 pg/day, 6.6 pg TEQ/day, and 13 pg TEQ/day, respectively. TCDD, PeCDF, and PCB 126 combined (24.6 pg TEQ/day) represent 40% of the estimated daily intake of DLCs from food sources.

This level of exposure together with the long half-life of DLCs in humans leads to persistent levels of DLCs in human tissues (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.

The average level of PCDDs, PCDFs, and dioxin-like PCBs, combined, on a TEQ basis, in human fat tissue is 68 pg TEQ/g lipid (USEPA, 2000b). The average levels of TCDD, PeCDF, and PCB 126 in human fat tissue are 5.5 pg/g, 5.0 pg TEQ/g, and 22.4 pg TEQ/g, respectively. Consequently, TCDD, PeCDF, and PCB 126 combined (32.9 pg TEQ/g lipid) represent 48% of the total level of DLCs present in human fat tissue.

Toxicokinetics

There is an extensive body of literature examining the absorption, distribution, metabolism, and excretion of

TCDD and related compounds (USEPA, 2000c); for the purpose of brevity, only pertinent information is provided here. Several studies have examined absorption of TCDD from the gastrointestinal (GI) tract (Piper *et al*., 1973; Rose *et al*., 1976). The absorption of TCDD from the GI tract in Sprague-Dawley rats given a single dose of 1 µg TCDD/kg body weight in corn oil:acetone (25:1) is 84% (range 66% to 93%). Similar results have been observed after repeated exposure (0.1 to 1 µg/kg per day) and higher doses.

The half-life of TCDD in rats varies depending on the organ examined and the dose used. In rats, the whole body elimination half-life has been estimated to be 12 to 31 days. In fat tissue in rats, TCDD exhibits first-order kinetics and has an elimination half-life of 24.5 days. In Wistar rats, liver exhibits a biphasic elimination pattern with half-lives of 11.5 days and 16.9 days for the shorter and longer term components. In female Sprague-Dawley rats, the half-life for TCDD in the liver is also biphasic with the half-life decreasing when the concentration of TCDD rose above 1,000 ppt wet weight (Walker *et al*., 2000). When the tissue concentration is in the range of 0 to 1,000 ppt, the half-life is estimated to be 33.1 days. When the concentration increases above 1,000 ppt, the half-life drops to just 16.7 days. In female rats, the half-life of TCDD in the lung is 39.7 days (95% confidence region of 34.4 to 45.8 days) (Tritscher *et al*., 2000). In serum, the half-life is estimated to be 44.6 days (95% confidence interval of 39.1 to 50.9 days). By comparison, it has been estimated that the whole body half-life of TCDD in humans is approximately 7 to 10 years.

The oral bioavailability of PeCDF in male Fischer rats is similar to that of TCDD (Brewster and Birnbaum, 1987) with more than 70% of the dose being absorbed. In addition, absorption is independent of dose. Once absorbed, DLCs are readily distributed throughout the body. In rats, whole body elimination half-life of PeCDF has been estimated to be 64 days. In fat 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 a dihydroxy-penta-chlorobiphenyl and a 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 toxicity associated with exposure to PCDDs and PCDFs is due to the parent compound and that metabolism is a detoxification mechanism.

Once absorbed, DLCs are transported primarily through the lymphatic system by chylomicrons and are readily distributed throughout the body. The main sites of distribution in rats within the first few days after exposure are primarily to the liver and fat tissue and lesser amounts to skin and muscle. DLC levels in the rat liver are generally higher than those in fat with the ratio increasing with both time and dose. The pattern of distribution in rats is due to the lipophilicity of DLCs and their binding to cytochrome P450 1A2 (Gillner *et al*., 1987; Diliberto *et al*., 1997). CYP1A2 is a known binding protein for DLCs and is also inducible via ligands of the AhR such as DLCs. Since CYP1A2 is inducible in the liver, DLCs tend to sequester in the liver at levels that would not be predicted based on their lipophilicity alone. The hepatic sequestration of DLCs is not observed in CYP1A2 knockout mice, demonstrating the critical involvement of CYP1A2 in this process (Diliberto *et al*., 1999).

In humans, it has been estimated that the median whole body half-life of TCDD is 7 years and that 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).

Toxic Equivalency Factor

The TEFs for TCDD, PeCDF, and PCB 126 are 1.0, 0.5, and 0.1, respectively (Van den Berg *et al*., 1998). Since the TEF mixture is composed of equal TEF-adjusted mass ratios of TCDD, PeCDF, and PCB 126, it is hypothesized that the predicted potency of the TEF mixture should be equivalent to TCDD alone.

Toxicity

Toxic effects observed with exposure to PCDDs, PCDFs and coplanar PCBs (CP-PCBs) (such as PCB 126) include developmental and reproductive alterations, immunotoxicity, teratogenicity, carcinogenicity, and lethality (Poland and Knutson, 1982; Birnbaum, 1994b; ATSDR, 1998; Grassman *et al*., 1998; USEPA, 2000c). Based on the high affinity of PCB 126 and PeCDF for the AhR, much of the toxicity of these compounds is

similar to that of the most potent dioxin, TCDD; therefore qualitatively, the toxicity of a mixture of these three compounds would be expected to be similar.

Different animal species vary widely in the 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 and PCB 126 is likely to be similar. The oral LD_{50} for PeCDF in guinea pigs (the most sensitive species for TCDD-induced lethality) is 10 µg/kg (Poland and Knutson, 1982). By comparison, the LD_{50} for TCDD is 1 µg/kg. The acute lethal doses of TCDD and related potent DLCs have latency periods of 1 to 2 weeks in all species tested, during which time animals exhibit a wasting syndrome. Other characteristic effects associated with exposure are chloracne, reduced body weight and body weight gain, porphyria, cleft palate, thymic atrophy, gastric hyperplasia/hypoplasia, hepatotoxicity, increased serum concentration 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

There have been no rodent carcinogenicity studies of defined mixtures of PCDDs, PCDFs, and PCBs. While mixtures of PCBs have been shown to be carcinogenic in rats and mice (Nagasaki *et al*., 1972; Ito *et al*., 1973; Kimbrough *et al*., 1975; Mayes *et al*., 1998) there have been no individual studies on the carcinogenicity of PCB 126 alone. Similarly, 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 inferred that the carcinogenicity of PeCDF and PCB 126 would be the same as TCDD, and therefore a mixture of these compounds would also be similar to that of TCDD alone.

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 induces tumors in male and female Osborne-Mendel rats and $B6C3F_1$ mice (NTP, 1982a). In the previous NTP (1982a) 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 (Kociba *et al*., 1978). 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 in that study. Increased incidences of hepatocellular 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) were observed. 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 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 or PCB 126 alone. Rather, exposure always has occurred as a mixture in combination with other structurally related compounds such as PCDDs, PCDFs, and PCBs.

Two accidental poisoning incidents in Japan and Taiwan were caused by high exposures to cooking oil contaminated with both 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). Recent follow-up studies do not show an increased mortality from cancer,

but mortality from liver disease was still elevated (Yu *et al*., 1997). Cancer mortality was also investigated in Swedish fisherman that consume fatty fish from the Baltic Sea. The predominant exposure to dioxin-like compounds was to PeCDF in addition to other PCDFs, and PCB exposure occurred. 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 that was primarily exposed to TCDD showed similar effects, indicating that exposure is associated with an increase in all cancers combined and with several specific cancers including rectal cancer, lung cancer, Hodgkins disease, non-Hodgkins lymphoma, and myeloid leukemia (Bertazzi *et al*., 2001).

Tumor Promotion Studies

In the liver, clonal expansion of genetically altered cells leads to the formation of putative preneoplastic altered hepatocellular focal lesions (AHF) identified by alterations in histomorphology or gene expression. These lesions are believed to be a precursor to the development of liver tumors (Pitot *et al*., 1991). Studies in Sprague-Dawley rats show that PeCDF and a mixture of PCDDs and PCDFs can enhance the development of AHF (Waern *et al*., 1991), indicative of a tumor promotion effect of a mixture. Numerous studies have also examined the promotion of putative preneoplastic liver lesions by TCDD within the framework of a two-stage initiation-promotion protocol (Dragan and Schrenk, 2000). These studies demonstrate that TCDD is a potent liver tumor promoter, that this effect is dose-dependent (Pitot *et al*., 1980; Maronpot *et al*., 1993; Teeguarden *et al*., 1999), and 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 on the promotion of preneoplastic lesions (Lucier *et al*., 1991; Wyde *et al*., 2001a, 2002). Studies in Sprague-Dawley rats show that PeCDF can enhance the development of AHF (Waern *et al*., 1991), indicative of a tumor promotion effect. Van der Plas *et al.* (1999) showed that a mixture of PCDDs, PCDFs, and PCBs leads to the increased development of putative preneoplastic altered hepatocellular focal lesions.

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 in these skin tumor promotion models (Poland *et al*., 1982).

Tumor promotion by PeCDF or PCB 126 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 papillomas in both male and female Tg.AC mice, supporting the conclusion 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, the induction of skin papillomas in this model occurred when TCDD was given by oral administration.

In addition to the liver and skin, TCDD is a tumor promoter in the lung (Anderson *et al*., 1991; Beebe *et al*., 1995). In contrast, no studies of PCB 126 have examined effects on tumor promotion in the lung. In Sprague-Dawley rats, which have a much lower spontaneous incidence rate of lung tumors, TCDD alone can promote 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 likely as a potent tumor promoter.

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, PCDFs, and CP-PCBs 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. Due to the lack of chlorine substitutions in either of the *ortho* positions on the phenyl rings, PCB 126 has a planar structure (Safe, 1990). As such, PCB 126 is the most potent PCB in terms of its ability to bind and activate the AhR. *In vitro* receptor binding assays show that PCB 126 has an affinity for the AhR of 1.2×10^{-7} M, approximately 10-fold lower than that of TCDD (1×10^{-8} M), the most potent AhR ligand. Therefore, much of the mechanism of action for dioxin-like effects of PCB 126 and 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, PCDFs, and CP-PCBs are powerful growth dysregulators (Birnbaum, 1994a). Since they are not directly genotoxic (Wassom *et al.*, 1977), it is believed that the pathological responses associated with exposure are fundamentally due to binding to and activation of the AhR, subsequent altered expression of AhR-regulated genes, and altered signaling of biological pathways that interact with the AhR signal transduction mechanism (Poland and Knutson, 1982).

Alterations in expression of dioxin-regulated genes occur via a mechanism that involves a high-affinity interaction of PCDDs, PCDFs, and CP-PCBs and related polycyclic and polyhalogenated aromatic hydrocarbons 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 receptor 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 AhR response to PCDDs, PCDFs, and CP-PCBs is the transcriptional induction of the cytochrome 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 nearly all tissues examined (Dolwick *et al.*, 1993) indicating that PCDDs, PCDFs, and CP-PCBs 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 ultimately 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. In addition, it has been shown that the hepatotoxic effects of TCDD in mice are dependent upon nuclear localization of the AhR (Bunger *et al.*, 2003).

The most studied response to PCDDs, PCDFs, and CP-PCBs is induction of CYP1A1 (Whitlock, 1999). CYP1A1 is induced in many tissues including liver, lung, kidney, nasal passages, and small intestine with the highest induction in the rat liver. Increased expression of CYP1A1 is a very sensitive response to PCDDs, PCDFs, and CP-PCBs and serves as a useful marker for activation of the AhR. PCDDs, PCDFs, and CP-PCBs induce CYP1A1 *in vivo* and *in vitro* in animal models and humans. CYP1A2 is constitutively expressed in the liver at low levels and inducible only 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. Induction of acetanilide-4-hydroxylase activity is a marker of CYP1A2 activity. In addition to the well characterized induction of CYP1A1 and CYP1A2, TCDD also induces 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, PCDFs, and CP-PCBs are believed to disrupt thyroid hormone homeostasis via the induction of the phase II enzymes, UDP-glucuronosyltransferases. 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, decreased serum T_4 levels via an induction in conjugation may lead to a decrease in the negative feedback inhibition on the pituitary gland. This would then lead to a rise in secreted TSH, resulting in chronic hyperstimulation of the thyroid gland follicular cells.

PCDDs, PCDFs, and CP-PCBs 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 part of pathways involved in cellular proliferation and differentiation. These include the glucocorticoid receptor tyrosine kinases, interleukin-1β, 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 microarray and proteomic analyses has increased the understanding of which genes/proteins are altered by TCDD *in vivo* and *in vitro* (Puga *et al*., 2000; Bruno *et al*., 2002; Kurachi *et al*., 2002; Martinez *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 expression of many genes have 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

In general, PCDDs, PCDFs, and PCBs are not mutagenic in standard short-term mutagenicity tests conducted *in vitro* or *in vivo*. 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 PCDF contaminated PCBs, sister chromatid exchange (SCE) frequencies and chromosomal aberrations were similar in control and exposed populations. However *alpha*-naphthoflavone induced SCEs 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 the 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 most likely through an indirect mechanism (Tritscher *et al*., 1996; Wyde *et al*., 2001b). TCDD has been shown to result in oxidative damage (Hassoun *et al*., 1998, 2000), and this is likely 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 their similarity to TCDD, it may be inferred that PeCDF and dioxin-like PCBs may have indirect genotoxic effects, depending upon the tissue or dose examined.

Study Rationale

The female Sprague-Dawley rat was selected as the model for the current study since 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 feed study conducted by Dow Chemical Company (Kociba *et al*., 1978). Moreover, the incidence of liver tumors in the female 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 day range used in the dosed feed study of TCDD where increased liver tumors were seen (Kociba *et al*., 1978). 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

TCDD

TCDD was obtained from IIT Research Institute (Chicago, IL) by Midwest Research Institute (Kansas City, MO) and provided to the study laboratory (Battelle Columbus Operations, Columbus, OH) by Research Triangle Institute (Research Triangle Park, NC) in one lot (CR82-2-2) that was used for the 2-year study. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute, and the study laboratory. Reports on analyses performed in support of the TEF mixture study are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot CR82-2-2 of the chemical, a white crystalline powder, was identified as TCDD by the analytical chemistry laboratory using infrared spectroscopy, proton nuclear magnetic resonance (NMR) spectroscopy, direct probe mass spectroscopy (MS), low resolution gas chromatography (GC) coupled with MS, and melting point determination. In addition, identity analysis was conducted by the study laboratory using proton NMR. All spectra were consistent with the structure of TCDD. Infrared and mass spectra matched reference spectra of TCDD, and although a reference proton NMR spectrum was not available, the observed chemical shift agreed with that reported in the literature (Gurka, *et al*., 1985; Ashley, *et al*., 1989). A precise melting point range was not determined as the chemical appeared to sublime at approximately 260° C.

The purity of lot CR82-2-2 was determined by the analytical chemistry laboratory and the study laboratory using GC. One purity profile detected two impurities with a combined relative area of 2.0% and another detected two impurities with a combined relative area of 1.6%. The major impurity detected by each system (1.5% of the major peak) was identified using GC/MS as 1,2,4-trichlorodibenzo-*p*-dioxin. A small peak eluting immediately after the main component was believed to be a dimethyl isomer of trichloro-*p*-dioxin (positional substitution unknown). Also, a trace amount of a higher molecular weight tetrachlorinated dioxin (parent ion = 426) was observed, but due to the relatively weak intensity of the signal, precise identification could not be made. The purity profile obtained by this study laboratory indicated that the test article had a purity of 101.6% relative to a reference sample of the same lot. The overall purity of lot CR82-2-2 was determined to be 98% or greater.

PeCDF

PeCDF was obtained from Cambridge Isotope Laboratories (Cambridge, MA) in one lot (080196) and was 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.

Lot 080196 of the chemical, a white powder, was identified by the analytical chemistry laboratory as PeCDF by proton and carbon-13 NMR spectroscopy. The spectrum of the purity analysis sample was compared to that of the frozen reference sample 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 NMR data.

The purity of lot 080196 was determined by the analytical chemistry laboratory and the study laboratory using GC. One purity profile 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. Two of the impurities (1%) had characteristics of other furans, while the other two impurities (1.4%) had none of the characteristics of polychlorinated biphenyls (PCBs), furans, or dioxins. The other profile 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.

PCB 126

PCB 126 was obtained from AccuStandard, Inc. (New Haven, CT), in one lot (130494) and was used in the 2-year study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Chemistry Support Services), and the study laboratory.

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

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

Formulation Materials

USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) in five 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% except one that had a single impurity of 0.125%. 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

TCDD

Lot CR82-2-2 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 10 µg/mL by dissolving approximately 10 mg of accurately weighed TCDD in 1,000 mL of acetone. A formulation stock solution was prepared at a target concentration of 15 µg/mL by dissolving approximately 15 mg of accurately weighed TCDD in 1,000 mL of acetone. Following analysis to confirm proper concentration, these solutions were used to prepare analytical standard stocks of 50 and 100 µg, frozen reference stocks and chemical reference stocks of 100 µg for periodic purity determinations, and dose formulation working stocks. They were prepared by transferring the required volumes of respective solutions into appropriately sized glass containers and evaporating the solvent. Dried aliquots were stored at room temperature and protected from light in amber glass bottles. Frozen reference stocks were stored at up to –20° C. Purity was monitored with periodic reanalysis by the study laboratory. No degradation was observed during the course of the study.

PeCDF

Using procedures similar to those described above for TCDD in the preparation of analytical standard stocks, frozen reference stocks, chemical reference stocks, and dose formulation working stocks, an analytical stock solution of lot 080196 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. Dried aliquots were stored at room temperature, protected from light in amber glass bottles sealed with Teflon®-lined lids. Frozen reference stocks were stored at up to –20° C. Purity was monitored with periodic reanalysis by the study laboratory. No degradation was observed during the course of the study.

PCB 126

Using procedures similar to those described above for TCDD in the preparation of analytical standard stocks, frozen reference stocks, chemical reference stocks, and dose formulation working stocks, an analytical stock solution of lot 130494 was prepared at a target concentration of 100 µg/mL by dissolving 10 mg of accurately weighed PCB 126 in 100 mL of acetone. A formulation stock solution was prepared at a target concentration of 125 µg/mL by dissolving 250 mg of accurately weighed PCB 126 in 2,000 mL of acetone. Dried aliquots were

stored at room temperature and protected from light in amber glass bottles sealed with Teflon®-lined lids. Frozen reference stocks were stored at up to –20° C. Purity was monitored with periodic reanalysis by the study laboratory. No degradation was observed during the course of the study.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving TCDD, PeCDF, and PCB 126 dose formulation working stocks in acetone and then diluting with corn oil such that the final dose formulations contained 1% acetone. 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 and stability studies of a low-dose formulation containing 1.32 ng/mL of TCDD, 2.64 ng/mL of PeCDF, and 13.32 ng/mL of PCB 126 and a homogeneity study of a high-dose formulation of 13.2 ng/mL TCDD, 26.4 ng/mL PeCDF, and 133.2 ng/mL PCB 126 were performed by the study laboratory using GC/MS. Homogeneity was confirmed, and stability was confirmed for 3 hours under simulated animal room conditions. Stability studies of the low-dose fomulation were performed by Midwest Research Institute using GC/MS. In these studies, stability was confirmed for at least 36 days for a dose formulation in corn oil containing 0.04% nonane stored in sealed amber glass containers at 5° C and at room temperature, and for up to 3 hours for formulations in a simulated dosing study. Gavagability was confirmed by the study laboratory for the high-dose formulation.

Periodic analyses of the dose formulations of the TEF mixture were conducted by the study laboratory using GC/MS. During the 2-year study, the dose formulations were analyzed at least every 3 months to determine the concentrations of TCDD, PeCDF, and PCB 126 in the mixture (Tables C3, C4, and C5). All 40 measurements of TCDD concentrations, 28 of 40 measurements of PeCDF concentrations, and 29 of 40 measurements of PCB 126 concentrations were within 10% of the target concentrations. In addition, all measurements of PeCDF and PCB 126 concentrations were within 15% of the targets. Fifteen of 16, 15 of 16, and 13 of 16 animal room sample measurements for TCDD, PeCDF, and PCB 126 concentrations, respectively, were within 10% of the target concentrations; all animal room samples were within 14% of the targets. All formulations were used in the study with the approval of the NTP.

2-Year Study

Study Design

Groups of 81 female rats received the TEF mixture in corn oil:acetone (99:1) by gavage at doses of 0, 10, 22, 46, or 100 ng TEQ/kg 5 days per week for up to 105 weeks (Table 2). The 0 ng TEQ/kg group received the corn oil:acetone (99:1) vehicle only and served as the vehicle control. Up to 10 female rats from each group were evaluated at 14, 31, and 53 weeks.

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 2
Exact Doses for the TEF Mixture

Dose Group	TCDD (ng/kg)	PeCDF (ng/kg)	PCB 126 (ng/kg)	TEQ[a] (ng/kg)
10 ng TEQ/kg	3.3	6.6	33.3	9.9
22 ng TEQ/kg	7.3	14.5	73.3	21.9
46 ng TEQ/kg	15.2	30.4	153.0	45.7
100 ng TEQ/kg	33.0	66.0	333.0	99.3

[a] TCDD equivalents (TEQ) dose is calculated as TCDD dose + (PeCDF dose × 0.5) + (PCB 126 dose × 0.1).

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 test compound. Rats were quarantined for 12 days before the beginning of the study. Rats 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 animals 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 100 ng TEQ/kg females at the end of the study.

Animal Maintenance

Male rats were housed three per cage, and female rats were housed five per cage. Feed and water were available *ad libitum*. Cages were changed twice weekly; racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 3. 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 again at necropsy. Body weights were recorded on the first day prior to dose initiation, at weekly intervals for the first 13 weeks of study, at monthly intervals thereafter, and again at necropsy.

At 14, 31, and 53 weeks, blood was taken from the retroorbital sinus of up to 10 female rats per group and processed to serum for thyroid hormone determinations. Radioimmunoassays were performed for thyroid stimulating hormone (TSH), triiodothyronine, and free thyroxine (T_4) using a Packard Cobra II gamma counter (Packard Instrument Company, Meriden, CT). The assay for total T_4 was performed on a Hitachi 911® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN) using a Boehringer Mannheim® enzyme immunoassay test system. Thyroid hormone data were summarized using the XYBION system (XYBION Medical Systems Corporation, Cedar Knolls, NJ).

For cell proliferation analysis at 14, 31, and 53 weeks, up to 10 female rats per group received drinking water *ad libitum* containing 40 mg BrdU/100 mL Milli-Q water for 5 days. The 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. The BrdU solutions were changed after 3 days, and water consumption was measured daily for 5 days. The cell turn-over rate in the liver of female rats following exposure to the TEF mixture was compared to the turnover rate in the liver of control rats by determining the incorporation of BrdU into liver cells. A sample of duodenum and liver was fixed in 10% neutral buffered formalin and then transferred to 70% ethanol. Representative sections of the liver and duodenum were trimmed and embedded, and two sections were cut. One of these sections was stained with hematoxylin and eosin, the other with anti-BrdU antibody complexed with avidin and biotin. At the 14-week interim evaluation, assessment of potential interlobular variation was determined in the vehicle control and 100 ng TEQ/kg groups by counting the stained cells in the left lobe and right median lobe. Interlobular variation greater than 25% was considered significant. For the remaining rats, stained cells were counted only in the left lobe. At least 2,000 labeled or unlabeled hepatocytes were counted using a 20× objective and ocular grid. The labeling index is expressed as the percentage of total nuclei that were labeled with BrdU.

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

For analysis of tissue concentrations of TCDD, PeCDF, and PCB 126, samples of fat, liver, lung, and blood were taken from up to 10 female rats per group at 14, 31, and 53 weeks and at 2 years. All samples were spiked with ^{13}C-labeled isotopes of the three target analytes for

analysis by isotope dilution with guidance from EPA Method 1613 (USEPA, 1994). Fat, liver, and lung tissue samples were sonicated in methylene chloride and then serially extracted with hexane. Blood samples were extracted in saturated aqueous ammonium sulfate:ethanol:hexane (1:1:1) using a liquid/liquid rotary (tumbling) extraction prior to serial extraction with hexane. For all tissue samples, the hexane extracts were combined, dried on a column of sodium sulfate, and completely exchanged into hexane using a nitrogen blow-down apparatus. The combined hexane extracts were quantitatively applied to a two-layer column of acid and neutral silica; the eluant from these columns dripped directly into secondary columns of activated carbon that trapped the analytes. The carbon columns were sequentially washed with methylene chloride:cyclohexane (1:1) and methylene chloride:methanol:benzene (15:4:1) to remove biological interferences. The carbon columns were then turned upside down and toluene was used to elute the adsorbed target compounds. Concentrations of TCDD, PeCDF, and PCB 126 in the tissue extracts were measured by high resolution capillary gas chromatography with high resolution mass spectrometry detection.

Complete necropsies and microscopic examinations were performed on all female rats. At the interim evaluations, the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid gland were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 3.

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 organs with potential chemical-related changes, which included the adrenal cortex, liver, lung, and pancreas.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the study laboratory pathologist, quality assessment pathologist, and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

To maintain consistency of diagnoses within and 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, this study of a mixture of TCDD, PeCDF, and PCB 126, along with three other studies (TCDD, PeCDF, and PCB 126; NTP, 2006a,b,c) were subjected to additional PWG reviews. Within many of these studies, there were hepatocellular proliferative lesions for which the criteria used for common diagnoses did not appear to fit. Furthermore, classification was sometimes confounded by significant liver damage (toxic hepatopathy) that was present in many animals from these studies. Therefore, a PWG 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 review panel utilizing 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 3
Experimental Design and Materials and Methods in the 2-Year Gavage Study of the TEF Mixture

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

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

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

Time Held Before Study
12 days

Average Age When Study Began
8 weeks

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

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

Date of Last Dose
June 12-13, 2000

Necropsy Dates
June 13-14, 2000

Average Age at Necropsy
112 weeks

Size of Study Groups
81

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

Animals per Cage
Male Rats: 3
Female Rats: 5

Method of Animal Identification
Tail tattoo

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

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

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

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

TABLE 3
Experimental Design and Materials and Methods in the 2-Year Gavage Study of the TEF Mixture

Racks
Stainless steel (Lab Products, Inc., Seaford, DE), changed and 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 ng TEQ/kg
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)

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

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

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

Cytochrome P450 Activities
At 14, 31, and 53 weeks, tissue samples from the liver were taken from up to 10 rats per group for 7-ethoxyresorufin-*O*-deethylase 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 fat, liver, lung, and blood were taken from up to 10 rats per group for analysis of TCDD, PeCDF, and PCB 126 concentrations.

Histopathology
Complete histopathology was performed on all animals 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 with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, skin, spleen, stomach (forestomach and glandular), thymus, thyroid gland, trachea, urinary bladder, and uterus. The adrenal gland, liver, lung, mammary gland, left ovary, pancreas, pituitary gland, spleen, stomach, thymus, thyroid gland, uterus, and vagina were examined in the vehicle control and 100 ng TEQ/kg rats at the 14-, 31-, and 53-week interim evaluations.

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 A1 and A5 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 (Table A3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Table A3 also gives 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-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and $B6C3F_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-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 TEF mixture; TCDD, PeCDF, PCB 126, PCB 153, the mixture of PCB 126 and PCB 153, and the binary mixture of PCB 126 and PCB 118; NTP, 2006a,b,c,d,e,f).

Quality Assurance Methods

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

RESULTS

2-Year Study

Survival

Estimates of 2-year survival probabilities for female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 1). Survival of all dosed groups of rats was similar to that of the vehicle control group.

Body Weights and Clinical Findings

Mean body weights of the 22 and 46 ng TEQ/kg study groups were less than those of the vehicle control group after week 69 of the study (Figure 2 and Table 5). Mean body weights of the 100 ng TEQ/kg group were less than those of the vehicle control group after week 37 of the study. With the exception of thin appearance most prominent in animals in the 100 ng TEQ/kg group, no clinical findings related to administration of the toxic equivalency factor (TEF) mixture were observed.

Table 4
Survival of Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Animals initially in study	81	81	81	81	81
14-Week interim evaluation[a]	10	10	10	10	10
31-Week interim evaluation[a]	10	10	10	10	10
53-Week interim evaluation[a]	8	8	8	8	8
Accidental deaths[a]	0	0	1	1	2
Moribund	26	25	22	25	33
Natural deaths	11	5	6	4	10
Animals surviving to study termination	16	23	24	23	8
Percent probability of survival at end of study[b]	30	43	46	44	16
Mean survival (days)[c]	616	645	646	640	588
Survival analysis[d]	P=0.012	P=0.195N	P=0.080N	P=0.160N	P=0.165

[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. A lower mortality in a dosed group is indicated by **N**.

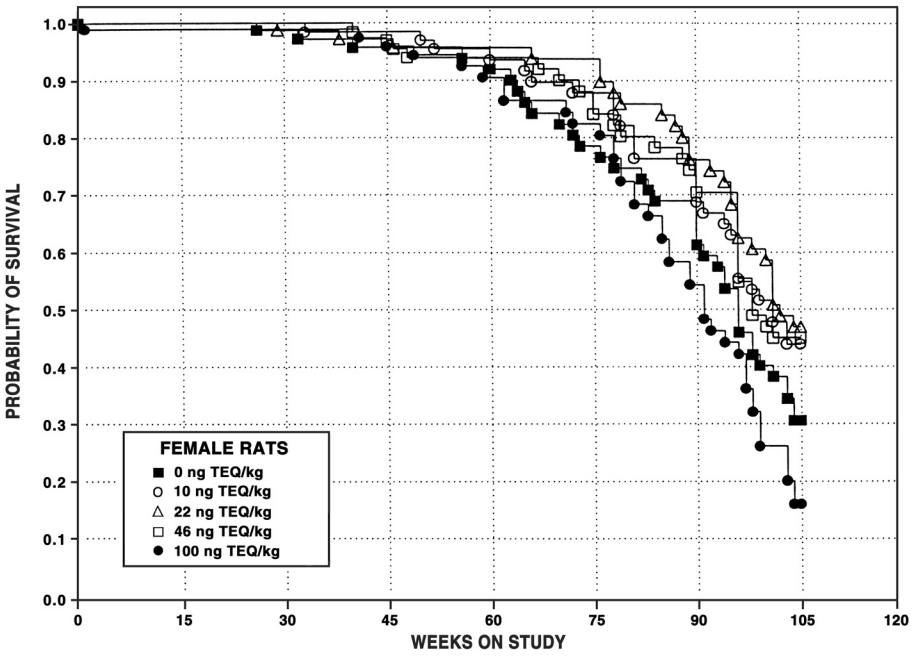


FIGURE 1
Kaplan-Meier Survival Curves for Female Rats Administered the TEF Mixture by Gavage for 2 Years

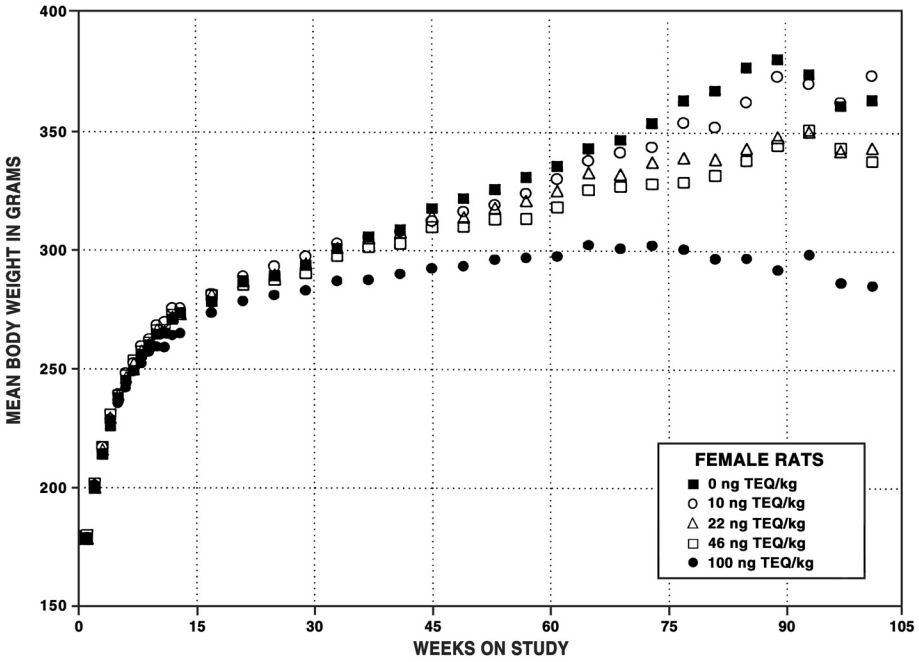


FIGURE 2
Growth Curves for Female Rats Administered the TEF Mixture by Gavage for 2 Years

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of the TEF Mixture

Weeks on Study	Vehicle Control		10 ng TEQ/kg			22 ng TEQ/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	179	98	179	100	98	178	100	98
2	200	98	201	101	98	200	100	98
3	214	98	217	101	98	216	101	98
4	226	98	229	101	98	230	102	98
5	238	98	240	101	98	239	101	98
6	245	98	249	101	98	247	101	98
7	250	98	253	101	98	252	101	98
8	256	98	260	101	98	258	101	97
9	260	98	263	101	98	261	100	97
10	265	98	269	102	98	267	101	97
11	265	98	270	102	98	266	100	97
12	271	98	276	102	98	272	100	97
13	274	98	276	101	98	273	100	97
17[a]	279	82	282	101	82	282	101	81
21	287	82	289	101	82	287	100	81
25	289	82	293	101	82	290	100	81
29	294	81	298	101	82	295	100	81
33[a]	301	64	303	101	66	301	100	64
37	306	64	306	100	65	305	100	64
41	309	63	308	100	65	308	100	63
45	318	63	313	98	65	315	99	63
49	322	63	317	98	65	314	98	62
53	326	63	320	98	63	318	98	62
57[a]	331	49	324	98	50	321	97	49
61	336	48	331	98	49	326	97	49
65	343	46	338	99	49	333	97	49
69	347	44	341	99	47	333	96	48
73	354	42	344	97	46	338	95	48
77	364	40	354	97	46	340	93	46
81	368	39	353	96	43	339	92	44
85	378	36	363	96	40	344	91	44
89	381	36	374	98	40	348	91	40
93	375	30	371	99	35	351	94	38
97	362	24	363	100	29	343	95	32
101	364	21	374	103	27	344	94	30
Mean for weeks								
1-13	242		245	101		243	101	
14-52	301		301	100		300	100	
53-101	356		350	98		337	95	

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of the TEF Mixture

Weeks on Study	46 ng TEQ/kg Av. Wt. (g)	46 ng TEQ/kg Wt. (% of controls)	46 ng TEQ/kg No. of Survivors	100 ng TEQ/kg Av. Wt. (g)	100 ng TEQ/kg Wt. (% of controls)	100 ng TEQ/kg No. of Survivors
1	180	101	98	179	100	98
2	202	101	98	201	101	97
3	217	101	98	215	100	97
4	231	102	98	228	101	97
5	239	100	98	236	99	97
6	247	101	98	242	99	97
7	254	102	98	249	100	97
8	258	101	98	253	99	97
9	261	100	98	258	99	97
10	267	101	98	260	98	97
11	267	101	98	259	98	97
12	273	101	98	265	98	97
13	273	100	98	265	97	97
17[a]	281	101	82	274	98	81
21	286	99	82	279	97	81
25	288	99	82	281	97	81
29	291	99	82	283	97	81
33[a]	298	99	66	287	95	65
37	302	99	66	288	94	65
41	303	98	65	290	94	65
45	310	98	65	293	92	64
49	310	96	62	294	91	63
53	313	96	62	296	91	62
57[a]	314	95	49	297	90	47
61	319	95	49	298	89	45
65	326	95	49	303	88	43
69	327	94	47	301	87	43
73	329	93	46	303	85	41
77	329	91	43	301	83	40
81	332	90	41	297	81	36
85	338	90	40	297	79	33
89	345	91	39	292	77	29
93	351	94	36	299	80	23
97	344	95	28	287	79	21
101	338	93	24	286	78	13
Mean for weeks						
1-13	244	101		239	99	
14-52	297	99		285	95	
53-101	331	93		297	84	

[a] Interim evaluations occurred during weeks 14, 31, and 53; until week 53, number of survivors included 17 special study animals that were not evaluated as part of the core study.

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. A downward trend in serum total T_4 concentrations with higher TEF mixture concentrations was evident at 14 weeks (Table 6). Total T_4 levels in all of the dosed groups were significantly lower than in the vehicle controls. A maximal suppression of total T_4 by 45.3% was observed in the 100 ng TEQ/kg group. Serum free T_4 concentrations in the 22, 46, and 100 ng TEQ/kg groups were significantly lower than in the vehicle controls by 30.3%, 28.2%, and 32.9%, respectively. Serum T_3 concentrations were elevated in all dosed groups, but were only significantly higher than that of the vehicle controls in the 46 and 100 ng TEQ/kg groups. These values exceeded the vehicle controls by 24.4% and 33.9%, respectively. Serum TSH concentrations in all dosed groups were elevated, but not significantly different, compared to the vehicle controls.

At 31 weeks, total T_4 concentrations were significantly lower in all TEF mixture-treated groups than in the vehicle controls; the differences were between 27% (46 ng TEQ/kg) and 36% (22 ng TEQ/kg). Serum free T_4 concentrations in the 22, 46, and 100 ng TEQ/kg groups were significantly lower than the vehicle controls by 23.9%, 21.4%, and 19.0%, respectively. There was a trend of increasing serum T_3 concentrations at 31 weeks, but a significant difference from the vehicle controls was only observed for the 100 ng TEQ/kg group. In the 100 ng TEQ/kg group, serum T_3 concentrations exceeded the vehicle controls by 15.2%. However, there were no statistically significant changes in serum TSH concentrations for any of the TEF mixture dosage groups compared to the vehicle controls at 31 weeks.

At the 53-week interim evaluation, a downward trend in serum total T_4 concentrations was evident. Statistically significant decreases in total T_4 concentrations of 24.0%, 30.5%, 51.1%, and 34.4% were observed in the 10, 22, 46, and 100 ng TEQ/kg groups, respectively, relative to the vehicle controls. Serum free T_4 concentration was significantly lower than the vehicle controls in 22 ng TEQ/kg or greater groups. These concentrations were 22.7%, 37.6%, and 34.7% lower than the vehicle controls for the 22, 46, and 100 ng TEQ/kg groups, respectively. Serum T_3 was increased in the 22 and 100 ng TEQ/kg groups compared to the vehicle controls, but these changes were not statistically significant. Serum TSH concentrations were significantly higher than vehicle controls only in the 22 ng TEQ/kg group.

Hepatic Cell Proliferation Data

Hepatocellular proliferation at the 14-, 31-, and 53-week interim evaluations is shown in Table 7. The consumption of the BrdU drinking water solution prior to each interim evaluation was similar across groups. At 14 weeks, the hepatocellular labeling index was similar in the dosed and vehicle control groups. At 31 weeks, the hepatocellular labeling index was significantly higher in the 46 and 100 ng TEQ/kg groups compared to the vehicle controls. The labeling index was 2.6-fold higher in these groups relative to the vehicle control group. The labeling index was elevated at 53 weeks in all dosed groups compared to the vehicle controls. Statistically significant increases in the labeling index were only observed in the 46 and 100 ng TEQ/kg groups, which were 2.6- and 5.7-fold higher than the vehicle controls.

TABLE 6
Serum Concentrations of Thyroid Hormones in Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Week 14					
n	10	10	10	10	10
Total T_4 (µg/dL)	6.000 ± 0.328	4.990 ± 0.255*	3.970 ± 0.133**	3.660 ± 0.318**	3.280 ± 0.210**
Free T_4 (ng/dL)	2.292 ± 0.181	1.988 ± 0.116	1.598 ± 0.123**	1.646 ± 0.152**	1.537 ± 0.116**
Total T_3 (ng/dL)	134.628 ± 6.983	148.361 ± 6.695	146.485 ± 9.517	167.542 ± 8.259**	180.231 ± 12.442**
TSH (ng/mL)	9.944 ± 0.552	10.932 ± 1.192	12.105 ± 1.331	13.206 ± 1.400	12.588 ± 1.222
Week 31					
n	10	10	10	10	10
Total T_4 (µg/dL)	3.830 ± 0.187	2.740 ± 0.138**	2.470 ± 0.138**	2.790 ± 0.195**	2.510 ± 0.144**
Free T_4 (ng/dL)	1.672 ± 0.052	1.481 ± 0.080	1.273 ± 0.043**	1.315 ± 0.083**	1.354 ± 0.105**
Total T_3 (ng/dL)	163.073 ± 5.695	166.727 ± 5.007	186.235 ± 6.868	177.232 ± 6.869	187.830 ± 9.700*
TSH (ng/mL)	15.907 ± 1.183	19.373 ± 1.264	14.165 ± 1.007	16.183 ± 1.555[b]	18.820 ± 1.674
Week 53					
n	8	8	8	8	8
Total T_4 (µg/dL)	3.275 ± 0.206	2.488 ± 0.200**	2.275 ± 0.182**	1.600 ± 0.291**	2.150 ± 0.130**
Free T_4 (ng/dL)	1.903 ± 0.105	1.606 ± 0.131	1.471 ± 0.103*	1.188 ± 0.133**	1.243 ± 0.092**
Total T_3 (ng/dL)	148.639 ± 6.810	152.788 ± 6.316	172.648 ± 9.000	139.929 ± 9.557	172.546 ± 10.165
TSH (ng/mL)	11.928 ± 0.912	12.593 ± 1.541[c]	16.621 ± 1.263*	14.155 ± 1.355	12.931 ± 1.033

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. T_4=thyroxine; T_3=triiodothyronine; TSH=thyroid stimulating hormone.
[b] n=9
[c] n=7

TABLE 7
Hepatic Cell Proliferation Data for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Labeling index (%)					
Week 14	1.374 ± 0.282	1.277 ± 0.221	1.437 ± 0.292	1.989 ± 0.329	1.600 ± 0.245
Week 31	0.645 ± 0.099	0.696 ± 0.106	0.898 ± 0.144	1.643 ± 0.308**	1.678 ± 0.226**
Week 53	0.591 ± 0.111	0.986 ± 0.227	1.039 ± 0.233	1.529 ± 0.324**	3.397 ± 0.820**

** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error.

Cytochrome P450 Enzyme Activities

At each interim evaluation, liver and lung samples were collected for determination of P450 enzyme activities. Microsomal suspensions were prepared from liver samples and were assayed for 7-ethoxyresorufin-*O*-deethylase (EROD, CYP1A1) and acetanilide-4-hydroxylase (A4H, CYP1A2) activities. Microsomal samples from lung were analyzed for EROD activity only.

At the 14-, 31-, and 53-week interim evaluations, hepatic EROD and A4H activities were significantly higher in all dosed groups compared to the vehicle controls (Table 8). Significant induction of hepatic P450 activities occurred at the lowest dose (10 ng TEQ/kg) and generally demonstrated an increasing dose-response. Hepatic EROD activities were maximally induced approximately 46-fold at 14 weeks (100 ng TEQ/kg), 36-fold at 31 weeks (46 and 100 ng TEQ/kg), and 43-fold at 53 weeks (100 ng TEQ/kg). Similarly, hepatic A4H activity increased with increasing doses of the TEF mixture. A4H activity in the liver of dosed rats maximally exceeded the vehicle controls by 4.4-fold at 14 weeks (100 ng TEQ/kg), 5.1-fold at 31 weeks (46 and 100 ng TEQ/kg), and 4.7-fold at 53 weeks (100 ng TEQ/kg).

EROD activities in the lung were significantly higher in all dosed groups compared to the vehicle controls at the 14-, 31-, and 53-week interim evaluations. At the lowest dose (10 ng TEQ/kg), pulmonary EROD activities were 14-fold higher than the vehicle controls at 14 weeks, 18-fold higher than the vehicle controls at 31 weeks, and 15-fold higher than the vehicle controls at 53 weeks. EROD activities in the lung generally increased with increasing doses of the TEF mixture. Maximal values for pulmonary EROD activities were 25-fold (22, 46, and 100 ng TEQ/kg), 47-fold (46 and 100 ng TEQ/kg), and 27-fold (46 and 100 ng TEQ/kg) higher than in the vehicle controls at 14, 31, and 53 weeks, respectively.

TABLE 8
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 the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Liver Microsomes					
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)					
Week 14	51.7 ± 1.9	1,135 ± 48**	1,715 ± 66**	2,029 ± 77**	2,402 ± 114**
Week 31	47.4 ± 2.4	1,226 ± 66**	1,593 ± 49**	1,746 ± 124**	1,710 ± 62**
Week 53	71.8 ± 4.4	1,707 ± 82**	2,326 ± 104**	2,710 ± 151**	3,078 ± 78**
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)					
Week 14	0.446 ± 0.013	1.027 ± 0.045**	1.314 ± 0.054**	1.565 ± 0.068**	1.956 ± 0.079**
Week 31	0.370 ± 0.013	1.169 ± 0.050**	1.636 ± 0.054**	1.893 ± 0.116**	1.873 ± 0.039**
Week 53	0.415 ± 0.020	1.302 ± 0.042**	1.586 ± 0.057**	1.641 ± 0.053**	1.934 ± 0.075**
Lung Microsomes					
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)					
Week 14	1.5 ± 0.2	20.5 ± 2.3**	31.9 ± 3.6**	36.7 ± 3.4**	34.6 ± 4.0**
Week 31	2.1 ± 0.2	37.5 ± 3.2**	64.3 ± 8.0**	92.8 ± 8.8**	99.9 ± 6.2**
Week 53	3.2 ± 0.4	46.9 ± 6.4**	68.9 ± 10.7**	84.9 ± 7.3**	80.7 ± 4.2**

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

Determinations of TCDD, PeCDF, and PCB 126 Concentrations in Tissues

Concentrations of TCDD, PeCDF, and PCB 126 were determined for all dose groups in fat, liver, lung, and blood at the 14-, 31-, and 53-week interim evaluations and at the end of the 2-year study (105 weeks). The highest concentrations of TCDD, PeCDF, and PCB 126 were observed in the liver, followed by fat (Tables 9, 10, and 11). In vehicle control liver, detectable concentrations of PeCDF and PCB 126 were observed at all time points. Mean liver concentrations of TCDD, PeCDF, and PCB 126 in vehicle controls were 8, 65, and 183 pg/g at 105 weeks, respectively. TCDD concentrations were undetectable in vehicle control liver at the interim evaluations, except in a single animal each at 14 and 53 weeks. Liver concentrations of TCDD, PeCDF, and PCB 126 at each timepoint increased with increasing doses of the TEF mixture, demonstrating a dose-related increase in liver burden. The concentrations of TCDD, PeCDF, and PCB 126 at each dose generally increased with duration of dosing, and the highest liver concentrations were observed at 105 weeks. Given the TEF values for TCDD, PeCDF, and PCB 126, of 1.0, 0.5, and 0.1, respectively, liver burdens on a total TEQ basis for the 100 ng TEQ/kg group were approximately 44,400, 91,300, 110,000, and 117,000 pg/g at 14, 31, 53, and 104 weeks, respectively.

In fat tissue of vehicle controls, PeCDF concentrations were below the experimental limit of quantitation at all of the interim evaluations, but a detectable concentration was observed in a single vehicle control animal at 105 weeks. Similarly, TCDD was not detected in fat from vehicle control rats, except one and two animals at 14 and 104 weeks, respectively. Detectable concentrations of PCB 126 in fat were observed in all vehicle control groups at each of the interim evaluations and at 105 weeks. In dosed groups, fat concentrations of TCDD, PeCDF, and PCB 126 at each timepoint increased with increasing doses of the TEF mixture, demonstrating a dose-related increase. The concentrations of TCDD, PeCDF, and PCB 126 in the 10, 22, and 100 ng TEQ/kg groups were higher following longer exposures. In the 46 ng TEQ/kg group, concentrations of TCDD, PeCDF, and PCB 126 were higher at 31 weeks than at 53 weeks. For all dosed groups, the highest fat concentrations of TCDD, PeCDF, and PCB 126 were observed at 105 weeks. The mean TEQ-calculated total fat burdens were approximtely 3,800, 8,000, 8,700, and 9,700 pg/g in the 100 ng TEQ/kg group at 14, 31, 53, and 105 weeks, respectively.

No measurable concentrations of TCDD, PeCDF, or PCB 126 were detected in the lung of vehicle control rats at any time point. At 14 weeks, lung concentrations of PeCDF were 10 pg/g in the 100 ng TEQ/kg group. Similar concentrations were observed in a single animal each in the 10 and 46 ng TEQ/kg groups. Measurable concentrations of TCDD and PCB 126 were observed at 14 weeks in the lung of all dosed groups with the highest concentrations observed in the 100 ng TEQ/kg group. At 31 weeks, lung concentrations of PeCDF were detectable in the 46 and 100 ng TEQ/kg groups. In dosed groups, lung concentrations of TCDD and PCB 126 increased with increasing doses of the TEF mixture, demonstrating a dose-related increase. On a TEQ basis, lung burden was 58 pg/g in the 100 ng TEQ/kg group. At 53 weeks, concentrations of TCDD, PeCDF, and PCB 126 in the lung generally increased with increasing doses of the TEF mixture. On a TEQ basis, lung burden was 73 pg/g in the 100 ng TEQ/kg group. At 104 weeks, PeCDF concentrations ranged from 14.5 pg/g (46 ng TEQ/kg) to 31 pg/g (100 ng TEQ/kg). In the dosed groups, TCDD and PCB 126 concentrations were the lowest in the 46 ng TEQ/kg group and the highest in the 22 ng TEQ/kg group. At 105 weeks, lung burdens for the 10, 22, 46, and 100 ng TEQ/kg groups on a TEQ basis were 85, 92, 46, and 74 pg/g, respectively.

No measurable concentrations of TCDD, PeCDF, or PCB 126 were detected in the blood of vehicle control animals at any time point. At 14 weeks, detectable concentrations of PeCDF were only observed in the 46 and 100 ng TEQ/kg groups. Blood concentrations of TCDD and PCB 126 at 14 weeks increased with increasing doses of the TEF mixture, demonstrating a dose-related increase. At 31 weeks, blood concentrations of PeCDF were detectable in 22 ng TEQ/kg or greater groups. Blood concentrations of TCDD and PCB 126 at 31 weeks increased with increasing doses of the TEF mixture, demonstrating a dose-related increase. At 53 and 105 weeks, blood concentrations of TCDD, PeCDF, and PCB 126 increased with increasing doses of the TEF mixture. The concentrations of TCDD, PeCDF, and PCB 126 at each dose were generally increased with longer dosing durations, and the highest concentrations in blood were observed at 105 weeks. Blood concentrations on a TEQ basis increased at each timepoint with increasing doses of the TEF mixture, and concentrations at each dose increased with longer dosing durations. Mean blood concentrations were 19, 31, 40, and 87 pg/g TEQ in the 100 ng TEQ/kg group at 14, 31, 53, and 105 weeks, respectively.

TABLE 9
Tissue Concentrations of TCDD in Female Rats in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Week 105	8	10	10	10	10
Fat					
Week 14	13.0[b]	305.4 ± 12.9	563.2 ± 44.2	704.7 ± 52.6	1,399.2 ± 103.3
Week 31	BLOQ	326.7 ± 12.3	646.5 ± 14.8	1,292.0 ± 37.9	2,610.0 ± 96.9
Week 53	BLOQ	345.25 ± 20.49	687.63 ± 44.42	1,098.88 ± 75.61	2,706.25 ± 95.02
Week 105	12.60 ± 0.40[c]	553.20 ± 33.88	856.59 ± 116.25	1616.00 ± 107.74	2823.75 ± 208.64[d]
Liver					
Week 14	1.47[b]	613.60 ± 33.06	1,675.50 ± 84.33	3,625.00 ± 166.72	6,660.00 ± 402.97
Week 31	BLOQ	727.2 ± 47.6	1,789.9 ± 130.9	3,383.0 ± 200.9	8,508.0 ± 495.0
Week 53	1.14[b]	779.63 ± 32.81	2,048.75 ± 103.76	3,836.25 ± 181.27	9,503.75 ± 647.26
Week 105	7.70 ± 6.00[e]	1,260.80 ± 100.66	2,611.00 ± 141.67	4,640.00 ± 336.96	10,518.75 ± 929.56[d]
Lung					
Week 14	BLOQ	12.82 ± 4.19[f]	9.57 ± 1.71[d]	14.76 ± 3.14[e]	21.20 ± 2.99[f]
Week 31	BLOQ	3.71 ± 0.65[g]	8.04 ± 2.34[g]	11.55 ± 3.11[h]	28.99 ± 9.27[e]
Week 53	BLOQ	6.89 ± 2.37[f]	11.80 ± 1.57[e]	19.61 ± 4.16[f]	34.73 ± 3.14
Week 105	BLOQ	34.60 ± 6.34	39.87 ± 8.28[f]	18.23 ± 2.52[d]	28.54 ± 8.04[f]
Blood					
Week 14	BLOQ	1.73 ± 0.18[h]	2.77 ± 0.14[f]	4.60 ± 0.19[d]	7.90 ± 0.41
Week 31	BLOQ	1.59 ± 0.13[d]	2.95 ± 0.13[d]	5.18 ± 0.36[i]	11.76 ± 0.59
Week 53	BLOQ	1..90 ± 0.08[h]	3.24 ± 0.20[f]	6.15 ± 0.54	14.44 ± 0.95
Week 105	BLOQ	2.28 ± 0.23[h]	5.05 ± 0.46[d]	8.24 ± 0.45	25.13 ± 7.02[d]

[a] Data are given in pg/g tissue (fat, liver, lung) or pg/mL (blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation. BLOQ=below the limit of quantitation; LOQ_{fat}=5 pg/g, LOQ_{liver}=1 pg/g, LOQ_{lung}=2.5 pg/g, LOQ_{blood}=1 pg/mL.
[b] n=1
[c] n=2
[d] n=8
[e] n=6
[f] n=7
[g] n=3
[h] n=5
[i] n=9

TABLE 10
Tissue Concentrations of PeCDF in Female Rats in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Week 105	8	10	10	10	10
Fat					
Week 14	BLOQ	140.44 ± 7.28[b]	226.89 ± 16.99[b]	307.50 ± 25.36	570.10 ± 40.79
Week 31	BLOQ	261.9 ± 5.8	485.7 ± 12.9	888.4 ± 27.5	1,740.0 ± 67.8
Week 53	BLOQ	335.38 ± 16.68	614.88 ± 40.40	861.88 ± 64.74	2,056.25 ± 67.74
Week 105	12.10[c]	502.50 ± 21.86	804.25 ± 108.02	1,508.00 ± 107.94	2,560.00 ± 208.13[d]
Liver					
Week 14	10.60 ± 1.01[b]	6,283.00 ± 298.65	15,000.00 ± 992.86	28,540.00 ± 1,275.16	47,790.00 ± 2,830.33
Week 31	10.79 ± 0.87[d]	14,250.00 ± 830.96	33,600.00 ± 2,389.84	60,260.00 ± 3,080.27	125,070.00 ± 7,313.31
Week 53	9.44 ± 0.61	13,762.50 ± 685.29	32,525.00 ± 1,542.58	62,187.50 ± 2,903.35	154,750.00 ± 7,798.24
Week 105	65.44 ± 51.33	21,840.00 ± 974.13	42,210.00 ± 2,625.58	85,770.00 ± 6,470.91	161,125.00 ± 8,197.21[d]
Lung					
Week 14	BLOQ	12.50[c]	BLOQ	10.90[c]	10.05 ± 1.56[e]
Week 31	BLOQ	BLOQ	BLOQ	8.32 ± 1.35[f]	17.10 ± 5.41[g]
Week 53	BLOQ	12.50[c]	8.61 ± 1.16[h]	11.94 ± 1.86[i]	23.03 ± 2.92[i]
Week 105	BLOQ	26.35 ± 6.88[d]	28.56 ± 4.86[i]	14.49 ± 2.24	31.03 ± 9.18[d]
Blood					
Week 14	BLOQ	BLOQ	BLOQ	2.21 ± 0.10[e]	3.24 ± 0.12[d]
Week 31	BLOQ	BLOQ	2.14 ± 0.07[f]	3.00 ± 0.16[b]	7.69 ± 0.51
Week 53	BLOQ	2.75 ± 0.32[j]	3.10 ± 0.015[i]	5.00 ± 0.42	12.18 ± 1.04
Week 105	BLOQ	2.34 ± 0.19[j]	4.53 ± 0.43[d]	8.66 ± 0.83	32.37 ± 14.24[i]

[a] Data are given in pg/g tissue (fat, liver, lung) or pg/mL (blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation. BLOQ=below the limit of quantitation; LOQ_{fat}=10 pg/g, LOQ_{liver}=2 pg/g, LOQ_{lung}=5 pg/g, LOQ_{blood}=2 pg/mL.
[b] n=9
[c] n=1
[d] n=8
[e] n=4
[f] n=3
[g] n=6
[h] n=5
[i] n=7
[j] n=2

TABLE 11
Tissue Concentrations of PCB 126 in Female Rats in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Week 105	8	10	10	10	10
Fat					
Week 14	108.91 ± 17.19	4,445.00 ± 179.47	8,037.00 ± 544.52	11,067.00 ± 806.23	20,920.00 ± 1,516.63
Week 31	89.93 ± 4.96	6,839.00 ± 173.75	14,050.00 ± 301.94	24,980.00 ± 1,032.24	45,400.00 ± 1,429.06
Week 53	105.98 ± 2.68	7,710.00 ± 357.72	14,450.00 ± 847.26	21,600.00 ± 1,494.63	49,887.50 ± 1,761.13
Week 105	205.7 ± 37.7	13,060.0 ± 529.6	19,339.7 ± 2,394.3	32,610.0 ± 1,815.0	55,712.5 ± 3,382.3
Liver					
Week 14	69.78 ± 5.38	11,839.00 ± 742.80	33,650.00 ± 1,564.34	73,230.00 ± 2,713.51	138,540.00 ± 8,812.77
Week 31	90.08 ± 6.74	22,030.00 ± 1,720.47	52,130.00 ± 3,517.48	84,660.00 ± 4,610.57	202,200.00 ± 11,693.11
Week 53	89.46 ± 2.92	22,250.00 ± 1,125.20	52,962.50 ± 2,772.63	98,287.50 ± 6,399.59	227,875.00 ± 14,479.59
Week 105	183.07 ± 74.11	35,470.00 ± 2,522.57	68,300.00 ± 3,771.85	116,050.00 ± 8,358.11	261,000.00 ± 17,227.47[c]
Lung					
Week 14	BLOQ	91.08 ± 35.31[b]	43.81 ± 6.25[c]	87.54 ± 28.38	113.08 ± 13.62
Week 31	BLOQ	32.10[d]	44.70 ± 8.01[e]	64.54 ± 9.47	201.89 ± 38.23[f]
Week 53	BLOQ	44.40 ± 20.28[e]	81.76 ± 20.92	145.53 ± 24.37	272.00 ± 31.42
Week 105	BLOQ	368.30 ± 130.60	373.58 ± 83.21	203.77 ± 51.97	303.50 ± 67.28[c]
Blood					
Week 14	BLOQ	17.19 ± 1.01[f]	30.53 ± 1.44	56.15 ± 3.08	92.59 ± 5.05
Week 31	BLOQ	21.69 ± 1.05	42.73 ± 1.90	75.21 ± 4.48	153.20 ± 7.51
Week 53	BLOQ	26.93 ± 1.34	47.90 ± 1.91	94.58 ± 8.24	198.00 ± 13.15
Week 105	BLOQ	45.55 ± 2.93	85.94 ± 6.29	153.80 ± 11.13	452.75 ± 125.78[c]

[a] Data are given in pg/g tissue (fat, liver, lung) or pg/mL (blood) as the mean ± standard error. Mean values do not include values that were below the experimental limit of quantitation. BLOQ=below the limit of quantitation; LOQ_{fat}=50 pg/g, LOQ_{liver}=10 pg/g, LOQ_{lung}=25 pg/g, LOQ_{blood}=10 pg/mL.
[b] n=4
[c] n=8
[d] n=1
[e] n=7
[f] n=9

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, lung, pancreas, adrenal cortex, oral mucosa, uterus, thymus, ovary, kidney, heart, bone marrow, urinary bladder, mesentery, thyroid gland, mammary gland, and pituitary gland (pars distalis). Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A.

Liver: Relative liver weights were significantly increased in all dosed groups of rats at 14, 31, and 53 weeks (Table B1). Absolute liver weights were significantly increased in 100 ng TEQ/kg rats at 14 weeks, in all dosed groups at 31 weeks, and in 46 and 100 ng TEQ/kg rats at 53 weeks.

At the 14-week interim evaluation, changes in the liver consisted of increased incidences of hepatocyte hypertrophy which occurred in all dosed groups and tended to correlate with increased liver weight (Tables 12 and A5). Single incidences of diffuse fatty change and multiple mixed cell focus were seen in the 46 and 100 ng TEQ/kg groups of rats, respectively.

At 31 weeks, increased incidences and/or severities of hepatocyte hypertrophy, pigmentation, and inflammation occurred in all dosed groups (Tables 12 and A5). Increased incidences of multinucleated hepatocytes and mixed cell focus were seen in the 46 and 100 ng TEQ/kg groups. Single incidences of diffuse fatty change and eosinophilic focus were seen in the 100 ng TEQ/kg group.

At 53 weeks, increased incidences and/or severities of hepatocyte hypertrophy, pigmentation, and mixed cell focus occurred in all dosed groups (Tables 12 and A5). The incidences of focal and diffuse fatty change were increased in the 100 ng TEQ/kg group. Increased incidences of multinucleated hepatocytes occurred in rats administered 22 or 100 ng TEQ/kg. The incidence of bile duct hyperplasia was significantly increased in 100 ng TEQ/kg rats, and single incidences of nodular hyperplasia and bile duct fibrosis were seen in the 100 ng TEQ/kg group. The incidences of toxic hepatopathy were increased in the 46 and 100 ng TEQ/kg groups.

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

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
14-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Hepatocyte, Hypertrophy[a]	0	1 (1.0)[b]	3 (1.0)	5* (1.2)	8** (1.4)
Fatty Change, Diffuse	0	0	0	1 (1.0)	0
Mixed Cell Focus, Multiple	0	0	0	0	1
31-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Hepatocyte, Hypertrophy	0	3 (1.0)	5* (1.0)	9** (1.3)	10** (1.4)
Hepatocyte, Multinucleated	0	0	0	1 (1.0)	8** (1.1)
Pigmentation	0	4* (1.0)	9** (1.1)	10** (1.6)	10** (1.6)
Inflammation	8 (1.0)	10 (1.0)	10 (1.1)	10 (1.3)	10 (1.6)
Mixed Cell Focus (includes multiple)	6	4	5	8	8
Fatty Change, Diffuse	0	0	0	0	1 (1.0)
Eosinophilic Focus	0	0	0	0	1
53-Week Interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Hepatocyte, Hypertrophy	0	5* (1.2)	7** (1.4)	8** (1.5)	8** (2.5)
Hepatocyte, Multinucleated	0	0	3 (1.0)	0	8** (1.8)
Pigmentation	0	4* (1.0)	8** (1.3)	8** (1.0)	8** (2.0)
Mixed Cell Focus (includes multiple)	3	4	7	6	7
Fatty Change, Focal	0	0	0	3 (1.0)	4* (1.3)
Fatty Change, Diffuse	0	0	0	0	2 (1.0)
Bile Duct, Hyperplasia	0	0	0	0	6** (1.0)
Hyperplasia, Nodular	0	0	0	0	1
Bile Duct, Fibrosis	0	0	0	0	1 (1.0)
Toxic Hepatopathy	0	0	0	3 (1.3)	8** (1.6)

* 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 2 years, dose-related increased incidences of cholangiocarcinoma were seen in groups administered 22 ng TEQ/kg or greater, and the incidences of this lesion were significantly increased in the 46 and 100 ng TEQ/kg groups (Tables 13, A1, and A3). The incidences of cholangiocarcinoma in groups administered 22 ng TEQ/kg or greater exceeded the historical control range (Tables 13 and A4a). The incidence of hepatocellular adenoma was significantly increased in the 100 ng TEQ/kg group and exceeded the historical control range.

Cholangiocarcinoma consisted of an irregular, relatively large, noncircumscribed lesion that replaced normal liver parenchyma. The lesion was characterized by fibrous connective tissue stroma containing numerous atypical bile ducts, which frequently contained mucinous material and cellular debris. The epithelium forming the atypical bile ducts was often discontinuous, consisted of large atypical cells, and displayed degenerative changes. Mitotic figures and localized invasion of adjacent liver parenchyma were also observed (Plates 1 and 2). The incidences of cholangiofibrosis increased

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Number Examined Microscopically	53	53	53	53	51
Hepatocyte, Hypertrophy[a]	1 (1.0)[b]	27** (1.1)	34** (1.2)	46** (1.5)	50** (3.0)
Hepatocyte, Multinucleated	0	12** (1.0)	10** (1.2)	39** (1.5)	51** (2.1)
Pigmentation	4 (1.5)	35** (1.3)	41** (1.2)	48** (1.9)	51** (2.2)
Inflammation	36 (1.1)	50** (1.1)	45* (1.2)	50** (1.3)	50** (1.3)
Fatty Change, Diffuse	3 (2.3)	5 (1.0)	14** (1.0)	34** (1.1)	36** (1.3)
Bile Duct, Hyperplasia	2 (1.5)	3 (1.3)	5 (1.4)	25** (1.4)	42** (1.9)
Oval Cell, Hyperplasia	0	1 (1.0)	1 (1.0)	26** (1.4)	42** (2.2)
Hyperplasia, Nodular	0	1	3	11**	38**
Eosinophilic Focus (includes multiple)	5	9	11	20**	19**
Cholangiofibrosis	0	2 (1.0)	3 (2.0)	4 (2.5)	17** (2.3)
Bile Duct, Cyst	1 (3.0)	3 (2.7)	3 (2.3)	4 (2.3)	9** (2.0)
Necrosis	3 (1.7)	1 (2.0)	9 (2.2)	3 (1.7)	15** (2.2)
Portal, Fibrosis	0	0	0	0	11** (1.9)
Mixed Cell Focus (includes multiple)	21	32	35*	36**	17
Toxic Hepatopathy	0	5* (1.0)	14** (1.0)	38** (1.6)	47** (3.1)
Hepatocellular Adenoma, Multiple	0	0	0	0	2
Hepatocellular Adenoma (includes multiple)[c]					
Overall rate[d]	0/53 (0%)	1/53 (2%)	1/53 (2%)	1/53 (2%)	11/51 (22%)
Adjusted rate[e]	0.0%	2.5%	2.4%	2.5%	31.0%
Terminal rate[f]	0/16 (0%)	1/23 (4%)	1/24 (4%)	1/23 (4%)	2/8 (25%)
First incidence (days)	—[h]	729 (T)	729 (T)	729 (T)	434 (T)
Poly-3 test[g]	P<0.001	P=0.520	P=0.526	P=0.517	P<0.001
Cholangiocarcinoma, Multiple	0	0	0	2	4
Cholangiocarcinoma (includes multiple)[i]					
Overall rate	0/53 (0%)	0/53 (0%)	2/53 (4%)	7/53 (13%)	9/51 (18%)
Adjusted rate	0.0%	0.0%	4.8%	17.4%	26.0%
Terminal rate	0/16 (0%)	0/23 (0%)	1/24 (4%)	5/23 (22%)	1/8 (13%)
First incidence (days)	—	—	669	521	548
Poly-3 test	P<0.001	—[j]	P=0.268	P=0.011	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (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. 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] Not applicable; no neoplasms in animal group
[i] Historical incidence: 0/371
[j] Value of statistic cannot be computed.

with increasing dose, and the incidence was significantly increased in the 100 ng TEQ/kg group. Cholangiofibrosis appeared similar to cholangiocarcinoma but was a much smaller, well demarcated lesion which did not show evidence of localized invasion (Plate 3).

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 in size or slightly larger than normal and were arranged in abnormal lobular patterns. The hepatic cords within an adenoma usually intersected the surrounding normal hepatic cords at an oblique angle or sometimes even at a right angle. A few small proliferating bile ducts or oval cells were sometimes seen but were not as numerous as in nodular hyperplasia. The uniform population of relatively normal sized, somewhat pleomorphic hepatocytes that were arranged in abnormal lobular patterns and the lack of proliferating bile ducts were important features differentiating adenoma from nodular hyperplasia.

At 2 years, the incidences of hepatocyte hypertrophy, multinucleated hepatocytes, pigmentation, inflammation, and diffuse fatty change were generally significantly greater than those in the vehicle controls and the severities of these lesions generally increased with increasing dose (Tables 13 and A5). The incidences of bile duct hyperplasia, oval cell hyperplasia, nodular hyperplasia, and eosinophilic focus were significantly increased in the 46 and 100 ng TEQ/kg groups, and the incidences of bile duct cyst, necrosis, and portal fibrosis were significantly increased in the 100 ng TEQ/kg group. Increased incidences of mixed cell focus were seen in all treated groups except the 100 ng TEQ/kg group. Dose-related increased incidences and severities of toxic hepatopathy occurred in all dosed groups.

Hepatocyte hypertrophy was characterized by hepatocytes that were enlarged with increased amounts of eosinophilic cytoplasm. Minimal hypertrophy affected periportal hepatocytes, and as severity increased, hepatocytes in other areas of the hepatic lobule were also affected. The hypertrophy usually was not confined to periportal hepatocytes, and therefore the general diagnosis of hepatocyte hypertrophy was used. Pigmentation consisted of light brown to golden pigment present within macrophages and occasionally hepatocytes. The pigmented macrophages were often seen in portal areas but were also seen scattered randomly within the liver. The pigment was shown to stain positive for iron with Perl's stain. Inflammation was generally a minor change consisting of accumulation of mononuclear cells (predominantly lymphocytes and plasma cells with occasional macrophages) most often within portal areas but also sometimes scattered randomly throughout the liver. 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.

Diffuse fatty change was generally a minimal to mild change consisting of discrete clear vacuoles (consistent with lipid) in the cytoplasm of hepatocytes involving foci of hepatocytes scattered diffusely throughout the liver. Bile duct hyperplasia consisted of increased numbers of bile duct nuclei within portal areas. Oval cell hyperplasia consisted of small ovoid cells with basophilic cytoplasm and round to ovoid nuclei. The ovoid cells were arranged in single or double rows and located predominantly in the portal areas.

Nodular hyperplasia was characterized by areas of focal hypertrophy and hyperplasia of hepatocytes that also contained proliferating bile ducts and oval cells, and was considered to be the result 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. 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 seen in lower dose animals in which toxic changes were minimal to inapparent. This suggested that nodular hyperplasia resulted from the presence of a heptocellular proliferative stimulus that may have been independent of the toxic changes, but that the severity of the nodular hyperplasia was increased by toxicity.

Nodular hyperplasia was also characterized by few to numerous, small to large, nodular foci generally composed of hepatocytes that were considerably larger than normal hepatocytes (hepatocyte hypertrophy) sometimes mixed with areas of increased numbers of small hepatocytes (hepatocyte hyperplasia) (Plate 4). Areas of nodular hyperplasia sometimes blended with the surrounding parenchyma, although often they had a distinct border. Large, focal to multifocal areas of nodular hyperplasia were sometimes seen that caused compression of surrounding tissue, and/or bulging of the capsular surface (Plate 5). The cells within nodular hyperplasia

generally were very large, larger than cells seen within adenomas and usually larger than cells seen within foci, with abundant eosinophilic cytoplasm and often with variable degrees of cytoplasmic vacuolization. In a few areas of nodular hyperplasia, however, the cells were of more normal size or sometimes slightly smaller than normal. The cells appeared to be arranged in normal cords, but the cells often were so large as to obscure the sinusoids between the cords giving the appearance of solid sheets of hepatocytes (Plate 6). 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. The presence of hypertrophic, vacuolated hepatocytes together with proliferating biliary epithelium were considered to be characteristics useful in differentiating nodular hyperplasia from adenoma.

Eosinophilic and mixed cell foci appeared similar and were characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic foci were composed of cells with eosinophilic cytoplasm (Plate 7). Mixed cell focus was composed of a mixture of cells with different staining properties, generally a mixture of eosinophilic cells and cells with clear cytoplasm (clear cells) (Plate 8). To be classified as an eosinophilic focus at least 80% of the cells within the focus had to be eosinophilic cells. Otherwise the focus was classified as a mixed cell focus. If two or more foci were present in a liver, it was qualified as "multiple."

There was a significant increase in the incidence of hepatocellular adenoma in the 100 ng TEQ/kg group. Hepatocellular adenoma was not observed in controls, but was seen in all dosed groups. 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 hepatocytes and lack of proliferating biliary epithelium were important features useful in differentiating adenoma from nodular hyperplasia.

Foci in control animals consisted of hepatocytes that were generally somewhat larger than normal but appeared otherwise normal and were arranged in a relatively normal lobular pattern. The hepatic cords at the periphery of these foci generally merged imperceptibly with the surrounding liver resulting in an indistinct border and little or no compression of the adjacent liver parenchyma. In contrast, foci in treated animals often had a more definite border, the cords within the focus often were not smoothly continuous with those in the surrounding parenchyma, and the foci consisted of cells that were often prominently enlarged with abundant eosinophilic or clear vacuolated cytoplasm. In addition, some larger foci caused variable degrees of compression of the surrounding hepatic parenchyma. The cells were arranged in a relatively normal lobular pattern, and foci sometimes contained large blood vessels and/or portal areas. The presence of proliferating bile ducts or oval cells was not considered characteristic of a focus. If proliferating bile ducts were present, this was considered indicative of nodular hyperplasia, described later.

Bile duct cysts were characterized by either single or multiple dilated bile ducts that were lined by attenuated epithelium. Necrosis consisted of scattered necrotic areas of hepatic parenchyma that were often randomly distributed, but occasionally, in more severe cases, were distributed more diffusely. Portal fibrosis consisted of fibrous connective tissue accumulation that extended between adjacent portal areas.

Toxic hepatopathy included all nonneoplastic liver changes under one overall term. The severity of toxic hepatopathy was graded in order to give one overall severity grade for the degree of toxicity in a liver. This was to allow for easier comparison of the degree of toxic change among different dosed groups than would be possible if the severities of all the individual nonneoplastic changes were compared among the different groups. This diagnosis was used in addition to, not instead of, any of the nonneoplastic diagnoses already made. The changes included under the diagnosis included hepatocyte hypertrophy, pigmentation, inflammation, multinucleated hepatocytes, diffuse fatty change, bile duct hyperplasia, oval cell hyperplasia,

nodular hyperplasia, focal cellular alteration, cholangiofibrosis, bile duct cyst, necrosis, portal fibrosis, and centrilobular degeneration. Some dosed animals occasionally had just a few of these changes present, but this was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy.

Lung: At the 53-week interim evaluation, increased incidences of bronchiolar metaplasia of the alveolar epithelium occurred in all dosed groups (Tables 14 and A5).

At 2 years, the incidences of single and multiple cystic keratinizing epithelioma were significantly increased in the 100 ng TEQ/kg group (Tables 14, A1, and A3). The incidences in the 46 and 100 ng TEQ/kg groups exceeded the historical control range (Tables 14 and A4b). Cystic keratinizing epithelioma sometimes occurred singly but more commonly occurred as multiple lesions within the same lung. The neoplasm ranged from relatively small to very large lesions that replaced much of the normal lung parenchyma. The epitheliomas were cystic structures consisting of a highly irregular wall of highly keratinized stratified squamous epithelium and a center filled with keratin. The outer portion of the lesion grew by expansion into the adjacent lung, but evidence of invasion was not observed (Plate 9).

At 2 years, significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium occurred in all dosed groups (Tables 14 and A5). Increased incidences of squamous metaplasia occurred in the 46 and 100 ng TEQ/kg groups, and significantly decreased incidences of alveolar epithelial hyperplasia occurred in groups administered 22 ng TEQ/kg or greater. Bronchiolar metaplasia of the alveolar epithelium consisted of replacement of the normal alveolar epithelium by cuboidal to columnar, sometimes ciliated cells, and was often accompanied by abundant mucus production in the affected area (Plate 10). The lesion generally diffusely affected the epithelium located at the bronchiolar-alveolar junction and adjacent alveoli (Plate 11). Aggregates of large alveolar macrophages were sometimes present in areas of bronchiolar metaplasia. This change was differentiated from alveolar epithelial hyperplasia that was seen in vehicle control animals. In alveolar epithelial hyperplasia, alveoli were lined by bronchiolar epithelium and unlike bronchiolar metaplasia in treated animals, prominent mucus production was not observed in alveolar epithelial hyperplasia. Very prominent inflammatory cell infiltrate, consisting of large aggregates of alveolar macrophages commonly mixed with focal aggregates of neutrophils, was usually associated with the affected areas. Squamous metaplasia of the alveolar epithelium was generally a minor change consisting of one or more small, irregular foci of keratinizing stratified squamous epithelium that had replaced the normal alveolar epithelium. The incidence of histiocyte cellular infiltration was significantly increased in the 100 ng TEQ/kg group; this was considered to be a secondary lesion in the lung.

TABLE 14
Incidences of Selected Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
53-Week Interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Alveolar Epithelium, Metaplasia, Bronchiolar[a]	0	1 (1.0)[b]	2 (1.5)	4* (1.0)	6** (1.3)
2-Year Study					
Number Examined Microscopically	53	53	53	53	53
Alveolar Epithelium, Metaplasia, Bronchiolar	0	20** (1.8)	33** (1.6)	41** (1.8)	40** (1.9)
Metaplasia, Squamous	2 (2.0)	0	2 (2.0)	8 (2.0)	11** (1.9)
Alveolar Epithelium, Hyperplasia	21 (1.3)	25 (1.0)	10** (1.3)	2** (1.0)	2** (1.5)
Infiltration Cellular, Histiocyte	43 (1.9)	50 (1.9)	48 (1.6)	48 (1.6)	50* (1.4)
Cystic Keratinizing Epithelioma, Multiple	0	0	0	0	11**
Cystic Keratinizing Epithelioma (includes multiple)[c]					
Overall rate[d]	0/53 (0%)	0/53 (0%)	0/53 (0%)	2/53 (4%)	20/53 (38%)
Adjusted rate[e]	0.0%	0.0%	0.0%	5.1%	54.7%
Terminal rate[f]	0/16 (0%)	0/23 (0%)	0/24 (0%)	1/23 (4%)	5/8 (63%)
First incidence (days)	—[h]	—[i]	—	697	542
Poly-3 test[g]	P<0.001	—	—	P=0.256	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study)
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 0/370
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed.

Pancreas: At the 14-week interim evaluation, two incidences of chronic active inflammation and a single incidence of acinar atrophy occurred in the 46 and 100 ng TEQ/kg groups, respectively (Tables 15 and A5). At 31 weeks, acinar cytoplasmic vacuolization occurred only in the 100 ng TEQ/kg group, and the increase was significant. In addition, two incidences of acinar atrophy occurred in the 22 ng TEQ/kg group in contrast to single incidences of this lesion in the vehicle control and 10 ng TEQ/kg groups. At 53 weeks, increased incidences of acinar cytoplasmic vacuolization occurred in the 46 and 100 ng TEQ/kg groups; single incidences of acinar atrophy and chronic active inflammation also occurred in these groups.

At 2 years, incidences of acinar adenoma and acinar carcinoma were seen in all dosed groups except those administered 100 ng TEQ/kg (Tables 15 and A1). The incidence of acinar adenoma in the 22 ng TEQ/kg group and the incidences of acinar carcinoma in the 10 and 46 ng TEQ/kg groups exceeded the historical control ranges (Tables 15 and A4c). Adenoma of the acinar cells

TABLE 15
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
14-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Inflammation, Chronic Active[a]	0	0	0	2 (1.0)[b]	0
Acinus, Atrophy	0	0	0	0	1 (1.0)
31-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Acinus, Vacuolization Cytoplasmic	0	0	0	0	5* (1.0)
Acinus, Atrophy	1 (1.0)	1 (1.0)	2 (1.0)	0	0
53-Week Interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Acinus, Vacuolization Cytoplasmic	0	0	0	2 (1.0)	7** (1.6)
Acinus, Atrophy	0	0	0	1 (2.0)	1 (2.0)
Inflammation, Chronic Active	0	0	0	1 (3.0)	1 (1.0)
2-Year Study					
Number Examined Microscopically	52	53	53	53	51
Artery, Inflammation, Chronic Active	0	6* (2.2)	3 (1.7)	8** (2.6)	14** (2.9)
Acinus, Vacuolization Cytoplasmic	1 (1.0)	0	3 (1.3)	15** (1.0)	30** (1.1)
Acinus, Atrophy	3 (1.3)	2 (1.0)	7 (1.7)	7 (1.7)	20** (2.0)
Inflammation, Chronic Active	3 (1.7)	1 (3.0)	6 (1.3)	7 (1.7)	16** (1.8)
Duct, Dilatation	0	0	0	0	5* (3.0)
Acinus, Adenoma[c]	0	0	2	0	0
Acinus, Carcinoma[d]	0	1	0	2	0
Acinus, Adenoma or Carcinoma[c]					
Overall rate[e]	0/52 (0%)	1/53 (2%)	2/53 (4%)	2/53 (4%)	0/51 (0%)
Adjusted rate[f]	0.0%	2.5%	4.8%	5.1%	0.0%
Terminal rate[g]	0/16 (0%)	1/23 (4%)	2/24 (8%)	2/23 (9%)	0/8 (0%)
First incidence (days)	—[i]	729 (T)	729 (T)	729 (T)	—
Poly-3 test[h]	P=0.533N	P=0.522	P=0.270	P=0.259	—[j]

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 1/366 (0.3% ± 0.7%), range 0%-2%
[d] Historical incidence: 0/366
[e] Number of animals with neoplasm per number of animals with lung examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend is indicated by **N**.
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

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

At 2 years, increased incidences and severities of arterial chronic active inflammation occurred in all dosed groups (Tables 15 and A5). Dose-related increased incidences of acinar cytoplasmic vacuolization, acinar atrophy, and chronic active inflammation occurred in groups administered 22 ng TEQ/kg or greater. Increased severity of acinar atrophy was seen with increasing dose. Dilatation of the pancreatic duct occurred only in 100 ng TEQ/kg rats, and the incidence was significantly increased.

Chronic active inflammation of the pancreatic artery was a focal to multifocal change characterized by a thick mantle of macrophages, lymphocytes, and plasma cells around the arteries, with infiltration into the muscular layers of the artery. There was often fibrinoid necrosis of the vessel, and the tunica intima was frequently thickened. Endothelial cells were swollen or decreased in number. This inflammatory reaction often extended into the surrounding parenchyma. Acinar cytoplasmic vacuolation 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. Acinar atrophy was a focal to multifocal to diffuse change consisting of a reduction in the amount of acinar tissue with an associated increase in stromal fibrous connective tissue. Chronic active inflammation was generally associated with atrophy and consisted of an infiltrate of mononuclear cells with occasional neutrophils within the stroma. Duct dilatation was characterized by a markedly ectatic duct with a thickened wall infiltrated with a population of mixed inflammatory cells.

Adrenal Cortex: Hyperplasia occurred in groups administered 22 ng TEQ/kg or greater at the 53-week interim evaluation (Tables 16 and A5). Increased incidences of hypertrophy occurred in the 10, 22, and 100 ng TEQ/kg groups.

At 2 years, single incidences each of cortical adenoma and cortical carcinoma occurred in the 10 and 22 ng TEQ/kg groups, and these incidences were at the upper end of the historical control ranges (Tables 16, A1, A3, and A4d). Cortical adenoma was a large, discrete lesion that replaced glandular parenchyma and caused compression of the remaining normal tissue. Adenoma was distinguished from hypertrophy or hyperplasia by the fact that adenoma consisted of somewhat atypical cortical cells that were arranged in abnormal patterns, rather than consisting of normal-appearing cells arranged in the normal cord pattern as seen with hypertrophy and hyperplasia. Large adenomas replaced much of the gland and caused enlargement of the gland. In contrast, cortical carcinoma was larger than adenoma, and consisted of highly atypical cells arranged in highly abnormal patterns. Invasion through the capsule into adjacent tissue was also present. Carcinomas replaced much of the gland and caused enlargement of the gland.

At 2 years, the incidences of adrenal cortical hyperplasia were significantly increased in all dosed groups (Tables 16 and A5). Increased incidences of cortical cystic degeneration occurred in all dosed groups and significantly increased in the 46 ng TEQ/kg group. Significantly increased incidence and severity of cortical atrophy occurred in the 100 ng TEQ/kg group; three incidences of this lesion occurred in the 10 ng TEQ/kg group, but their relationship to treatment is unclear. The incidence of cortical cytoplasmic vacuolization was significantly increased in the 100 ng TEQ/kg group.

Cortical hyperplasia was a focal to multifocal change, generally located in the zona fasciculata, consisting of a discrete area containing increased numbers of cortical cells. The hyperplastic cells were the same size or somewhat smaller than surrounding normal cortical cells, and had slightly basophilic cytoplasm. In some cases, especially with large lesions, there was compression of the surrounding tissue. However, these were distinguishable as hyperplasia by the fact that the cells still formed normal cords, particularly in the upper zona fasciculata. 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 zona fasciculata and zona reticularis. Larger lesions compressed

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Cortex in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
53-Week Interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Hyperplasia[a]	0	0	2 (2.0)[b]	1 (1.0)	2 (2.0)
Hypertrophy	3 (1.7)	7 (1.0)	5 (1.0)	3 (1.0)	5 (1.6)
2-Year Study					
Number Examined Microscopically	52	53	53	53	51
Hyperplasia	12 (2.5)	26** (2.4)	23* (2.8)	25* (2.6)	21* (2.5)
Degeneration, Cystic	9 (2.4)	15 (2.3)	19 (2.3)	25** (2.3)	16 (2.1)
Atrophy	0	3 (2.3)	0	0	18** (2.7)
Cytoplasmic Vacuolization	6 (1.2)	13 (1.5)	11 (1.5)	7 (1.7)	15* (1.5)
Adenoma[c]	0	1	1	0	0
Carcinoma[c]	0	1	1	0	0

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± standard deviation): 2/369 (0.5% ± 0.9%), range 0%-2%

or replaced adjacent parenchyma. Some lesions were very large, replaced much of the gland, and caused enlargement of the gland. 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 vacuolation occurred most commonly within foci of hypertrophy. Cortical atrophy was a locally extensive to diffuse change characterized by loss of cortical epithelial cells within the zona fasciculata and zona reticularis with a subsequent reduction in cortical thickness. The zona glomerulosa was spared. The remaining cells were sometimes vacuolated, especially in the more severe lesions. In severe cases the entire cortex was considerably reduced in thickness resulting in a smaller gland that often was surrounded by a thickened capsule.

Oral Mucosa: At 2 years, two incidences of gingival squamous cell carcinoma occurred in the 100 ng TEQ/kg group in contrast to single incidences of this lesion in the vehicle control and 10 ng TEQ/kg groups. The incidence in the 100 ng TEQ/kg group exceeded the historical control range (Tables 17, A1, and A4e). Gingival 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. The gingival squamous cell carcinomas seen in the treated animals had a comparable structure and location to that seen in the vehicle control animals in the present study, and in vehicle control animals in other studies conducted as part of the dioxin TEF evaluation.

At 2 years, significantly increased incidences of gingival squamous hyperplasia occurred in all dosed groups (Tables 17 and A5). Gingival 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

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Oral Mucosa in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Number Necropsied	53	53	53	53	53
Gingival, Hyperplasia, Squamous[a]	8 (1.5)[b]	17* (1.5)	18* (1.4)	26** (1.6)	30** (1.6)
Squamous Cell Carcinoma[c]					
Overall rate[d]	1/53 (2%)	1/53 (2%)	0/53 (0%)	0/53 (0%)	2/53 (4%)
Adjusted rate[e]	2.7%	2.5%	0.0%	0.0%	6.0%
Terminal rate[f]	0/16 (0%)	0/23 (0%)	0/24 (0%)	0/23 (0%)	0/8 (0%)
First incidence (days)	668	563	—[h]	—	434
Poly-3 test[g]	P=0.261	P=0.737N	P=0.475N	P=0.484N	P=471

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups (mean ± 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. 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] Not applicable; no neoplasms in animal group

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 gingival squamous hyperplasia and gingival squamous cell carcinoma.

Uterus: At 2 years, a single incidence of squamous cell carcinoma occurred in the 22 ng TEQ/kg group (0/52, 0/53, 1/53, 0/53, 0/51; Table A1). 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. A single case of squamous cell papilloma was seen in the vehicle control group. One uterine squamous cell carcinoma was seen in 371 current historical vehicle control females.

At 2 years, non-dose-related increased incidences of squamous metaplasia occurred in all dosed groups, and the survival-adjusted incidences were significantly increased in the 46 and 100 ng TEQ/kg groups (Tables 18 and A5). The significance of this finding was unclear. Squamous metaplasia was generally a minimal to mild, multifocal change consisting of tubular structures within the endometrium that were lined by stratified squamous epithelium.

Thymus: The thymus weights of 46 and 100 ng TEQ/kg rats were significantly decreased at 14 weeks (Table B1). Increased incidences of atrophy occurred in all dosed groups at the 14-, 31-, and 53-week interim evaluations except in the 10 ng TEQ/kg group at 31 weeks

TABLE 18
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
14-Week Interim Evaluation					
Thymus[a]	10	10	10	10	10
Atrophy[b]	0	2 (1.0)[c]	3 (1.0)	4* (1.0)	7** (1.1)
Ovary	10	10	10	10	10
Atrophy	0	1 (3.0)	2 (3.0)	3 (2.7)	4* (3.5)
31-Week Interim Evaluation					
Thymus	10	10	10	10	9
Atrophy	0	0	3 (1.3)	7** (1.9)	7** (2.1)
53-Week Interim Evaluation					
Thymus	8	8	8	8	6
Atrophy	3 (2.0)	7 (1.7)	8* (2.0)	8* (3.4)	6* (3.7)
2-Year Study					
Uterus	52	53	53	53	51
Metaplasia, Squamous	21 (2.0)	32 (2.2)	32 (2.3)	35* (2.4)	30* (2.4)
Thymus	52	48	50	53	50
Atrophy	32 (2.3)	43* (2.9)	45** (3.3)	50** (3.7)	48** (3.9)
Ovary	52	52	53	53	51
Inflammation, Chronic Active	0	2 (3.0)	1 (4.0)	2 (3.5)	5* (3.6)
Kidney	52	53	53	53	51
Nephropathy	26 (1.1)	41** (1.2)	40** (1.3)	47** (1.3)	49** (2.1)
Transitional Epithelium, Hyperplasia	5 (2.0)	5 (1.8)	8 (1.9)	10 (2.0)	8 (2.5)
Heart	53	53	53	52	53
Cardiomyopathy	11 (1.0)	26** (1.0)	31** (1.1)	30** (1.1)	32** (1.1)
Bone Marrow	53	53	53	53	53
Hyperplasia	36 (3.1)	36 (2.8)	34 (2.7)	41 (3.0)	48** (3.0)
Urinary Bladder	52	52	53	53	50
Transitional Epithelium, Hyperplasia	0	0	1 (2.0)	3 (2.7)	4* (1.8)
Mesentery	52	53	53	53	51
Artery, Inflammation, Chronic Active	0	1 (4.0)	0	3 (3.7)	6* (3.7)

* 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 except mesentery is number necropsied.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

(Tables 18 and A5). At 2 years, atrophy occurred in all groups including the vehicle controls, and the incidences were significantly increased in all dosed groups. The severity of this lesion increased with increasing dose. Atrophy consisted of varying degrees of loss of lymphoid cells from the cortex resulting in reduction of cortical thickness.

Ovary: At 14 weeks and 2 years, respectively, increased incidences of moderate to marked atrophy and moderate to marked chronic active inflammation occurred, and the incidences were significantly increased in the 100 ng TEQ/kg group (Tables 18 and A5). Atrophy was characterized by overall reduction in size and absence of ovarian structures, primarily corpora lutea, but also lack of follicles in some cases. Chronic active inflammation consisted of encapsulated abscesses. In some cases, the inflammation extended into adjacent tissue.

Kidney: At 2 years, significantly increased incidences of nephropathy occurred in all dosed groups (Tables 18 and A5). Severity was increased in the 100 ng TEQ/kg group. Increased incidences of transitional epithelial hyperplasia occurred in groups administered 22 ng TEQ/kg or greater. Nephropathy was generally a minimal to mild change, although sometimes moderate to marked nephropathy was seen. The appearance of this lesion was typical of that seen in aging rats, and was similar to that observed in 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. Transitional epithelial hyperplasia was sometimes focal to multifocal, but generally a diffuse, usually minimal to mild change consisting of varying degrees of thickening of the renal pelvic or papillary epithelium up to approximately 1.5 to 2 times the normal thickness. The significance of this change was unclear; it did not appear to correlate with the increased severity of nephropathy, since the animals with hyperplasia often had minimal nephropathy.

Heart: At 2 years, significantly increased incidences of cardiomyopathy occurred in all dosed groups (Tables 18 and A5). Cardiomyopathy had the typical microscopic appearance of this lesion seen in aging rats and appeared similar to the cardiomyopathy seen in aging F344/N rats (MacKenzie and Alison, 1990). It was a multifocal, generally minimal to mild lesion consisting of hyper-eosinophilic myofibers that lacked cross striations, infiltrates of mononuclear cells, separation of myofibers by myxomatous material (bluish material on H&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.

Bone Marrow: At 2 years, the incidence of hyperplasia was significantly increased in the 100 ng TEQ/kg group (Tables 18 and A5). Bone marrow hyperplasia was graded as follows: grade 4 (marked) was used when the entire marrow cavity was filled with dense marrow. Grade 3 (moderate) hyperplasia was recorded when marrow elements composed about 90% of the cavity (the remaining 10% was fat). Grade 2 (mild) hyperplasia was recorded when marrow elements composed approximately 60% to 90% of the marrow cavity, and grade 1 (minimal) hyperplasia was rarely recorded because of the normal variation in the amount of bone marrow. Normal bone marrow was diagnosed when the distal end of the femur section contained 20% to 60% marrow.

Urinary Bladder: At 2 years, the incidence of transitional epithelial hyperplasia was significantly increased in the 100 ng TEQ/kg group (Tables 18 and A5). Transitional epithelial hyperplasia was characterized by diffuse hypercellularity and thickening of the transitional epithelium up to approximately three to four times normal thickness. It was noted that in nearly every case inflammation was present with the hyperplasia and it appeared that the hyperplasia was secondary to the inflammation. Hyperplasia of the transitional epithelium of the urinary bladder often occurred in animals that also had hyperplasia of the transitional epithelium of the renal pelvis.

Mesentery: At 2 years, the incidence of marked chronic active inflammation of the mesenteric artery was significantly increased in the 100 ng TEQ/kg group (Tables 18 and A5). This change in the mesenteric artery (Plate 12) appeared similar to that seen in the pancreatic arteries.

Thyroid Gland: At the 14-week interim evaluation, the incidences of follicular cell hypertrophy were significantly increased in groups administered 22 ng TEQ/kg or greater (Tables 19 and A5). At the 31-week interim

evaluation, the incidences of this lesion were increased in the 10, 46, and 100 ng TEQ/kg groups but there was no clear dose-related pattern. At the 53-week interim evaluation, the severity of follicular cell hypertrophy was increased in the 100 ng TEQ/kg group. At 2 years, the incidences of follicular cell hypertrophy were increased in all dosed groups. Follicular cell hypertrophy was a localized to diffuse change, characterized by follicles that were decreased in size and contained decreased amounts of colloid in which aggregates of amphophilic, flocculant appearing material were often present. The affected follicles were lined by large, prominent cuboidal follicular epithelial cells that were approximately two to three times normal size, usually with abundant pale cytoplasm containing small, clear, vacuoles (Plates 13 and 14). Since some degree of this change can occur spontaneously, the severity grade of minimal was recorded when 50% to 60% of the follicles were involved, mild when 60% to 75% of the follicles were involved, moderate when 75% to 90% of the follicles were involved, and marked when over 90% of the follicles were involved. Significantly decreased incidences of thyroid gland (C-cell) adenoma occurred in the 10, 46, and 100 ng TEQ/kg groups.

Mammary Gland and Pituitary Gland (pars distalis): At 2 years, the incidences of mammary gland fibroadenoma were significantly decreased in groups administered 10 and 100 ng TEQ/kg (vehicle control, 34/53; 10 ng TEQ/kg, 27/53; 22 ng TEQ/kg, 35/53; 46 ng TEQ/kg, 28/53; 100 ng TEQ/kg, 18/53; Table A3). The incidence of pituitary gland (pars distalis) adenoma was significantly decreased in the 100 ng TEQ/kg group at 2 years (28/53, 29/53, 22/53, 20/53, 10/53; Table A3).

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
14-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Follicular Cell, Hypertrophy[a]	0	3 (1.0)[b]	7** (1.0)	10** (1.4)	7** (1.4)
31-Week Interim Evaluation					
Number Examined Microscopically	10	10	10	10	10
Follicular Cell, Hypertrophy	1 (1.0)	5 (1.8)	1 (1.0)	4 (2.0)	3 (1.0)
53-Week Interim Evaluation					
Number Examined Microscopically	8	8	8	8	8
Follicular Cell, Hypertrophy	2 (1.0)	2 (1.5)	3 (1.0)	3 (1.7)	4 (2.3)
2-Year Study					
Number Examined Microscopically	53	53	51	52	51
Follicular Cell, Hypertrophy	4 (1.5)	13* (1.1)	12 (1.5)	18** (1.7)	23** (1.9)
C-Cell Adenoma, Bilateral	2	2	3	1	0
C-Cell Adenoma (includes bilateral)					
Overall rate[c]	17/53 (32%)	10/53 (19%)	18/51 (35%)	9/52 (17%)	5/51 (10%)
Adjusted rate[d]	44.4%	24.5%	42.7%	22.2%	14.7%
Terminal rate[e]	7/16 (44%)	6/23 (26%)	10/24 (42%)	6/23 (26%)	0/8 (0%)
First incidence (days)	568	627	617	548	542
Poly-3 test[f]	P=0.005N	P=0.045N	P=0.531N	P=0.027N	P=0.004N

* 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 lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 3=moderate, 4=marked
[c] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by **N**.

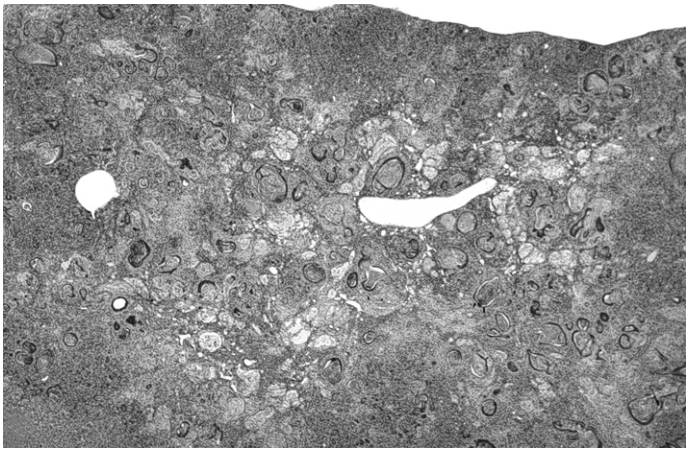


PLATE 1
Cholangiocarcinoma in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. In contrast to cholangiofibrosis, cholangiocarcinoma is larger in size and widely invasive of the hepatic parenchyma. H&E; 5x

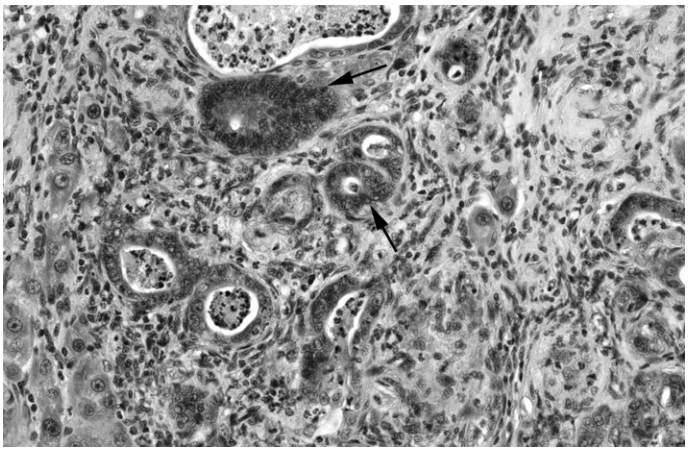


PLATE 2
Higher magnification of Plate 1. Note that the lesion consists of fibrous connective tissue stroma containing numerous atypical bile ducts (arrows). H&E; 66x

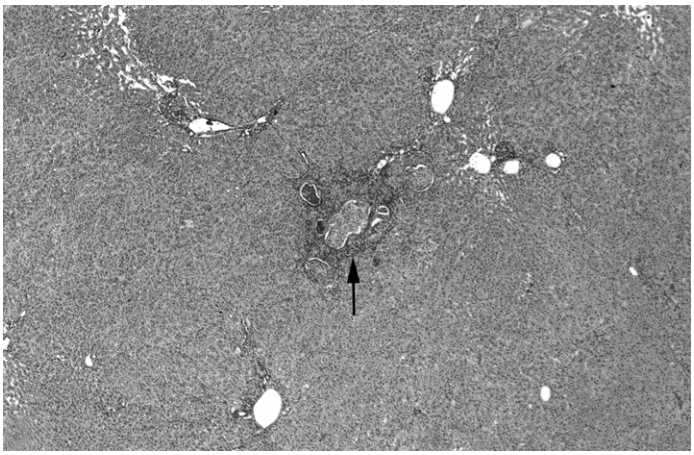


PLATE 3
Cholangiofibrosis (grade 2) in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the well-circumscribed lesion (arrow). H&E; 6.6x

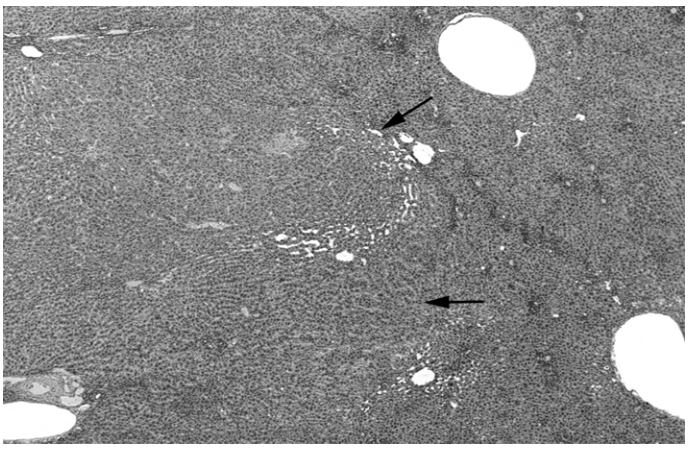


PLATE 4
Nodular hyperplasia (arrows) in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the presence of multiple, small to large, nodular foci composed of hepatocytes (arrows). H&E; 6.6x

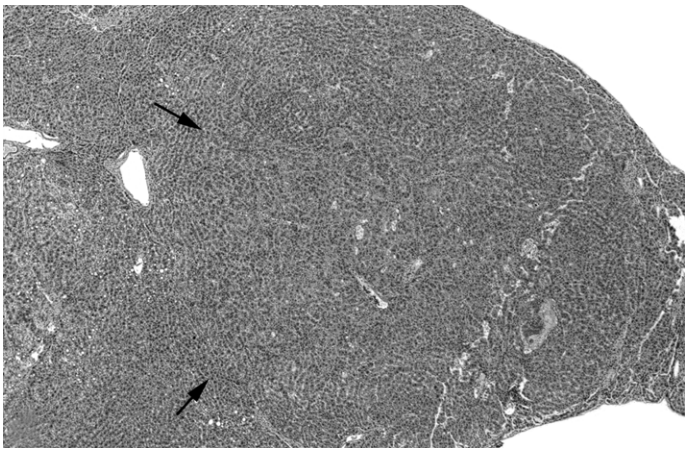


Plate 5
Nodular hyperplasia in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the nodular mass that is larger than a focus, having a distinct border (arrows), and producing compression of surrounding normal parenchyma. H&E; 6.6x

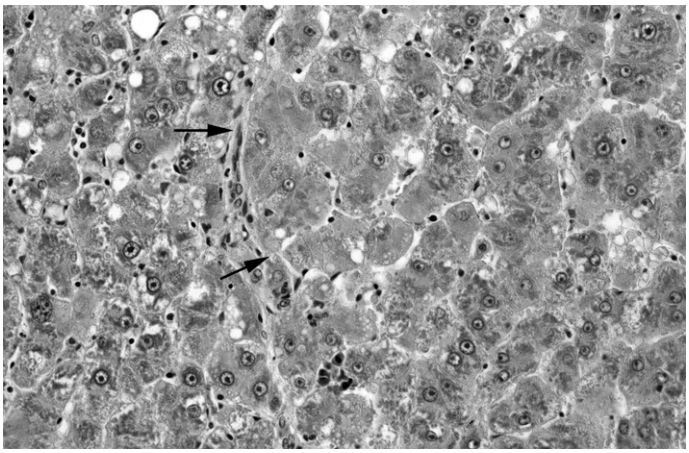


Plate 6
Nodular hyperplasia (arrows) in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the presence of a distinct border, producing compression of surrounding normal parenchyma. The cells are large and arranged in normal cords, giving the appearance of solid sheets of hepatocytes. H&E; 66x

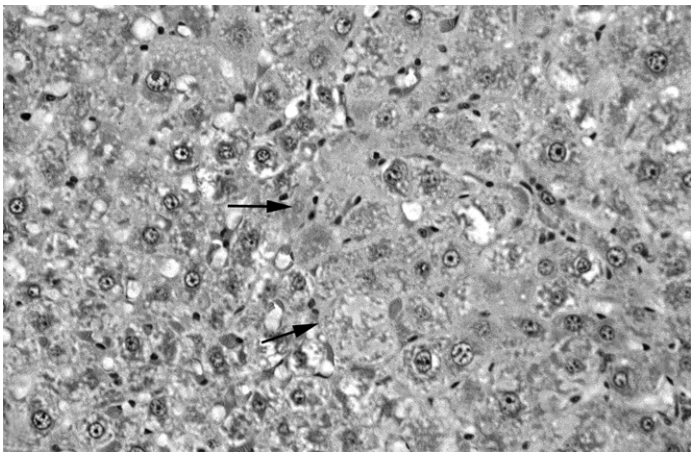


Plate 7
Eosinophilic focus (arrows) in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note that the hepatocytes within foci are generally larger, having increased amounts of ground glass-appearing cytoplasm. H&E; 66x

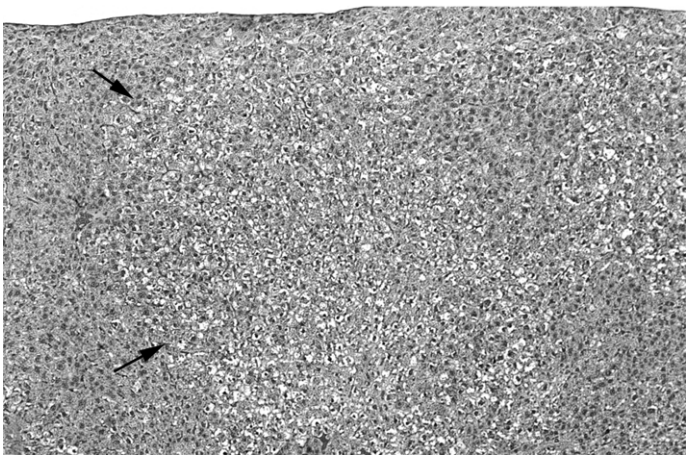


Plate 8
Mixed cell focus (arrows) in the liver of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note that the hepatocytes are composed of a mixture of cells, including cells with clear cytoplasm (clear cells). The margins of the focus are distinct, but the hepatic cords merge imperceptibly with the surrounding hepatic cords. H&E; 16x

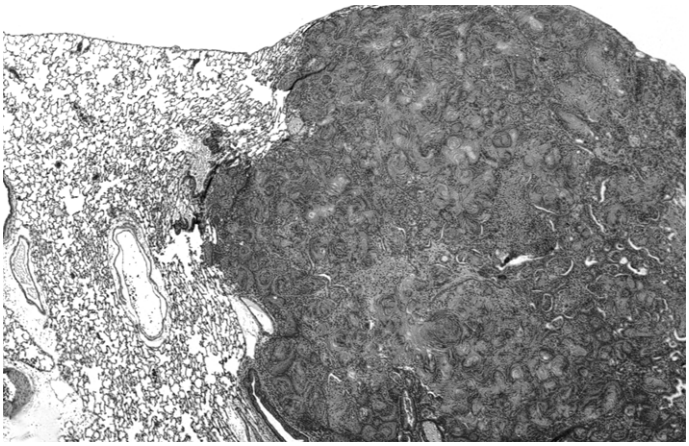


PLATE 9
Cystic keratinizing epithelioma in the lung of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the cystic structure consisting of an irregular wall of highly keratinized stratified squamous epithelium and a center filled with keratin. The outer portion of the lesion grows by expansion into the adjacent lung, but there is no evidence of invasion. H&E; 6.6x

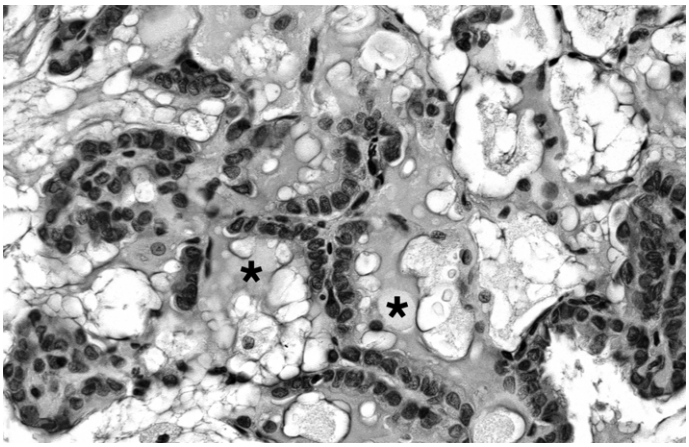


PLATE 10
Alveolar epithelial-bronchiolar metaplasia in the lung of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the replacement of the normal alveolar epithelium by cuboidal to columnar, sometimes ciliated cells, accompanied by abundant mucus production (asterisks) in the affected area. H&E; 100x

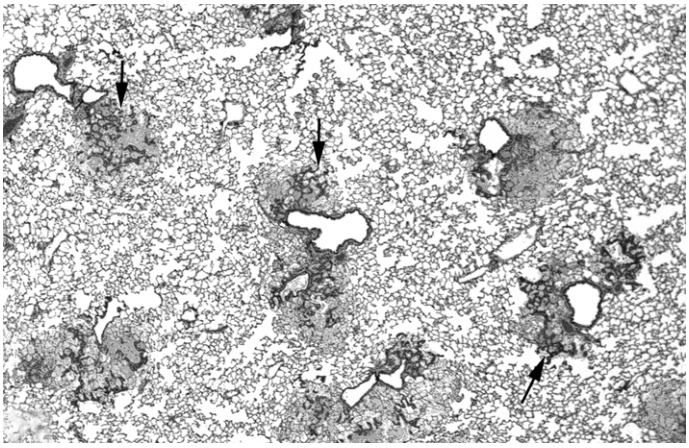


PLATE 11
Alveolar epithelial-bronchiolar metaplasia (arrows) in the lung of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the multiple foci of this change, located at the bronchiolar-alveolar junction and adjacent alveoli. H&E; 5x

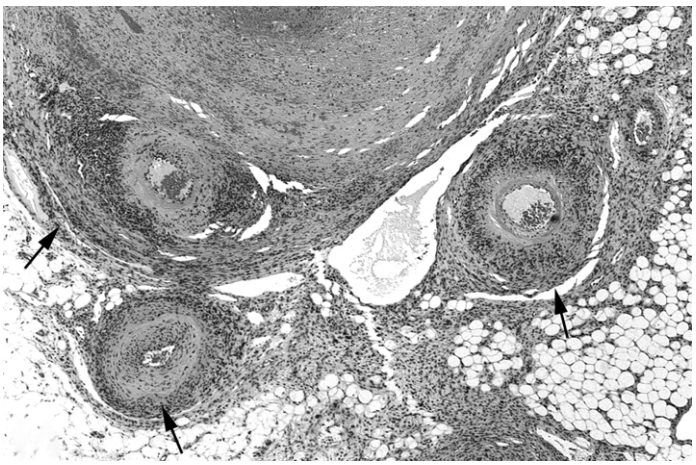


PLATE 12
Chronic active inflammation in multiple mesenteric arteries of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note the presence of a thick mantle of inflammatory infiltrate around the arteries (arrows), fibrinoid necrosis of the vessels, and the presence of thrombosis in one of the affected vessels. H&E; 13.2x

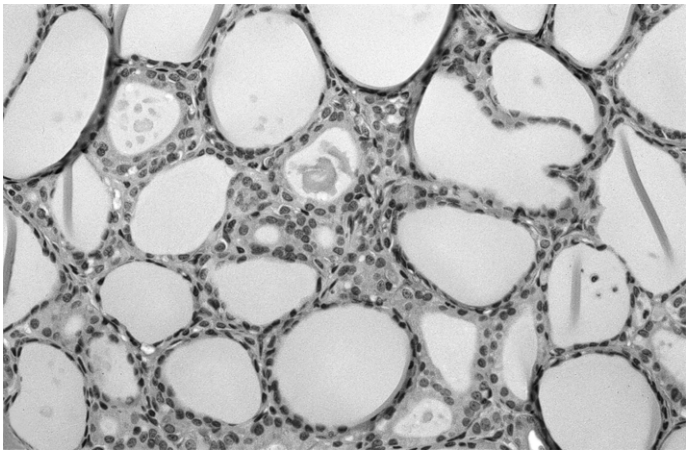


PLATE 13

Normal aspect of thyroid gland follicles in a control female rat from the 2-year TEF mixture study. Note that the follicles are distended with homogeneous colloid and the lining epithelium is flattened. H&E; 66x

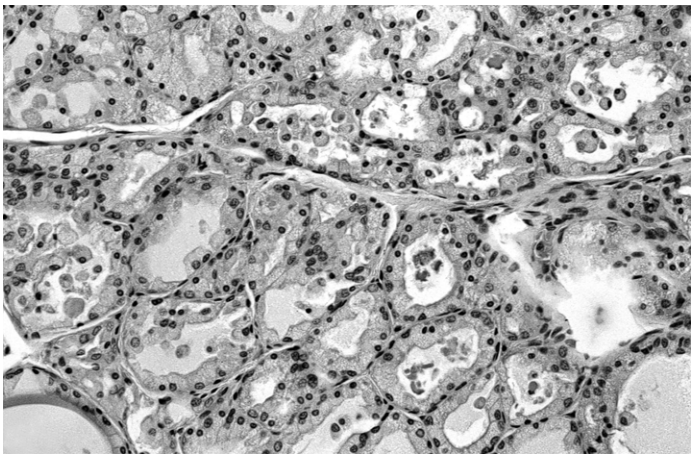


PLATE 14

Follicular hypertrophy in the thyroid gland of a female rat administered 100 ng TEQ/kg TEF mixture by gavage for 2 years. Note that the follicles are smaller in size, lined by cuboidal epithelium and contain decreased amounts of colloid in which aggregates of amphophilic, flocculant-appearing material are present (compare with Plate 9). H&E; 66x

DISCUSSION AND CONCLUSIONS

This 2-year study of the chronic toxicity and carcinogenicity of a mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) [henceforth referred to as the toxic equivalency factor (TEF) mixture] in female Harlan Sprague-Dawley rats is one in a series of studies carried out as part of the dioxin TEF evaluation 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 TEF evaluation was an analysis of the comparative carcinogenicity of TCDD, PeCDF, and PCB 126, in this Technical Report only the results of the TEF mixture toxicology and carcinogenicity study are described and where appropriate a qualitative comparison to neoplastic responses seen in the gavage study of TCDD (NTP, 2006a) conducted as part of the dioxin TEF evaluation. A quantitative analysis of the effects observed in this study to responses observed with other compounds studied as part of the dioxin TEF evaluation are presented elsewhere (Toyoshiba *et al.*, 2004; Walker *et al*., 2005).

The TEF mixture was designed so that the total TCDD toxic equivalents (TEQ) would be the same as those used in the TCDD study (NTP, 2006a) conducted as part of the dioxin TEF evaluation (10, 22, 46, and 100 ng TEQ/kg). The mass ratio of TCDD, PeCDF, and PCB126 in the mixture was 1:2:10. This was such that each contributed one-third of the total TEQ of the mixture, calculated using the current World Health Organization (WHO) TEFs of 1.0 for TCDD, 0.1 for PCB 126, and 0.5 for PeCDF. Dose selection in the range of 10 to 100 ng TEQ/kg for this study was based on prior observations made in a 2-year dosed-feed study of TCDD conducted by Kociba *et al*. (1978). In that study, Spartan Sprague-Dawley rats were exposed to doses of 1, 10, and 100 ng/kg body weight per day; increased incidences of liver neoplasms were observed at 10 and 100 ng/kg.

In the present study, there was no significant effect on survival in any dosed group. Daily administration of doses above 22 ng TEQ/kg led to a reduction in body weight gain over the course of the 2-year study with a greater magnitude of reduction observed with increasing dose and over time. Reduction in body weight gain is a characteristic toxic response to treatment with DLCs. The reduction in body weight gain seen in the present study was comparable to that observed in the dioxin TEF evaluation TCDD study (NTP, 2006a) where the most pronounced reduction in body weight gain occurred in the 100 ng/kg group.

The principal findings of this study were significantly increased incidences of cystic keratinizing epithelioma (CKE) in the lung and cholangiocarcinoma and hepatocellular adenoma in the liver. In addition, marginally increased incidences of neoplasms of the pancreatic acinus may have been related to administration of the TEF mixture. Treatment-related increased incidences of cystic keratinizing epithelioma, cholangiocarcinoma, and hepatocellular adenoma were all observed in the TCDD, PeCDF, and PCB 126 studies (NTP, 2006a,b,c) conducted as part of the dioxin TEF evaluation.

The principal nonneoplastic finding in this study was significant hepatotoxicity. In addition, numerous organs exhibited increased incidences of nonneoplastic lesions, notably in the lung, pancreas, adrenal cortex, oral mucosa, uterus, thymus, ovary, kidney, heart, bone marrow, urinary bladder, mesentery, and thyroid gland.

Chronic exposure led to significant accumulation of TCDD, PCB 126, and PeCDF in the liver, fat, and lung and detectable levels in blood. The significant accumulation in fat is consistent with the lipophilic nature of these compounds. Previous studies of DLCs indicate that the liver and fat are the main depots for DLCs in rodents and together comprise approximately 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 that is inducible by DLCs in the liver (Diliberto *et al*., 1997). Using the WHO TEFs, liver burden on a total TEQ basis for the 10 and 100 ng TEQ/kg doses were 16 and 117 ng TEQ/g, respectively at the end of the 2-year study. The relative contribution of each compound to the total TEQ in the 100 ng TEQ/kg group (117 ng TEQ/g) was 9% for TCDD (11 ng/g), 22% for PCB 126 (26 ng TEQ/g), and 69% for PeCDF (81 ng TEQ/g). Because CYP1A2 is

responsible for the hepatic sequestration of DLCs, the observed difference in relative proportion of each compound (on a TEQ basis) in the liver compared to the proportion in the administered materials is likely due to relative differences in binding of DLCs to CYP1A2.

By comparison, in the Kociba *et al*. (1978) study, terminal liver TCDD levels were 5.1 ng/g at the 10 ng/kg per day dose and 24 ng/g at the 100 ng/kg per day dose. By comparison, terminal liver levels in the NTP (2006a) TCDD study were 2 ng/g and 9 ng/g at the 10 ng/kg and 100 ng/kg groups, respectively. In the present study therefore, the 10 and 100 ng TEQ/kg groups had liver TEQ levels that were 3- and 5-fold higher, respectively, than the TEQ levels seen in the Kociba *et al.* (1978) study and 8- and 13-fold higher, respectively compared to the NTP (2006a) TCDD study. The high TEQ levels in the present study compared to the NTP (2006a) study are mainly due to the significant accumulation of PeCDF. PeCDF accounts for almost 70% of the total TEQ in the livers in this study. In the NTP (2006b) PeCDF study, the TEQ levels of PeCDF were also significantly higher than expected based on the levels observed in the TCDD study.

Levels of TCDD, PeCDF, and PCB 126 were measurable in tissues of vehicle control animals. All experimental treatments are made on top of a background of exposure to DLCs that are present in feed; therefore, the vehicle control group exposure is not zero (Vanden Heuvel *et al*., 1994; Feeley and Jordan, 1998; Jordan and Feeley, 1999). However, given that terminal levels of DLCs are 100- to 1,000-fold lower than levels seen in the lowest administered dose, the contribution of this background exposure rate to the observed responses is likely to be negligible. Levels of PCDDs, PCDFs, and PCBs 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. TCDD and PCB 126 were below the limits of detection.

Increased expressions of CYP1A1 and CYP1A2 are characteristic responses to DLCs and are directly linked to binding and activation of the aryl hydrocarbon receptor (AhR) by DLCs (Whitlock, 1993). In many cases the relative potency for induction of CYP1A1 *in vivo* is an appropriate surrogate for the dioxin-like activity of a given compound and provides the basis for many TEFs (Van den Berg *et al*., 1998). In this study, increased CYP1A1 and CYP1A2 enzyme activity as a result of exposure to the TEF mixture was observed at all time points and at all doses used. The finding that the liver was a target following exposure to these DLCs was expected. It was also expected that exposure would lead to increases in these specific dioxin-like responses. While not discussed in this Technical Report, data on altered expression of CYP1A1 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 for 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 site for the action of these compounds. In the present study, the principal neoplasms with increased incidences in the liver were hepatocellular adenoma and cholangiocarcinoma. These increases are consistent with observations made in the prior studies of each of these individual compounds conducted as part of the dioxin TEF evaluation. In the TCDD and PCB 126 studies (NTP, 2006a,c), there were significant increases in the incidences of cholangiocarcinoma and hepatocellular adenoma. Increased incidences of hepatocellular adenoma and cholangiocarcinoma were also observed in the PeCDF gavage study (NTP, 2006b), and were considered to be related to treatment.

The incidence and pattern of hepatic toxicity exhibited a clear dose and duration dependence and preceded neoplastic effects in the liver. In this study, there was a significant increase in hepatic toxicity with increases in severity occurring at higher doses and longer durations of treatment. Hepatic toxicity was characterized by foci of 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. A comprehensive term of toxic hepatopathy was also used, reflecting the overall severity grade of the nonneoplastic effects. The purpose of the use 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. Some treated animals occasionally had just a few of these changes present, but this was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy.

Treatment-related effects observed at the 14-week interim evaluation were restricted to hepatocyte hypertrophy. At 31 weeks, there was increased severity of hypertrophy, and a broader spectrum of effects, including multinucleated hepatocytes and pigmentation deposits, was observed. These changes continued to be

observed at 53 weeks together with fatty change, bile duct hyperplasia, and toxic hepatopathy. At the end of the 2-year study, there were additional toxic changes including foci of cellular alteration, bile duct cysts, cholangiofibrosis, inflammation, necrosis, oval cell hyperplasia, portal fibrosis, and nodular hyperplasia.

The increases in the incidence and severity of hepatotoxicity and incidence of hepatocellular adenoma are consistent with previously observed effects of TCDD and hexachlorodioxins on the liver (Kociba *et al.*, 1978; NCI, 1980; NTP, 1982a,b). Moreover, the Poly-3 survival-adjusted incidence of hepatocellular adenoma in the 100 ng TEQ/kg group in the present study (31%) is almost identical to that seen in the comparable 100 ng/kg group (30%) in the TCDD study (NTP, 2006a). In contrast, the Poly-3 survival-adjusted incidence of cholangiocarcinoma in the 100 ng TEQ/kg group (26%) was markedly lower than in the 100 ng/kg group (55%) in the TCDD study.

The incidences of hepatic neoplasms in the TEF mixture study were lower than expected given the disposition data and the observations of increased incidence of cholangiocarcinoma and hepatocellular adenoma of the liver observed in the TCDD study (NTP, 2006a). Terminal liver TEQ levels in the 100 ng TEQ/kg group (117 ng TEQ/g) were 13-fold higher than the TCDD levels seen in animals dosed with 100 ng TCDD/kg (9.3 ng/g), where significantly increased incidences of cholangiocarcinoma and hepatocellular adenoma were observed (NTP, 2006a). Indeed, based on the higher mean liver levels of TEQs, one would expect that the incidence of neoplasms in the present study would have been higher. Based on the lower magnitude of reduction in body weight and lower severity of hepatotoxicity in the highest dose in the present study compared to the high dose used in the TCDD study (NTP, 2006a), it is likely that the highest doses in these two studies were not equally effective and higher doses of TCDD, PeCDF, or PCB 126 would need to be have been used to see similar results. Moreover, given the low incidences of neoplasms in the PeCDF study (NTP, 2006b), it is likely that the lower incidences of cholangiocarcinoma and hepatocellular adenoma in the present study compared to the TCDD study (NTP, 2006a) may be due in part to the high tissue levels of PeCDF observed in the present study. A formal statistical analysis of the potential relative contributions of each individual congener to the neoplastic and nonneoplastic responses observed in the present study will not be dealt with here but is presented elsewhere (Toyoshiba *et al*., 2004; Walker *et al*., 2005).

Pathology nomenclature for liver neoplasms changed since the Kociba *et al.* (1978) study indicated a 47% incidence of "hepatocellular hyperplastic nodules" in the 100 ng TCDD/kg body weight group compared to a 9% incidence in control animals. Subsequent to that study, there was an evolution of nomenclature for hepatocellular proliferative lesions and a reevaluation of the slides from that study. In that evaluation, neoplastic lesions were classified as adenoma or carcinoma. Using the newer nomenclature, the incidence of hepatocellular adenoma at the highest dose of 100 ng TCDD/kg body weight was 31% (Goodman and Sauer, 1992). A summary of the pathology reevaluation is provided in the NTP (2006a) Technical Report for TCDD. It is clear from the pathology reevaluations that some of the hyperplastic nodules originally seen in the Kociba *et al.* (1978) study were indeed nonneoplastic. Significant hepatotoxicity was noted in the Kociba *et al.* (1978) TCDD study, in the NCI (1980) TCDD study, and in the NTP (1982a) TCDD study.

In the reevaluation of the Kociba *et al.* (1978) study, the incidences of hepatocellular adenoma were 2/86, 9/50, and 14/45 in the 0, 10, and 100 ng/kg groups, respectively (Goodman and Sauer, 1992). In addition, 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 body weight per day, similar to the current study. The incidence of neoplastic nodules or hepatocellular carcinoma was 14/49 (29%). There was no significant effect in male rats. In the NCI 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%), or 30/50 (60%) at doses of 0, 1,250, 2,500, or 5,000 ng/kg per week, respectively (NCI, 1980). 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 used in the present gavage study.

The spectrum of hepatocellular proliferative lesions observed in the present study is not common in NTP studies, and there is a lack of biological information relative to the progression and behavior of these lesions. These lesions generally occurred on a background of toxic hepatopathy, the components of which have been

listed and described in the results section. It is generally accepted that in the rat, hepatocellular adenoma and hepatocellular carcinoma represent a morphological and biological continuum (Narama *et al.*, 2003; Hailey *et al.*, 2005). Foci of cellular alteration are often part of that continuum, but not always. In the high dose group, 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 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 normal-sized or slightly smaller or larger than normal. In contrast, foci in the livers of dosed animals generally had discrete borders, produced some compression of the adjacent parenchyma, and consisted of large, hypertrophic, often vacuolated cells. The significantly increased incidences of hypertrophy 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 hepatocellular and biliary cells, and therefore, these lesions were considered to have progressed beyond a simple focus of cellular alteration. However, because of the somewhat normal hepatic structure and the dual cellular composition, 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. In the dioxin TEF evaluation studies, nodular hyperplasia was seen most commonly in the higher dose groups in which prominent toxic changes were present. However, a lesser degree of nodular hyperplasia was sometimes seen in lower dose animals in which the only evidence of liver pathology may have been 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 toxic hepatopathy. This does not explain however, these responses in animals that lack toxic hepatopathy. This indicates that some type of stimulus, other than regeneration secondary to degeneration and necrosis of the hepatic parenchyma, may have contributed to the proliferative lesions observed in this study.

Dealing with the potential pathogenesis for the foci and nodular hyperplasia, the earliest treatment-related hepatocellular change seen in these studies, noted at the 14-, 31-, and 53-week interim evaluations, was a diffuse hepatocyte hypertrophy. 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 that 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 generally absent. The

lack of proliferating biliary epithelium or oval cells was an important feature used in differentiating adenoma from nodular hyperplasia.

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

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

Cholangiofibrosis was the term used to describe small lesions that were less aggressive in appearance. Cholangiofibrosis often originated in the portal area and tended to have a more mature fibrous connective tissue component and less atypia associated with the epithelial cells. Most often, cholangiofibrosis and cholangiocarinomas 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 the incidence of lesions similar to those observed in the present study. In the Maronpot *et al.* (1991) furan study, the lesions appeared more aggressive, yet even in that study, where there was nearly a 100% incidence in treated animals, there were few metastases. In this study, it appears that the cholangiocarcinomas were slow growing neoplasms of relatively low-grade malignancy. Transplantation studies done in the furan study were positive for growth and metastases. Transplantation studies were not done with lesions from the TEF mixture study.

The mechanism underlying the increase in incidence of cholangiocarcinoma is likely to be multifactorial. The TEF mixture 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 observed bile duct proliferation may represent a process of excessive and long term repair following specific damage to hepatocytes and leading to the death of hepatocytes and perhaps also of the bile duct epithelium. The proliferative response may be a reparative response of proliferating hepatocytes, bile duct cells, and scarring tissue (cholangiofibrosis). The inflammation also observed can produce oxidative stress that may also result in promotion of DNA damage. Consequently, the oxidative stress may be only a secondary phenomenon due to the ongoing response to the toxic hepatopathy. In addition, there may also be a direct stimulatory effect on the oval cells themselves, which is supported by the observed increases in oval cell hyperplasia. The oval cells may differentiate into hepatocytes or biliary cells. Consequently, this may explain why both hepatocellular proliferative and biliary lesions are associated with exposure.

There has been a considerable amount of research examining the potential mode of action of DLCs in the liver.

There is a general scientific consensus that almost all responses of TCDD and related compounds require initial binding to the AhR. Recent data 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).

With regard to the increased incidence of hepatocellular adenoma, numerous studies have shown in initiation-promotion models of hepatocarcinogenesis that TCDD, PCB 126, and PeCDF are promoters of altered hepatic foci. Given that TCDD and related compounds are not direct-acting genotoxic agents and are potent growth dysregulators, it is believed that their predominant mode of action is 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 net growth rate of initiated cells due to selective growth advantage or decreased rate of cell death via suppression of apoptosis. In the NTP (2006a) study and prior studies with TCDD, there were 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 Bohnenberger *et al*. (2001) have also shown a suppression of apoptosis by TCDD and PCBs. In addition, altered growth regulation may be due to alterations in intercellular communication that have also been observed in the livers of rats exposed to DLCs (Baker *et al*., 1995; Warngard *et al*., 1996; Bager *et al*., 1997). While DLCs are not direct-acting genotoxic agents, there is 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 AhR-dependent induction of CYP1 family cytochromes P450 that leads to induction of oxidative stress either due to inefficient electron transfer during P450 metabolism (Park *et al*., 1996) or to 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 induction of oxidative stress and DNA damage by high-dose acute exposure to TCDD (Stohs *et al*., 1990). Induction of lipid peroxidation and single stranded DNA breaks was also observed in tissues from the present study (Hassoun *et al*., 2001) and from other studies conducted as part of the dioxin TEF evaluation (Hassoun *et al*., 2000, 2002). Other studies on the female specific tumor promotion response in rats have shown 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, there was a significantly increased incidence of CKE of the lung associated with exposure to the TEF mixture. Histopathologically, these lesions varied in size and number and appeared as cystic structures consisting of a complex wall of keratinizing stratified squamous epithelium, with a center filled with keratin. These lesions were absent in vehicle control animals but observed in 55% of animals treated at the highest dose (100 ng TEQ/kg). In addition, there was an increase in the incidence of alveolar squamous metaplasia in the 100 ng TEQ/kg group.

Significantly increased incidences of CKE were also observed in the TCDD and PCB 126 studies conducted as part of the dioxin TEF evaluation (NTP, 2006a,c). In the 2-year feed study of TCDD conducted by Kociba *et al*. (1978), an increased incidence of keratinizing squamous cell carcinoma of the lung was observed following exposure to 100 ng TCDD/kg body weight per day. In the dioxin TEF evaluation studies, squamous cell carcinoma was distinguished from CKE by the presence of areas of solid growth and evidence of invasion. While no direct comparison has been made between CKE and the keratinizing squamous cell carcinoma observed in the Kociba *et al.* (1978) study, given the keratinizing nature of the CKE, it is possible that these may represent the same lesions; CKE was not a diagnostic term consistently in use at the time of that evaluation. Diagnostic criteria for classification of CKE as a lesion distinct from squamous cell carcinoma were later developed at a workshop held in the mid 1990s (Boorman *et al.*, 1996). In contrast to the present study, a recent carcinogenicity study of the high TEQ PCB mixture Aroclor 1254 demonstrated no increases in the incidence of any type of lung tumor (Mayes *et al*., 1998). While Aroclor 1254 contains a significant TEQ contribution by PCB 126, this mixture also contains mono-*ortho*- and di-*ortho*-PCBs.

In addition to increased incidences of CKE in the present study, there were significant increases in the incidences of bronchiolar metaplasia of the alveolar

epithelium at both 53 weeks and 2 years. The incidences of this lesion were significantly higher in all dosed groups at 2 years. These findings are consistent with prior observations of increases in the incidences of alveolar-bronchiolar metaplasia following exposure to TCDD within the framework of a two stage initiation-promotion model in Sprague-Dawley rats (Tritscher *et al*., 2000).

Alveolar ducts and alveoli are normally lined by 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. Interestingly, there were significantly decreased incidences of alveolar epithelial hyperplasia in the groups administered 22 ng TEQ/kg or greater.

The TEF mixture induced a multifocal lesion that was found throughout the lung at the junction of the terminal bronchioles and alveolar ducts. The epithelium was cuboidal to columnar and ciliated in contrast to type II alveolar epithelial cells. Also, scattered throughout the ciliated cells were dome-shaped nonciliated cells, consistent with Clara cells. Clara cells are normally found in the lining of the bronchioles, but not alveoli or alveolar ducts. Histochemical analyses of mucin and GSTPi in lung tissue from the dioxin TEF evaluation studies indicates that this 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).

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

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

Single gingival squamous cell carcinomas of the oral mucosa occurred in the vehicle control and 10 ng TEQ/kg groups, and two were seen in the 100 ng TEQ/kg group. In both the TCDD and PCB 126 studies conducted as part of the dioxin TEF evaluation, there were significant increases in the incidences of gingival squamous cell carcinoma of the oral mucosa (NTP, 2006a,c). Similarly, in the TCDD feed study by Kociba *et al*. (1978), there were increases in stratified squamous cell carcinoma of the hard palate/nasal turbinates in male and female rats. The location of the gingival squamous cell carcinomas in the studies conducted during the dioxin TEF evaluation was adjacent to the molars and invaded into the hard palate/nasal turbinate areas. This suggests that the lesions seen in the present studies and the Kociba *et al*. (1978) study are similar. While the incidence of gingival squamous cell carcinoma in the 100 ng TEQ/kg group in the present study was not significantly elevated over concurrent vehicle controls, the prior observations of significant increases in the incidences of this neoplasm with TCDD and PCB 126 (NTP, 2006a,c) suggest that the observed neoplasms in the 100 ng TEQ/kg group may have been due to treatment with the TEF mixture. That the oral mucosa was a target organ in this study is supported by the observation that the incidences of gingival squamous hyperplasia were increased in all dosed groups at the end of the

2-year study. In addition, as noted above for the effects on the lung, the squamous lesions in the oral cavity may also be related to the alteration in retinoid homeostasis in the liver that is known to be induced by DLCs.

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

In the present study, there were increases in the incidences of adrenal cortical atrophy and cytoplasmic vacuolization at the 100 ng TEQ/kg dose at 2 years. In addition, there were significantly increased incidences of adrenocortical hyperplasia in all dosed groups. Single occurrences of adrenal cortical adenoma and carcinoma were each observed in the 10 and 22 ng TEQ/kg groups. In the Kociba *et al.* (1978) TCDD study, there was a significant increase in the incidence of adrenal cortical adenoma in male but not female rats at 100 ng/kg. In the NTP (2006a) TCDD study, there were sporadic cases of adenoma of the adrenal cortex in both vehicle control and treated animals but no significant TCDD-related increase in incidence. The observed neoplasms in the present study were not considered to be related to treatment. The cortical atrophy seen in the present study was a prominent effect and may reflect the continued stress in these animals, leading to depletion of corticosteroid hormones (Sapolsky *et al*., 1987). It may also be due to other unknown mechanisms.

A significant reduction in the incidence of benign pheochromocytoma of the adrenal gland was previously observed in male rats in the 2-year study by Kociba *et al*. (1978) (33% and 14% in vehicle controls and 100 ng TCDD/kg, respectively). There was no significant effect on the incidence of benign pheochromocytoma in the dioxin TEF evaluation TCDD study (NTP, 2006a) or the present TEF mixture study.

In the present study, the incidences of active inflammation of the pancreas, atrophy of the pancreatic acinus, acinar cytoplasmic vacuolization, chronic active inflammation of the pancreatic artery, and dilatation of the pancreatic ducts were all significantly higher than those in the vehicle controls after administration of 100 ng TEQ/kg per day for 2 years. In addition, pancreatic acinar neoplasms were observed in the 10, 22, and 46 ng TEQ/kg groups. Pancreatic adenoma and carcinoma were absent in vehicle controls and were only observed in dosed animals. Only a single pancreatic adenoma and no pancreatic carcinomas have been observed in the pooled historical control groups in the seven dioxin TEF evaluation studies. In addition, Majeed *et al*. (1997) showed that acinar pancreatic carcinoma is a rare tumor in female Sprague-Dawley CD rats, with a spontaneous background rate of 0.02%. Increased incidences of pancreatic acinar neoplasms were observed in the NTP (2006a) TCDD study and may have been related to treatment. Due to the rarity of these neoplasms and the fact that they were only observed in treated animals, it is believed that the acinar neoplasms may have been related to treatment with the TEF mixture.

Acinar atrophy of the pancreas may be related to the down regulation of cholecystokinin (CCK). As shown by Lee *et al*. (2000) in samples from the PCB 126 study conducted as part of the dioxin TEF evaluation, intestinal CCK is reduced by PCB 126 exposure. Down-regulation of CCK is likely due to a general endocrine effect as a result of the reduction in body weight gain following exposure to 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 are observed in OLETF rats that lack the CCK-A receptor gene (Jimi *et al*., 1997). In addition, antagonism of CCK action can lead to reduced pancreatic growth (Ohlsson *et al*., 1995). The relationship between acinar atrophy and cytoplasmic vacuolization and the development of pancreatic neoplasms is unclear because these lesions were also observed in the PCB 126 study, yet there were no increases in pancreatic neoplasms in that study (NTP, 2006c).

In the present study, the incidences of kidney nephropathy in all dosed groups were significantly higher than that in the vehicle control group. While it is known that the kidney is directly responsive to AhR agonists, the kidney historically has not been a target for DLC-induced neoplasia.

In this study, there were significantly increased incidences of cardiomyopathy in all dosed groups. Similarly, increased incidences of cardiomyoptahy were seen in the other studies conducted as part of the dioxin TEF evaluation (Jokinen *et al*., 2003). However, the average severity of cardiomyopathy was unaffected. Cardiomyopathy is a common, spontaneously occurring degenerative change of myocardial fibers that is seen in rats as they age. Its cause in the rat is unknown, but age of onset and severity are affected by diet, environment, and stress. The microscopic appearance of cardiomyopathy was the same in both the vehicle control and dosed 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 Kociba *et al*. (1978) TCDD study, there was an increase of myocardial degenerative change above background levels in females only.

In this study, the incidences of thymic atrophy were increased in a time- and dose-dependent manner and were significantly increased in all dosed groups 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). Thymic atrophy may in part be related to the reduction in body weight gain observed in these animals as seen in short-term feed restriction studies (Levin *et al*., 1993).

In the present study, there were increased incidences of thyroid gland follicular cell hypertrophy in all dosed groups at the end of the 2-year study. However, there were no increases in the incidences of thyroid gland follicular cell neoplasms. This is consistent with the TCDD study (NTP, 2006a). By comparison, in the 2-year gavage study of TCDD in Osborne-Mendel rats, there was a significant increase in the incidence of thyroid gland follicular cell adenomas in male rats and a nonsignificant increase in females (NTP, 1982a).

Alteration in thyroid hormone homeostasis by PCB 126 and TCDD is well established (Van Birgelen *et al*., 1994, 1995b; Schmidt *et al*., 2003). Analyses of thyroid hormones in the present study confirmed the alterations in thyroid hormone homeostasis. Significant reductions in thyroxine (T_4) were seen at all the interim timepoints. Increases in triiodothyronine (T_3) and thyroid stimulating hormone (TSH) were seen at 14 and 31 weeks, but at 53 weeks, there was no effect on 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 result of increased hepatic expression of UDPGT. This leads to a decreased negative feedback inhibition of the thyroid gland leading to overexpression 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). In the present study, it was observed that despite alterations in T_4 and TSH at the early timepoints, the effects on TSH were not seen at 53 weeks despite a significant effect on T_4. It is possible, therefore, that the lack of follicular cell neoplasia in this study reflects a lack of a sustained long-term increase on TSH sufficient to promote the neoplasia.

In this 2-year study, there was a significantly lower adjusted incidence of C-cell adenomas in rats administered 100 ng TEQ/kg. Similarly, there was a lower incidence of C-cell adenomas in the NTP (2006a) TCDD study and in male rats in the Kociba *et al*. (1978) TCDD study. It is unclear if the reduction in incidence of this neoplasm is related to the reduction in body weight gain seen after exposure to DLCs. In the present study and the TCDD study, there was a reduction in body weight gain at the higher doses. Similar reductions in the incidences of neoplasms that are often associated with body weight, such as mammary gland and pituitary gland neoplasms, were also observed at the higher doses. However, in the present study, a significant reduction in the incidence of C-cell adenoma was observed at the 10 ng TEQ/kg dose where there was no effect on body weight. This suggests that the observed reductions in incidence may not have been due to body weight effects.

In this study, there was a significantly lower incidence of fibroadenoma of the mammary gland following exposure to the TEF mixture. Fibroadenoma is a spontaneous lesion in female Sprague-Dawley rats and occurred at a high incidence in the vehicle controls (65%). The incidence of fibroadenoma in the 100 ng TEQ/kg group was 34%. In addition, there was a significantly lower incidence of spontaneous pituitary gland adenoma or carcinoma following dosing.

It is believed that the lower incidences of mammary gland and pituitary gland tumors in exposed rodents are 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 tumors has been observed in many NTP studies of F344 rats (Seilkop, 1995). Significantly lower incidences of mammary gland and pituitary gland tumors were also observed in animals exposed to 100 ng TCDD/kg in the 2-year feed study of Kociba *et al*. (1978) and in the TCDD and PCB 126 studies conducted as part of the dioxin TEF evaluation (NTP, 2006a,c).

Reductions in IGF-1 may underlie the inhibitory effect of reduced body weight gain on tumor development. It is known that caloric restriction leads to lower levels of IGF-1 and reduction in background tumor rates (Hursting *et al*., 2003). One of the major intestinal hormones expressed in the proximal gastrointestinal tract is CCK. CCK regulates gallbladder contraction, pancreatic secretion, stomach emptying, intestinal motility and can also inhibit food intake. In an analysis of intestinal tissue obtained from the present study, Lee *et al.* (2000) showed lower levels of intestinal CCK and an induction of IGFBP3 by TCDD in the dioxin TEF evaluation study. Alterations in CCK-processing enzymes by TCDD were also observed in cultured intestinal cells suggesting direct effects of TCDD on intestinal cells. The authors hypothesized that alterations in CCK may be due to alterations in processing enzymes and lower IGF-1 levels as a result of alterations in IGFBP3.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of the mixture of TCDD, PeCDF, and PCB 126 in female Harlan Sprague-Dawley rats based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and cystic keratinizing epithelioma of the lung. Neoplasms of the pancreatic acinus may have been related to administration of the mixture of TCDD, PeCDF, and PCB 126.

Administration of the mixture of TCDD, PeCDF, and PCB 126 caused increased incidences of nonneoplastic lesions of the liver, lung, pancreas, adrenal cortex, oral mucosa, uterus, thymus, ovary, kidney, heart, bone marrow, urinary bladder, mesenteric artery, and thyroid gland 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 THE TEF MIXTURE

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Table A1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Disposition Summary					
Animals initially in study	81	81	81	81	81
14-Week interim evaluation	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8
Early deaths					
Accidental deaths			1	1	2
Moribund	26	25	22	25	33
Natural deaths	11	5	6	4	10
Survivors					
Terminal sacrifice	16	23	24	23	8
Animals examined microscopically	81	81	81	81	81

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)			(10)
Fibroadenoma		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 A1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
53-Week Interim Evaluation					
Endocrine System					
Thyroid gland	(8)	(8)	(8)	(8)	(8)
C-cell, adenoma	2 (25%)				
Integumentary System					
Mammary gland	(8)	(1)	(1)	(1)	(8)
Fibroadenoma		1 (100%)	1 (100%)	1 (100%)	2 (25%)
Skin			(1)		
Subcutaneous tissue, fibrosarcoma			1 (100%)		
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					
2-Year Study					
Alimentary System					
Intestine large, colon	(53)	(52)	(53)	(53)	(51)
Carcinoma	1 (2%)				
Intestine large, rectum	(52)	(53)	(53)	(53)	(52)
Polyp adenomatous			1 (2%)		
Schwannoma malignant, metastatic, uterus				1 (2%)	
Intestine large, cecum	(51)	(53)	(53)	(53)	(51)
Intestine small, duodenum	(52)	(53)	(53)	(53)	(52)
Carcinoma			1 (2%)		
Intestine small, jejunum	(52)	(53)	(53)	(53)	(50)
Leiomyosarcoma	1 (2%)		1 (2%)		
Intestine small, ileum	(52)	(53)	(53)	(53)	(50)
Liver	(53)	(53)	(53)	(53)	(51)
Cholangiocarcinoma			2 (4%)	5 (9%)	5 (10%)
Cholangiocarcinoma, multiple				2 (4%)	4 (8%)
Hepatocellular adenoma		1 (2%)	1 (2%)	1 (2%)	9 (18%)
Hepatocellular adenoma, multiple					2 (4%)
Histiocytic sarcoma					1 (2%)
Mesentery		(1)	(2)	(6)	(8)
Lipoma				1 (17%)	
Oral mucosa	(10)	(18)	(19)	(29)	(31)
Gingival, squamous cell carcinoma	1 (10%)	1 (6%)			2 (6%)
Pancreas	(52)	(53)	(53)	(53)	(51)
Acinus, adenoma			2 (4%)		
Acinus, carcinoma		1 (2%)		2 (4%)	

Table A1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Alimentary System (continued)					
Salivary glands	(53)	(53)	(53)	(53)	(53)
Carcinoma		1 (2%)			
Schwannoma malignant, metastatic, skin					1 (2%)
Stomach, forestomach	(53)	(53)	(53)	(53)	(52)
Stomach, glandular	(53)	(53)	(53)	(53)	(52)
Tongue	(1)				(1)
Squamous cell carcinoma, metastatic, oral mucosa	1 (100%)				
Tooth	(24)	(23)	(27)	(37)	(30)
Peridontal tissue, fibrosarcoma	1 (4%)				
Cardiovascular System					
Blood vessel	(53)	(53)	(53)	(52)	(53)
Aorta, adventitia, carcinoma, metastatic, mammary gland	1 (2%)				
Heart	(53)	(53)	(53)	(52)	(53)
Fibrous histiocytoma, metastatic, skeletal muscle		1 (2%)			
Schwannoma malignant	2 (4%)			1 (2%)	3 (6%)
Endocrine System					
Adrenal cortex	(52)	(53)	(53)	(53)	(51)
Adenoma		1 (2%)	1 (2%)		
Carcinoma		1 (2%)	1 (2%)		
Adrenal medulla	(52)	(53)	(53)	(53)	(51)
Pheochromocytoma benign	4 (8%)	3 (6%)	4 (8%)	4 (8%)	1 (2%)
Bilateral, pheochromocytoma benign	1 (2%)	1 (2%)			
Islets, pancreatic	(52)	(53)	(53)	(53)	(51)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Carcinoma	1 (2%)				
Parathyroid gland	(50)	(50)	(49)	(47)	(52)
Adenoma	1 (2%)				
Pituitary gland	(53)	(53)	(53)	(53)	(53)
Carcinoma			1 (2%)		
Pars distalis, adenoma	28 (53%)	29 (55%)	22 (42%)	20 (38%)	10 (19%)
Thyroid gland	(53)	(53)	(51)	(52)	(51)
Bilateral, C-cell, adenoma	2 (4%)	2 (4%)	3 (6%)	1 (2%)	
Bilateral, follicular cell, carcinoma			1 (2%)		
C-cell, adenoma	15 (28%)	8 (15%)	15 (29%)	8 (15%)	5 (10%)
C-cell, carcinoma	2 (4%)			1 (2%)	1 (2%)
Follicular cell, adenoma			1 (2%)		1 (2%)
General Body System					
None					
Genital System					
Ovary	(52)	(52)	(53)	(53)	(51)
Fibrous histiocytoma, metastatic, skeletal muscle		1 (2%)			
Granulosa cell tumor malignant			1 (2%)		1 (2%)
Granulosa cell tumor benign			1 (2%)	1 (2%)	

Table A1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Genital System (continued)					
Uterus	(52)	(53)	(53)	(53)	(51)
Carcinoma		1 (2%)	2 (4%)	2 (4%)	1 (2%)
Hemangiosarcoma					1 (2%)
Leiomyoma			2 (4%)		
Leiomyosarcoma	1 (2%)				
Polyp stromal	3 (6%)	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Polyp stromal, multiple	1 (2%)		1 (2%)		
Schwannoma malignant	1 (2%)		1 (2%)	2 (4%)	1 (2%)
Squamous cell carcinoma			1 (2%)		
Squamous cell papilloma	1 (2%)				
Cervix, carcinoma	1 (2%)				
Cervix, squamous cell carcinoma		1 (2%)			1 (2%)
Serosa, carcinoma, metastatic, uterus	1 (2%)				
Vagina				(1)	
Schwannoma malignant				1 (100%)	
Hematopoietic System					
Bone marrow	(53)	(53)	(53)	(53)	(53)
Lymph node	(4)	(1)	(2)	(9)	(11)
Deep cervical, carcinoma, metastatic, thyroid gland				1 (11%)	
Lymph node, mandibular	(53)	(53)	(53)	(53)	(53)
Lymph node, mesenteric	(52)	(53)	(53)	(53)	(50)
Spleen	(52)	(53)	(53)	(53)	(51)
Thymus	(52)	(48)	(50)	(53)	(50)
Fibrosarcoma, metastatic, skin	1 (2%)				
Integumentary System					
Mammary gland	(53)	(53)	(53)	(53)	(52)
Adenoacanthoma			1 (2%)		
Adenolipoma			1 (2%)		
Adenoma		1 (2%)			
Carcinoma	5 (9%)	6 (11%)	3 (6%)	5 (9%)	1 (2%)
Carcinoma, multiple	1 (2%)				
Fibroadenoma	21 (40%)	17 (32%)	19 (36%)	20 (38%)	15 (29%)
Fibroadenoma, multiple	13 (25%)	10 (19%)	16 (30%)	8 (15%)	3 (6%)
Skin	(53)	(53)	(53)	(53)	(53)
Fibroma	1 (2%)	2 (4%)	2 (4%)	3 (6%)	
Fibrosarcoma	1 (2%)			1 (2%)	
Pilomatrixoma				1 (2%)	
Schwannoma malignant					1 (2%)
Schwannoma malignant, metastatic, uterus				1 (2%)	
Squamous cell papilloma			1 (2%)		

Table A1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Musculoskeletal System					
Skeletal muscle		(1)			
Fibrous histiocytoma		1 (100%)			
Nervous System					
Brain	(53)	(53)	(53)	(53)	(53)
Carcinoma, metastatic, kidney	1 (2%)				
Carcinoma, metastatic, pituitary gland			1 (2%)		
Granular cell tumor malignant			1 (2%)		
Medulloblastoma malignant		1 (2%)	1 (2%)		
Oligodendroglioma malignant			1 (2%)		
Meninges, meningioma malignant		1 (2%)			
Spinal cord	(1)	(1)			
Respiratory System					
Lung	(53)	(53)	(53)	(53)	(53)
Alveolar/bronchiolar adenoma			1 (2%)	1 (2%)	
Carcinoma, metastatic, mammary gland	3 (6%)	1 (2%)		2 (4%)	
Carcinoma, metastatic, uterus	1 (2%)				
Carcinoma, metastatic, adrenal cortex			1 (2%)		
Cystic keratinizing epithelioma				2 (4%)	9 (17%)
Cystic keratinizing epithelioma, multiple					11 (21%)
Fibrosarcoma, metastatic, skin	1 (2%)				
Fibrous histiocytoma, metastatic, skeletal muscle		1 (2%)			
Histiocytic sarcoma					1 (2%)
Schwannoma malignant, metastatic, skin					1 (2%)
Mediastinum, carcinoma, metastatic, mammary gland	1 (2%)				
Mediastinum, fibrous histiocytoma, metastatic, skeletal muscle		1 (2%)			
Nose	(53)	(53)	(53)	(53)	(53)
Schwannoma malignant			1 (2%)		
Special Senses System					
Harderian gland	(53)	(53)	(52)	(53)	(53)
Urinary System					
Kidney	(52)	(53)	(53)	(53)	(51)
Hemangiosarcoma			1 (2%)		
Nephroblastoma	1 (2%)				
Bilateral, renal tubule, carcinoma	1 (2%)				
Renal tubule, carcinoma		1 (2%)			
Transitional epithelium, papilloma				1 (2%)	
Systemic Lesions					
Multiple organs[b]	(53)	(53)	(53)	(53)	(53)
Adenolipoma			1 (2%)		
Histiocytic sarcoma					1 (2%)
Lymphoma malignant	2 (4%)	1 (2%)		1 (2%)	2 (4%)

Table A1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Neoplasm Summary					
Total animals with primary neoplasms[c]					
31-Week interim evaluation		2			
53-Week interim evaluation	2	1	2	1	2
2-Year study	51	46	50	50	45
Total primary neoplasms					
31-Week interim evaluation		2			
53-Week interim evaluation	2	1	2	1	2
2-Year study	115	94	118	99	93
Total animals with benign neoplasms					
31-Week interim evaluation		2			
53-Week interim evaluation	2	1	1	1	2
2-Year study	45	43	46	43	39
Total benign neoplasms					
31-Week interim evaluation		2			
53-Week interim evaluation	2	1	1	1	2
2-Year study	92	77	97	75	68
Total animals with malignant neoplasms					
53-Week interim evaluation			1		
2-Year study	18	14	19	21	21
Total malignant neoplasms					
53-Week interim evaluation			1		
2-Year study	23	17	21	23	25
Total animals with metastatic neoplasms					
2-Year study	7	2	2	4	1
Total metastatic neoplasms					
2-Year study	11	5	2	5	2
Total animals with uncertain neoplasms-benign or malignant					
2-Year study				1	
Total uncertain neoplasms					
2-Year study				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 metatstatic neoplasms

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

Number of Days on Study	176	218	276	387	416	435	444	454	456	484	500	508	526	540	568	575	587	625	625	626	627	631	645	655	658	666	668	668
Carcass ID Number	021	034	017	032	035	050	028	070	004	084	013	032	005	002	062	030	066	024	096	015	027	063	026	049	056	018	001	023
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma										X																		
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																												
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oral mucosa				+						+		+				+				+	+		+		+			+
Gingival, squamous cell carcinoma																												X
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																												+
Squamous cell carcinoma, metastatic, oral mucosa																												X
Tooth		+		+	+			+		+		+				+				+		+	+		+		+
Peridontal tissue, fibrosarcoma																						X						
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aorta, adventitia, carcinoma, metastatic, mammary gland													X															
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant													X															
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																												
Bilateral, pheochromocytoma benign																												
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X				
Carcinoma																												
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X	X					X					X		X			X	X		X	X	X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																												
C-cell, adenoma															X								X		X		X	X
C-cell, carcinoma																X												

+: 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 the TEF Mixture: Vehicle Control

Number of Days on Study	669	682	682	693	702	715	721	723	727	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	
Carcass ID Number	085	057	060	003	074	083	046	020	080	029	031	058	059	061	065	067	073	078	082	019	025	033	076	077	081		Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Carcinoma																											1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Leiomyosarcoma												X															1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Oral mucosa																	+										10
Gingival, squamous cell carcinoma																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Tongue																											1
Squamous cell carcinoma, metastatic, oral mucosa																											1
Tooth	+		+				+		+				+	+	+	+	+			+				+	+		24
Peridontal tissue, fibrosarcoma																											1
Cardiovascular System																											
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Aorta, adventitia, carcinoma, metastatic, mammary gland																											1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Schwannoma malignant												X															1
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Pheochromocytoma benign				X		X											X				X						4
Bilateral, pheochromocytoma benign												X															1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		52
Adenoma																											1
Carcinoma																						X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+		50
Adenoma											X																1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Pars distalis, adenoma	X	X		X	X	X	X	X			X	X		X	X	X	X	X	X		X			X	X		28
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Bilateral, C-cell, adenoma											X							X									2
C-cell, adenoma		X	X		X		X		X			X	X	X					X				X				15
C-cell, carcinoma														X													2

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

Number of Days on Study	176	218	276	387	416	435	444	454	456	484	500	508	526	540	568	575	587	625	625	626	627	631	645	655	658	666	668	668
Carcass ID Number	021	034	017	032	035	050	028	070	004	084	013	072	005	002	062	030	066	024	096	015	027	063	026	049	056	018	010	023
General Body System																												
None																												
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																												
Polyp stromal																					X							
Polyp stromal, multiple																											X	
Schwannoma malignant														X														
Squamous cell papilloma																												
Cervix, carcinoma																									X			
Serosa, carcinoma, metastatic, uterus																									X			
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node										+		+													+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																										X		
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma		X						X					X															
Carcinoma, multiple																												
Fibroadenoma	X			X		X	X		X					X	X		X		X	X	X	X	X	X				
Fibroadenoma, multiple																X									X		X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																												
Fibrosarcoma																										X		
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney																X												
Peripheral nerve												+																
Spinal cord												+																

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

Number of Days on Study	669	682	682	693	702	715	721	723	727	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	
Carcass ID Number	085	057	060	003	074	083	046	020	080	029	031	058	059	061	065	067	073	078	082	019	025	033	076	077	081	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Leiomyosarcoma																		X								1
Polyp stromal							X														X					3
Polyp stromal, multiple																										1
Schwannoma malignant																										1
Squamous cell papilloma																								X		1
Cervix, carcinoma																										1
Serosa, carcinoma, metastatic, uterus																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node			+																							4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Fibrosarcoma, metastatic, skin																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma				X																		X				5
Carcinoma, multiple												X														1
Fibroadenoma		X		X			X				X					X						X			X	21
Fibroadenoma, multiple	X							X	X			X	X	X				X	X		X			X		13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma				X																						1
Fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, kidney																										1
Peripheral nerve																										1
Spinal cord																										1

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

Number of Days on Study	176	218	276	387	416	435	444	445	456	484	500	508	526	540	568	575	587	625	625	626	627	631	645	655	658	666	668	668
Carcass ID Number	021	034	017	032	035	050	028	070	004	084	013	072	005	002	062	030	066	024	096	015	027	063	026	049	056	018	001	023
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland		X											X															
Carcinoma, metastatic, uterus																									X			
Fibrosarcoma, metastatic, skin																										X		
Mediastinum, carcinoma, metastatic, mammary gland													X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																												
Ear																								+				
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma			X																									
Bilateral, renal tubule, carcinoma																X												
Ureter																									+			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant												X																

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

Number of Days on Study	669	682	682	693	702	715	721	723	727	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	
Carcass ID Number	085	057	060	003	074	083	046	020	080	029	031	058	059	061	065	067	073	078	082	019	025	033	076	077	081	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, mammary gland				X																						3
Carcinoma, metastatic, uterus																										1
Fibrosarcoma, metastatic, skin																										1
Mediastinum, carcinoma, metastatic, mammary gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																										
Ear																										1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Nephroblastoma																										1
Bilateral, renal tubule, carcinoma																										1
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymphoma malignant																X										2

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 10 ng TEQ/kg

Number of Days on Study	227	350	362	416	455	456	498	540	546	548	562	563	563	626	627	627	627	631	652	659	668	668	669	669	683	689	702	702
Carcass ID Number	158	146	121	149	180	105	140	102	189	117	112	126	163	177	123	153	161	111	128	148	127	145	104	129	103	119	186	187
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, carcinoma																												
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								X				
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth				+				+	+	+		+	+				+			+			+		+	+	+	+
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skeletal muscle			X																									
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Carcinoma																												
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																					X	X						
Bilateral, pheochromocytoma benign						X																						
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X		X			X			X	X	X	X	X	X	X	X		X	X	X		X	X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																					X							
C-cell, adenoma																	X					X						
General Body System																												
None																												

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 10 ng TEQ/kg

Number of Days on Study	718	721	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	120	122	101	114	130	139	150	151	179	188	190	196	107	108	110	113	115	124	136	143	152	162	165	176	178	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
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				+		+						+			+	+								+		18
Gingival, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Acinus, carcinoma																						X				1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth				+	+	+				+		+		+	+	+		+				+				23
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibrous histiocytoma, metastatic, skeletal muscle																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma							X																			1
Carcinoma																									X	1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign																									X	3
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																						X				1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma	X	X		X			X		X					X	X	X		X		X	X	X	X			29
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma														X												2
C-cell, adenoma		X	X				X		X	X											X					8
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 10 ng TEQ/kg

Number of Days on Study	227	350	362	416	455	456	498	540	546	548	562	563	563	626	627	627	631	652	659	668	668	669	669	683	689	702	702
Carcass ID Number	158	146	121	149	180	105	140	102	189	117	112	126	163	177	123	153	161	111	128	148	127	145	104	129	103	119	186	187
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastic, skeletal muscle			X																									
Oviduct																												+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																												
Polyp stromal																												
Cervix, squamous cell carcinoma																												
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									X							
Fibroadenoma					X	X							X	X	X	X	X	X				X		X				X
Fibroadenoma, multiple			X					X	X	X										X		X					X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																						X						
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																									
Fibrous histiocytoma			X																									
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Medulloblastoma malignant						X																						
Meninges, meningioma malignant		X																										
Peripheral nerve																			+									
Spinal cord																			+									
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland	X																											
Fibrous histiocytoma, metastatic, skeletal muscle			X																									
Mediastinum, fibrous histiocytoma, metastatic, skeletal muscle			X																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 10 ng TEQ/kg

Number of Days on Study	718	721	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	120	122	101	114	130	139	150	151	179	188	190	196	107	108	110	113	115	124	136	143	152	162	165	176	178	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Fibrous histiocytoma, metastatic, skeletal muscle																										1
Oviduct																					+					2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																									X	1
Polyp stromal																			X							1
Cervix, squamous cell carcinoma																					X					1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node							+																			1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	M	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma	X																									1
Carcinoma				X						X													X			6
Fibroadenoma			X			X						X			X			X					X			17
Fibroadenoma, multiple		X						X	X																	10
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma												X														2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																										1
Fibrous histiocytoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Medulloblastoma malignant																										1
Meninges, meningioma malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, mammary gland																										1
Fibrous histiocytoma, metastatic, skeletal muscle																										1
Mediastinum, fibrous histiocytoma, metastatic, skeletal muscle																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 10 ng TEQ/kg

Number of Days on Study	227	350	362	416	455	456	498	540	546	548	562	563	563	626	627	627	627	631	652	659	668	668	669	669	683	689	702	702
Carcass ID Number	158	146	121	149	180	105	140	102	189	117	112	126	163	177	223	253	161	111	128	148	127	145	104	129	103	119	186	187
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, carcinoma																								X				
Urinary bladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																													

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 10 ng TEQ/kg

Number of Days on Study	718	721	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	120	122	101	114	130	139	150	151	179	188	190	196	107	108	110	113	115	124	136	143	152	162	165	176	178	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymphoma malignant															X											1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 22 ng TEQ/kg

Number of Days on Study	046	203	263	322	456	526	529	540	548	592	609	616	617	621	639	652	659	659	666	669	669	683	699	702	702	702	703	709
Carcass ID Number	243	292	244	298	222	252	229	290	245	260	266	225	282	256	212	294	291	295	227	202	224	287	257	214	269	289	246	223
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous					X																							
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma					X																							
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																												
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																				X								
Hepatocellular adenoma																												
Mesentery																			+									
Oral mucosa							+			+		+		+	+		+					+	+		+		+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																												
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth						+	+			+		+			+		+			+	+	+	+	+	+	+	+	
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Carcinoma																X												
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign					X																	X		X				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								X				
Pars distalis, adenoma										X		X		X				X	X	X		X			X		X	
Thyroid gland	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma													X															
Bilateral, follicular cell, carcinoma																												
C-cell, adenoma														X	X			X	X				X				X	
Follicular cell, adenoma																		X										
General Body System																												
None																												

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 22 ng TEQ/kg

Number of Days on Study	723	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	226	201	204	205	241	249	258	268	280	285	288	203	242	247	250	254	255	259	267	270	276	278	281	283	297	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp adenomatous																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leiomyosarcoma																	X									1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cholangiocarcinoma												X														2
Hepatocellular adenoma																									X	1
Mesentery									+																	2
Oral mucosa			+	+		+			+			+	+		+		+		+							19
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Acinus, adenoma							X									X										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth			+	+		+			+	+	+	+	+						+	+	+		+	+		27
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma															X											1
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign		X																								4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																		X								1
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																										1
Pars distalis, adenoma	X			X		X		X	X		X	X		X	X		X		X			X			X	22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Bilateral, C-cell, adenoma												X	X													3
Bilateral, follicular cell, carcinoma								X																		1
C-cell, adenoma	X									X					X	X		X		X		X		X	X	15
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 22 ng TEQ/kg

Number of Days on Study	046	203	263	322	456	526	529	540	548	592	609	616	617	621	639	652	659	659	666	669	669	683	699	702	702	702	703	709
Carcass ID Number	243	292	244	298	222	252	229	290	245	260	266	225	282	256	212	294	291	295	227	202	224	287	257	214	269	289	246	223
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																												
Granulosa cell tumor benign														X														
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																												
Leiomyoma																		X										
Polyp stromal																										X		
Polyp stromal, multiple																												
Schwannoma malignant											X																	
Squamous cell carcinoma																												
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node														+										+				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma					X																							
Adenolipoma																				X								
Carcinoma																										X		
Fibroadenoma			X	X			X					X	X							X			X	X				
Fibroadenoma, multiple						X		X	X	X				X			X	X			X	X			X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma					X																							
Squamous cell papilloma																				X								
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																								X				
Granular cell tumor malignant																											X	
Medulloblastoma malignant															X													
Oligodendroglioma malignant		X																										
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																												
Carcinoma, metastatic, adrenal cortex																X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																												X
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 22 ng TEQ/kg

Number of Days on Study	723	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	226	201	204	205	241	249	258	268	280	285	288	203	242	247	250	254	255	259	267	270	276	278	281	283	297	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granulosa cell tumor malignant		X																								1
Granulosa cell tumor benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma															X										X	2
Leiomyoma																						X				2
Polyp stromal																						X				2
Polyp stromal, multiple																							X			1
Schwannoma malignant																										1
Squamous cell carcinoma																		X								1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoacanthoma																										1
Adenolipoma																										1
Carcinoma	X														X											3
Fibroadenoma	X	X					X				X				X	X		X		X		X		X	X	19
Fibroadenoma, multiple			X	X				X					X	X			X									16
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma											X															2
Squamous cell papilloma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, pituitary gland																										1
Granular cell tumor malignant																										1
Medulloblastoma malignant																										1
Oligodendroglioma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Alveolar/bronchiolar adenoma																									X	1
Carcinoma, metastatic, adrenal cortex																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 22 ng TEQ/kg

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

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 22 ng TEQ/kg

Number of Days on Study	723	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	226	201	204	205	241	249	258	268	280	285	288	203	242	247	250	254	255	259	267	270	276	278	281	283	297	Total Tissues/ Tumors
Special Senses System																										
Ear																										1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Hemangiosarcoma																							X			1
Ureter										+																1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenolipoma																										1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 46 ng TEQ/kg

Number of Days on Study	276	315	322	323	465	469	490	505	521	521	542	548	585	616	619	626	626	668	668	668	669	669	669	669	669	682	682	682	682
Carcass ID Number	373	389	361	382	306	314	333	309	323	324	319	393	318	320	334	317	379	359	364	374	310	311	321	325	344	312	341	391
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, uterus															X														
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma										X												X							
Cholangiocarcinoma, multiple																													
Hepatocellular adenoma																													
Mesentery								+											+										
Lipoma																			X										
Oral mucosa		+				+	+	+	+	+	+	+		+					+	+		+			+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, carcinoma																													
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth		+		+		+	+	+	+	+	+	+				+	+	+	+	+	+	+	+		+		+	+
Cardiovascular System																													
Blood vessel	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant					X																								
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																						X					X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																													
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X			X								X											
Thyroid gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																													
C-cell, adenoma												X				X										X			
C-cell, carcinoma																													
General Body System																													
None																													

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 46 ng TEQ/kg

Number of Days on Study	697	702	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	331	375	307	322	339	345	358	362	377	384	385	390	316	335	337	340	343	356	372	381	386	387	392	394	395	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant, metastatic, uterus																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cholangiocarcinoma				X						X	X															5
Cholangiocarcinoma, multiple									X					X												2
Hepatocellular adenoma																							X			1
Mesentery			+								+			+									+			6
Lipoma																										1
Oral mucosa				+		+	+		+	+	+			+		+			+	+	+	+			+	29
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Acinus, carcinoma												X												X		2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth		+		+			+		+	+	+	+		+		+		+	+	+	+	+	+	+	+	37
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Schwannoma malignant																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign												X				X										4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma					X																					1
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma		X		X	X	X	X	X			X			X	X		X			X	X	X	X	X	X	20
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Bilateral, C-cell, adenoma														X												1
C-cell, adenoma								X				X	X						X					X		8
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 the TEF Mixture: 46 ng TEQ/kg

Number of Days on Study	276	315	322	323	465	469	490	505	521	521	542	548	585	616	619	626	626	668	668	668	669	669	669	669	669	682	682	682
Carcass ID Number	373	389	361	382	306	314	333	309	323	324	319	393	318	320	334	317	379	359	364	374	310	311	321	325	344	312	341	391
Genital System																												
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																												
Oviduct																												
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma								X																				
Polyp stromal																												
Schwannoma malignant															X											X		
Vagina												+																
Schwannoma malignant												X																
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+				+					+		+	+	+												
Deep cervical, carcinoma, metastatic, thyroid gland																												
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma	X		X										X															
Fibroadenoma				X			X		X		X	X		X					X		X	X		X	X		X	X
Fibroadenoma, multiple																	X			X			X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma											X					X												
Fibrosarcoma																											X	
Pilomatrixoma																												
Schwannoma malignant, metastatic, uterus															X													
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																												
Carcinoma, metastatic, mammary gland	X												X															
Cystic keratinizing epithelioma																												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 46 ng TEQ/kg

Number of Days on Study	697	702	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	331	375	307	322	339	345	358	362	377	384	385	390	316	335	337	340	343	356	372	381	386	387	392	394	395	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granulosa cell tumor benign															X											1
Oviduct		+																		+						2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma											X															2
Polyp stromal	X																X						X			3
Schwannoma malignant																										2
Vagina																										1
Schwannoma malignant																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node	+								+														+			9
Deep cervical, carcinoma, metastatic, thyroid gland									X																	1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma					X										X											5
Fibroadenoma		X			X								X	X	X		X					X				20
Fibroadenoma, multiple	X			X			X	X			X															8
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma																								X		3
Fibrosarcoma																							X			1
Pilomatrixoma																										1
Schwannoma malignant, metastatic, uterus																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Alveolar/bronchiolar adenoma			X																							1
Carcinoma, metastatic, mammary gland																										1
Cystic keratinizing epithelioma	X															X										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 46 ng TEQ/kg

Number of Days on Study	2	3	3	3	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	7	1	2	3	6	6	9	0	2	2	4	4	8	1	1	2	2	6	6	6	6	6	6	6	6	8	8	8
	6	5	2	3	5	9	0	5	1	1	2	8	5	6	9	6	6	8	8	8	9	9	9	9	9	2	2	2
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	3	3	3
	7	8	6	8	0	1	3	0	2	2	1	9	1	2	3	1	7	5	6	7	1	1	2	2	4	1	4	9
	3	9	1	2	6	4	3	9	3	4	9	3	8	0	4	7	9	9	4	4	0	1	1	5	4	2	1	1
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																													
Ureter																													
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 the TEF Mixture: 46 ng TEQ/kg

Number of Days on Study	697	702	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	331	375	307	322	339	345	358	362	377	384	385	390	316	335	337	340	343	356	372	381	386	387	392	394	395	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Transitional epithelium, papilloma										X																1
Ureter																+										1
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 the TEF Mixture: 100 ng TEQ/kg

Number of Days on Study	002	028	312	339	387	392	399	409	434	434	497	498	526	542	546	548	548	562	563	575	589	592	598	598	617	623	631	633
Carcass ID Number	419	469	487	464	417	496	443	422	432	470	441	483	406	434	438	446	448	420	439	408	463	425	407	482	474	445	442	480
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	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Intestine small, ileum	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Liver	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																	X	X									X	
Cholangiocarcinoma, multiple																												
Hepatocellular adenoma															X				X			X						X
Hepatocellular adenoma, multiple										X																		
Histiocytic sarcoma			X																									
Mesentery			+																									
Oral mucosa				+			+		+		+	+	+	+	+	+				+	+	+			+	+	+	+
Gingival, squamous cell carcinoma									X													X						
Pancreas	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, skin																												X
Stomach, forestomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+																					
Tooth				+		+	+		+	+	+	+	+		+					+	+	+		+	+	+	+	+
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant			X							X		X																
Endocrine System																												
Adrenal cortex	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																												
Islets, pancreatic	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																												X
Thyroid gland	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma														X						X				X				
C-cell, carcinoma																												
Follicular cell, adenoma																												
General Body System																												
None																												

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 100 ng TEQ/kg

Number of Days on Study	634	639	658	666	674	674	674	682	682	688	689	689	715	718	721	728	728	729	729	729	729	729	729	729	729	
Carcass ID Number	449	476	485	488	472	478	481	418	486	436	668	467	489	465	440	421	444	409	410	416	423	435	437	467	479	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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Cholangiocarcinoma	X		X																							5
Cholangiocarcinoma, multiple							X							X		X				X						4
Hepatocellular adenoma				X					X						X	X							X			9
Hepatocellular adenoma, multiple																				X						2
Histiocytic sarcoma																										1
Mesentery	+							+							+	+				+			+		+	8
Oral mucosa		+						+	+	+		+	+	+	+	+	+	+	+	+				+	+	31
Gingival, squamous cell carcinoma																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant, metastatic, skin																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Tongue																										1
Tooth	+	+	+					+				+	+	+	+	+	+	+	+					+		30
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant																										3
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Pheochromocytoma benign							X																			1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	52
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma						X		X				X	X			X			X			X		X	X	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
C-cell, adenoma			X												X											5
C-cell, carcinoma						X																				1
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 the TEF Mixture: 100 ng TEQ/kg

Number of Days on Study	002	287	312	339	387	392	399	409	434	434	497	498	526	542	546	548	548	562	563	575	589	592	598	598	617	623	631	633
Carcass ID Number	419	469	487	464	417	496	443	422	432	470	441	483	406	434	438	446	448	420	439	408	463	425	407	482	474	445	442	480
Genital System																												
Clitoral gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Ovary	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																												
Oviduct												+			+									+		+		
Uterus	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																		X										
Hemangiosarcoma															X													
Polyp stromal																		X										
Schwannoma malignant								X																				
Cervix, squamous cell carcinoma																												
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+	+						+			+				+					+				+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Spleen	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																												
Mammary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																	X											
Fibroadenoma								X				X			X								X					
Fibroadenoma, multiple													X							X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																												X
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystic keratinizing epithelioma																			X		X		X					
Cystic keratinizing epithelioma, multiple														X		X									X			
Histiocytic sarcoma			X																									
Schwannoma malignant, metastatic, skin																												X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 100 ng TEQ/kg

Number of Days on Study	634	639	658	666	674	674	674	682	682	688	689	689	715	718	721	728	728	729	729	729	729	729	729	729	729	
Carcass ID Number	449	476	485	488	472	478	481	418	486	436	468	467	489	465	440	421	444	409	410	416	423	435	437	467	479	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Granulosa cell tumor malignant								X																		1
Oviduct			+																							5
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Carcinoma																										1
Hemangiosarcoma																										1
Polyp stromal						X																				2
Schwannoma malignant																										1
Cervix, squamous cell carcinoma																X										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node		+			+																+			+		11
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma																										1
Fibroadenoma				X	X			X			X	X						X	X		X	X	X		X	15
Fibroadenoma, multiple																X										3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cystic keratinizing epithelioma			X					X	X					X							X			X		9
Cystic keratinizing epithelioma, multiple							X			X		X			X		X	X	X						X	11
Histiocytic sarcoma																										1
Schwannoma malignant, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 100 ng TEQ/kg

Number of Days on Study	0	2	3	3	3	3	3	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6
	0	8	1	3	8	9	9	0	3	3	9	9	2	4	4	4	4	6	6	7	8	9	9	9	1	2	3	3
	2	7	2	9	7	2	9	9	4	4	7	8	6	2	6	8	8	2	3	5	9	2	8	8	7	3	1	3
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	4	4	4
	1	6	8	6	1	9	4	2	3	7	4	8	0	3	3	4	4	2	3	0	6	2	0	8	7	4	4	8
	9	9	7	4	7	6	3	2	2	0	1	3	6	4	8	6	8	0	9	8	3	5	7	2	4	5	2	0
Special Senses System																												
Ear																												
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																												
Kidney	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter							+																					
Urinary bladder	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																									
Lymphoma malignant					X																							

Table A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of the TEF Mixture: 100 ng TEQ/kg

Number of Days on Study	634	639	658	666	674	774	774	822	822	888	889	899	715	718	721	728	728	729	729	729	729	729	729	729	729	
Carcass ID Number	449	476	485	488	472	478	481	418	486	436	468	467	489	465	440	421	444	409	410	416	423	435	437	467	479	Total Tissues/ Tumors
Special Senses System																										
Ear																		+								1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Histiocytic sarcoma																										1
Lymphoma malignant					X																					2

TABLE A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Adrenal Medulla: Pheochromocytoma Benign					
Overall rate[a]	5/52 (10%)	4/53 (8%)	4/53 (8%)	4/53 (8%)	1/51 (2%)
Adjusted rate[b]	13.9%	9.7%	9.5%	10.0%	3.1%
Terminal rate[c]	3/16 (19%)	1/23 (4%)	1/24 (4%)	2/23 (9%)	0/8 (0%)
First incidence (days)	693	456	456	669	674
Poly-3 test[d]	P=0.128N	P=0.414N	P=0.398N	P=0.435N	P=0.122N
Heart: Schwannoma Malignant					
Overall rate	2/53 (4%)	0/53 (0%)	0/53 (0%)	1/52 (2%)	3/53 (6%)
Adjusted rate	5.4%	0.0%	0.0%	2.5%	8.6%
Terminal rate	1/16 (6%)	0/23 (0%)	0/24 (0%)	0/23 (0%)	0/8 (0%)
First incidence (days)	526	—[e]	—	465	312
Poly-3 test	P=0.090	P=0.219N	P=0.212N	P=0.471N	P=0.472
Liver: Hepatocellular Adenoma					
Overall rate	0/53 (0%)	1/53 (2%)	1/53 (2%)	1/53 (2%)	11/51 (22%)
Adjusted rate	0.0%	2.5%	2.4%	2.5%	31.0%
Terminal rate	0/16 (0%)	1/23 (4%)	1/24 (4%)	1/23 (4%)	2/8 (25%)
First incidence (days)	—	729 (T)	729 (T)	729 (T)	434
Poly-3 test	P<0.001	P=0.520	P=0.526	P=0.517	P<0.001
Liver: Cholangiocarcinoma					
Overall rate	0/53 (0%)	0/53 (0%)	2/53 (4%)	7/53 (13%)	9/51 (18%)
Adjusted rate	0.0%	0.0%	4.8%	17.4%	26.0%
Terminal rate	0/16 (0%)	0/23 (0%)	1/24 (4%)	5/23 (22%)	1/8 (13%)
First incidence (days)	—	—	669	521	548
Poly-3 test	P<0.001	—[f]	P=0.268	P=0.011	P<0.001
Lung: Cystic Keratinizing Epithelioma					
Overall rate	0/53 (0%)	0/53 (0%)	0/53 (0%)	2/53 (4%)	20/53 (38%)
Adjusted rate	0.0%	0.0%	0.0%	5.1%	54.7%
Terminal rate	0/16 (0%)	0/23 (0%)	0/24 (0%)	1/23 (4%)	5/8 (63%)
First incidence (days)	—	—	—	697	542
Poly-3 test	P<0.001	—	—	P=0.256	P<0.001
Mammary Gland: Fibroadenoma					
Overall rate	34/53 (64%)	27/53 (51%)	35/53 (66%)	28/53 (53%)	18/53 (34%)
Adjusted rate	74.5%	56.6%	71.9%	61.1%	48.7%
Terminal rate	11/16 (69%)	8/23 (35%)	16/24 (67%)	10/23 (44%)	6/8 (75%)
First incidence (days)	176	362	263	333	409
Poly-3 test	P=0.024N	P=0.047N	P=0.477N	P=0.115N	P=0.008N
Mammary Gland: Fibroadenoma or Adenoma					
Overall rate	34/53 (64%)	28/53 (53%)	35/53 (66%)	28/53 (53%)	18/53 (34%)
Adjusted rate	74.5%	58.7%	71.9%	61.1%	48.7%
Terminal rate	11/16 (69%)	8/23 (35%)	16/24 (67%)	10/23 (44%)	6/8 (75%)
First incidence (days)	176	362	263	333	409
Poly-3 test	P=0.018N	P=0.072N	P=0.477N	P=0.115N	P=0.008N
Mammary Gland: Carcinoma					
Overall rate	6/53 (11%)	6/53 (11%)	3/53 (6%)	5/53 (9%)	1/53 (2%)
Adjusted rate	15.5%	14.2%	7.2%	12.0%	3.0%
Terminal rate	2/16 (13%)	3/23 (13%)	1/24 (4%)	2/23 (9%)	0/8 (0%)
First incidence (days)	218	227	702	276	548
Poly-3 test	P=0.081N	P=0.559N	P=0.207N	P=0.446N	P=0.084N

Table A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Mammary Gland: Adenoma or Carcinoma					
Overall rate	6/53 (11%)	7/53 (13%)	3/53 (6%)	5/53 (9%)	1/53 (2%)
Adjusted rate	15.5%	16.5%	7.2%	12.0%	3.0%
Terminal rate	2/16 (13%)	3/23 (13%)	1/24 (4%)	2/23 (9%)	0/8 (0%)
First incidence (days)	218	227	702	276	548
Poly-3 test	P=0.062N	P=0.570	P=0.207N	P=0.446N	P=0.084N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma					
Overall rate	37/53 (70%)	33/53 (62%)	36/53 (68%)	31/53 (58%)	19/53 (36%)
Adjusted rate	77.1%	65.9%	73.7%	64.4%	50.6%
Terminal rate	11/16 (69%)	10/23 (44%)	16/24 (67%)	10/23 (44%)	6/8 (75%)
First incidence (days)	176	227	263	276	409
Poly-3 test	P=0.008N	P=0.151N	P=0.441N	P=0.116N	P=0.005N
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	28/53 (53%)	29/53 (55%)	22/53 (42%)	20/53 (38%)	10/53 (19%)
Adjusted rate	67.2%	62.5%	50.4%	48.4%	30.0%
Terminal rate	11/16 (69%)	11/23 (48%)	12/24 (50%)	15/23 (65%)	4/8 (50%)
First incidence (days)	416	416	592	490	633
Poly-3 test	P<0.001N	P=0.401N	P=0.075N	P=0.052N	P<0.001N
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma or Carcinoma					
Overall rate	28/53 (53%)	29/53 (55%)	23/53 (43%)	20/53 (38%)	10/53 (19%)
Adjusted rate	67.2%	62.5%	52.5%	48.4%	30.0%
Terminal rate	11/16 (69%)	11/23 (48%)	12/24 (50%)	15/23 (65%)	4/8 (50%)
First incidence (days)	416	416	592	490	633
Poly-3 test	P<0.001N	P=0.401N	P=0.110N	P=0.052N	P<0.001N
Skin: Fibroma					
Overall rate	1/53 (2%)	2/53 (4%)	2/53 (4%)	3/53 (6%)	0/53 (0%)
Adjusted rate	2.8%	5.0%	4.8%	7.4%	0.0%
Terminal rate	0/16 (0%)	1/23 (4%)	1/24 (4%)	1/23 (4%)	0/8 (0%)
First incidence (days)	693	668	456	542	—
Poly-3 test	P=0.363N	P=0.535	P=0.550	P=0.343	P=0.524N
Skin: Fibroma or Fibrosarcoma					
Overall rate	2/53 (4%)	2/53 (4%)	2/53 (4%)	4/53 (8%)	0/53 (0%)
Adjusted rate	5.5%	5.0%	4.8%	9.9%	0.0%
Terminal rate	0/16 (0%)	1/23 (4%)	1/24 (4%)	1/23 (4%)	0/8 (0%)
First incidence (days)	666	668	456	542	—
Poly-3 test	P=0.320N	P=0.661N	P=0.644N	P=0.384	P=0.265N
Thyroid Gland (C-Cell): Adenoma					
Overall rate	17/53 (32%)	10/53 (19%)	18/51 (35%)	9/52 (17%)	5/51 (10%)
Adjusted rate	44.4%	24.5%	42.7%	22.2%	14.7%
Terminal rate	7/16 (44%)	6/23 (26%)	10/24 (42%)	6/23 (26%)	0/8 (0%)
First incidence (days)	568	627	617	548	542
Poly-3 test	P=0.005N	P=0.045N	P=0.531N	P=0.027N	P=0.004N
Thyroid Gland (C-Cell): Adenoma or Carcinoma					
Overall rate	18/53 (34%)	10/53 (19%)	18/51 (35%)	10/52 (19%)	6/51 (12%)
Adjusted rate	46.4%	24.5%	42.7%	24.7%	17.5%
Terminal rate	7/16 (44%)	6/23 (26%)	10/24 (42%)	7/23 (30%)	0/8 (0%)
First incidence (days)	568	627	617	548	542
Poly-3 test	P=0.011N	P=0.029N	P=0.457N	P=0.031N	P=0.006N

Table A3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Uterus: Polyp Stromal					
Overall rate	4/53 (8%)	1/53 (2%)	3/53 (6%)	3/53 (6%)	2/53 (4%)
Adjusted rate	10.9%	2.5%	7.3%	7.6%	6.0%
Terminal rate	1/16 (6%)	1/23 (4%)	2/24 (8%)	2/23 (9%)	0/8 (0%)
First incidence (days)	627	729 (T)	702	697	562
Poly-3 test	P=0.512N	P=0.153N	P=0.437N	P=0.461N	P=0.387N
All Organs: Benign Neoplasms					
Overall rate	45/53 (85%)	43/53 (81%)	46/53 (87%)	43/53 (81%)	39/53 (74%)
Adjusted rate	93.1%	86.5%	91.6%	91.1%	90.1%
Terminal rate	14/16 (88%)	18/23 (78%)	22/24 (92%)	21/23 (91%)	8/8 (100%)
First incidence (days)	176	362	263	333	409
Poly-3 test	P=0.550N	P=0.211N	P=0.539N	P=0.506N	P=0.433N
All Organs: Malignant Neoplasms					
Overall rate	18/53 (34%)	14/53 (26%)	19/53 (36%)	21/53 (40%)	21/53 (40%)
Adjusted rate	41.6%	31.1%	42.5%	45.9%	50.6%
Terminal rate	5/16 (31%)	7/23 (30%)	8/24 (33%)	9/23 (39%)	1/8 (13%)
First incidence (days)	218	227	203	276	312
Poly-3 test	P=0.080	P=0.204N	P=0.553	P=0.423	P=0.264
All Organs: Benign or Malignant Neoplasms					
Overall rate	51/53 (96%)	46/53 (87%)	50/53 (94%)	50/53 (94%)	45/53 (85%)
Adjusted rate	96.2%	88.8%	96.2%	97.4%	95.6%
Terminal rate	14/16 (88%)	18/23 (78%)	22/24 (92%)	22/23 (96%)	8/8 (100%)
First incidence (days)	176	227	203	276	312
Poly-3 test	P=0.319	P=0.136N	P=0.686N	P=0.591	P=0.649N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, heart, liver, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

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

Study	Incidence in Controls	
	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 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 A4c
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 A4d
Historical Incidence of Adrenal Cortex Neoplasms in Vehicle Control Female Sprague-Dawley Rats[a]

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

[a] Data as of February 24, 2005

Table A4e
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 A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
Disposition Summary					
Animals initially in study	81	81	81	81	81
14-Week interim evaluation	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8
Early deaths					
Accidental deaths			1	1	2
Moribund	26	25	22	25	33
Natural deaths	11	5	6	4	10
Survivors					
Terminal sacrifice	16	23	24	23	8
Animals examined microscopically	81	81	81	81	81
14-Week Interim Evaluation					
Alimentary System					
Liver	(10)	(10)	(10)	(10)	(10)
Clear cell focus					1 (10%)
Fatty change, diffuse				1 (10%)	
Inflammation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	8 (80%)
Mixed cell focus, multiple					1 (10%)
Hepatocyte, hypertrophy		1 (10%)	3 (30%)	5 (50%)	8 (80%)
Pancreas	(10)	(10)	(10)	(10)	(10)
Basophilic focus					1 (10%)
Inflammation, chronic active				2 (20%)	
Acinus, atrophy					1 (10%)
Stomach, forestomach	(10)				(10)
Hyperkeratosis	1 (10%)				
Hyperplasia, squamous	1 (10%)				
Inflammation	1 (10%)				
Stomach, glandular	(10)				(10)
Cyst					1 (10%)
Endocrine System					
Adrenal cortex	(10)	(10)	(10)	(10)	(10)
Hyperplasia					1 (10%)
Hypertrophy	1 (10%)				
Thyroid gland	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy		3 (30%)	7 (70%)	10 (100%)	7 (70%)
Genital System					
Ovary	(10)	(10)	(10)	(10)	(10)
Atrophy		1 (10%)	2 (20%)	3 (30%)	4 (40%)
Uterus	(10)	(10)	(10)	(10)	(10)
Metaplasia, squamous		1 (10%)	1 (10%)		2 (20%)
Endometrium, hyperplasia, cystic	2 (20%)				2 (20%)

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

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
14-Week Interim Evaluation (continued)					
Hematopoietic System					
Spleen	(10)				(10)
Pigmentation	10 (100%)				10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)
Atrophy		2 (20%)	3 (30%)	4 (40%)	7 (70%)
Respiratory System					
Lung	(10)	(10)	(10)	(10)	(10)
Hemorrhage			1 (10%)	1 (10%)	
Infiltration cellular, histiocyte			1 (10%)		
Inflammation, chronic active					1 (10%)
Alveolar epithelium, hyperplasia				1 (10%)	
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)
Clear cell focus			1 (10%)		
Eosinophilic focus					1 (10%)
Fatty change, diffuse					1 (10%)
Inflammation	8 (80%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus	3 (30%)	2 (20%)	1 (10%)	4 (40%)	2 (20%)
Mixed cell focus, multiple	3 (30%)	2 (20%)	4 (40%)	4 (40%)	6 (60%)
Pigmentation		4 (40%)	9 (90%)	10 (100%)	10 (100%)
Hepatocyte, hypertrophy		3 (30%)	5 (50%)	9 (90%)	10 (100%)
Hepatocyte, multinucleated				1 (10%)	8 (80%)
Pancreas	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	1 (10%)				1 (10%)
Acinus, atrophy	1 (10%)	1 (10%)	2 (20%)		
Acinus, vacuolization cytoplasmic					5 (50%)
Stomach, glandular	(10)				(10)
Glands, ectasia					2 (20%)
Endocrine System					
Adrenal cortex	(10)	(10)	(10)	(10)	(10)
Degeneration, cystic	1 (10%)				
Hyperplasia	1 (10%)				2 (20%)
Hypertrophy	5 (50%)	1 (10%)	1 (10%)	1 (10%)	3 (30%)
Vacuolization cytoplasmic	1 (10%)				

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
31-Week Interim Evaluation (continued)					
Endocrine System (continued)					
Pituitary gland	(10)				(10)
Hyperplasia	1 (10%)				
Hypertrophy					1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)	(10)
C-cell, hyperplasia	1 (10%)				
Follicular cell, hypertrophy	1 (10%)	5 (50%)	1 (10%)	4 (40%)	3 (30%)
Genital System					
Ovary	(10)	(10)	(10)	(10)	(10)
Atrophy	10 (100%)	9 (90%)	9 (90%)	8 (80%)	9 (90%)
Uterus	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative	4 (40%)		1 (10%)		2 (20%)
Metaplasia, squamous	8 (80%)	9 (90%)	8 (80%)	8 (80%)	8 (80%)
Endometrium, hyperplasia, cystic	2 (20%)		1 (10%)		2 (20%)
Hematopoietic System					
Lymph node			(1)		
Metaplasia, squamous			1 (100%)		
Spleen	(10)				(10)
Pigmentation	10 (100%)				10 (100%)
Thymus	(10)	(10)	(10)	(10)	(9)
Atrophy			3 (30%)	7 (70%)	7 (78%)
Respiratory System					
Lung	(10)	(10)	(10)	(10)	(10)
Hemorrhage				1 (10%)	
Infiltration cellular, histiocyte	1 (10%)	1 (10%)	1 (10%)		1 (10%)
Urinary System					
Kidney				(1)	
Inflammation, chronic active				1 (100%)	
Systems Examined with No Lesions Observed					
Cardiovascular System					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
53-Week Interim Evaluation					
Alimentary System					
Liver	(8)	(8)	(8)	(8)	(8)
Basophilic focus	1 (13%)				1 (13%)
Cholangiofibrosis				2 (25%)	1 (13%)
Clear cell focus	1 (13%)	1 (13%)			1 (13%)
Eosinophilic focus			1 (13%)		1 (13%)
Eosinophilic focus, multiple		1 (13%)			
Fatty change, diffuse					2 (25%)
Fatty change, focal				3 (38%)	4 (50%)
Inflammation	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Mixed cell focus	2 (25%)	2 (25%)	4 (50%)	1 (13%)	
Mixed cell focus, multiple	1 (13%)	2 (25%)	3 (38%)	5 (63%)	7 (88%)
Pigmentation		4 (50%)	8 (100%)	8 (100%)	8 (100%)
Regeneration					1 (13%)
Toxic hepatopathy				3 (38%)	8 (100%)
Bile duct, fibrosis					1 (13%)
Bile duct, hyperplasia					6 (75%)
Bile duct, inflammation, chronic active				1 (13%)	1 (13%)
Hepatocyte, hypertrophy		5 (63%)	7 (88%)	8 (100%)	8 (100%)
Hepatocyte, multinucleated			3 (38%)		8 (100%)
Pancreas	(8)	(8)	(8)	(8)	(8)
Basophilic focus					1 (13%)
Inflammation, chronic active				1 (13%)	1 (13%)
Acinus, atrophy				1 (13%)	1 (13%)
Acinus, vacuolization cytoplasmic				2 (25%)	7 (88%)
Stomach, glandular	(8)			(1)	(8)
Cyst, squamous	1 (13%)				
Developmental malformation				1 (100%)	
Endocrine System					
Adrenal cortex	(8)	(8)	(8)	(8)	(8)
Degeneration, cystic	1 (13%)				2 (25%)
Hyperplasia			2 (25%)	1 (13%)	2 (25%)
Hypertrophy	3 (38%)	7 (88%)	5 (63%)	3 (38%)	5 (63%)
Vacuolization cytoplasmic					1 (13%)
Thyroid gland	(8)	(8)	(8)	(8)	(8)
C-cell, hyperplasia			1 (13%)		
Follicular cell, hypertrophy	2 (25%)	2 (25%)	3 (38%)	3 (38%)	4 (50%)
Genital System					
Ovary	(8)	(8)	(8)	(8)	(8)
Atrophy	8 (100%)	8 (100%)	8 (100%)	6 (75%)	8 (100%)
Cyst				1 (13%)	
Inflammation, suppurative	1 (13%)				
Uterus	(8)	(8)	(8)	(8)	(8)
Inflammation, suppurative	1 (13%)				
Metaplasia, squamous	8 (100%)	8 (100%)	8 (100%)	6 (75%)	7 (88%)
Endometrium, hyperplasia, cystic	6 (75%)				2 (25%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
53-Week Interim Evaluation (continued)					
Hematopoietic System					
Spleen	(8)				(8)
Pigmentation	8 (100%)				8 (100%)
Thymus	(8)	(8)	(8)	(8)	(6)
Atrophy	3 (38%)	7 (88%)	8 (100%)	8 (100%)	6 (100%)
Integumentary System					
Mammary gland	(8)	(1)	(1)	(1)	(8)
Cyst	3 (38%)				
Hyperplasia	1 (13%)				1 (13%)
Skin			(1)		
Ulcer			1 (100%)		
Respiratory System					
Lung	(8)	(8)	(8)	(8)	(8)
Infiltration cellular, histiocyte	6 (75%)	5 (63%)	5 (63%)	4 (50%)	6 (75%)
Inflammation		1 (13%)			
Inflammation, chronic active				1 (13%)	
Alveolar epithelium, metaplasia, bronchiolar		1 (13%)	2 (25%)	4 (50%)	6 (75%)
Urinary System					
Kidney			(1)	(1)	
Inflammation, chronic active			1 (100%)	1 (100%)	
Nephropathy			1 (100%)		
Systems Examined with No Lesions Observed					
Cardiovascular System					
General Body System					
Musculoskeletal System					
Nervous System					
Special Senses System					

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study					
Alimentary System					
Esophagus	(53)	(53)	(53)	(53)	(53)
Muscularis, inflammation		2 (4%)	1 (2%)	1 (2%)	
Intestine large, colon	(53)	(52)	(53)	(53)	(51)
Parasite metazoan			1 (2%)		
Intestine large, rectum	(52)	(53)	(53)	(53)	(52)
Parasite metazoan	2 (4%)	4 (8%)	5 (9%)	1 (2%)	
Artery, inflammation, chronic active				2 (4%)	3 (6%)
Serosa, inflammation					1 (2%)
Intestine large, cecum	(51)	(53)	(53)	(53)	(51)
Artery, inflammation, chronic active					1 (2%)
Intestine small, duodenum	(52)	(53)	(53)	(53)	(52)
Serosa, inflammation, chronic active					1 (2%)
Liver	(53)	(53)	(53)	(53)	(51)
Angiectasis	3 (6%)	2 (4%)			3 (6%)
Basophilic focus	9 (17%)	14 (26%)	10 (19%)	5 (9%)	8 (16%)
Basophilic focus, multiple	15 (28%)	12 (23%)	3 (6%)	7 (13%)	8 (16%)
Cholangiofibrosis		2 (4%)	3 (6%)	4 (8%)	17 (33%)
Clear cell focus		1 (2%)	1 (2%)		
Clear cell focus, multiple	1 (2%)			1 (2%)	
Degeneration, cystic	1 (2%)			1 (2%)	2 (4%)
Eosinophilic focus	4 (8%)	2 (4%)	5 (9%)	5 (9%)	
Eosinophilic focus, multiple	1 (2%)	7 (13%)	6 (11%)	15 (28%)	19 (37%)
Fatty change, diffuse	3 (6%)	5 (9%)	14 (26%)	34 (64%)	36 (71%)
Fatty change, focal	3 (6%)	4 (8%)	7 (13%)	2 (4%)	1 (2%)
Hematopoietic cell proliferation	16 (30%)	18 (34%)	19 (36%)	8 (15%)	9 (18%)
Hepatodiaphragmatic nodule			1 (2%)		
Hyperplasia, nodular		1 (2%)	3 (6%)	11 (21%)	38 (75%)
Inflammation	36 (68%)	50 (94%)	45 (85%)	50 (94%)	50 (98%)
Karyomegaly				1 (2%)	
Mixed cell focus	4 (8%)	5 (9%)	5 (9%)	1 (2%)	
Mixed cell focus, multiple	17 (32%)	27 (51%)	30 (57%)	35 (66%)	17 (33%)
Necrosis	3 (6%)	1 (2%)	9 (17%)	3 (6%)	15 (29%)
Pigmentation	4 (8%)	35 (66%)	41 (77%)	48 (91%)	51 (100%)
Toxic hepatopathy		5 (9%)	14 (26%)	38 (72%)	47 (92%)
Bile duct, cyst	1 (2%)	3 (6%)	3 (6%)	4 (8%)	9 (18%)
Bile duct, dilatation	1 (2%)				1 (2%)
Bile duct, fibrosis	3 (6%)	2 (4%)	1 (2%)	5 (9%)	1 (2%)
Bile duct, hyperplasia	2 (4%)	3 (6%)	5 (9%)	25 (47%)	42 (82%)
Centrilobular, degeneration	3 (6%)	2 (4%)	1 (2%)	1 (2%)	6 (12%)
Centrilobular, fibrosis					1 (2%)
Hepatocyte, hypertrophy	1 (2%)	27 (51%)	34 (64%)	46 (87%)	50 (98%)
Hepatocyte, multinucleated		12 (23%)	10 (19%)	39 (74%)	51 (100%)
Oval cell, hyperplasia		1 (2%)	1 (2%)	26 (49%)	42 (82%)
Portal, fibrosis					11 (22%)
Serosa, fibrosis					1 (2%)
Serosa, inflammation, chronic	1 (2%)				

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Alimentary System (continued)					
Mesentery		(1)	(2)	(6)	(8)
Inflammation, chronic active					2 (25%)
Necrosis			1 (50%)		1 (13%)
Artery, inflammation, chronic active		1 (100%)		3 (50%)	6 (75%)
Fat, necrosis			1 (50%)	2 (33%)	
Oral mucosa	(10)	(18)	(19)	(29)	(31)
Gingival, hyperplasia, squamous	8 (80%)	17 (94%)	18 (95%)	26 (90%)	30 (97%)
Pancreas	(52)	(53)	(53)	(53)	(51)
Degeneration	1 (2%)				
Inflammation, chronic active	3 (6%)	1 (2%)	6 (11%)	7 (13%)	16 (31%)
Inflammation, granulomatous				1 (2%)	
Acinus, atrophy	3 (6%)	2 (4%)	7 (13%)	7 (13%)	20 (39%)
Acinus, hyperplasia	2 (4%)		1 (2%)		
Acinus, vacuolization cytoplasmic	1 (2%)		3 (6%)	15 (28%)	30 (59%)
Artery, inflammation, chronic active		6 (11%)	3 (6%)	8 (15%)	14 (27%)
Duct, cyst			1 (2%)		
Duct, dilatation					5 (10%)
Duct, inflammation, chronic active					2 (4%)
Salivary glands	(53)	(53)	(53)	(53)	(53)
Atrophy	1 (2%)				
Inflammation	2 (4%)			1 (2%)	
Mineralization				1 (2%)	
Stomach, forestomach	(53)	(53)	(53)	(53)	(52)
Cyst				1 (2%)	1 (2%)
Diverticulum				1 (2%)	
Edema	1 (2%)		1 (2%)		
Erosion			1 (2%)		
Hyperkeratosis			2 (4%)	2 (4%)	
Hyperplasia, squamous	5 (9%)	1 (2%)	4 (8%)	9 (17%)	6 (12%)
Inflammation	2 (4%)		2 (4%)	1 (2%)	3 (6%)
Mineralization	1 (2%)		4 (8%)	2 (4%)	
Ulcer	3 (6%)		2 (4%)	1 (2%)	2 (4%)
Artery, inflammation, chronic active				2 (4%)	1 (2%)
Stomach, glandular	(53)	(53)	(53)	(53)	(52)
Erosion	1 (2%)				
Mineralization	2 (4%)	4 (8%)	3 (6%)	3 (6%)	1 (2%)
Artery, inflammation, chronic active					2 (4%)
Tongue	(1)				(1)
Infiltration cellular					1 (100%)
Tooth	(24)	(23)	(27)	(37)	(30)
Peridontal tissue, inflammation	23 (96%)	21 (91%)	22 (81%)	34 (92%)	30 (100%)
Cardiovascular System					
Blood vessel	(53)	(53)	(53)	(52)	(53)
Aorta, mineralization				1 (2%)	3 (6%)
Heart	(53)	(53)	(53)	(52)	(53)
Cardiomyopathy	11 (21%)	26 (49%)	31 (58%)	30 (58%)	32 (60%)
Inflammation, suppurative	1 (2%)				1 (2%)
Mineralization				1 (2%)	
Necrosis					1 (2%)
Thrombosis	1 (2%)				1 (2%)
Artery, degeneration				1 (2%)	1 (2%)
Artery, inflammation, chronic active				1 (2%)	1 (2%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Endocrine System					
Adrenal cortex	(52)	(53)	(53)	(53)	(51)
Angiectasis	15 (29%)	20 (38%)	18 (34%)	17 (32%)	8 (16%)
Atrophy		3 (6%)			18 (35%)
Degeneration, cystic	9 (17%)	15 (28%)	19 (36%)	25 (47%)	16 (31%)
Hematopoietic cell proliferation			1 (2%)		1 (2%)
Hyperplasia	12 (23%)	26 (49%)	23 (43%)	25 (47%)	21 (41%)
Hypertrophy	44 (85%)	45 (85%)	47 (89%)	46 (87%)	45 (88%)
Inflammation				1 (2%)	
Mineralization		1 (2%)			1 (2%)
Necrosis	2 (4%)		2 (4%)		2 (4%)
Thrombosis		1 (2%)			
Vacuolization cytoplasmic	6 (12%)	13 (25%)	11 (21%)	7 (13%)	15 (29%)
Capsule, inflammation				1 (2%)	1 (2%)
Adrenal medulla	(52)	(53)	(53)	(53)	(51)
Hyperplasia	10 (19%)	21 (40%)	12 (23%)	15 (28%)	9 (18%)
Islets, pancreatic	(52)	(53)	(53)	(53)	(51)
Hyperplasia			1 (2%)	2 (4%)	
Pituitary gland	(53)	(53)	(53)	(53)	(53)
Angiectasis	6 (11%)		1 (2%)	2 (4%)	3 (6%)
Cyst	1 (2%)		1 (2%)	2 (4%)	
Cytoplasmic alteration		3 (6%)			
Hemorrhage	1 (2%)				
Vacuolization cytoplasmic		1 (2%)	2 (4%)	3 (6%)	1 (2%)
Pars distalis, hyperplasia	18 (34%)	19 (36%)	24 (45%)	20 (38%)	19 (36%)
Pars intermedia, cyst			1 (2%)		
Thyroid gland	(53)	(53)	(51)	(52)	(51)
C-cell, hyperplasia	24 (45%)	24 (45%)	20 (39%)	18 (35%)	14 (27%)
Follicular cell, hyperplasia	1 (2%)				
Follicular cell, hypertrophy	4 (8%)	13 (25%)	12 (24%)	18 (35%)	23 (45%)
General Body System					
None					
Genital System					
Clitoral gland	(51)	(53)	(53)	(51)	(50)
Hyperplasia, squamous				1 (2%)	
Inflammation	42 (82%)	40 (75%)	37 (70%)	33 (65%)	29 (58%)
Duct, cyst	39 (76%)	41 (77%)	44 (83%)	39 (76%)	44 (88%)
Ovary	(52)	(52)	(53)	(53)	(51)
Atrophy	44 (85%)	44 (85%)	43 (81%)	49 (92%)	40 (78%)
Cyst	8 (15%)	16 (31%)	20 (38%)	14 (26%)	7 (14%)
Fibrosis		1 (2%)			
Hemorrhage					1 (2%)
Inflammation, chronic active		2 (4%)	1 (2%)	2 (4%)	5 (10%)
Artery, inflammation, chronic active					1 (2%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Genital System (continued)					
Oviduct		(2)		(2)	(5)
Cyst		2 (100%)			1 (20%)
Inflammation, chronic active				2 (100%)	4 (80%)
Uterus	(52)	(53)	(53)	(53)	(51)
Adenomyosis		1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)				1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Inflammation, suppurative	6 (12%)	5 (9%)	9 (17%)	13 (25%)	6 (12%)
Metaplasia, squamous	21 (40%)	32 (60%)	32 (60%)	35 (66%)	30 (59%)
Necrosis					1 (2%)
Thrombosis				1 (2%)	
Ulcer		1 (2%)		1 (2%)	
Cervix, hyperplasia, stromal	1 (2%)				
Endometrium, fibrosis					1 (2%)
Endometrium, hyperplasia, cystic	37 (71%)	35 (66%)	34 (64%)	33 (62%)	23 (45%)
Epithelium, necrosis	1 (2%)				
Hematopoietic System					
Bone marrow	(53)	(53)	(53)	(53)	(53)
Hyperplasia	36 (68%)	36 (68%)	34 (64%)	41 (77%)	48 (91%)
Lymph node	(4)	(1)	(2)	(9)	(11)
Lumbar, ectasia			1 (50%)		
Lumbar, hemorrhage			1 (50%)		
Lumbar, hyperplasia, lymphoid			1 (50%)		1 (9%)
Lumbar, hyperplasia, plasma cell	1 (25%)			3 (33%)	
Mediastinal, ectasia		1 (100%)		4 (44%)	2 (18%)
Mediastinal, fibrosis				1 (11%)	
Mediastinal, hemorrhage				1 (11%)	2 (18%)
Mediastinal, hyperplasia, histiocytic				1 (11%)	1 (9%)
Mediastinal, hyperplasia, lymphoid					2 (18%)
Mediastinal, hyperplasia, plasma cell					3 (27%)
Pancreatic, ectasia	1 (25%)				
Pancreatic, hyperplasia					1 (9%)
Pancreatic, hyperplasia, histiocytic					1 (9%)
Popliteal, hyperplasia, plasma cell				1 (11%)	
Renal, hyperplasia, histiocytic	1 (25%)				
Lymph node, mandibular	(53)	(53)	(53)	(53)	(53)
Ectasia		1 (2%)	1 (2%)	2 (4%)	
Hemorrhage				1 (2%)	
Hyperplasia, plasma cell	31 (58%)	42 (79%)	30 (57%)	22 (42%)	27 (51%)
Necrosis, focal				1 (2%)	
Lymph node, mesenteric	(52)	(53)	(53)	(53)	(50)
Ectasia	1 (2%)				
Hemorrhage	1 (2%)				1 (2%)
Hyperplasia					1 (2%)
Hyperplasia, histiocytic				1 (2%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)		
Pigmentation				1 (2%)	

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Hematopoietic System (continued)					
Spleen	(52)	(53)	(53)	(53)	(51)
Hematopoietic cell proliferation	43 (83%)	48 (91%)	42 (79%)	44 (83%)	42 (82%)
Hemorrhage					1 (2%)
Inflammation, suppurative					1 (2%)
Necrosis	1 (2%)				1 (2%)
Pigmentation	45 (87%)	50 (94%)	52 (98%)	47 (89%)	46 (90%)
Lymphoid follicle, atrophy	1 (2%)	2 (4%)	3 (6%)	2 (4%)	3 (6%)
Red pulp, atrophy		1 (2%)	3 (6%)		
Thymus	(52)	(48)	(50)	(53)	(50)
Atrophy	32 (62%)	43 (90%)	45 (90%)	50 (94%)	48 (96%)
Cyst	2 (4%)				
Ectopic thyroid				1 (2%)	
Hemorrhage				1 (2%)	
Integumentary System					
Mammary gland	(53)	(53)	(53)	(53)	(52)
Cyst		3 (6%)	1 (2%)	1 (2%)	
Hyperplasia	22 (42%)	7 (13%)	14 (26%)	12 (23%)	9 (17%)
Inflammation, granulomatous	3 (6%)	4 (8%)	1 (2%)	3 (6%)	1 (2%)
Inflammation, suppurative				1 (2%)	
Skin	(53)	(53)	(53)	(53)	(53)
Cyst epithelial inclusion		2 (4%)	1 (2%)	3 (6%)	3 (6%)
Hyperkeratosis			1 (2%)		
Inflammation, suppurative				1 (2%)	
Ulcer				1 (2%)	1 (2%)
Hair follicle, atrophy		1 (2%)			
Musculoskeletal System					
Bone	(53)	(53)	(53)	(53)	(53)
Mineralization			1 (2%)		
Nervous System					
Brain	(53)	(53)	(53)	(53)	(53)
Edema				1 (2%)	3 (6%)
Gliosis	1 (2%)				1 (2%)
Hemorrhage		1 (2%)			
Hydrocephalus	1 (2%)	4 (8%)	2 (4%)	2 (4%)	
Inflammation, granulomatous			1 (2%)		
Mineralization	1 (2%)		1 (2%)	1 (2%)	
Necrosis					3 (6%)
Artery, degeneration					1 (2%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Respiratory System					
Lung	(53)	(53)	(53)	(53)	(53)
Congestion			1 (2%)	1 (2%)	
Cyst					1 (2%)
Edema			2 (4%)	2 (4%)	1 (2%)
Hemorrhage			1 (2%)		1 (2%)
Infiltration cellular, histiocyte	43 (81%)	50 (94%)	48 (91%)	48 (91%)	50 (94%)
Inflammation	4 (8%)	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Metaplasia, squamous	2 (4%)		2 (4%)	8 (15%)	11 (21%)
Mineralization				1 (2%)	
Alveolar epithelium, hyperplasia	21 (40%)	25 (47%)	10 (19%)	2 (4%)	2 (4%)
Alveolar epithelium, metaplasia, bronchiolar		20 (38%)	33 (62%)	41 (77%)	40 (75%)
Perivascular, inflammation, chronic active					1 (2%)
Nose	(53)	(53)	(53)	(53)	(53)
Inflammation	1 (2%)	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Goblet cell, hyperplasia			2 (4%)	1 (2%)	
Goblet cell, septum, hyperplasia				1 (2%)	
Nasolacrimal duct, inflammation				1 (2%)	
Respiratory epithelium, hyperplasia		1 (2%)		4 (8%)	
Turbinate, cyst				1 (2%)	
Turbinate, respiratory epithelium, hyperplasia					1 (2%)
Special Senses System					
Eye	(53)	(53)	(53)	(53)	(53)
Anterior chamber, ciliary body, iris, inflammation, suppurative					1 (2%)
Cornea, inflammation, suppurative					1 (2%)
Lens, degeneration		1 (2%)			
Retina, atrophy	1 (2%)	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Harderian gland	(53)	(53)	(52)	(53)	(53)
Inflammation	8 (15%)	11 (21%)	4 (8%)	5 (9%)	7 (13%)
Inflammation, chronic active					1 (2%)
Necrosis					1 (2%)
Urinary System					
Kidney	(52)	(53)	(53)	(53)	(51)
Calculus microscopic observation only	9 (17%)	5 (9%)	8 (15%)	4 (8%)	1 (2%)
Casts protein			1 (2%)	1 (2%)	
Cyst		1 (2%)		1 (2%)	
Developmental malformation					1 (2%)
Fibrosis					1 (2%)
Inflammation, chronic				1 (2%)	
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)		
Inflammation, suppurative	3 (6%)	2 (4%)	3 (6%)	3 (6%)	6 (12%)
Mineralization	41 (79%)	48 (91%)	47 (89%)	42 (79%)	35 (69%)
Nephropathy	26 (50%)	41 (77%)	40 (75%)	47 (89%)	49 (96%)
Pelvis, dilatation	2 (4%)		1 (2%)	1 (2%)	1 (2%)
Pelvis, inflammation	2 (4%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Renal tubule, hyperplasia				2 (4%)	
Renal tubule, necrosis	1 (2%)		1 (2%)		
Transitional epithelium, hyperplasia	5 (10%)	5 (9%)	8 (15%)	10 (19%)	8 (16%)

Table A5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
2-Year Study (continued)					
Urinary System (continued)					
Ureter	(1)		(1)	(1)	(1)
Cyst	1 (100%)				
Inflammation			1 (100%)		
Metaplasia, squamous				1 (100%)	
Transitional epithelium, hyperplasia			1 (100%)	1 (100%)	
Urinary bladder	(52)	(52)	(53)	(53)	(50)
Edema				1 (2%)	
Hemorrhage					1 (2%)
Inflammation	12 (23%)	3 (6%)	4 (8%)	8 (15%)	5 (10%)
Metaplasia, squamous				1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	3 (6%)	4 (8%)

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 Evalutions in the 2-Year Gavage Study of the TEF Mixture 146

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 the TEF Mixture[a]

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Necropsy body wt					
Week 14	299 ± 8	280 ± 7*	282 ± 4	277 ± 6*	260 ± 6**
Week 31	291 ± 6	297 ± 5	300 ± 7	294 ± 8	295 ± 6
Week 53	324 ± 5	308 ± 9	316 ± 9	320 ± 11	288 ± 5**
L. Kidney					
Week 14					
Absolute	0.773 ± 0.018	0.818 ± 0.021	0.788 ± 0.011	0.771 ± 0.014	0.764 ± 0.020
Relative	2.594 ± 0.052	2.920 ± 0.046**	2.798 ± 0.035**	2.785 ± 0.050**	2.943 ± 0.038**
Week 31					
Absolute	0.817 ± 0.018	0.906 ± 0.027*	0.843 ± 0.020	0.856 ± 0.025	0.840 ± 0.016
Relative	2.816 ± 0.052	3.052 ± 0.085*	2.821 ± 0.066	2.915 ± 0.048	2.852 ± 0.043
Week 53					
Absolute	0.964 ± 0.030	0.922 ± 0.028	0.906 ± 0.029	1.011 ± 0.024	0.877 ± 0.026
Relative	2.972 ± 0.056	3.005 ± 0.098	2.868 ± 0.066	3.193 ± 0.168	3.052 ± 0.086
Liver					
Week 14					
Absolute	9.323 ± 0.264	10.071 ± 0.275	9.774 ± 0.337	10.109 ± 0.318	10.715 ± 0.368*
Relative	31.197 ± 0.387	35.933 ± 0.646**	34.649 ± 1.005**	36.414 ± 0.750**	41.180 ± 0.744**
Week 31					
Absolute	8.936 ± 0.378	10.695 ± 0.272**	10.653 ± 0.225**	11.557 ± 0.514**	11.986 ± 0.423**
Relative	30.685 ± 0.827	35.967 ± 0.481**	35.609 ± 0.531**	39.235 ± 0.994**	40.616 ± 0.904**
Week 53					
Absolute	10.382 ± 0.280	11.271 ± 0.388	11.299 ± 0.410	14.083 ± 0.709**	12.421 ± 0.481**
Relative	32.052 ± 0.760	36.607 ± 0.633*	35.764 ± 1.111*	44.007 ± 1.581**	43.212 ± 1.493**
Lung					
Week 14					
Absolute	1.831 ± 0.087	1.859 ± 0.098	1.854 ± 0.073	1.929 ± 0.110	1.849 ± 0.051
Relative	6.108 ± 0.183	6.614 ± 0.290	6.570 ± 0.222	6.958 ± 0.374*	7.128 ± 0.165**
Week 31					
Absolute	1.913 ± 0.105	1.803 ± 0.036[b]	2.071 ± 0.070	2.118 ± 0.104	2.102 ± 0.075
Relative	6.574 ± 0.305	6.097 ± 0.184[b]	6.934 ± 0.249	7.212 ± 0.323	7.144 ± 0.256
Week 53					
Absolute	2.248 ± 0.163	2.116 ± 0.082	2.186 ± 0.093	2.245 ± 0.048[c]	2.387 ± 0.093
Relative	6.923 ± 0.458	6.904 ± 0.303	6.967 ± 0.410	7.057 ± 0.183[c]	8.326 ± 0.371*
L. Ovary					
Week 14					
Absolute	0.063 ± 0.003	0.059 ± 0.007	0.057 ± 0.005	0.056 ± 0.004	0.052 ± 0.004
Relative	0.210 ± 0.009	0.209 ± 0.021	0.201 ± 0.017	0.202 ± 0.013	0.198 ± 0.014
Week 31					
Absolute	0.050 ± 0.002	0.056 ± 0.005	0.050 ± 0.002[b]	0.057 ± 0.005	0.054 ± 0.003
Relative	0.172 ± 0.006	0.188 ± 0.015	0.169 ± 0.005[b]	0.192 ± 0.012	0.183 ± 0.007
Week 53					
Absolute	0.053 ± 0.004	0.052 ± 0.002	0.049 ± 0.003	0.063 ± 0.007	0.045 ± 0.003
Relative	0.165 ± 0.012	0.169 ± 0.008	0.155 ± 0.005	0.195 ± 0.019	0.156 ± 0.010

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 the TEF Mixture

	Vehicle Control	10 ng TEQ/kg	22 ng TEQ/kg	46 ng TEQ/kg	100 ng TEQ/kg
n					
Week 14	10	10	10	10	10
Week 31	10	10	10	10	10
Week 53	8	8	8	8	8
Necropsy body wt					
Week 14	299 ± 8	208 ± 7*	282 ± 4	277 ± 6*	260 ± 6**
Week 31	291 ± 6	297 ± 5	300 ± 7	294 ± 8	295 ± 6
Week 53	324 ± 5	308 ± 9	316 ± 9	320 ± 11	288 ± 5**
Spleen					
Week 14					
Absolute	0.580 ± 0.013	0.561 ± 0.025	0.557 ± 0.024	0.519 ± 0.013*	0.472 ± 0.017**
Relative	1.946 ± 0.041	2.000 ± 0.072	1.974 ± 0.077	1.872 ± 0.039	1.813 ± 0.031
Week 31					
Absolute	0.512 ± 0.019	0.499 ± 0.017	0.503 ± 0.013	0.516 ± 0.022	0.482 ± 0.022
Relative	1.764 ± 0.062	1.678 ± 0.044	1.682 ± 0.031	1.754 ± 0.056	1.633 ± 0.054
Week 53					
Absolute	0.540 ± 0.011	0.494 ± 0.025	0.499 ± 0.017	0.553 ± 0.055	0.432 ± 0.019
Relative	1.670 ± 0.045	1.606 ± 0.076	1.581 ± 0.052	1.732 ± 0.163	1.504 ± 0.066
Thymus					
Week 14					
Absolute	0.225 ± 0.009	0.200 ± 0.015	0.194 ± 0.007*	0.161 ± 0.007**	0.151 ± 0.006**
Relative	0.759 ± 0.039	0.713 ± 0.048	0.689 ± 0.029	0.582 ± 0.025**	0.586 ± 0.027**
Thyroid gland					
Week 14					
Absolute	0.027 ± 0.001	0.025 ± 0.001	0.031 ± 0.002	0.028 ± 0.003	0.023 ± 0.002
Relative	0.089 ± 0.003	0.088 ± 0.003	0.111 ± 0.007	0.101 ± 0.010	0.088 ± 0.005
Week 31					
Absolute	0.023 ± 0.001	0.024 ± 0.002	0.027 ± 0.001	0.024 ± 0.001	0.026 ± 0.001
Relative	0.081 ± 0.005	0.080 ± 0.007	0.090 ± 0.004	0.082 ± 0.004	0.089 ± 0.005
Week 53					
Absolute	0.023 ± 0.003	0.019 ± 0.001	0.020 ± 0.001	0.018 ± 0.001	0.016 ± 0.001*
Relative	0.070 ± 0.010	0.060 ± 0.003	0.064 ± 0.004	0.055 ± 0.003	0.056 ± 0.003

* 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

TCDD

TCDD was obtained from IIT Research Institute (Chicago, IL) by Midwest Research Institute (Kansas City, MO) and provided to the study laboratory (Battelle Columbus Operations, Columbus, OH) by Research Triangle Institute (Research Triangle Park, NC) in one lot (CR82-2-2) that was used for the 2-year study. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute, and the study laboratory. Reports on analyses performed in support of the TEF mixture study are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot CR82-2-2 of the chemical, a white crystalline powder, was identified as TCDD by the analytical chemistry laboratory using infrared spectroscopy, proton nuclear magnetic resonance (NMR) spectroscopy, direct probe mass spectroscopy (MS), low resolution gas chromotography (GC) coupled with MS by system A (Table C1), and melting point determination. In addition, identity analysis was conducted by the study laboratory using proton NMR. All spectra were consistent with the structure of TCDD. Infrared and mass spectra matched reference spectra of TCDD, and although a reference proton NMR spectrum was not available, the observed chemical shift agreed with that reported in the literature (Gurka *et al*., 1985; Ashley *et al*., 1989). A precise melting point range was not determined as the chemical appeared to sublime at approximately 260° C. The infrared, proton NMR, and mass spectra are presented in Figures C1, C2, and C3, respectively.

The purity of lot CR82-2-2 was determined by the analytical chemistry laboratory using GC systems A, B, and C and by the study laboratory using GC system D. The purity profile obtained by system B detected two impurities with a combined relative area of 2.0%, and that obtained by system C detected two impurities with a combined relative area of 1.6%. The major impurity detected by each system (1.5% of the major peak) was identified using GC/MS by system A as 1,2,4-trichlorodibenzo-*p*-dioxin. A small peak eluting immediately after the main component was believed to be a dimethyl isomer of trichloro-*p*-dioxin (positional substitution unknown). Also, a trace amount of a higher molecular weight tetrachlorinated dioxin (parent ion = 426) was observed, but due to the relatively weak intensity of the signal, precise identification could not be made. The purity profile obtained by system D indicated that the test article had a purity of 101.6% relative to a reference sample of the same lot. The overall purity of lot CR82-2-2 was determined to be 98% or greater.

PeCDF

PeCDF was obtained from Cambridge Isotope Laboratories (Cambridge, MA) in one lot (080196) and was 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.

Lot 080196 of the chemical, a white powder, was identified by the analytical chemistry laboratory as PeCDF by proton and carbon-13 NMR spectroscopy. The spectrum of the purity analysis sample was compared to that of the frozen reference sample 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 NMR data. The NMR spectra are presented in Figures C4 and C5.

The purity of lot 080196 was determined by the analytical chemistry laboratory using GC by system E and by the study laboratory using GC by system F. The purity profile obtained by system E 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. Two of the impurities (1%) had characteristics of other furans, while the other two impurities (1.4%) had none of the

characteristics of polychlorinated biphenyls (PCBs), furans, or dioxins. Gas chromatography by system F 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.

PCB 126

PCB 126 was obtained from AccuStandard, Inc. (New Haven, CT), in one lot (130494) and was used in the 2-year study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Chemistry Support Services), and the study laboratory.

Lot 130494 of the chemical, a white powder, was identified as PCB 126 by proton and carbon-13 NMR spectroscopy and melting point determination. All spectra were consistent with the structure of a pentachlorobiphenyl, and the melting point (156.9° C) determined by differential scanning calorimetry agreed with the literature value (Bolgar *et al*., 1995). Proton and carbon-13 NMR spectra are presented in Figures C6 and C7.

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

Formulation Materials

USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) in five 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 using system I 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% except one that had a single impurity of 0.125%. 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

TCDD

Lot CR82-2-2 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 10 µg/mL by dissolving approximately 10 mg of accurately weighed TCDD in 1,000 mL of acetone. Frozen reference stocks were stored at up to –20° C. A formulation stock solution was prepared at a target concentration of 15 µg/mL by dissolving approximately 15 mg of accurately weighed TCDD in 1,000 mL of acetone. Following analysis to confirm proper concentration, these solutions were used to prepare analytical standard stocks of 50 and 100 µg, frozen reference stocks and chemical reference stocks of 100 µg for periodic purity determinations, and dose formulation working stocks. They were prepared by transferring the required volumes of respective solutions into appropriately sized glass containers and evaporating the solvent. Dried aliquots were stored at room temperature and protected from light in amber glass bottles. Purity was monitored with periodic reanalysis by the study laboratory using system D. No degradation was observed during the course of the study.

PeCDF

Using procedures similar to those described above for TCDD in the preparation of analytical standard stocks, frozen reference stocks, chemical reference stocks, and dose formulation working stocks, an analytical stock solution of lot 080196 was prepared at a target concentration of 100 µg/mL by dissolving 10 mg of PeCDF in 100 mL of acetone. Frozen reference stocks were stored at up to –20° C. 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. Dried aliquots were stored at room temperature, protected from light in amber glass bottles sealed with Teflon®-lined lids. Purity was monitored with periodic reanalysis by the study laboratory using system F. No degradation was observed during the course of the study.

PCB 126

Using procedures similar to those described above for TCDD in the preparation of analytical standard stocks, frozen reference stocks, chemical reference stocks, and dose formulation working stocks, an analytical stock solution of lot 130494 was prepared at a target concentration of 100 µg/mL by dissolving 10 mg of accurately weighed PCB 126 in 100 mL of acetone. Frozen reference stocks were stored at up to –20° C. A formulation stock solution was prepared at a target concentration of 125 µg/mL by dissolving 250 mg of accurately weighed PCB 126 in 2,000 mL of acetone. Dried aliquots were stored at room temperature and protected from light in amber glass bottles sealed with Teflon®-lined lids. Purity was monitored with periodic reanalysis by the study laboratory using system H. No degradation was observed during the course of the study.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving TCDD, PeCDF, and PCB 126 dose formulation working stocks in acetone and then diluting with corn oil such that the final dose formulations contained 1% acetone (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 and stability studies of a low-dose formulation containing 1.32 ng/mL TCDD, 2.64 ng/mL PeCDF, and 13.32 ng/mL PCB 126 and a homogeneity study of a high-dose formulation of 13.2 ng/mL TCDD, 26.4 ng/mL PeCDF, and 133.2 ng/mL PCB 126 were performed by the study laboratory using GC/MS by systems J (TCDD and PeCDF) and K (PCB 126) (Table C1). Homogeneity was confirmed, and stability was confirmed for 3 hours under simulated animal room conditions. Stability studies of the low-dose fomulation were performed by Midwest Research Institute using GC/MS by system L or a similar system. In these studies, stability was confirmed for at least 36 days for a dose formulation in corn oil containing 0.04% (v:v) nonane stored in sealed amber glass containers at 5° C and at room temperature, and for up to 3 hours for formulations exposed to ambient air and room light conditions in a simulated dosing study. Gavagability was confirmed by the study laboratory for the high-dose formulation.

Periodic analyses of the dose formulations of the TEF mixture were conducted by the study laboratory using GC/MS by systems J and K. During the 2-year study, the dose formulations were analyzed at least every 3 months to determine the concentrations of TCDD, PeCDF, and PCB 126 in the mixture (Tables C3, C4, and C5). All (40/40) measurements of TCDD concentrations, 70% (28/40) of the measurements of PeCDF concentrations, and 73% (29/40) of the measurements of PCB 126 concentrations were within 10% of the target concentrations. In addition, all measurements of PeCDF and PCB 126 concentrations were within 15% of the targets. Of the animal room sample measurements for TCDD, PeCDF, and PCB 126 concentrations, 15/16, (94%), 15/16 (94%), and 13/16 (81%), respectively, were within 10% of the target concentrations; all animal room samples were within 14% of the targets. All formulations were used in the study with the approval of the NTP.

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

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry, selected ion recording	J&W DB-5 MS, 30 m × 0.32 mm, 0.5-µm film thickness (J&W Scientific, Folsom, CA)	Helium at 2 mL/minute	150° C to 300° C at 10° C/minute, then held for 45 minutes
System B			
Electron capture	J&W DB-5 MS, 30 m × 0.32 mm, 0.5-µm film thickness (J&W Scientific)	Helium at 2.1 mL/minute	150° C to 300° C at 10° C/minute, then held for 45 minutes
System C			
Flame ionization	J&W DB-5 MS, 30 m × 0.32 mm, 0.5-µm film thickness (J&W Scientific)	Helium at 1.1 mL/minute	150° C to 300° C at 10° C/minute, then held for 15 minutes
System D			
Electron capture	Supelco PTE-5, 30 m × 0.32 mm, 1-µm film thickness (Supelco, Inc., Bellefonte, PA)	Helium at 1.5 mL/minute	200° C to 300° C at 10° C/minute, then held for 8 minutes
System E			
Mass spectrometry	DB-5 (MS), 15 m × 0.25 mm fused silica, 0.25-µm film thickness (Restek, Bellefonte, PA)	Helium at 4 psi	50° C for 1 minute, increased to 300° C at 8° C/minute, then held for 10 minutes
System F			
Flame ionization	PTE-5 (QTM), 15 m × 0.53 mm, 0.5-µm film thickness (Supelco, Inc.)	Helium at 5 mL/minute	45° C for 5 minutes, increased to 300° C at 15° C/minute, then held for 5 minutes
System G			
Mass spectrometry	DB-5MS 15 m × 0.25 mm, 0.25-µm film thickness (Restek)	Helium at 6 mL/minute	50° C for 1 minute, increased to 300° C at 10° C/minute, then held for 10 minutes
System H			
Flame ionization	Supelco PTE-5, 15 m × 0.53 mm, 0.5-µm film thickness (Supelco, Inc.)	Helium at 15 mL/minute	45° C for 5 minutes, increased to 300° C at 15° C/minute
System I			
Flame ionization	Supelco 20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcoport, 2.4 m × 2 mm (Supelco, Inc.)	Nitrogen at 30 mL/minute	40° C for 4 minutes, increased to 170° C at 10° C/minute
System J			
Mass spectrometry, selected ion recording	J&W DB-5 MS, 15 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium at 1 mL/minute	100° C to 310° C at 15° C/minute

Table C1
Gas Chromatography Systems Used in the 2-Year Gavage Study of the TEF Mixture

Detection System	Column	Carrier Gas	Oven Temperature Program
System K			
Mass spectrometry, selected ion recording	J&W DB-5 MS, 15 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium at 1 mL/minute	100° C for 1 minute, increased to 240° C at 15° C/minute, then increased to 285° C at 40° C/minute, then held for 2 minutes
System L			
Mass spectrometry	J&W DB-5 MS, 60 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium	150° C for 2 minutes, increased to 230° C at 50° C/minute, held for 2 minutes, increased to 235° C at 1° C/minute, held for 2 minutes, increased to 320° C at 15° C/minute, then held for 3 minutes

[a] Gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA) (systems A, B, C, D, F, H, I, and L), and Carlo Erba/Fisons (Valencia, CA) (systems E, G, J, and K). Mass spectrometers were manufactured by Hewlett-Packard (system A), VG (Cheshire, UK) (systems E, G, J, and K), and Fisons (system L).

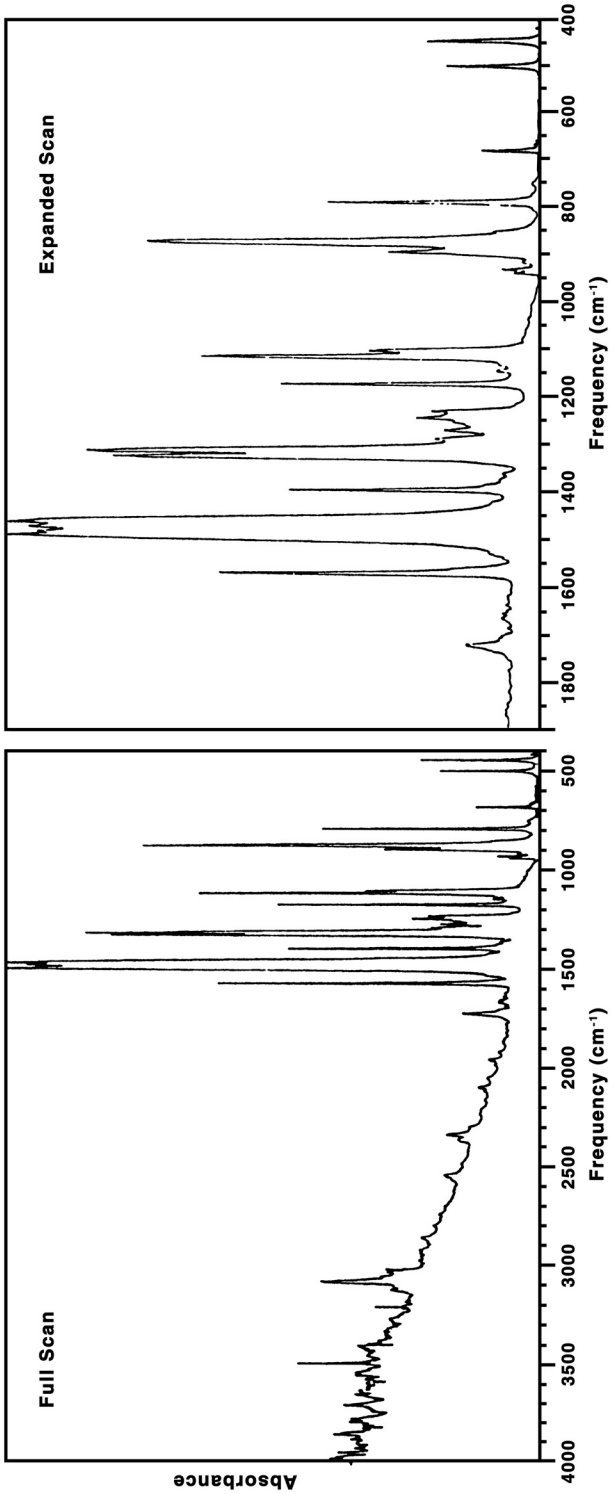

FIGURE C1
Infrared Spectrum of TCDD

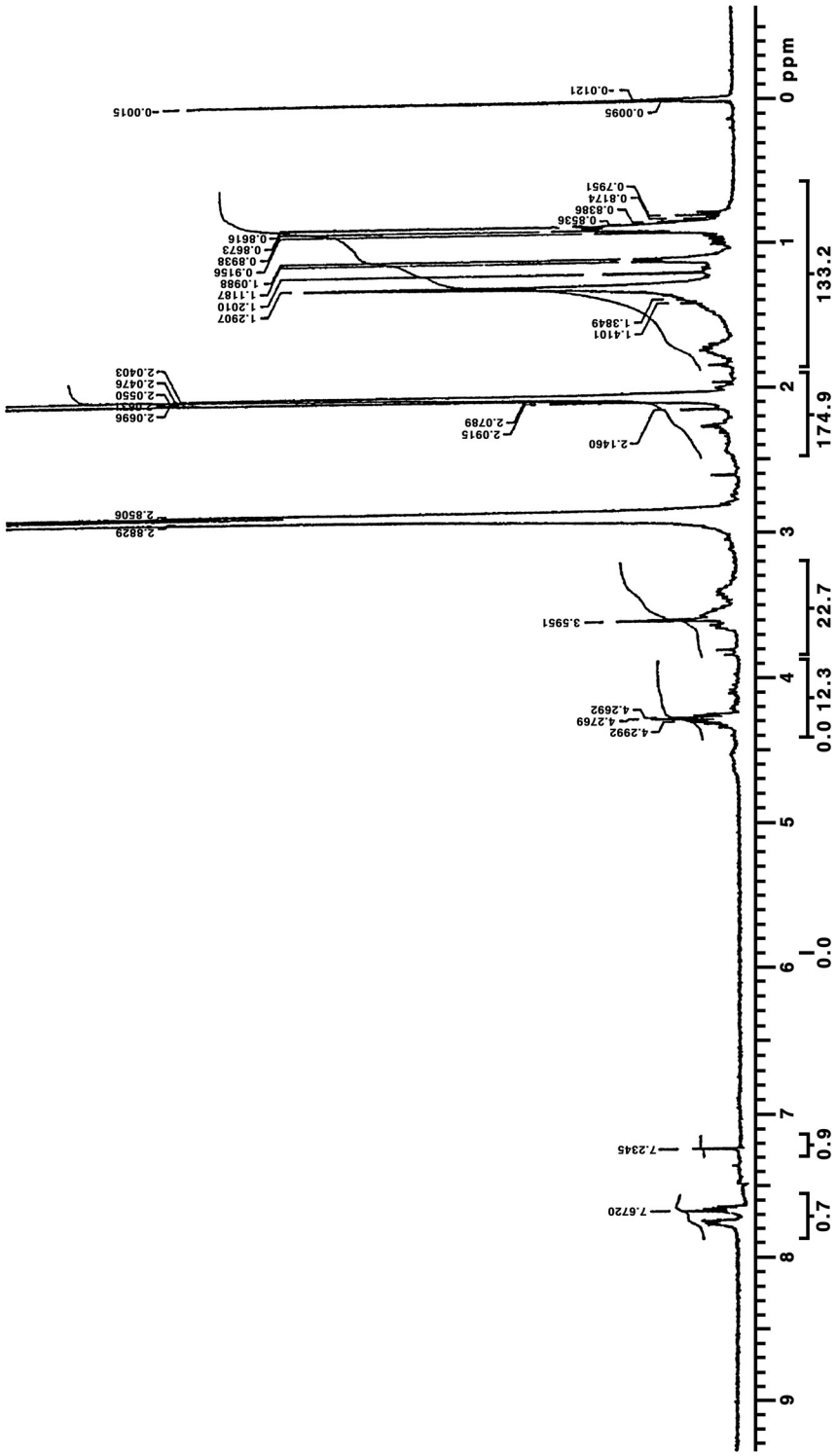

FIGURE C2
Proton Nuclear Magnetic Resonance Spectrum of TCDD

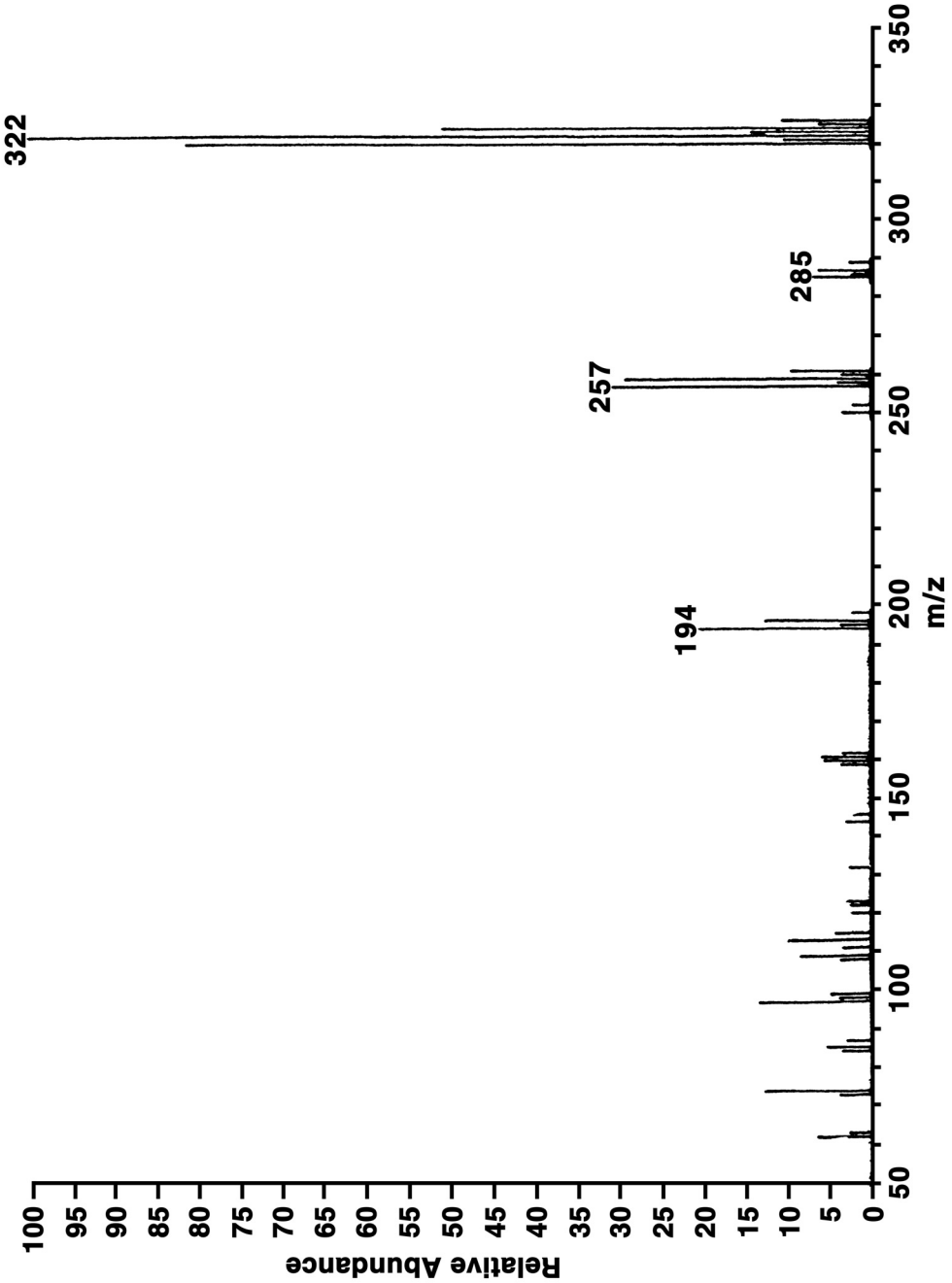

FIGURE C3
Direct Probe Mass Spectrum of TCDD

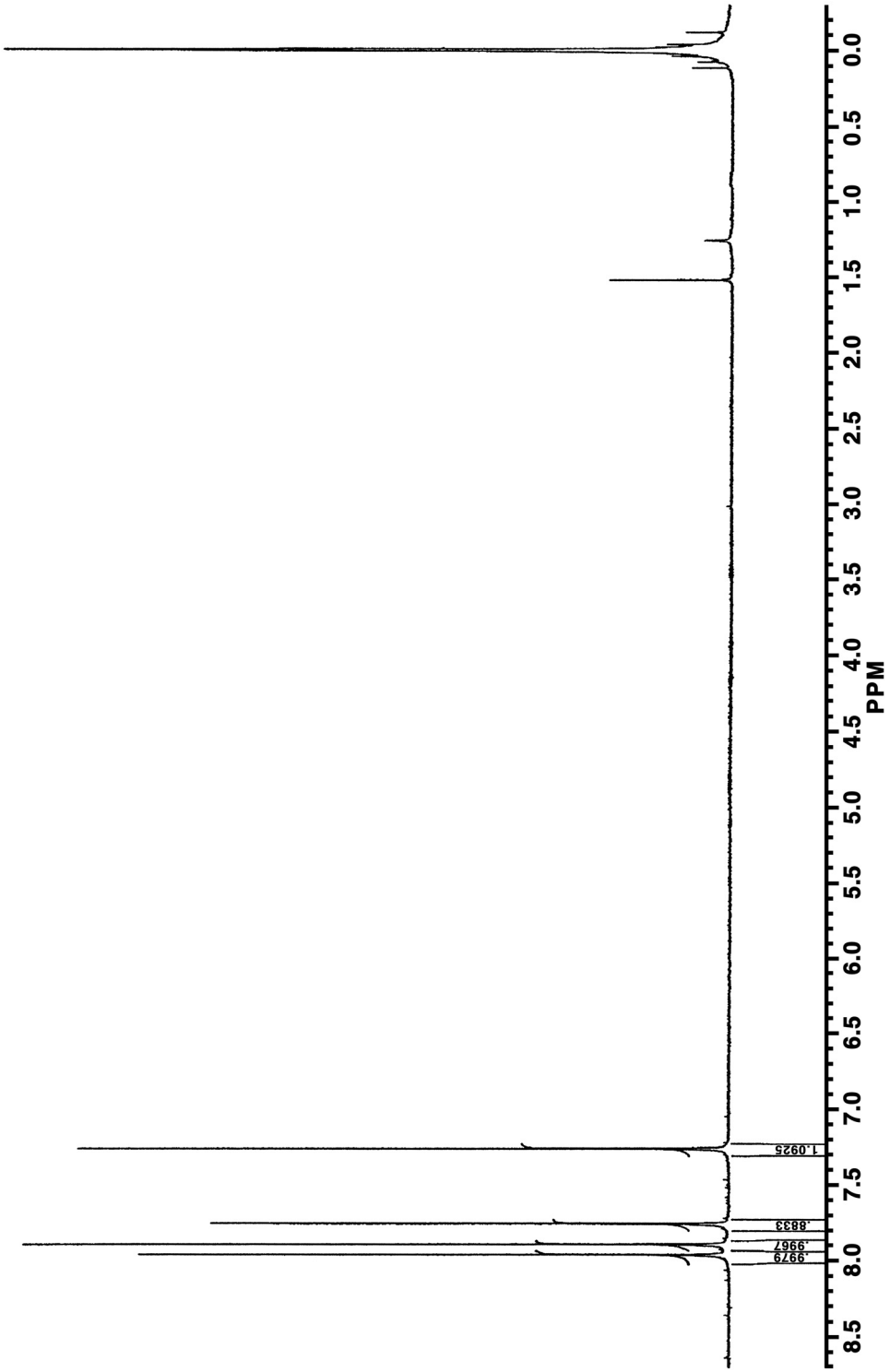

FIGURE C4
Proton Nuclear Magnetic Resonance Spectrum of PeCDF

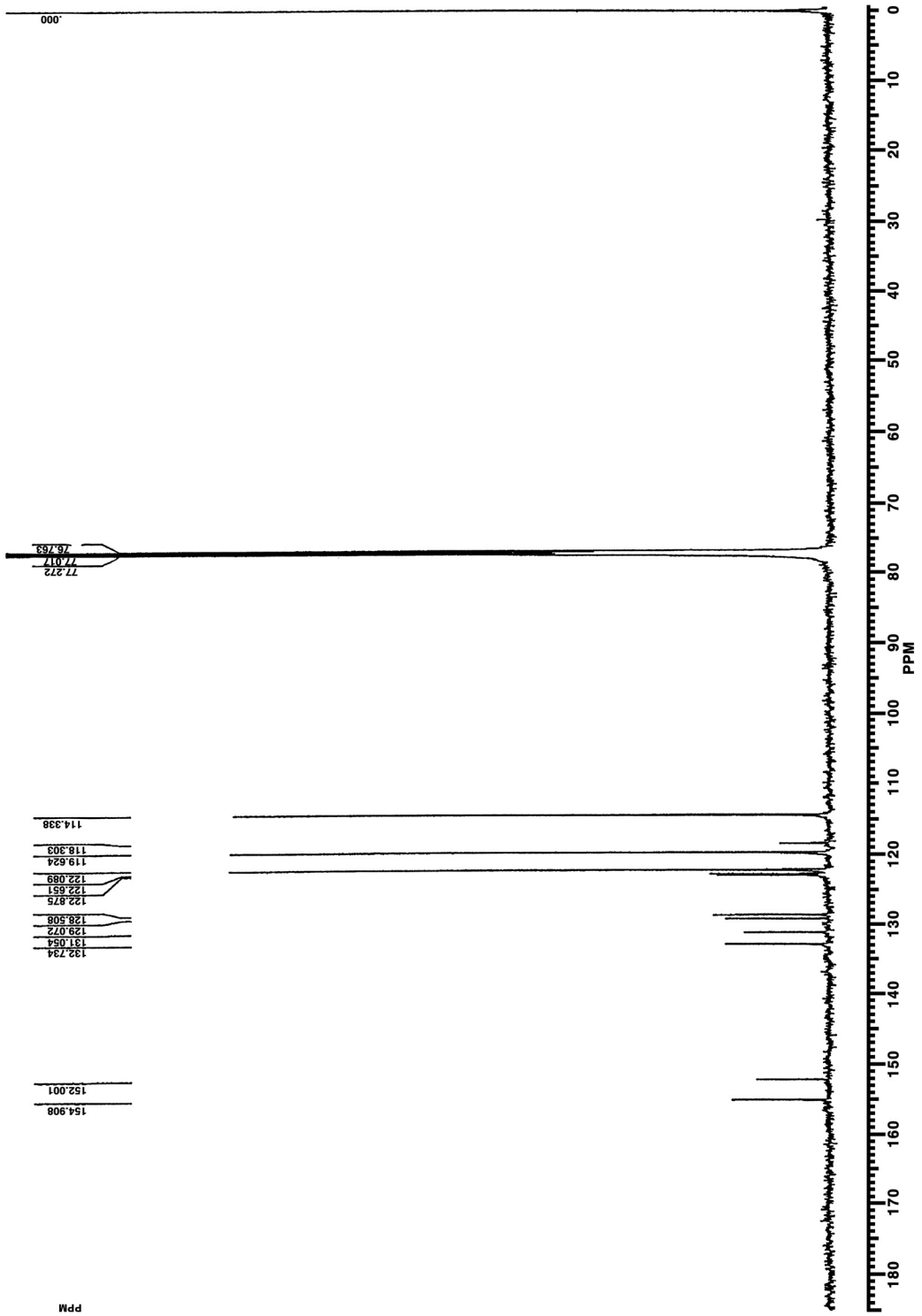

FIGURE C5
Carbon-13 Nuclear Magnetic Resonance Spectrum of PeCDF

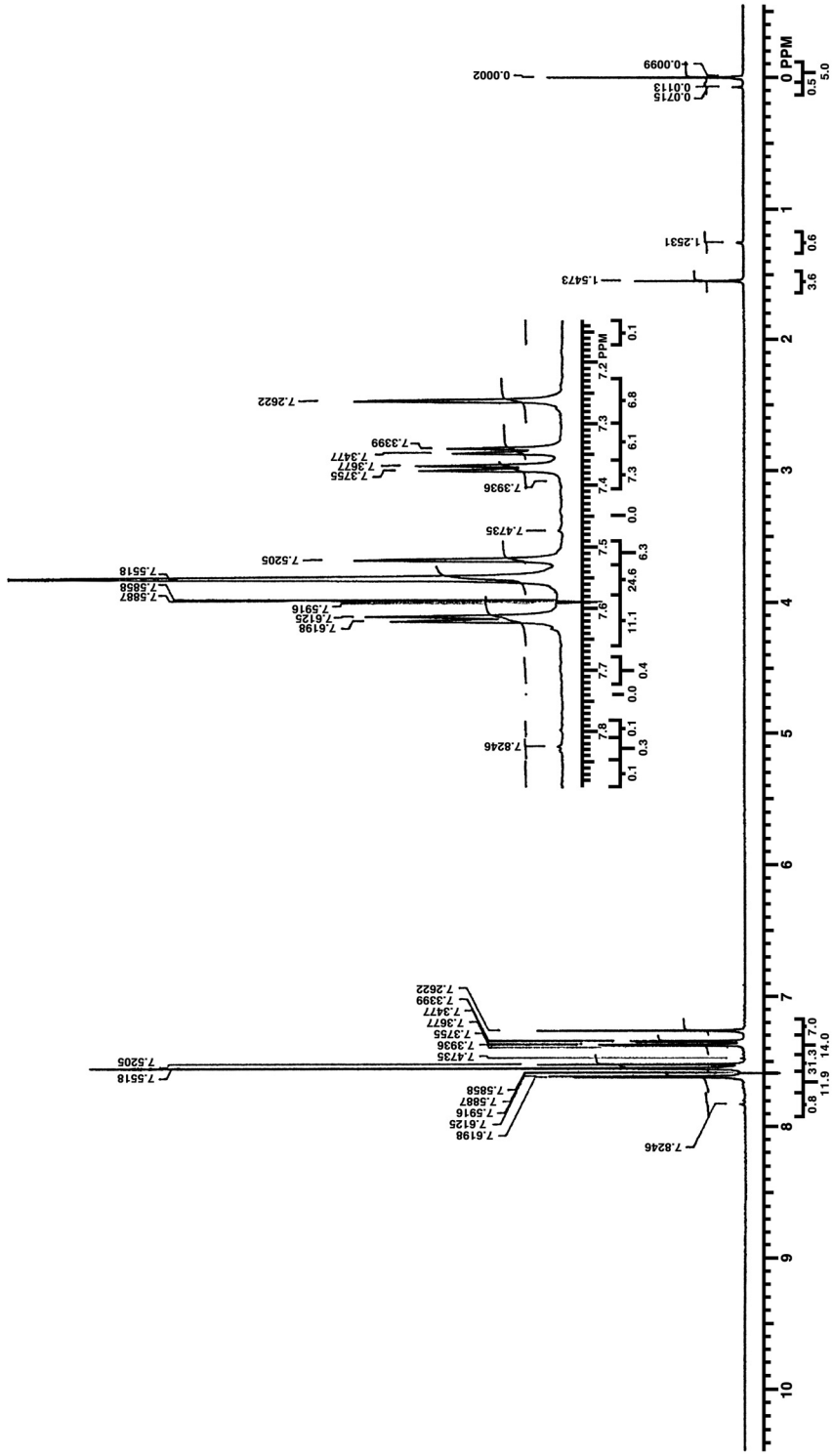

FIGURE C6
Proton Nuclear Magnetic Resonance Spectrum of PCB 126

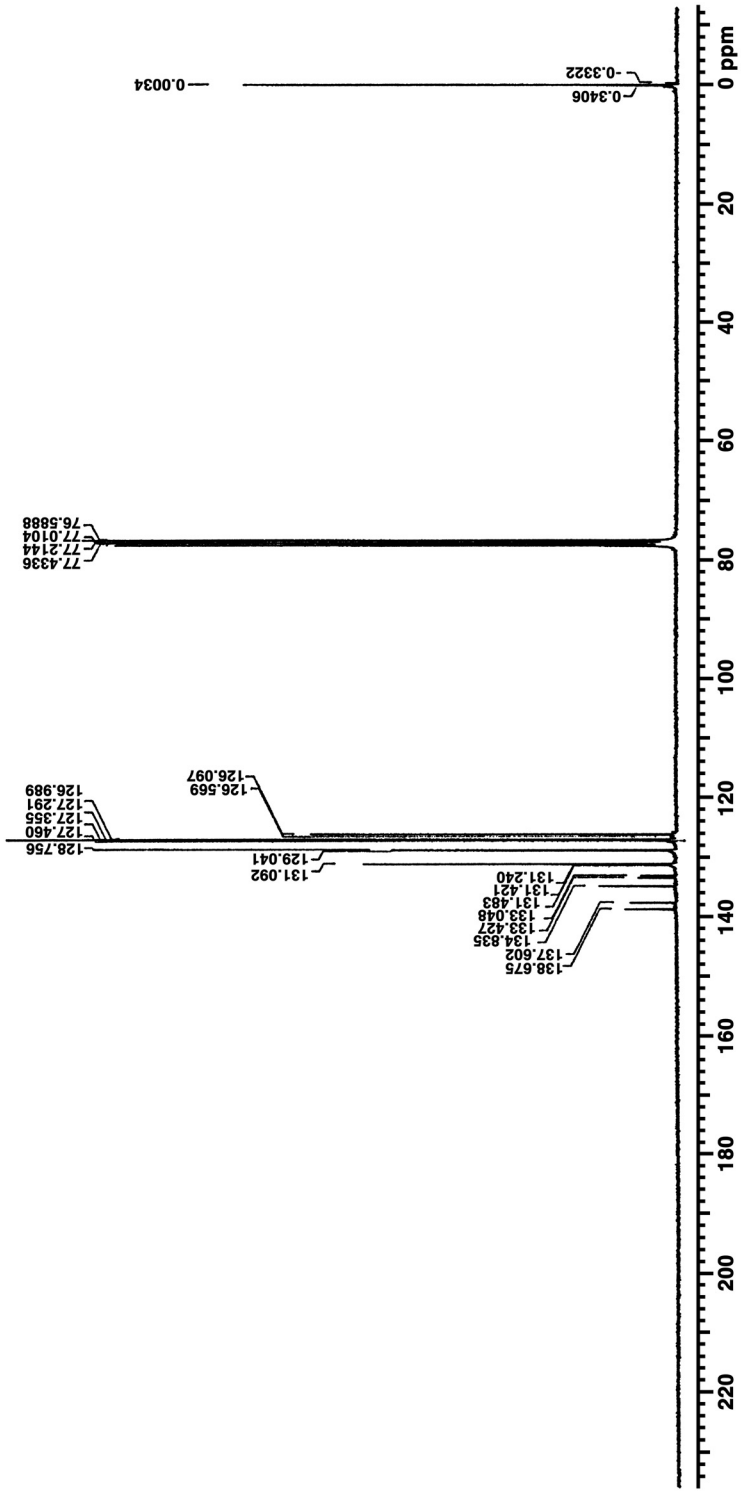

FIGURE C7
Carbon-13 Nuclear Magnetic Resonance Spectrum of PCB 126

Table C2
Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of the TEF Mixture

Preparation
Dose formulation working stocks were prepared by transferring the appropriate volumes of 15 µg/mL (TCDD), 80 µg/mL (PeCDF), and 125 µg/mL (PCB 126) formulation stock solutions into 15 mL amber glass bottles, evaporating the acetone, and sealing the bottles with Teflon®-lined lids.

To prepare the dose formulations, 10 mL of acetone was added to a dose formulation working stock bottle, vortexed for about 2 minutes, sonicated for about 30 minutes, and transferred to a 2 L volumetric flask containing 1 L of corn oil. The dose formulation working stock bottle was rinsed twice with 5 mL of acetone and vortexed for about 2 minutes and the acetone rinses were added to the volumetric flask. Volumetric flask contents were diluted to volume with corn oil, capped, and stirred on a stirplate for at least 3 hours, with periodic inverting and shaking.

Chemical Lot Numbers
TCDD: CR82-2-2
PeCDF: 080196
PCB 126: 130494

Maximum Storage Time
35 days

Storage Conditions
Dose formulation working stocks of the TEF mixture were stored in 15 mL amber glass vials, sealed with Teflon®-lined lids at room temperature (approximately 25° C). Dose formulations were stored in 120 mL amber glass screw-cap bottles with Teflon®-lined lids at room temperature.

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

TABLE C3
Results of Analyses of TCDD Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of the TEF Mixture

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration[a] (ng/mL)	Difference from Target (%)
June 4, 1998	June 9, 1998	1.32	1.221	–8
		2.92	2.761	–5
		6.08	5.841	–4
		13.2	13.06	–1
	July 15, 1998[b]	1.32	1.247	–6
		2.92	2.723	–7
		6.08	5.870	–3
		13.2	13.95	+6
July 30, 1998	August 4, 1998	1.32	1.290	–2
		2.92	2.900	–1
		6.08	6.085	0
		13.2	12.50	–5
October 22, 1998	October 26-27, 1998	1.32	1.275	–3
		2.92	2.769	–5
		6.08	6.226	+2
		13.2	12.87	–3
January 11, 1999	January 15-16, 1999	1.32	1.261	–4
		2.92	2.714	–7
		6.08	5.827	–4
		13.2	12.43	–6
	February 26-27, 1999[b]	1.32	1.343	+2
		2.92	3.014	+3
		6.08	6.137	+1
		13.2	12.71	–4
April 5, 1999	April 8, 1999	1.32	1.205	–9
		2.92	2.856	–2
		6.08	6.070	0
		13.2	12.88	–2
June 28, 1999	July 1, 1999	1.32	1.289	–2
		2.92	2.747	–6
		6.08	6.030	–1
		13.2	12.33	–7
September 20, 1999	September 23-24, 1999	1.32	1.260	–5
		2.92	2.799	–4
		6.08	5.935	–2
		13.2	12.69	–4
	November 8, 1999[b]	1.32	1.370	+4
		2.92	2.670	–9
		6.08	5.859	–4
		13.2	12.07	–9

TABLE C3
Results of Analyses of TCDD Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of the TEF Mixture

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration (ng/mL)	Difference from Target (%)
December 28, 1999	January 5, 2000	1.32	1.203	–9
		2.92	2.705	–7
		6.08	5.818	–4
		13.2	11.95	–9
March 27, 2000	April 3-4, 2000	1.32	1.326 ± 0.032	0
		2.92	2.643 ± 0.015	–9
		6.08	5.820 ± 0.043	–4
		13.2	12.19 ± 0.070	–8
May 12, 2000	May 17-18, 2000	1.32	1.325 ± 0.015	0
		2.92	2.728 ± 0.016	–7
		6.08	5.989 ± 0.053	–1
		13.2	12.53 ± 0.130	–5
	June 23-24, 2000[b]	1.32	1.472 ± 0.058[c]	+12
		2.92	3.035 ± 0.171	+4
	June 21-22, 2000[b]	6.08	6.117 ± 0.060	+1
		13.2	14.48 ± 0.230	+10

[a] Reported value is the average of duplicate analyses or the average ± standard deviation of quadruplicate analyses.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration; NTP approved the use of the formulation.

TABLE C4
Results of Analyses of PeCDF Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of the TEF Mixture

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration[a] (ng/mL)	Difference from Target (%)
June 4, 1998	June 9, 1998	2.64	2.264[c]	–14
		5.8	5.164[c]	–11
		12.16	11.02	–9
		26.4	24.45	–7
	July 15, 1998[b]	2.64	2.404	–9
		5.8	5.256	–9
		12.16	11.16	–8
		26.4	24.38	–8
July 30, 1998	August 4, 1998	2.64	2.365	–10
		5.8	5.255	–9
		12.16	11.76	–3
		26.4	22.78[c]	–14
October 22, 1998	October 26-27, 1998	2.64	2.506	–5
		5.8	5.713	–2
		12.16	11.39	–6
		26.4	26.72	+1
January 11, 1999	January 15-16, 1999	2.64	2.391	–9
		5.8	5.261	–9
		12.16	11.11	–9
		26.4	23.82	–10
	February 26-27, 1999[b]	2.64	2.731	+3
		5.8	5.702	–2
		12.16	11.87	–2
		26.4	25.42	–4
April 5, 1999	April 8, 1999	2.64	2.423	–8
		5.8	5.523	–5
		12.16	11.29	–7
		26.4	23.89	–10
June 28, 1999	July 1, 1999	2.64	2.283[c]	–14
		5.8	4.954[c]	–15
		12.16	11.13	–8
		26.4	22.54[c]	–15
September 20, 1999	September 23-24, 1999	2.64	2.347[c]	–11
		5.8	5.149[c]	–11
		12.16	11.53	–5
		26.4	24.07	–9
	November 8, 1999[b]	2.64	2.344[c]	–11
		5.8	5.501	–5
		12.16	12.10	0
		26.4	24.54	–7

TABLE C4
Results of Analyses of PeCDF Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of the TEF Mixture

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration (ng/mL)	Difference from Target (%)
December 28, 1999	January 5, 2000	2.64	2.26[c]	–14
		5.8	5.158[c]	–11
		12.16	10.85[c]	–11
		26.4	22.6[c]	–14
March 27, 2000	April 3-4, 2000	2.64	2.401 ± 0.026	–9
		5.8	5.243 ± 0.088	–10
		12.16	11.75 ± 0.165	–3
		26.4	24.31 ± 0.380	–8
May 12, 2000	May 17-18, 2000	2.64	2.503 ± 0.028	–5
		5.8	5.368 ± 0.080	–7
		12.16	12.404 ± 0.160	+2
		26.4	23.98 ± 0.407	–9
	June 21-22, 2000[b]	2.64	2.853 ± 0.144	+8
		5.8	5.445 ± 0.110	–6
		12.16	12.21 ± 0.269	0
		26.4	24.96 ± 0.277	–6

[a] Reported value is the average of duplicate analyses or the average ± standard deviation of quadruplicate analyses.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration; NTP approved the use of the formulation.

TABLE C5
Results of Analyses of PCB 126 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of the TEF Mixture

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration[a] (ng/mL)	Difference from Target (%)
June 4, 1998	June 8-9, 1998	13.32	11.67[c]	–12
		29.32	25.69[c]	–12
		61.2	54.01[c]	–12
		133.2	118.0[c]	–11
	July 13, 1998[b]	13.32	12.66	–5
		29.32	27.83	–5
		61.2	54.67[c]	–11
		133.2	119.2[c]	–11
July 30, 1998	August 3, 1998	13.32	12.88	–3
		29.32	26.64	–9
		61.2	60.25	–2
		133.2	122.6	–8
October 22, 1998	October 27-28, 1998	13.32	12.37	–7
		29.32	27.05	–8
		61.2	52.75[c]	–14
		133.2	120.6	–9
January 11, 1999	January 14-15, 1999	13.32	12.11	–9
		29.32	26.79	–9
		61.2	55.15	–10
		133.2	115.9[c]	–13
	February 23-24, 1999[b]	13.32	12.19	–8
		29.32	26.68	–9
		61.2	56.76	–7
		133.2	125.1	–6
April 5, 1999	April 8-9, 1999	13.32	12.61	–5
		29.32	27.38	–7
		61.2	57.80	–6
		133.2	123.4	–7
June 28, 1999	July 8, 1999	13.32	12.07	–9
		29.32	26.86	–8
		61.2	56.72	–7
		133.2	120.2	–10
September 20, 1999	September 28-29, 1999	13.32	12.32	–8
		29.32	27.18	–7
		61.2	57.85	–5
		133.2	121.6	–9
	November 2 and 10, 1999[b]	13.32	12.38	–7
		29.32	26.42	–10
		61.2	59.02	–4
		133.2	113.9[c]	–14

TABLE C5
Results of Analyses of PCB 126 Concentrations in Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of the TEF Mixture

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration (ng/mL)	Difference from Target (%)
December 28, 1999	January 6 and 9, 2000	13.32	11.57[c]	–13
		29.32	28.01	–4
		61.2	54.81	–10
		133.2	113.8[c]	–15
March 27, 2000	April 4-5, 2000	13.32	12.22 ± 0.29	–8
		29.32	26.00 ± 0.45[c]	–11
		61.2	55.58 ± 0.92	–9
		133.2	118.5 ± 0.30[c]	–11
May 12, 2000	May 18-19, 2000	13.32	11.96 ± 0.07	–10
		29.32	26.28 ± 0.48	–10
		61.2	57.51 ± 0.28	–6
		133.2	119.1 ± 1.1[c]	–11
	June 19-20, 2000[b]	13.32	12.52 ± 0.34	–6
		29.32	27.84 ± 1.40	–5
		61.2	66.09 ± 6.71	+8
		133.2	129.8 ± 12.0	–3

[a] Reported value is the average of duplicate analyses or the average ± standard deviation of quadruplicate analyses.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration; NTP approved the use of the formulation.

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)	13.3 ± 0.41	12.7 – 14.5	25
Crude fat (% by weight)	8.1 ± 0.23	7.6 – 8.6	25
Crude fiber (% by weight)	9.1 ± 0.60	7.9 – 10.0	25
Ash (% by weight)	4.9 ± 0.17	4.7 – 5.4	25
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)	5,785 ± 922	4,220 – 7,790	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)[b]	7.9 ± 0.84	6.1 – 9.3	25
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 (%)	0.986 ± 0.041	0.903 – 1.060	25
Phosphorus (%)	0.555 ± 0.025	0.505 – 0.592	25
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)	0.16 ± 0.079	0.10 – 0.37	25
Cadmium (ppm)	0.04 ± 0.006	0.04 – 0.07	25
Lead (ppm)	0.09 ± 0.052	0.05 – 0.25	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.19 ± 0.033	0.15 – 0.28	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	10.8 ± 2.95	9.04 – 21.1	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	<10		25
Coliform (MPN/g)	0		25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.6 ± 1.54	2.1 – 8.8	25
N-Nitrosodimethylamine (ppb)[e]	1.9 ± 0.91	1.0 – 5.1	25
N-Nitrosopyrrolidine (ppb)[e]	2.7 ± 1.00	1.0 – 5.6	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.138 ± 0.127	0.020 – 0.499	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.225 ± 0.215	0.020 – 0.826	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

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

TABLE 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 male and female sentinel rats at 1 month, male sentinel rats at 6, 12, and 18 months, and from randomly selected 100 ng TEQ/kg female rats at the end of the study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to MA Bioservices/BioReliance (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
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	1, 6, 12, and 18 months, and study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	1, 6, 12, and 18 months, and study termination
Sendai	1, 6, 12, and 18 months, and study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, and study termination
M. arthritidis	Study termination

RESULTS

All test results were negative.

APPENDIX F
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.

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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.