NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

(CAS NO. 1746-01-6)

IN FEMALE HARLAN SPRAGUE-DAWLEY RATS

(GAVAGE STUDIES)

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

April 2006

NTP TR 521

NIH Publication No. 06-4468

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.

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CONTENTS

SUMMARY

Background
2,3,7,8-Tetrachlorobenzo-*p*-dioxin (TCDD) is one of a large family of hydrocarbons containing chlorine known as 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 TCDD on female rats for comparison with the potency of other chemicals in that family.

Methods
We exposed groups of 53 or 54 female rats by depositing solutions of TCDD dissolved in corn oil through tubes directly into their stomachs five days a week for two years. Daily doses of TCDD were 3, 10, 22, 46, or 100 nanograms (ng) per kilogram of body weight. Animals receiving corn oil alone served as the control group. Tissues from more than 40 sites were examined for every animal.

Results
Exposure to TCDD caused a variety of diseases in several organs. Cancers of the liver, lung, mouth, and uterus were seen in female rats exposed to TCDD. A variety of other toxic lesions observed in exposed animals included hypertrophy, hyperplasia, fibrosis, and necrosis of the liver, hyperplasia of the oral mucosa, metaplasia of the lung, inflammation and atrophy of the pancreas, kidney nephropathy, cardiomyopathy of the heart, atrophy and hyperplasia of the adrenal gland, atrophy of the thymus, hyperplasia of the forestomach, hypertrophy of the thyroid gland, and inflammation of the mesentery.

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

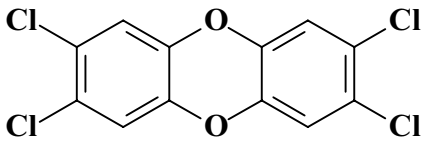
ABSTRACT

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 tissue, 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

TCDD is not manufactured commercially other than for scientific research purposes. The main sources of TCDD releases into the environment are from combustion and incineration; metal smelting, refining, and processing; chemical manufacturing and processing; biological and photochemical processes; and existing reservoir sources that reflect past releases. TCDD (dioxin) was selected for study by the National Toxicology Program as a part of the dioxin TEF evaluation to assess the cancer risk posed by complex mixtures of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PCBs. The dioxin TEF evaluation includes conducting multiple 2-year rat bioassays to evaluate the relative chronic toxicity and carcinogenicity of DLCs, structurally related PCBs, and mixtures of these compounds. While one of the aims of the dioxin TEF evaluation was a comparative analysis across studies, in this Technical Report, only the TCDD results are presented and discussed. TCDD was included because it is the reference compound for the dioxin TEF methodology. Female Harlan Sprague-Dawley rats were administered TCDD (at least 98% pure) in corn oil:acetone (99:1) by gavage for 14, 31, or 53 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse bone marrow cells.

2-Year Study

Groups of 81 or 82 female rats were administered 3, 10, 22, 46, or 100 ng TCDD/kg body weight in corn oil:acetone (99:1) by gavage, 5 days per week, for up to 105 weeks; a group of 81 vehicle control female rats received the corn oil/acetone vehicle alone. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. A stop-exposure group of 50 female rats was administered 100 ng/kg TCDD in corn oil:acetone (99:1) by gavage for 30 weeks and then just the vehicle for the remainder of the study.

Survival of dosed groups was similar to that of the vehicle control group. Mean body weights of 100 ng/kg core study and stop-exposure groups were less than those of the vehicle control group after week 13 of the study. Mean body weights of 46 ng/kg rats were less than those of the vehicle controls during year 2 of the study, and those of 22 ng/kg rats were less than those of the vehicle controls the last 10 weeks of the study.

Thyroid Hormone Concentrations

Alterations in serum thyroid hormone levels were evaluated at the 14-, 31- and 53-week interim evaluations. At 14 weeks, there were significant decreases in serum total and free thyroxine (T_4) levels and increases in serum total triiodothyronine (T_3) and thyroid stimulating

hormone (TSH). At 31 weeks, there were significant decreases in serum total and free T_4 levels and increases in serum total T_3 but no significant effect on TSH. At 53 weeks, there were significant decreases in serum total T_4 levels and increases in serum total T_3. There were no significant effects on total T_4 or TSH levels.

Hepatic Cell Proliferation Data

To evaluate hepatocyte replication, analysis of labeling of replicating hepatocytes with 5-bromo-2′-deoxyuridine was conducted at the 14-, 31-, and 53-week interim evaluations. The hepatocellular labeling index was significantly higher in the 22 ng/kg group compared to vehicle controls at 14 weeks. At the 31-week interim evaluation, the labeling indices in hepatocytes were significantly higher in all dosed groups than in the vehicle controls. At 53 weeks, labeling indices were significantly higher in the 46 and 100 ng/kg groups than in 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 14, 31, and 53 weeks. In addition, pentoxyresorufin-*O*-deethylase (PROD) activity was analyzed. Hepatic EROD, PROD, and A4H activities were significantly higher in all dosed groups relative to vehicle controls at the 14-, 31-, and 53-week interim evaluations. Pulmonary EROD was also significantly higher in all dosed groups compared to vehicle controls at 14, 31, and 53 weeks.

Determinations of TCDD Concentrations in Tissues

The tissue disposition of TCDD was analyzed in the liver, lung, fat, and blood of all animals in each group at the 14-, 31-, and 53-week interim evaluations and in 10 animals per group at the end of the 2-year study (105 weeks). The highest concentrations of TCDD were observed in the liver, followed by fat tissue. Liver and fat tissue concentrations of TCDD increased with increasing doses of TCDD. No measurable concentrations of TCDD were observed in blood from vehicle control or treated rats at any of the interim evaluations. Mean levels of TCDD in the liver and fat in the 100 ng/kg group at the end of the 2-year study were 9.3 and 3.2 ng/g, respectively. In liver tissue from the stop-exposure group, TCDD concentrations were slightly higher than those observed in the 3 ng/kg group. In the stop-exposure group, TCDD concentrations in fat were below the limits of quantitation.

Pathology and Statistical Analyses

Absolute and/or relative liver weights were increased at 14, 31, and 53 weeks, with more severe effects occurring in the higher dosed groups. Increased liver weights correlated with increased incidences of hepatocyte hypertrophy at 14, 31, and 53 weeks.

Exposure led to a treatment-related increase in hepatic toxicity with a broad spectrum of lesions. Incidences and severities of lesions increased at higher doses and longer durations of exposure. The earliest effects were increased incidences and severities of hepatocyte hypertrophy at 14 weeks. At 2 years, there was a significant increase in toxic hepatopathy characterized by increased incidences of numerous nonneoplastic liver lesions including hepatocyte hypertrophy, multinucleated hepatocytes, altered hepatocellular foci, inflammation, pigmentation, diffuse fatty change, necrosis, portal fibrosis, oval cell hyperplasia, bile duct hyperplasia, bile duct cysts, cholangiofibrosis, and nodular hyperplasia

At 2 years, the incidence of hepatocellular adenoma was significantly increased in the 100 ng/kg core study group. Dose-related increased incidences of cholangiocarcinoma were seen in core study rats administered 22 ng/kg or greater. The highest incidence of cholangiocarcinoma was seen in the 100 ng/kg core study group and included a significant number of animals with multiple cholangiocarcinomas. Two cholangiocarcinomas and two hepatocellular adenomas were seen in the 100 ng/kg stop-exposure group. Two hepatocholangiomas were seen in the 100 ng/kg core study group, and one cholangioma was seen in the 100 ng/kg stop-exposure group.

In the lung, the incidence of cystic keratinizing epithelioma of the lung was significantly increased at 2 years in the 100 ng/kg core study group. Nonneoplastic effects in the lung included increased incidences of bronchiolar metaplasia.

The incidence of gingival squamous cell carcinoma of the oral mucosa was significantly increased in the 100 ng/kg core study group at 2 years and was accompanied by an increased incidence of gingival squamous hyperplasia.

At 2 years, the incidence of squamous cell carcinoma of the uterus in the 46 ng/kg group was significantly increased, and there were two squamous cell carcinomas in the 100 ng/kg stop-exposure group.

At 2 years, one acinar adenoma and two acinar cell carcinomas of the pancreas were seen in the 100 ng/kg core

study group; one acinar carcinoma was seen in the 100 ng/kg stop-exposure group. The incidences of acinar cell adenoma or carcinoma (combined) exceeded the historical vehicle control range. Nonneoplastic effects in the lung included acinar cytoplasmic vacuolization, chronic active inflammation, acinar atrophy, and arterial chronic active inflammation.

Numerous nonneoplastic effects were seen in other organs including thymic atrophy, adrenal cortex atrophy, adrenal cortex hyperplasia, cardiomyopathy, mesenteric artery inflammation, clitoral gland cysts, nephropathy, squamous hyperplasia of the forestomach, and thyroid gland follicular cell hypertrophy. A decrease in the incidence of ovarian atrophy was also observed.

Genetic Toxicology

TCDD was not mutagenic in any of several *in vitro* and *in vivo* short-term tests. No induction of gene mutations was seen in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 exposed to TCDD with or without S9 activation enzymes. No induction of trifluorothymidine resistance (gene mutations) was observed in L5178Y $tk^{+/-}$ mouse lymphoma cells tested with or without S9 activation. In cytogenetic tests with cultured Chinese hamster ovary cells, no consistently reproducible induction of sister chromatid exchanges or chromosomal aberrations were noted after culturing with TCDD with or without S9. *In vivo* tests in male *D. melanogaster* showed no induction of sex-linked recessive lethal mutations in germ cells following treatment of adult flies by injection with TCDD. In male $B6C3F_1$ mice, no increases in the frequency of chromosomally aberrant cells were seen in bone marrow samples taken at two different posttreatment sampling times.

Conclusions

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of TCDD in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma and hepatocellular adenoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. The increased incidence of squamous cell carcinoma of the uterus was also considered to be related to TCDD administration. The marginally increased incidences of pancreatic acinar neoplasms and occurrences of hepatocholangioma and cholangioma of the liver may have been related to TCDD administration.

TCDD administration caused increased incidences of nonneoplastic lesions of the liver, lung, oral mucosa, pancreas, thymus, adrenal cortex, heart, clitoral gland, kidney, forestomach, mesentery, 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 Report Review Subcommittee comments and public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of TCDD in Female Sprague-Dawley Rats

Doses in corn oil/acetone by gavage
0, 3, 10, 22, 46, or 100 ng/kg, and 100 ng/kg (stop-exposure)

Body weights
22, 46, and 100 ng/kg core study and 100 ng/kg stop-exposure groups less than the vehicle control group

Survival rates
25/53, 21/54, 23/53, 19/53, 22/53, 21/53, 21/50

Nonneoplastic effects
Liver:
hepatocyte hypertrophy (0/53, 19/54, 19/53, 42/53, 41/53, 52/53, 22/50);
multinucleated hepatocyte (0/53, 0/54, 16/53, 26/53, 36/53, 51/53, 32/50);
eosinophilic focus (11/53, 14/54, 21/53, 27/53, 27/53, 44/53, 27/50);
inflammation (33/53, 46/54, 47/53, 50/53, 52/53, 49/53, 43/50);
pigmentation (4/53, 9/54, 34/53, 48/53, 52/53, 53/53, 45/50);
diffuse fatty change (0/53, 2/54, 12/53, 17/53, 30/53, 48/53, 10/50);
necrosis (1/53, 4/54, 4/53, 8/53, 10/53, 17/53, 8/50);
oval cell hyperplasia (0/53, 4/54, 3/53, 20/53, 38/53, 53/53, 1/50);
bile duct hyperplasia (5/53, 4/54, 7/53, 22/53, 40/53, 53/53, 7/50);
bile duct cyst (3/53, 1/54, 2/53, 2/53, 0/53, 21/53, 6/50);
nodular hyperplasia (0/53, 0/54, 0/53, 3/53, 7/53, 36/53, 0/50);
portal fibrosis (0/53, 0/54, 0/53, 0/53, 5/53, 27/53, 1/50);
toxic hepatopathy (0/53, 2/54, 8/53, 30/53, 45/53, 53/53, 16/50);
cholangiofibrosis (1/53, 1/54, 2/53, 1/53, 11/53, 31/53, 1/50)
Lung:
alveolar epithelium, metaplasia, bronchiolar (2/53, 19/54, 33/53, 35/52, 45/53, 46/52, 31/50)
Oral Mucosa:
gingival squamous hyperplasia (1/53, 7/54, 14/53, 13/53, 15/53, 16/53, 8/50)
Pancreas:
acinar cytoplasmic vacuolization (1/51, 0/54, 0/52, 1/53, 15/52, 42/51, 0/49);
chronic active inflammation (0/51, 0/54, 2/52, 2/53, 3/52, 6/51, 4/49);
acinar atrophy (1/51, 2/54, 4/52, 4/53, 4/52, 9/51, 4/49);
arterial chronic active inflammation (0/51, 1/54, 1/52, 2/53, 2/52, 7/51, 2/49)
Thymus:
atrophy (36/51, 41/52, 44/52, 41/49, 44/46, 42/42, 45/49);
severity of atrophy (2.6, 2.7, 3.0, 3,1, 3.6, 3.9, 3.3)
Adrenal Cortex:
atrophy (2/53, 0/54, 4/53, 5/53, 5/53, 27/53, 4/50);
hyperplasia (16/53, 16/54, 18/53, 25/53, 29/53, 30/53, 20/50)
Heart:
cardiomyopathy (10/53, 12/54, 22/53, 25/52, 32/53, 36/52, 22/50)
Clitoral Gland:
cystic duct (34/50, 37/52, 41/53, 42/52, 41/51, 48/53, 35/49)
Kidney:
nephropathy (34/53, 26/54, 32/53, 36/53, 39/53, 52/53, 41/50);
severity of nephropathy (1.2, 1.1, 1.3, 1.4, 1.4, 2.2, 1.4)
Forestomach:
squamous hyperplasia (3/53, 4/54, 4/53, 2/53, 7/53, 11/53, 5/50)
Mesentery
chronic active artery inflammation (0/53, 1/54, 0/53, 0/53, 4/53, 7/53, 1/50)
Thyroid Gland:
follicular cell hypertrophy (3/52, 4/54, 4/53, 7/51, 10/53, 17/52, 6/49)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of TCDD in Female Sprague-Dawley Rats

Neoplastic effects

Liver:
hepatocellular adenoma (0/53, 0/54, 0/53, 0/53, 1/53, 13/53, 2/50);
cholangiocarcinoma (0/53, 0/54, 0/53, 1/53, 4/53, 25/53, 2/50)

Lung:
cystic keratinizing epithelioma (0/53, 0/54, 0/53, 0/52, 0/53, 9/52, 0/50)

Oral Mucosa:
gingival squamous cell carcinoma (1/53, 2/54, 1/53, 0/53, 4/53, 10/53, 5/50)

Uterus:
squamous cell carcinoma (0/53, 0/54, 0/53, 0/53, 5/53, 0/53, 2/50)

Equivocal findings

Pancreas:
acinar adenoma or carcinoma (0/51, 0/54, 0/52, 0/53, 0/52, 3/51, 1/49)

Liver:
hepatocholangioma (0/53, 0/54, 0/53, 0/53, 0/53, 2/53, 0/50);
cholangioma (0/53, 0/54, 0/53, 0/53, 0/53, 0/53, 1/50)

Level of evidence of carcinogenic activity

Clear evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537, with and without S9
Mouse lymphoma gene mutations:	Negative with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro:*	Negative with and without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro:*	Negative with and without S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	No induction of sex-linked recessive lethal mutations
Chromosomal aberrations	
Mouse bone marrow *in vivo:*	Negative

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 TCDD on February 17, 2004, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 17, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD) 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, Research Triangle Park, NC.

Dr. N.J. Walker, NIEHS, introduced the toxicology and carcinogenesis studies of TCDD by noting that the chemical is the benchmark base of reference for the toxic equivalency factor (TEF) methodology. He described the study design and the spectrum of hormonal and histopathologic alterations seen in the liver, lung, oral mucosa, and pancreas as well as nonneoplastic lesions in a variety of other tissues. The proposed conclusion was *clear evidence of carcinogenic activity* of TCDD in female Harlan Sprague-Dawley rats.

Dr. Elwell, the first principal reviewer, said the study was well designed and included a number of useful mechanistic studies. He suggested the inclusion of inflammation of the mesenteric artery as another nonneoplastic effect in the conclusions.

Dr. Gasiewicz, the second principal reviewer, suggested that references to increases or decreases in lesion incidences implied statistical significance, and differences that were not statistically significant should be so specified. He inquired about variations in control values for thyroid hormones and the BrdU labeling index at different time points and whether more quantitative criteria could be assigned for severity grades for nonneoplastic lesions. He also questioned inclusion of squamous cell carcinoma of the uterus in one dose group as treatment related in the conclusions.

Dr. Boekelheide, the third principal reviewer, also suggested attempts to quantify the diagnostic criteria for histopathologic diagnoses.

Dr. Walker explained that the measurements for clinical chemistry parameters were done sequentially at the time of analysis with emphasis on comparison between dose groups, so seeming differences between different time points might be artifactual. Differences in water consumption could also have been a factor.

Dr. Walker said that carcinomas of the uterus were also observed in the stop-exposure study, which lent support to their being chemical related. Dr. Elwell added that the occurrence of five such tumors in one dose group seemed sufficiently significant, particularly since another database of industry studies reported only two such tumors in 900 historical control female Sprague-Dawley rats. Dr. A. Nyska, NIEHS, said that fuller diagnostic criteria for nonneoplastic lesions would be included in the final document.

Dr. W.T. Allaben, NCTR, inquired about the choice of corn oil as the gavage vehicle. Dr. Walker replied that corn oil was used for more ready comparison with other studies in the literature, which used the same route.

Dr. Thrall suggested including bone marrow smears along with histopathology routinely in studies of chemicals associated with lymphoproliferative or myeloproliferative.

Dr. Elwell moved that the conclusions be accepted as written, with the inclusion of inflammation of the mesenteric artery to the conclusions and mention of ovarian atrophy in the text of the Abstract. Dr. Boekelheide seconded the motion, which was approved 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 were 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 re-reviewed in depth in this Technical Report.

Mechanism of Action via the Aryl Hydrocarbon Receptor

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

specific genes such as CYP1A1. Binding of the AhR-ARNT heterodimer to these elements leads to increased transcription of the specific gene. The characteristic response to TCDD is the transcriptional induction of CYP1A1, which is mediated by binding of the heterodimer to DREs present in the 5′ flanking region of the gene. The AhR is expressed in all tissues with a definite tissue specificity in terms of level of expression and diversity of response. TCDD has been shown to modulate numerous growth factor, cytokine, hormone, and metabolic pathways in animals and experimental systems. Many, if not all, are parts of pathways involved in cellular proliferation and differentiation; 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 expressions of many genes have 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 powerful growth dysregulators. The effect of DLCs on growth regulation may be manifested through alterations in genes involved in cellular growth and homeostasis. Although the relationship between these effects and carcinogenesis can only be inferred, all of these effects are involved in cellular growth and differentiation; disruption of normal cellular processes could be a risk factor for carcinogenicity.

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

Polyhalogenated Aromatic Hydrocarbon Mixtures and Toxic Equivalency Factors

PHAHs always exist in the environment as complex mixtures; therefore, normal background human exposure to PHAHs always occurs as a complex mixture. The toxic equivalency factor (TEF) approach has been developed to assess risks 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.

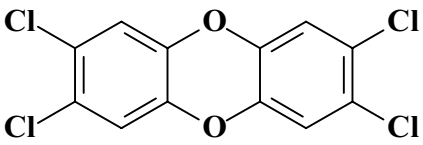
One of the implicit assumptions in the use of TEFs is that the TEQ for different compounds is dose additive. While dose additivity is supported for certain mixtures, this may not be true for some biological endpoints in some models. As outlined by Van den Berg *et al.* (1998), the TEF methodology is 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 the female Sprague-Dawley rat.
3. The carcinogenicity of a dioxin-like, non-ortho-substituted PCB is not altered by the presence of a mono-ortho- or di-ortho-substituted PCB.
4. Relative potencies for DLCs are dose additive.
5. The relative potencies for activation of biochemical endpoints, such as CYP1A1 induction, in the 2-year studies are equivalent to the relative potency for induction of carcinogenesis when estimated based on administered dose.
6. The relative potencies for activation of biochemical endpoints, such as CYP1A1 induction, in the 2-year studies are equivalent to the relative potency for induction of carcinogenesis when estimated based on target tissue dose.
7. The relative potencies for alteration of a given response are the same, regardless of the dose metric used (e.g., administered dose, serum or whole blood concentrations, or tissue dose).

Individual Compounds, Mixtures, and Rationale for Choice

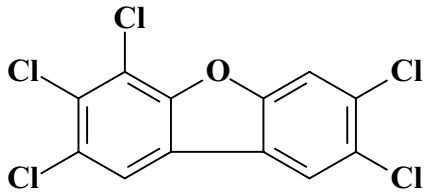


2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD

CAS No. 1746-01-6

Chemical Formula:	$C_{12}H_4Cl_4O_2$
Molecular weight:	321.97

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.

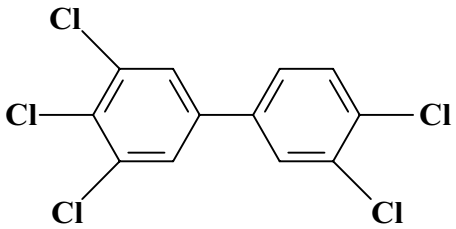


2,3,4,7,8-Pentachlorodibenzofuran
PeCDF

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.

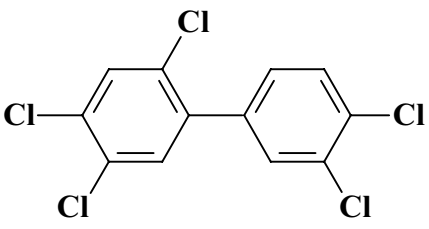


3,3′,4,4′,5-Pentachlorobiphenyl
PCB 126

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.

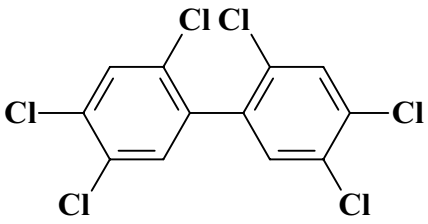


2,3′,4,4′,5-Pentachlorobiphenyl
PCB 118

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, PCB 126, and PeCDF

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

Binary mixture study of PCB 126 and PCB 153

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

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

Binary mixture of PCB 118 and PCB 126

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

Study Design, Species, and Dose Selection Rationale

These studies were conducted in female Harlan Sprague-Dawley rats based on the prior observations by Kociba *et al*. (1978) of the carcinogenicity of TCDD in 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 Sprague-Dawley rats with TCDD. Animals were dosed by oral gavage because the majority of human exposure is oral.

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

known effects on body weight and liver toxicity seen with TCDD at this dose level. Prior studies of TCDD suggest that this dose is at or near the predicted maximum tolerated dose.

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

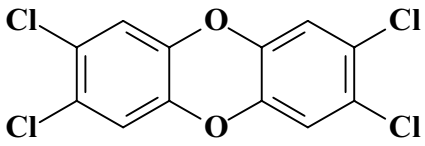
Additional "special study" animals were included at each interim necropsy. Tissues from these animals were provided to specific extramural grantees to facilitate the conduct of additional mechanistic studies. These animals were not evaluated as part of the core study.

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

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

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

INTRODUCTION



2,3,7,8-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

Chemical and Physical Properties

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

TCDD is a colorless to white crystalline powder with a melting point of 305° C. It is insoluble in water, slightly soluble in n-octanol, methanol, and lard oil, and soluble in organic solvents including *o*-dichlorobenzene, chlorobenzene, benzene, and chloroform (IARC, 1997; Lewis, 1997; ATSDR, 1998). TCDD is stable in DMSO, 95% ethanol, or acetone. TCDD can undergo a slow photochemical and bacterial degradation, but it is normally extremely stable. Although TCDD is nonflammable, it degrades when heated in excess of 500° C (Sittig, 1985; Lewis, 1988).

Production, Use, and Human Exposure

TCDD is not manufactured commercially other than for scientific research purposes. The main sources of TCDD 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). Chemical reactions that result in the formation of TCDD are used in the production of phenoxy herbicides (e.g., 2,4,5-trichlorophenoxyacetic acid), hexachlorophene, chlorophenols, chlorodiphenyl ether herbicides [e.g., 1,3,4-trichloro-2-(4-nitrophenoxy)benzene], hexachlorobenzene, pulp bleaching, and the manufacture of dyes and pigments. TCDD is formed as an unwanted combustion by-product during the incineration of municipal waste, sewage sludge, hospital waste, polyvinyl chloride, and wood and from automobile emissions. Photochemical dechlorination of dioxins has been shown to produce TCDD. Biochemical reactions that occur in sewage sludge can also result in the formation of dioxins.

Due to high lipophilicity and low solubility of TCDD in aqueous media, TCDD accumulates in the fat tissues of animals. The highest concentrations of TCDD 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 TCDD and related DLCs (Schecter *et al*., 1994a). It is estimated that 90% of human exposure to TCDD and related dioxins occurs through ingestion of food contaminated with these compounds. A small fraction of exposure occurs via inhalation or dermal absorption.

Adult daily intake of DLCs, including PCDDs, PCDFs, and dioxin-like PCBs, from all sources is estimated to be approximately 70 pg toxic equivalents (TEQ)/day, where TEQ reflects the potency adjusted mass of all DLCs covered by the World Health Organization 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 TEQ/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. TCDD alone represents 8% of the estimated daily intake of DLCs.

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 on dietary practices and proximity to specific sources of exposure, some populations may have additional exposure/body burdens above this level. 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 adipose tissue is 68 pg TEQ/g lipid (USEPA, 2000b). The average levels of TCDD, PeCDF, and PCB 126 in human adipose tissues are 5.5 pg TEQ/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 adipose tissue. TCDD alone represents 8% of the DLCs present in human adipose tissue.

TOXICOKINETICS

There is an extensive body of literature examining the toxicokinetics 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 tract (Piper *et al*., 1973; Rose *et al*., 1976). The absorption of TCDD from the gastrointestinal 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. Once absorbed, TCDD is transported primarily through the lymphatic system by chylomicrons and is readily distributed throughout the body. The main sites of distribution in rats within the first few days after exposure are to the liver and adipose tissue; lesser amounts are distributed to skin and the thyroid gland (Pohjanvirta *et al*., 1990). In blood, TCDD is associated mainly with lipoproteins, serum lipids, and a smaller fraction of albumin and cellular components. The pattern of distribution of TCDD in rats is due to its lipophilicity and binding to cytochrome P450 1A2 (Gillner *et al*., 1987; Diliberto *et al*., 1997). Cytochrome P450 1A2 is a known TCDD-binding protein that is also inducible by TCDD. Because CYP1A2 is inducible only in the liver and nasal passages (Goldstein and Linko, 1984), TCDD tends to sequester in the liver at levels that would not be predicted based on its lipophilicity alone. The hepatic sequestration by TCDD is not seen in CYP1A2 knockout mice, demonstrating the critical involvement of CYP1A2 in this process (Diliberto *et al*., 1999).

Estimates of the half-life of TCDD in rats depend on the organs examined and doses used. Whole-body elimination half-life in rats has been estimated as 12 to 31 days (USEPA, 2000c). In rat adipose tissue, TCDD exhibits first-order kinetics with an elimination half-life of 24.5 days. In Wistar rat liver, TCDD exhibits a biphasic elimination pattern with half-lives of 11.5 days and 16.9 days for the short- and long-term components. The elimination of TCDD from the liver of female Sprague-Dawley rats is also biphasic, and the half-lives decrease when the concentration of TCDD is greater than 1,000 ppt wet weight (Walker *et al*., 2000). For example, when the tissue concentration is below 1,000 ppt, the half-life is estimated to be 33.1 days, but it drops to just 16.7 days when the tissue concentration is above 1,000 ppt. Similarly, for animals that began TCDD exposure 18 weeks following diethylnitrosamine

initiation, when the tissue concentration was below 1,000 ppt, the half-life was estimated to be 45.3 days, but it dropped to just 19.2 days [95% confidence interval (CI) 9.1 to 66.0 days] when the tissue concentration was above 1,000 ppt.

In the lung of female Sprague-Dawley rats, the half-life of TCDD is 39.7 days (95% CI 34.4 to 45.8 days) (Tritscher *et al*., 2000). In serum, the half-life is estimated to be 44.6 days (95% CI 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.

TCDD Toxic Equivalency Factor

TCDD is the reference compound for the dioxin toxic equivalency factor (TEF) scheme and therefore has a TEF of 1.0 (Van den Berg *et al*., 1998).

TOXICITY

TCDD is one of the most studied environmental toxicants, and a vast body of literature is available on toxicities associated with TCDD exposure in multiple species (Poland and Knutson, 1982; Birnbaum, 1994b; ATSDR, 1998; Grassman *et al*., 1998; USEPA, 2000c). Consequently, it is beyond the scope of this Technical Report to fully describe all the toxicities in all species that have been observed. Some of the toxic effects observed include developmental and reproductive alterations, immunotoxicity, teratogenicity, carcinogenicity, and lethality. Different animal species vary widely in sensitivity to the lethal toxicity of TCDD; the oral LD_{50} of the chemical varies from 1 µg/kg (guinea pigs) to 5,000 µg/kg (hamsters). In rats, the LD_{50} is 26 µg/kg (male) or 22 µg/kg (female), and in monkeys, the LD_{50} is 70 µg/kg (Poland and Knutson, 1982). An acute lethal dose of TCDD has a latency period of 1 to 2 weeks in all species tested, during which time the animals exhibit a wasting syndrome. Characteristic effects associated with TCDD exposure are chloracne, reduced body weight and body weight gain, porphyria, thymic atrophy, gastric hyperplasia/hypoplasia, hepatotoxicity, increased serum concentrations of liver enzymes, hypertriglyceridemia, increased liver weight, hepatic Vitamin A depletion, altered thyroid homeostasis, and increased metabolizing enzymes (Poland and Knutson, 1982).

CARCINOGENICITY

Experimental Animals

The carcinogenicity of TCDD has been clearly established in rodents by the dermal, dosed feed, and gavage routes of administration (Table 2) (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 NTP (1982a) study, significantly increased incidences of thyroid gland follicular cell adenoma were observed in high dose male and female rats and high dose female mice. TCDD gavage administration also produced an increased incidence of neoplastic liver nodules in high dose female mice and an increased incidence 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 NTP (1982b) studies, there is substantial evidence of carcinogenicity of TCDD in male and female rats and mice (Table 3).

TABLE 2
Tissue Sites of Increased Neoplasm Incidences in Rodent Bioassays of TCDD

Species/Strain	Route of Administration	Sex	Tissue Site	Reference
Rats/Sprague-Dawley	Dosed feed	Male	Tongue Nasal turbinates/hard palate	Kociba *et al.*, 1978
		Female	Lung Nasal turbinates/hard palate Liver	
Rats/Osborne-Mendel	Gavage	Male	Thyroid gland	NTP, 1982a
		Female	Liver	
Mice/B6C3F$_1$	Gavage	Male	Liver	NTP, 1982a
		Female	Liver Thyroid gland	
Mice/Swiss Webster	Dermal	Female	Integumentary fibrosarcoma	NTP, 1982b
Mice/B6C3 and B6C	Gavage	Male	Thymic lymphomas	Della Porta *et al.*, 1987
		Female	Liver	
Mice/Swiss	Gavage	Male	Liver	Toth *et al.*, 1979
Hamsters/Syrian Golden	Dermal	Male	Facial skin carcinoma	Rao *et al.*, 1988

TABLE 3
Levels of Evidence for Carcinogenicity of TCDD in NTP Rodent Bioassays

Sex/Species	Route of Administration	
	Gavage[a]	Dermal[b]
Male/Rats[c]	Positive	Not Tested
Female/Rats[c]	Positive	Not Tested
Male/Mice[d]	Positive	Equivocal
Female/Mice[d]	Positive	Positive

[a] NTP, 1982a
[b] NTP, 1982b
[c] Osborne-Mendel rats
[d] B6C3F$_1$ mice (gavage); Swiss-Webster mice (dermal)

One of the most highly cited carcinogenicity studies for TCDD is a 2-year study conducted by Dow Chemical Company (Kociba *et al*., 1978). In that study, increased incidences of tumors were seen at multiple sites in Sprague-Dawley rats administered up to 100 ng TCDD/kg per day in feed for 2 years (Table 4). 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 the 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, pituitary gland adenoma (females), and acinar adenoma of the pancreas (males). Two evaluations of the pathology of the female liver tumor data confirmed significant increases in the incidences of hepatocellular adenoma and hepatocellular carcinoma (Squire, 1980; Goodman and Sauer, 1992; Table 5).

TABLE 4
Incidences of Tumors in Sprague-Dawley Rats in a 2-Year Feed Study of TCDD[a]

	Male				Female			
	0 ng/kg	1 ng/kg	10 ng/kg	100 ng/kg	0 ng/kg	1 ng/kg	10 ng/kg	100 ng/kg
Liver[b]	85	50	50	50	86	50	50	49
Hepatocellular Hyperplastic Nodules[c]	6	0	3	2	8	3	18*	23*
Hepatocellular Carcinoma	2	0	0	1	1	0	2	11*
Lung	85	50	50	50	86	50	50	49
Keratinizing Squamous Cell Carcinoma	0	0	0	1	0	0	0	7*
Nose, Hard Palate	85	50	50	50	86	50	50	49
Stratified Squamous Cell Carcinoma	0	0	0	4*	0	0	1	4*
Tongue	85	50	50	50	86	50	50	49
Stratified Squamous Cell Carcinoma	0	1	1	3*	1	0	0	2
Adrenal Gland	85	50	50	50	86	50	50	49
Adrenal Cortex Adenoma	0	0	2	5*	9	6	2	5
Adrenal Medulla, Pheochromocytoma	28	6	10	4*	7	2	1	3
Skin	85	50	50	50	86	50	50	49
Subcutaneous, Lipoma, Fibroma, or Fibroadenoma	10	1*	5	6	1	1	0	0
Uterus					86	50	50	49
Benign Neoplasms					28	12	11	7*
Mammary Gland					86	50	50	49
Benign Neoplasms					73	35	36	24*
Carcinoma					8	4	4	0*
Pituitary Gland	85	50	50	50	86	50	50	49
Adenoma	26	6	11	13	43	18	13	12*
Pancreas	85	50	50	50	86	50	50	49
Acinar Adenoma	14	7	5	2*	0	1	0	1

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
[a] Data presented by Kociba *et al*., 1978
[b] Number of animals with tissue examined microscopically (only sites where significant changes were observed are shown)
[c] Number of animals with lesion

Table 5
Incidences of Liver Tumors in Female Sprague-Dawley Rats Exposed to TCDD in Feed for 2 Years[a]

Evaluation	Tumor Classification	TCDD (ng/kg per day)			
		0 ng/kg	1 ng/kg	10 ng/kg	100 ng/kg
Kociba *et al*. (1978)[b]	Hyperplastic Nodule	8/86▲▲	3/50	18/50**	23/49**
	Hepatocellular Carcinoma	1/86▲▲	0/50	2/50	11/49**
	Hyperplastic Nodule or Hepatocellular Carcinoma	9/86▲▲	3/50	18/50**	34/48**
Squire (1980)	Neoplastic Nodule or Hepatocellular Carcinoma	16/86▲▲	8/50	27/50**	33/47**
Goodman and Sauer (1992)	Hepatocellular Adenoma	2/86▲▲	1/50	9/50**	14/45**
	Hepatocellular Carcinoma	0/86▲▲	0/50	0/50	4/45**
	Hepatocellular Adenoma or Carcinoma	2/86▲▲	1/50	9/50**	18/45**

** Significantly different (P≤0.01) from the control group by the Fisher exact test
▲▲ Significant (P≤0.01) Mantel-Haenszel trend test (Huff *et al.,* 1991).
[a] Hyperplastic nodule, neoplastic nodule and hepatocellular adenoma are interchangeable descriptions for the same lesion.
[b] Incidence data for hyperplastic nodule or hepatocellular carcinoma (combined) in this study are as described by Huff *et al.,* 1991.

Humans

Cancer incidence has been evaluated in several human populations with elevated dioxin exposures (USEPA, 2000c). These are either occupational cohort studies of phenoxy herbicide workers who were exposed to mixtures of DLCs or studies of a population in Seveso, Italy, that was accidentally exposed to TCDD after an explosion at a chemical plant in 1976. The most recent follow-up of the Seveso cohort that was primarily exposed to TCDD indicates that exposure is associated with an increase in all cancers combined and with several specific cancers: rectal cancer, lung cancer, Hodgkins disease, non-Hodgkins lymphoma, and myeloid leukemia (Bertazzi *et al*., 2001). TCDD has been classified as a known human carcinogen by the IARC and the NTP based on an evaluation of all the human and rodent cancer data with consideration of the role of the aryl hydrocarbon receptor (AhR) in the mechanism of action of TCDD.

TUMOR PROMOTION STUDIES

Numerous studies have examined the promotion of putative preneoplastic liver lesions by TCDD within the framework of a two-stage initiation-promotion protocol (Dragan and Schrenk, 2000). In the liver, clonal expansion of genetically altered cells leads to the formation of putative preneoplastic altered hepatocellular focal lesions identified by alterations in histomorphology or gene expression. These lesions are believed to be precursors in the development of liver tumors (Pitot *et al*., 1991). These studies demonstrate that TCDD is a potent liver tumor promoter and that this effect is dose-dependent (Pitot *et al*., 1980; Maronpot *et al*., 1993; Teeguarden *et al*., 1999), duration-of-exposure dependent, and reversible (Dragan *et al*., 1992; Walker *et al*., 1998, 2000). Studies also show that TCDD promotes more tumors in female rat liver than in male rat liver, and that this is due to the enhancing effect of estrogens on the promotion of preneoplastic lesions (Lucier *et al*., 1991; Wyde *et al*., 2001a, 2002). Moreover, this may explain why TCDD is a hepatocarcinogen in female but not male rats (Kociba *et al*., 1978).

Tests of the tumor initiating and promoting capacity of TCDD have also been conducted in two-stage (initiation-TCDD promotion) models of mouse skin tumorgenesis (IARC, 1997; Dragan and Schrenk, 2000; USEPA, 2000c). Similar studies demonstrate that TCDD is at least two orders of magnitude more potent than the prototypical tumor promoter tetradecanoyl phorbol acetate (Poland *et al*., 1982) in those skin tumor promotion models. Studies looking at AhR-active congeners indicate that TCDD-mediated skin tumor promotion is likely to be AhR-dependent (Hebert *et al*., 1990). Overall, there appears to be insufficient evidence that TCDD is a tumor initiator in mouse skin (Dragan and Schrenk, 2000). Transgenic models have also been used to examine the mechanism of 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. These data provide further support for the potent tumor promoting and weak initiating activities of TCDD.

In addition to the liver and skin, TCDD is a tumor promoter in the lung (Anderson *et al*., 1991; Beebe *et al*., 1995). An increased trend in the incidence of lung tumors was observed in male mice in the NTP carcinogenesis bioassay of TCDD (NTP, 1982a). In subsequent studies, control or N-nitrosodimethylamine- (NDMA) initiated male Swiss mice were given single (1.6, 16, or 48 µg/kg) or multiple (0.05 µg/kg per week for 20 weeks) intraperitoneal injections of TCDD and sacrificed at 52 weeks of age (Anderson *et al.*, 1991). A 100% incidence rate of lung tumors in NDMA-initiated mice was observed in the absence of TCDD treatment. Tumor multiplicity was significantly increased in two NDMA/TCDD treatment groups (1.6 and 16 µg/kg) when compared to NDMA-initiated controls. 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 TCDD's dominant mode of action for carcinogenesis is likely as a potent tumor promoter.

Mechanistic Studies

TCDD is generally classified as nongenotoxic and nonmutagenic. The common mechanism of action of TCDD and related compounds involves an initial binding to the AhR (Poland and Knutson, 1982; Safe, 1990; Whitlock, 1990; Schmidt and Bradfield, 1996). The broad spectrum of TCDD effects on hormone and growth factor systems, cytokines, and other signal transducer pathways indicates that TCDD is an extremely powerful growth dysregulator (Birnbaum, 1994a; Table 6). Because TCDD is not directly genotoxic (Wassom *et al*., 1977), it is believed that the pathological responses associated with exposure to TCDD are fundamentally due to binding to and activation of the AhR, subsequent alterations in expression of TCDD-regulated genes, and altered signaling of biological pathways that interact with the AhR signal transduction mechanism (Poland and Knutson, 1982).

Alterations in expression of TCDD-regulated genes occur via a mechanism that involves a high-affinity interaction of TCDD and related polycyclic and polyhalogenated aromatic hydrocarbons with an intracellular receptor 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 translocates to the nucleus (Whitlock, 1993). At some point subsequent to ligand binding, the AhR associates with an aromatic hydrocarbon nuclear translocator protein (ARNT) to form the nuclear DNA-binding and transcriptionally active AhR complex. Both the AhR and ARNT proteins are members of the basic helix-loop-helix family of transcription factors (Hoffman *et al*., 1991; Burbach *et al*., 1992; Ema *et al*., 1992). The AhR-ARNT heterodimer binds with high affinity to a specific DNA sequence termed the dioxin response element (DRE). DREs have been identified in the enhancer regions of genes encoding several drug-metabolizing enzymes (Lai *et al*., 1996). The characteristic response to TCDD 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 all tissues examined (Dolwick *et al*., 1993), with a definite tissue specificity in terms of level of expression and diversity of response, indicating that TCDD is likely to have some effect in every tissue. However, even with the same receptor and the same ligand, there are both qualitative and quantitative differences in response, and these differences are likely to be involved in the tissue- and species-specificity of the response to TCDD. It is still not known how alterations in gene expression ultimately lead to the development of pathologies and adverse health effects associated with TCDD exposure. However, it is generally accepted that most, if not all, TCDD responses require an initial step of binding to the AhR.

The most studied response to TCDD is induction of the CYP1A class of cytochromes P450 (Whitlock, 1999). CYP1A1 is induced in many tissues including the liver, lung, kidney, nasal passages, and small intestine, with the highest induction in rats occurring in the liver.

TABLE 6
Biochemical Effects of TCDD[a]

Enzyme Induction	Modulation of Hormones and Receptors	Modulation of Growth Factors and Receptors
CYP1A1	Androgens	Vitamin A
CYP1B1	Estrogens	EGF
CYP1A2	Estrogen receptor	TGFα
Quinone reductase	Glucocorticoids	EGF receptor
UDP-glucuronosyltransferase	Glucocorticoid receptor	TGFβ
Glutathione-*S*-transferase	Insulin	TNFα
Aldehyde dehydrogenase	Gastrin	IL-1β
Ornithine decarboxylase	Thyroid hormones	c-*Ras*
Tyrosine kinase	Melatonin	c-*ErbA*
Terminal deoxynucleotidyltransferase		
Phosphoenolpyruvate carboxykinase		
Plasminogen activator inhibitor-2		

[a] Adapted from Birnbaum, 1994a

CYP1A1 induction is a very sensitive response to TCDD and, as such, serves as a useful marker for exposure to TCDD. TCDD induces CYP1A1 *in vivo* and *in vitro* in animal models and humans. Induction of 7-ethoxyresorufin-*O*-deethylase activity is a marker of CYP1A1 activity. CYP1A2 is constitutively expressed in the liver at low levels and is inducible by TCDD only in the liver and possibly nasal turbinates of rats (Goldstein and Linko, 1984). CYP1A2 is induced by TCDD, and induction of acetanilide-4-hydroxylase activity is a marker of CYP1A2 activity. In addition to the well-characterized induction of CYP1A1 and CYP1A2, TCDD 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).

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

TCDD has 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, are part of pathways involved in cellular proliferation and differentiation. These include the glucocorticoid receptor tyrosine kinases, interleukin-1-beta, plasminogen activator inhibitor-2, urokinase type plasminogen activator, tumor necrosis factor-alpha, gonadotrophin releasing hormone, testosterone, and prostaglandin endoperoxide H synthase-2. More recently, the application of toxicogenomic analyses has increased our understanding of the number of genes/proteins altered by TCDD both *in vitro* (Puga *et al*., 2000; Martinez *et al*., 2002) and *in vivo* (Bruno *et al*., 2002; Kurachi *et al.*, 2002; Zeytun *et al*., 2002). Most of the molecular details for induction of gene expression via the AhR have been characterized for the transcriptional activation of the CYP1A1 gene (Whitlock, 1999). While TCDD has been shown to affect the expression of many genes, there is detailed characterization of transcriptional activation through the AhR for only a few of these.

GENETIC TOXICOLOGY

In general, polychlorinated biphenyl compounds are not mutagenic in standard short-term mutagenicity tests conducted *in vitro* or *in vivo*. TCDD follows this same activity pattern. TCDD (tested up to the limits of toxicity) was not mutagenic in *Salmonella typhimurium* with

or without exogenous liver S9 metabolic activation enzymes (Mortelmans *et al*., 1984; Blevins, 1991). No induction of mutations was seen at the trifluorothymidine locus in mouse lymphoma L5178Y $tk^{+/-}$ cells treated with up to 1.0 µg/mL TCDD with or without S9 (McGregor *et al*., 1991). In tests for induction of chromosomal damage in cultured Chinese hamster ovary cells, TCDD did not induce sister chromatid exchanges or chromosomal aberrations when tested up to toxic levels (0.8 µg/mL) with or without S9 (Galloway *et al*., 1987). In *in vivo* tests, no induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* following injection of adult flies with 50, 250, or 500 ppm TCDD (Zimmering *et al*., 1985). TCDD, administered for 6 weeks at a dose of 2 µg/kg to male and female Big Blue transgenic rats, did not alter the frequency or spectrum of mutations (Thornton *et al*., 2001). There is one report of a positive result with TCDD (and two other polychlorinated aromatic hydrocarbons) in a test that measured induction of chromosomal deletions, resulting from intrachromosomal recombination, in mouse embryos *in vivo* (Schiestl *et al*., 1997); in this study, the two other compounds, Aroclor 1221 and Aroclor 1260, also induced deletions in yeast cells. An earlier study of the potential for TCDD-induced cytogenetic damage in laboratory mice showed no increase in the frequencies of sister chromatid exchanges, chromosomal aberrations, or micronuclei in bone marrow cells of either C57Bl/6J or DBA/21 mice, following administration of a single dose of up to 150 µg TCDD/kg (Meyne *et al*., 1985). In summary, no mutagenic activity was detected with TCDD in a variety of *in vitro* and *in vivo* short-term tests.

Study Rationale

The female Sprague-Dawley rat was selected as the model for the current study because this sex and species has been used frequently in chronic and subchronic studies of dioxins. In addition, this was the model in which TCDD was demonstrated as a carcinogen in a dosed-feed study conducted by the Dow Chemical Company (Kociba *et al*., 1978). Moreover, the incidence of liver tumors in 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 for the current study were based on the 1 to 100 ng/kg per day range used in the Kociba *et al*. (1978) feed study where increased incidences of liver tumors were seen. These studies were not specifically designed to determine a no-observed-adverse-effect level or lowest-observed-adverse-effect level; rather, doses used in the present study were selected to increase dose-response data density in the 10 to 100 ng/kg range, where increases in liver and lung tumors were expected, to facilitate derivation of relative potency factors for carcinogenesis. Male rats were not studied due to the lack of induction of liver and lung tumors in the previous studies of Sprague-Dawley rats.

MATERIALS AND METHODS

Procurement and Characterization of TCDD

TCDD was obtained from IIT Research Institute (Chicago, IL) 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 (Research Triangle Park, NC), and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the TCDD study are on file at the National Institute of Environmental Health Sciences.

The chemical, a white crystalline powder, was identified as TCDD by infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, direct probe mass spectroscopy (MS), low resolution gas chromotography (GC) coupled with MS, and melting point determination. A precise melting point range was not determined as the chemical appeared to sublime at approximately 260° C. The purity was determined by GC. The purity profiles detected two impurities with a combined relative area of 2.0% and two impurities with a combined relative area of 1.6%. The major impurity in each profile (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 overall purity was determined to be 98% or greater.

Preparation of Stock Samples

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. Details concerning the preparation and use of these stock solutions are provided in Appendix D. The test article was stored at room temperature and protected from light in amber glass bottles. Purity was monitored with periodic reanalysis using GC. No degradation was observed during the course of the study.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing TCDD with acetone and then diluting the mixture in corn oil (Spectrum Quality Products) such that the final concentration contained 1.0% acetone (Table D2). The dose formulations were stored at room temperature in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids, for up to 35 days.

Homogeneity studies of 1.2 and 40 ng/mL dose formulations and simulated animal room stability studies of a 1.2 ng/mL dose formulation were performed by the study laboratory using GC/MS. Homogeneity was confirmed, and stability was confirmed for 3 hours under simulated animal room conditions. An additional stability study of a 0.2 ng/mL dose formulation was performed by the analytical laboratory, and stability was confirmed for at least 35 days for the dose formulation stored in sealed glass containers at 5° C and room temperature. Gavagability was confirmed by the study laboratory for the 40 ng/mL formulation.

Periodic analyses of the dose formulations of TCDD were conducted by the study laboratory using GC/MS. During the 2-year study, the dose formulations were analyzed at least every 3 months (Table D3). Of the dose formulations analyzed, 56 of 58 were within 10% of the target concentrations, and 19 of 21 animal room samples were within 10% of the target concentrations. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

2-YEAR STUDY

Study Design

Groups of 81 or 82 (3 ng/kg) female rats received TCDD in corn oil:acetone (99:1) by gavage at doses of 3, 10, 22, 46, or 100 ng/kg 5 days per week for up to 105 weeks; a group of 81 female rats received the corn oil:acetone (99:1) vehicle alone. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. For stop-exposure evaluation, a group of 50 female rats was given 100 ng/kg for 30 weeks and then the vehicle for the remainder of the study.

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

Source and Specification of Animals

Male and female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN), for use in the 2-year study. Sufficient male rats were included in this study to ensure normal estrous cycling of female rats. Male rats were not administered the test compound. Rats were quarantined for 11 days before and were approximately 8 weeks old at the beginning of the study. Rats were evaluated for parasites and gross observation of disease, and the health of the rats was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F). Sentinel rats included five males and five females at 1 month, five males at 6, 12, and 18 months, and five 100 ng/kg females at the end of the study.

Animal Maintenance

Male rats were housed three per cage, and female rats were housed three or five per cage. Feed 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 7. Information on feed composition and contaminants is provided in Appendix E.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded on day 29, monthly thereafter, and at the end of the study. Body weights were recorded on the first day of the study, weekly for 13 weeks, monthly thereafter, and at the end of the study.

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

For cell proliferation analysis at 14, 31, and 53 weeks, up to 10 female rats per group (except stop-exposure) received drinking water containing 40 mg BrdU/100 mL Milli-Q water for 5 days. BrdU solutions were administered in amber glass water bottles (Allentown Caging Equipment Company, Inc., Allentown, NJ) equipped with Teflon®-lined lids and stainless steel sipper tubes. BrdU solutions were changed after 3 days, and water consumption was measured daily for 5 days. Cell turnover rate in the liver of dosed female rats was compared to the turnover rate in the vehicle control rats by determining the incorporation of BrdU into hepatocytes. A sample of duodenum and liver was fixed in 10% neutral buffered formalin for 18 to 24 hours then transferred to 70% ethanol. Representative sections of the duodenum and liver were trimmed and embedded, and two sections were cut. One of these sections was stained with hematoxylin and eosin and the other with anti-BrdU antibody complexed with avidin and biotin. At the 14-week interim evaluation, potential interlobular variation was determined in the vehicle control and 100 ng/kg groups by counting stained cells in the left lobe and right median lobe. Interlobular variation greater than 25% was considered significant. For the remaining rats, stained cells were counted only in the left lobe. Two thousand labeled or unlabeled hepatocyte nuclei were counted using a 20× objective and ocular grid. The labeling index is expressed as the percentage of total nuclei that were labeled with BrdU.

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

Coomassie Plus Protein Assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. Cytochrome P450 1A1 (CYP1A1)-associated 7-ethoxyresorufin-*O*-deethylase (EROD), CYP1A2-associated acetanilide-4-hydroxylase (A4H), and CYP2B-associated pentoxyresorufin-*O*-deethylase (PROD) activities were determined in microsomal proteins, isolated from frozen liver or lung tissue according to established procedures. Data are shown as pmol/minute per mg (EROD and PROD) or nmol/minute per mg (A4H) microsomal protein.

For analysis of tissue concentrations of TCDD, 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. Analysis of fat, lung, and blood samples included overnight saponification with potassium hydroxide (blood samples were sonicated following digestion), extraction of the saponificate with hexanes:acetone (1:4 for fat and blood, 1:1 for lung) using vortexing and centrifugation, sample extract cleanup using open-bed silica gel/sodium sulfate columns, and elution with hexanes. Liver samples were homogenized in saturated aqueous sodium sulfate, extracted with hexanes:acetone (1:9), and cleaned and eluted using the same techniques as for the other tissues. Concentrations of TCDD in the tissue extracts were measured by capillary gas chromatography with high resolution mass spectrometry detection.

Complete necropsies and microscopic examinations were performed on all rats. At the interim evaluations, the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid gland were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 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 7.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the adrenal cortex, liver, lung, oral mucosa, pancreas, thymus, thyroid gland, and uterus.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the 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 TCDD, along with three other studies (PCB126, the TEF mixture, and PeCDF), were subjected to additional PWG reviews. Within many of these studies, there were hepatocellular proliferative lesions for which the criteria used for common diagnoses did not appear to fit. Furthermore, classification was sometimes confounded by significant liver damage (toxic hepatopathy) that was present in many animals from these studies. With the consecutive pathology peer review of each of these

studies, the morphological spectrum of proliferative lesions became more apparent to those involved and the diagnostic criteria for the proliferative lesion further refined. Therefore, a PWG review was held to ensure that these important proliferative lesions were sufficiently and consistently categorized across all seven studies for which data are to be compared. PWG participants for this review were primarily those involved in previous PWGs. A different group of pathologists was also convened to provide additional guidance on the most appropriate classification of the hepatocellular proliferative lesions from these studies of DLCs (Hailey *et al.,* 2005). 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 7
Experimental Design and Materials and Methods in the 2-Year Gavage Study of TCDD

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
11 days

Average Age When Study Began
8 weeks

Date of First Dose (female rats only)
June 1, 1998

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

Date of Last Dose
May 30-June 1, 2000 (core study), December 30, 1998 (stop-exposure group)

Necropsy Dates
May 31-June 2, 2000

Average Age at Necropsy
112 weeks

Size of Study Groups
81 (vehicle control, 10, 22, 46, and 100 ng/kg), 82 (3 ng/kg), or 50 (100 ng/kg stop-exposure)

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

Animals per Cage
3 males; 3 or 5 females

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, except via amber glass bottles during BrdU administration, available *ad libitum*

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

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

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

TABLE 7
Experimental Design and Materials and Methods in the 2-Year Gavage Study of TCDD

Racks
Stainless steel (Lab Products, Inc., Maywood, NJ), 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, 3, 10, 22, 46, or 100 ng/kg

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

Method of Sacrifice
Carbon dioxide asphyxiation

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

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

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

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

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

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

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1a, A1b, A1c, A5a, A5b, and A5c 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 A3a and A3b) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the numbers of animals on which a necropsy was performed. Tables A3a and A3b also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-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 dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Thyroid hormone, cell proliferation, and cytochrome P450 data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957)

were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. For female Sprague-Dawley rats, the NTP historical database is currently limited to the seven gavage studies conducted as part of the dioxin TEF evaluation (the current TCDD study, PCB 126, the TEF mixture, PeCDF, PCB 153, the PCB 126/PCB 153 mixture, and the PCB 126/PCB 118 mixture; 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 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.

Genetic Toxicology

The genetic toxicity of TCDD was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, mutations in L5178Y mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, sex-linked recessive lethal mutations in *Drosophila melanogaster,* and micronucleated erythrocytes in mouse bone marrow. The protocols for these studies and the results are given in Appendix B.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.*, 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for female rats are shown in Table 8 and in the Kaplan-Meier survival curves (Figure 1). Survival of dosed groups of rats was similar to that of the vehicle control group.

Body Weights and Clinical Findings

Mean body weights of the 100 ng/kg core study group and the 100 ng/kg stop-exposure group were less than those of the vehicle control group after week 13 of the study (Figure 2 and Table 9). Mean body weights of the 46 ng/kg group were less than those of the vehicle control group during year 2 of the study, and mean body weights of 22 ng/kg rats were less than those of the vehicle controls the last 10 weeks of the study. No clinical findings related to TCDD administration were observed.

TABLE 8
Survival of Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
Animals initially in study	81	82	81	81	81	81	50
14-Week interim evaluation[a]	10	10	10	10	10	10	
31-Week interim evaluation[a]	10	10	10	10	10	10	
53-Week interim evaluation[a]	8	8	8	8	8	8	
Accidental deaths[a]	0	0	5	0	0	0	0
Moribund	19	27	15	19	17	17	16
Natural deaths	9	6	10	15	14	15	13
Animals surviving to study termination	25	21	23	19	22	21	21
Percent probability of survival at end of study[b]	47	39	49	36	42	40	42
Mean survival (days)[c]	637	598	619	599	634	672	650
Survival analysis[d]	P=0.736N	P=0.289	P=0.840N	P=0.184	P=0.728	P=0.807	P=0.860

[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 negative trend or a lower mortality in a dosed group is indicated by **N**. The trend test does not include the 100 ng/kg stop-exposure group.

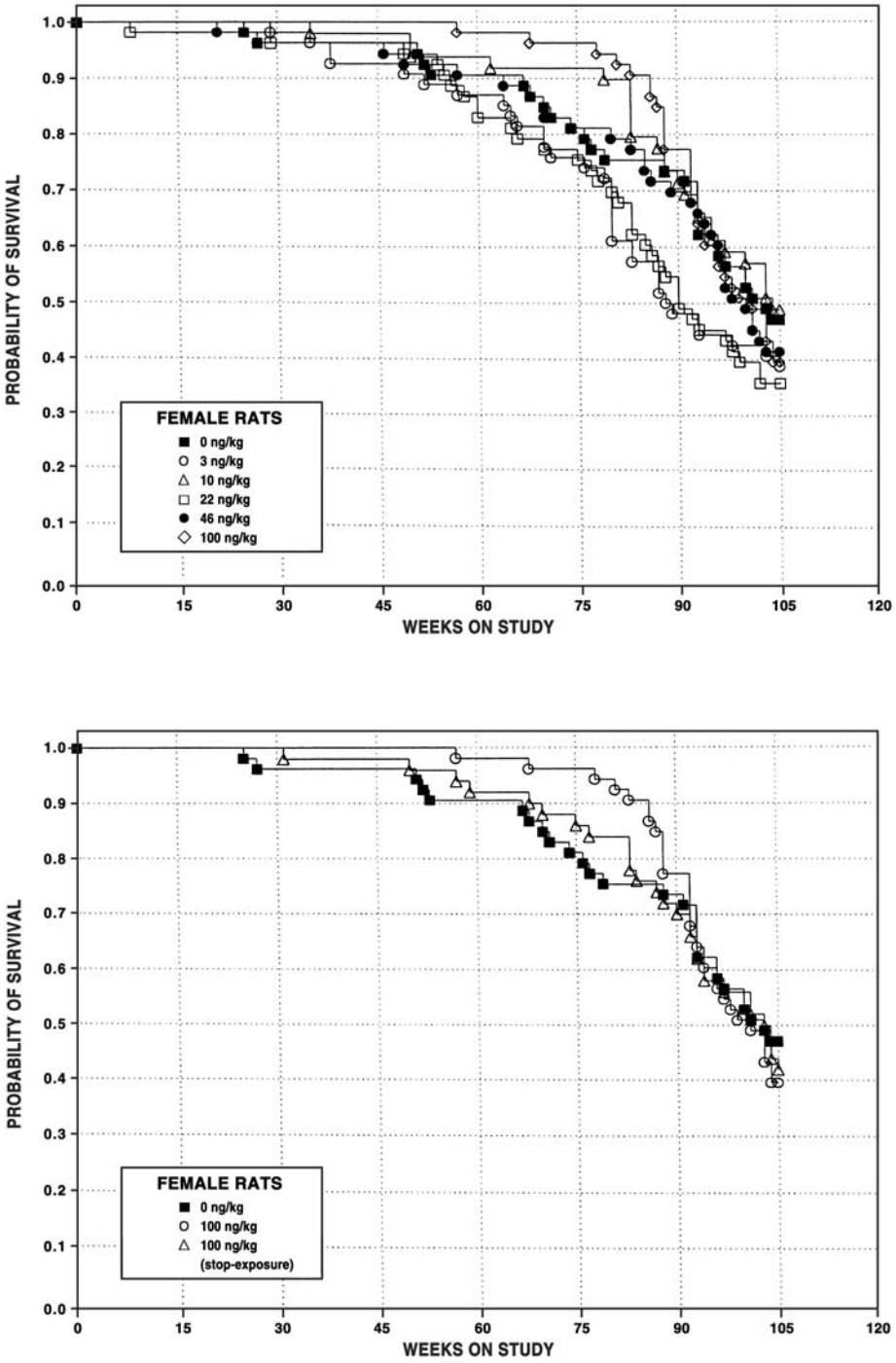


FIGURE 1
Kaplan-Meier Survival Curves for Female Rats Administered TCDD by Gavage for 2 Years

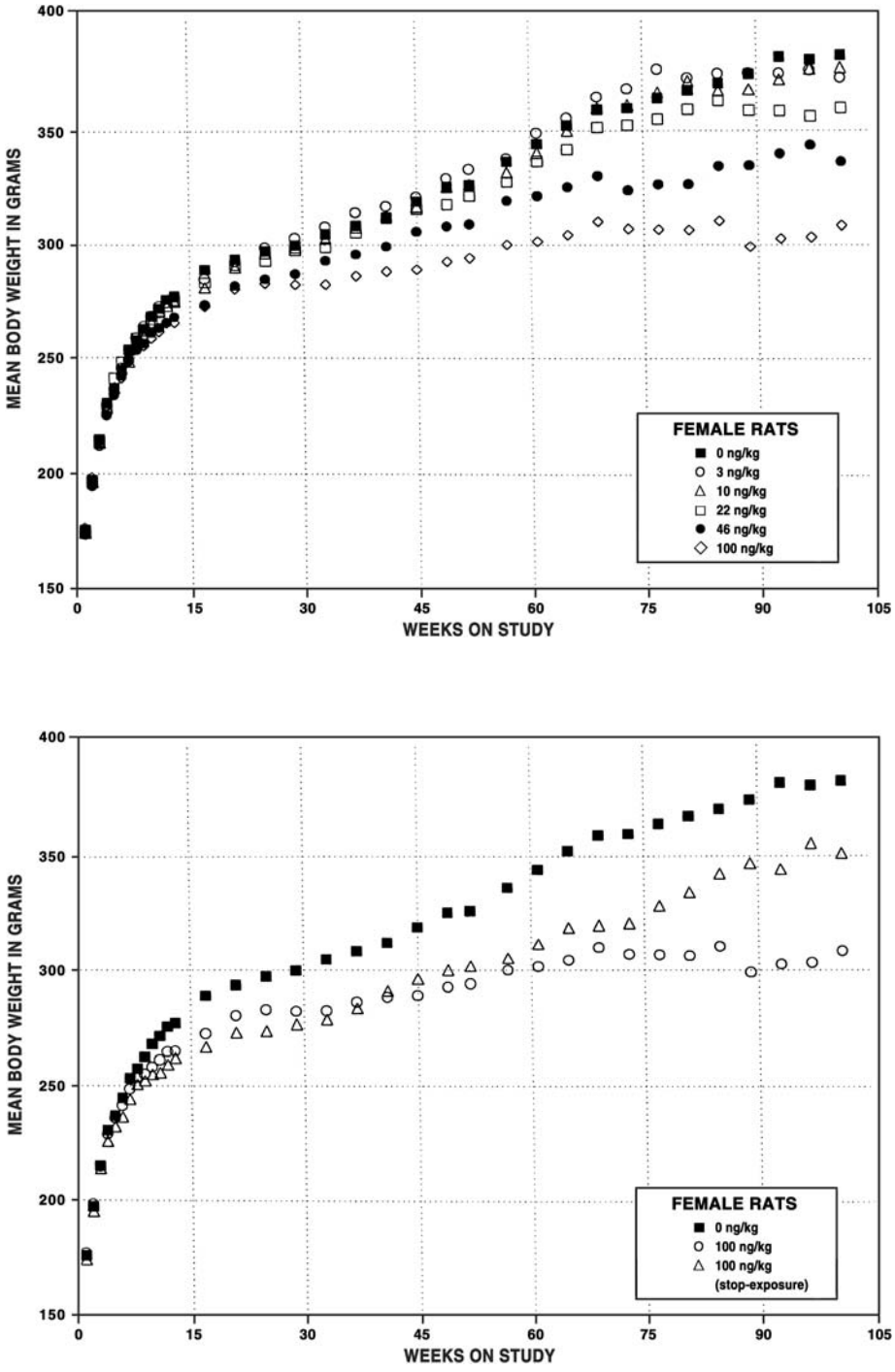


FIGURE 2
Growth Curves for Female Rats Administered TCDD by Gavage for 2 Years

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of TCDD

Weeks on Study	Vehicle Control		3 ng/kg			10 ng/kg			22 ng/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	177	98	175	99	98	175	99	98	175	99	98
2	198	98	196	99	98	198	100	98	196	99	98
3	216	98	214	99	98	216	100	98	214	99	98
4	231	98	229	99	98	231	100	98	229	99	98
5	237	98	237	100	98	241	102	98	241	102	98
6	247	98	245	99	98	248	101	98	246	100	98
7	255	98	249	98	98	252	99	98	252	99	98
8	260	98	258	99	98	260	100	97	258	99	98
9	265	98	262	99	98	262	99	96	260	98	97
10	270	98	267	99	98	268	99	96	265	98	97
11	274	98	271	99	98	273	100	96	268	98	97
12	278	98	273	98	98	275	99	96	273	98	97
13	279	98	275	99	98	278	100	96	275	98	97
17[a]	290	82	284	98	82	284	98	80	280	97	81
21	296	82	292	99	82	292	99	80	288	97	81
25	300	81	298	100	82	298	99	80	290	97	81
29	302	80	302	100	82	300	100	79	295	98	80
33[a]	306	64	308	101	65	304	99	63	298	97	64
37	311	64	314	101	64	309	99	61	304	98	64
41	315	64	318	101	62	314	100	61	309	98	64
45	321	64	322	100	62	319	99	61	314	98	64
49	328	64	330	101	62	328	100	61	317	97	64
52	329	62	334	102	60	330	100	59	320	97	63
57[a]	337	48	338	100	48	332	99	46	328	97	47
61	345	48	349	101	47	341	99	46	337	98	44
65	353	48	356	101	45	350	99	45	342	97	44
69	359	46	365	102	44	360	100	45	352	98	42
73	360	44	368	102	41	361	100	45	353	98	41
77	364	41	377	103	40	367	101	45	355	98	39
81	368	40	373	102	33	372	101	44	359	98	36
85	371	40	375	101	31	368	99	39	363	98	33
89	375	39	375	100	27	368	98	36	359	96	29
93	382	37	375	98	26	372	97	33	359	94	25
97	381	31	376	99	24	377	99	30	356	94	24
101	383	27	373	98	23	377	99	28	360	94	21
Mean for weeks											
1-13	245		242	99		244	100		242	99	
14-52	310		310	100		308	99		302	97	
53-101	365		367	101		363	99		352	96	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of TCDD

Weeks on Study	46 ng/kg			100 ng/kg			100 ng/kg (Stop-Exposure)		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	175	99	98	176	100	98	174	99	50
2	197	99	98	198	100	98	195	98	50
3	216	100	98	215	99	98	214	99	50
4	229	99	98	228	98	98	226	98	50
5	237	100	98	234	99	98	232	98	50
6	244	99	98	240	97	98	237	96	50
7	250	98	98	247	97	98	244	96	50
8	256	98	98	252	97	98	251	96	50
9	260	98	98	254	96	98	253	95	50
10	263	97	98	257	95	98	255	95	50
11	265	97	98	260	95	98	256	93	50
12	268	96	98	264	95	98	260	94	50
13	270	97	98	264	95	98	262	94	50
17[a]	276	95	82	272	94	82	267	92	50
21	284	96	82	279	95	82	273	92	50
25	286	96	81	282	94	82	274	91	50
29	288	96	80	281	93	82	277	92	50
33[a]	293	96	64	282	92	66	279	91	49
37	297	95	64	286	92	66	284	91	49
41	300	95	64	288	92	66	291	93	49
45	306	95	64	289	90	66	296	92	49
49	309	94	63	293	89	66	300	92	49
52	310	94	62	294	89	66	302	92	48
57[a]	320	95	49	300	89	53	306	91	48
61	322	93	48	302	88	52	312	91	46
65	326	92	47	305	86	52	319	91	46
69	330	92	46	310	86	51	320	89	45
73	324	90	44	307	85	51	321	89	44
77	327	90	43	307	84	51	329	90	43
81	327	89	42	307	83	49	335	91	42
85	335	90	40	311	84	48	343	92	38
89	335	89	38	299	80	41	347	93	36
93	340	89	35	303	79	36	345	90	31
97	344	90	31	304	80	29	356	93	29
101	337	88	25	309	81	26	351	92	28
Mean for weeks									
1-13	241	98		238	97		235	96	
14-52	295	95		285	92		284	92	
53-101	331	91		305	84		332	91	

[a] Interim evaluations occurred during weeks 14, 31, and 53; until week 53, number of survivors includes 17 or 16 (3 ng/kg group) special study animals (except stop-exposure group).

Thyroid Hormone Concentrations

Assays for total thyroxine (T_4), free T_4, total triiodothyronine (T_3), and thyroid stimulating hormone (TSH) were conducted at the 14-, 31-, and 53-week interim evaluations (Table 10). A downward trend in serum total T_4 concentrations with higher TCDD concentrations was evident at 14, 31, and 53 weeks. At 14, 31, and 53 weeks, all dosed groups showed a decrease in mean total T_4 concentrations relative to the vehicle controls. At 14 weeks, the T_4 concentrations in the 22, 46, and 100 ng/kg groups were significantly lower than in vehicle controls by 25.1%, 28.1%, and 31.7%, respectively. At 31 weeks, total T_4 concentrations in the 22, 46, and 100 ng/kg groups were significantly lower than those in the vehicle controls. These decreases were 34.4%, 33.9%, and 21.8% lower relative to vehicle controls in the 22, 46, and 100 ng/kg groups, respectively. At 53 weeks, significant depressions in mean total T_4 concentrations relative to vehicle controls of 46.3% and 32.7% were observed in the 46 and 100 ng/kg groups, respectively.

At 14 weeks, a significant decrease in serum free T_4 concentrations relative to the vehicle controls was observed at doses of 22 ng/kg or greater with the high dose group showing the greatest change (25.0%). At 31 weeks, serum free T_4 concentrations were lower at doses greater than 10 ng/kg compared to vehicle controls. These changes were significant for the 22, 46, and 100 ng/kg groups, which were 25.1%, 16.0%, and 26.8% lower than vehicle controls, respectively. At the 53-week interim evaluation, no significant differences in free T_4 concentrations were observed between vehicle control and TCDD-treated groups, although there was a slight trend toward lower levels at the higher doses.

There was a trend for serum concentrations of T_3 to increase with increasing dose in the 10 ng/kg or greater groups at 14 weeks. In the 46 and 100 ng/kg groups, these increases were significantly higher than vehicle controls by 18.3% and 33.0%, respectively. At 31 weeks, serum T_3 was significantly higher in the 46 and 100 ng/kg groups than in the vehicle controls by 30.0% and 22.6%, respectively. At the 53-week interim evaluation, an increasing trend in serum T_3 with higher TCDD concentrations was observed. These increases were dose-dependent and significant for the 10, 22, 46, and 100 ng/kg groups. Serum T_3 concentrations were 13.8%, 23.6%, 27.9%, and 35.4% higher than vehicle controls in the 10, 22, 46, and 100 ng/kg groups, respectively.

There was a trend toward increased serum TSH levels at doses of 10 ng/kg or greater at 14 weeks and 3 ng/kg or greater at 31 weeks. At 14 weeks, serum TSH levels were significantly higher in the 46 and 100 ng/kg groups relative to vehicle controls. No significant differences were observed in serum TSH levels at 31 or 53 weeks. At the 53-week interim evaluation, the mean serum TSH level in the 100 ng/kg group was 43.5% higher, but not significantly, than the vehicle controls.

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Week 14						
n	10	10	10	10	10	10
Total T_4 (µg/dL)	6.370 ± 0.372	5.940 ± 0.321	5.480 ± 0.371	4.770 ± 0.310**	4.580 ± 0.309**	4.350 ± 0.289**
Free T_4 (ng/dL)	2.478 ± 0.175	2.404 ± 0.130	2.513 ± 0.242	1.983 ± 0.144*	2.040 ± 0.130*	1.858 ± 0.131*
Total T_3 (ng/dL)	155.685 ± 9.739	149.141 ± 4.180	163.995 ± 4.995	159.066 ± 4.888	184.171 ± 6.961*	207.026 ± 6.149**
TSH (ng/mL)	14.981 ± 0.949	13.282 ± 0.718	16.018 ± 1.047	17.434 ± 0.737	19.765 ± 1.587*	18.836 ± 1.656*
Week 31						
n	10	10	10	10	10	10
Total T_4 (µg/dL)	3.810 ± 0.414	3.650 ± 0.201	3.260 ± 0.178	2.500 ± 0.153**	2.520 ± 0.237**	2.980 ± 0.205**
Free T_4 (ng/dL)	1.734 ± 0.171	1.750 ± 0.094	1.573 ± 0.105	1.299 ± 0.072**	1.457 ± 0.124*	1.269 ± 0.080**
Total T_3 (ng/dL)	138.281 ± 4.658	134.433 ± 6.867	137.681 ± 4.858	142.253 ± 3.073	179.900 ± 7.440**	169.465 ± 8.805**
TSH (ng/mL)	12.321 ± 0.552[b]	14.828 ± 1.008	15.740 ± 0.864	13.654 ± 0.998	16.640 ± 1.988	16.172 ± 1.685
Week 53						
n	8	8	8	8	8	8
Total T_4 (µg/dL)	3.213 ± 0.236	3.038 ± 0.204	2.550 ± 0.200	2.800 ± 0.089	1.725 ± 0.161**	2.163 ± 0.167**
Free T_4 (ng/dL)	1.553 ± 0.153	1.593 ± 0.149	1.441 ± 0.109	1.781 ± 0.149	1.221 ± 0.144	1.329 ± 0.076
Total T_3 (ng/dL)	129.815 ± 3.540	134.334 ± 7.868	147.760 ± 3.393**	160.403 ± 7.226**	166.051 ± 8.173**	175.767 ± 12.596**
TSH (ng/mL)	13.193 ± 1.107	12.562 ± 0.902	12.390 ± 0.936	12.113 ± 0.939	13.318 ± 1.251	18.930 ± 1.985

* 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

Hepatic Cell Proliferation Data

Hepatocellular proliferation data from the 14-, 31-, and 53-week interim evaluations are shown in Table 11. The consumption of the BrdU drinking water solutions prior to each interim evaluation was similar across groups. Labeling indexes in controls varied from 0.327 to 1.410. Treatment-related effects in dosed groups were observed relative to duration-of-exposure-matched vehicle controls. At 14 weeks, the hepatocellular labeling index was significantly higher in the 22 ng/kg group compared to the vehicle controls. A 2-fold increase in the 22 ng/kg group was observed relative to vehicle controls. The labeling index was significantly higher in all dose groups at the 31-week interim evaluation than in the vehicle controls. A 2- to 3-fold increase was observed at doses of 3, 10, and 22 ng/kg compared to the vehicle controls. The hepatocyte labeling index was 4-fold higher in the 46 ng/kg group and 11-fold higher in the 100 ng/kg group compared to vehicle controls. At 53 weeks, the labeling index was elevated by nearly 3-fold at 10 ng/kg; however, this increase was not statistically different than vehicle controls due to a large variance in response. The labeling index was significantly higher in the 46 and 100 ng/kg groups than in vehicle controls at the 53-week interim evaluation. The labeling index was nearly 2-fold higher at 46 ng/kg and greater than 5-fold higher at 100 ng/kg relative to vehicle controls.

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	9	10	10	10	10	10
Week 53	8	8	8	8	8	8
Labeling index (%)						
Week 14	1.410 ± 0.271	1.675 ± 0.471	1.916 ± 0.251	2.858 ± 0.395*	2.405 ± 0.485	1.694 ± 0.313
Week 31	0.327 ± 0.063	0.852 ± 0.206*	0.956 ± 0.233*	0.792 ± 0.146*	1.333 ± 0.355**	3.846 ± 0.974**
Week 53	0.948 ± 0.190	0.976 ± 0.121	2.736 ± 1.159	1.289 ± 0.192	1.844 ± 0.293*	4.896 ± 0.883**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error.

Cytochrome P450 Enzyme Activities

At each interim evaluation, liver and lung samples were collected for determinations of P450 enzyme activity. Microsomal suspensions were prepared from liver samples and were assayed for 7-ethoxyresorufin-*O*-deethylase (EROD, CYP1A1) activity, 7-pentoxyresorufin-*O*-deethylase (PROD, CYP2B) activity, and acetanilide-4-hydroxylase (A4H, CYP1A2) activity. Microsomal samples from lung were analyzed for EROD activity only (Table 12).

Hepatic EROD, PROD, and A4H activities were significantly higher in all groups administered TCDD relative to the vehicle controls at the 14-, 31-, and 53-week interim evaluations. Significant induction of hepatic P450 activities occurred at the lowest dose (3 ng/kg) for all three sampling times (14, 31, and 53 weeks). Hepatic EROD activities were maximally induced approximately 70- and 62-fold over vehicle controls in the 100 ng/kg group at 14 and 53 weeks, respectively, and 82-fold over vehicle controls in the 46 ng/kg dose group at 31 weeks. Similarly, hepatic PROD activity generally increased with increasing dose and was significantly higher in all dosed groups relative to vehicle controls at all three interim evaluations (14, 31, and 53 weeks). Hepatic PROD activities were maximally induced approximately 8- and 6-fold over vehicle controls in the 100 ng/kg group at 14 and 53 weeks, respectively, and 8.5-fold over vehicle controls in the 46 ng/kg group at 31 weeks. Hepatic A4H activity generally increased with increasing dose. Hepatic A4H activities were maximally induced approximately 6- and 4.5-fold over vehicle controls in the 100 ng/kg group at 14 and 53 weeks, respectively, and 5.5-fold over vehicle controls in the 46 ng/kg group at 31 weeks.

Pulmonary EROD activities were approximately 10-fold lower than hepatic EROD activities in the vehicle control groups. EROD activity in the lung was significantly higher in all dosed groups compared to vehicle controls at the 14-, 31-, and 53-week interim evaluations. The maximum degree of induction relative to the vehicle control group was approximately 20-fold at 14 weeks (100 ng/kg), 24-fold at 31 weeks (22, 46, and 100 ng/kg), and 14-fold (10, 46, and 100 ng/kg) at 53 weeks.

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Liver Microsomes						
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Week 14	0.448 ± 0.017	0.787 ± 0.052**	1.004 ± 0.078**	1.391 ± 0.092**	2.340 ± 0.122**	2.660 ± 0.087**
Week 31	0.394 ± 0.020	0.728 ± 0.045**	0.791 ± 0.059**	1.207 ± 0.071**	2.174 ± 0.172**	1.882 ± 0.103**
Week 53	0.531 ± 0.023	0.882 ± 0.032**	1.484 ± 0.073**	1.711 ± 0.133**	2.193 ± 0.179**	2.385 ± 0.141**
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	30.523 ± 1.475	925.193 ± 80.785**	1,550.31 ± 116.116**	1,819.68 ± 108.267**	2,064.69 ± 134.623**	2,129.73 ± 88.569**
Week 31	30.932 ± 1.263	926.292 ± 64.816**	1,238.36 ± 74.002**	1,700.49 ± 101.296**	2,548.92 ± 161.798**	2,074.85 ± 156.032**
Week 53	30.215 ± 1.590	569.377 ± 24.622**	1,280.00 ± 95.303**	1,551.16 ± 112.364**	1,726.81 ± 107.575**	1,871.47 ± 109.140**
7-Pentoxyresorufin-*O*-deethylase (PROD) (pmol/minute per mg microsomal protein)						
Week 14	3.784 ± 0.246	9.143 ± 0.514**	16.571 ± 0.843**	23.772 ± 0.960**	27.346 ± 1.443**	30.914 ± 0.847**
Week 31	3.261 ± 0.260	9.634 ± 0.413**	14.769 ± 0.502**	20.421 ± 0.905**	27.717 ± 1.248**	25.257 ± 1.674**
Week 53	3.614 ± 0.172	7.271 ± 0.197**	14.760 ± 0.569**	17.281 ± 0.562**	20.581 ± 1.078**	21.213 ± 1.352**
Lung Microsomes						
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Week 14	3.004 ± 0.509	27.396 ± 1.602**	38.483 ± 2.463**	44.194 ± 3.493**	44.309 ± 3.794**	62.214 ± 4.899**
Week 31	2.071 ± 0.307	25.342 ± 0.806**	30.387 ± 1.844**	50.190 ± 2.745**	49.066 ± 4.399**	48.421 ± 2.825**
Week 53	3.011 ± 0.560	27.149 ± 1.863**	42.847 ± 3.943**	36.569 ± 4.591**	43.746 ± 6.558**	43.713 ± 2.235**

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

Determinations of TCDD Concentrations in Tissues

Concentrations of TCDD were determined in liver, lung, fat, and blood at the 14-, 31-, and 53-week interim evaluations and at the end of the 2-year study (105 weeks) (Table 13). The highest concentrations of TCDD were observed in the liver, followed by fat tissue. In vehicle control liver, TCDD concentrations were below the limits of quantitation (113 pg/g) at 14, 31, and 105 weeks. At the 53-week interim evaluation, liver concentrations of TCDD in vehicle control liver were 249 pg/g. Hepatic concentrations increased with increasing doses of TCDD. The highest hepatic concentrations were observed in the 100 ng/kg group with 18,280, 21,180, 15,763, and 9,325 pg/g at 14, 31, 53, and 105 weeks, respectively. In the liver tissue from the stop-exposure group, TCDD concentrations were 737 pg/g, which was slightly higher than levels observed in the 3 ng/kg group (681 pg/g).

In fat tissue of controls, TCDD concentrations were below the experimental limit of quantitation (200 pg/g) at all of the interim evaluations. In treated groups, fat concentrations increased with increasing doses of TCDD. The highest concentrations were observed in the 100 ng/kg group with 6,395, 7,848, 5,816, and 3,177 pg/g at 14, 31, 53, and 105 weeks, respectively. In the fat tissue of the stop-exposure group, TCDD concentrations were below the limits of quantitation.

No measurable concentrations of TCDD were observed in blood from vehicle control or treated rats at any of the interim evaluations. No measurable concentrations of TCDD were observed in the lung at 14, 53, or 105 weeks. At 31 weeks, TCDD concentrations of 10,500 and 963 pg/g were observed in the lungs of single rats in the 10 and 46 ng/kg groups, respectively. Given the high levels, this may have been due to accidental inhalation of dosing material during gavage. No measurable concentrations of TCDD were observed in the lungs of rats from the other TCDD-treated groups or the vehicle control group.

TABLE 13
Tissue Concentrations of TCDD in Female Rats in the 2-Year Gavage Study of TCDD[a]

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
n							
Week 14	10	10	10	10	10	10	
Week 31	10	10	10	10	10	10	
Week 53	8	8	8	8	8	8	
Week 105	10	8	10	10	10	10	10
Fat							
Week 14	BLOQ	295.33 ± 27.31[b]	708.60 ± 45.87	1,274.80 ± 120.20	2,620.00 ± 131.39	6,395.00 ± 390.03	
Week 31	BLOQ	378.10 ± 48.00	767.50 ± 23.74	1,572.00 ± 72.00	3,089.00 ± 140.98	7,848.00 ± 554.10	
Week 53	BLOQ	304.29 ± 26.95[c]	633.50 ± 41.03	1,272.63 ± 66.08	2,502.50 ± 126.25	5,816.25 ± 417.12	
Week 105	BLOQ	505.38 ± 91.94	753.00 ± 67.60[b]	1,403.80 ± 117.01	1,996.00 ± 122.68	3,177.00 ± 505.59	BLOQ
Liver							
Week 14	BLOQ	676.20 ± 23.37	2,166.67 ± 85.52[b]	4,931.00 ± 212.01	9,970.00 ± 675.44	18,280.00 ± 805.37	
Week 31	BLOQ	598.90 ± 28.62	2,079.00 ± 130.82	4,489.00 ± 193.12	9,522.00 ± 334.03	21,180.00 ± 817.01	
Week 53	249.00[d]	499.38 ± 27.15	1,940.00 ± 124.57	4,437.50 ± 132.07	7,818.75 ± 367.21	15,762.50 ± 437.09	
Week 105	BLOQ	680.71 ± 57.95[c]	2,213.00 ± 163.28	4,364.00 ± 267.99	6,413.00 ± 241.93	9,325.00 ± 1,163.11	737.00 ± 270.04[e]
Lung							
Week 14	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	
Week 31	BLOQ	BLOQ	10,500[d]	BLOQ	963[d]	BLOQ	
Week 53	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	
Week 105	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Blood							
Week 14	NA	NA	NA	NA	BLOQ	BLOQ	
Week 31	NA	NA	NA	NA	NA	BLOQ	
Week 53	NA	NA	NA	NA	NA	NA	
Week 105	NA	NA	NA	NA	NA	NA	NA

[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. NA=not analyzed. BLOQ=below the limit of quantitation; LOQ_{fat}=200 pg/g, LOQ_{liver}=113 pg/g, LOQ_{lung}=500 pg/g, LOQ_{blood}=103 pg/mL.
[b] n=9
[c] n=7
[d] n=1
[e] n=5

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

Liver: Liver weights were significantly increased in all dosed groups at 14 and 31 weeks (Table C1). At 53 weeks, relative liver weights of rats administered 10 ng/kg or greater were significantly increased as was the absolute liver weight of 100 ng/kg rats. At the 14-week interim evaluation, changes in the liver consisted of increased incidences and/or severities of hepatocellular hypertrophy in groups administered 10 ng/kg or greater (Tables 14 and A5a); the increased incidences of hypertrophy tended to correlate with increased liver weight. Multinucleated hepatocytes and diffuse fatty change were seen in 100 ng/kg rats.

At 31 weeks, hepatocellular hypertrophy occurred in all dosed groups and tended to correlate with increased liver weight (Tables 14 and A5a). Dose-related increased incidences of multinucleated hepatocytes were seen in the 46 and 100 ng/kg groups. Increased incidences of pigmentation occurred in rats administered 10 ng/kg or greater, and the severity was increased in the 100 ng/kg group. Mixed cell focus, toxic hepatopathy, and diffuse fatty change were significantly increased in the 100 ng/kg group. The severities of inflammation were increased in all dosed groups.

At the 53-week interim evaluation, hepatocellular hypertrophy occurred in all dosed groups and tended to correlate with increased liver weight (Tables 14 and A5a). The incidences and severities of multinucleated hepatocytes and bile duct hyperplasia were increased in the 100 ng/kg group. The incidences of pigmentation were increased in rats administered 10 ng/kg or greater, and the severity of this lesion increased with increasing dose. Increased incidences of multiple mixed cell focus occurred in the 46 and 100 ng/kg groups. Dose-related increased incidences of toxic hepatopathy and diffuse fatty change occurred in the 46 and 100 ng/kg groups. The severities of inflammation were increased in rats administered 22 ng/kg or greater. Cholangiofibrosis and bile duct fibrosis were seen in the 46 and 100 ng/kg groups.

At 2 years, the incidence of hepatocellular adenoma was significantly increased in the 100 ng/kg core study group and exceeded the historical vehicle control range (Tables 15, A3a, and A4a). Dose-related increased incidences of cholangiocarcinoma were seen in the core study rats administered 22 ng/kg or greater. The highest incidence of cholangiocarcinoma was seen in the 100 ng/kg core study group and included a significant number of animals with multiple cholangiocarcinomas. No cholangiocarcinomas have occurred in the historical vehicle controls (Tables 15 and A4a). Two cholangiocarcinomas and two hepatocellular adenomas were seen in the 100 ng/kg stop-exposure group. Two hepatocholangiomas were seen in the 100 ng/kg core study group, and one cholangioma occurred in a 100 ng/kg stop-exposure rat (Tables 15, A1b, and A1c); no hepatocholangiomas or cholangiomas have occurred in the historical vehicle controls (Tables 15 and A4a). The incidences of cholangiofibrosis were significantly increased in the 46 and 100 ng/kg core study groups, and the incidence in the 100 ng/kg stop-exposure group was significantly lower than the 100 ng/kg core study group (Tables 15, A5b, and A5c).

Hepatocellular adenoma was a nodular mass that usually was larger than a focus, had a distinct border, and produced more compression of surrounding normal parenchyma (Plate 1). 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.

TABLE 14
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 TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
14-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy[a]	1 (1.0)[b]	1 (1.0)	4 (1.3)	7** (1.0)	10** (1.7)	10** (2.3)
Hepatocyte, Multinucleated	0	0	0	0	0	3 (1.0)
Fatty Change, Diffuse	0	0	0	0	0	2 (1.0)
31-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	2 (1.0)	3 (1.0)	6** (1.2)	9** (1.3)	10** (2.7)
Hepatocyte, Multinucleated	0	0	0	0	5* (1.0)	9** (2.0)
Pigmentation	0	1 (1.0)	8** (1.0)	9** (1.0)	10** (1.0)	10** (2.1)
Mixed Cell Focus (includes multiple)	3	7	4	2	4	10**
Toxic Hepatopathy	0	0	0	0	0	10** (2.0)
Fatty Change, Diffuse	0	0	0	0	0	6** (1.0)
Inflammation	9 (1.0)	9 (1.3)	10 (1.3)	10 (1.5)	10 (1.5)	10 (1.8)
Oval Cell, Hyperplasia						1 (1.0)
Bile Duct, Hyperplasia						1 (2.0)
53-Week Interim Evaluation						
Number Examined Microscopically	8	8	8	8	8	8
Hepatocyte, Hypertrophy	0	4 (1.0)	7** (1.1)	8** (1.3)	8** (2.0)	8** (3.3)
Hepatocyte, Multinucleated	0	0	0	0	2 (1.0)	8** (1.6)
Pigmentation	0	0	5* (1.0)	8** (1.0)	8** (1.8)	8** (2.6)
Mixed Cell Focus (includes multiple)	2	5	0	6	7*	8**
Toxic Hepatopathy	0	0	1 (1.0)	0	6** (1.5)	8** (3.4)
Fatty Change, Diffuse	0	0	0	0	2 (1.0)	6** (1.7)
Bile Duct, Fibrosis	0	0	0	0	1 (1.0)	2 (2.0)
Bile Duct, Hyperplasia	0	0	1 (1.0)	2 (1.0)	3 (1.3)	8** (1.9)
Inflammation	8 (1.0)	8 (1.0)	8 (1.0)	8 (1.5)	8 (1.5)	8 (2.0)
Cholangiofibrosis	0	0	0	0	1 (3.0)	1 (2.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 15
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
Number Examined							
Microscopically	53	54	53	53	53	53	50
Hepatocyte, Hypertrophy[a]	0	19** (1.2)[b]	19** (1.2)	42** (1.5)	41** (2.0)	52** (3.2)	22**▲▲ (1.7)
Hepatocyte, Multinucleated	0	0	16** (1.2)	26** (1.2)	36** (1.4)	51** (1.8)	32**▲▲ (1.3)
Eosinophilic Focus (includes multiple)	11	14	21*	27**	27**	44**	27**▲
Inflammation	33 (1.1)	46* (1.2)	47** (1.1)	50** (1.4)	52** (1.2)	49** (1.3)	43** (1.2)
Pigmentation	4 (1.0)	9 (1.2)	34** (1.4)	48** (1.7)	52** (2.0)	53** (2.2)	45** (1.8)
Fatty Change, Diffuse	0	2 (1.0)	12** (1.1)	17** (1.3)	30** (1.2)	48** (1.9)	10**▲▲ (1.4)
Necrosis	1 (2.0)	4 (2.3)	4 (2.0)	8* (2.3)	10** (1.9)	17** (2.5)	8* (1.8)
Oval Cell, Hyperplasia	0	4* (1.0)	3 (1.3)	20** (1.3)	38* (1.5)	53** (2.3)	1▲▲ (1.0)
Bile Duct, Hyperplasia	5 (1.6)	4 (1.5)	7 (1.1)	22** (1.3)	40** (1.4)	53** (2.1)	7▲▲ (1.3)
Bile Duct, Cyst	3 (3.3)	1 (3.0)	2 (2.0)	2 (1.5)	0	21** (2.9)	6▲▲ (2.3)
Hyperplasia, Nodular	0	0	0	3	7**	36**	0▲▲
Portal, Fibrosis	0	0	0	0	5* (1.8)	27** (2.0)	1▲▲ (2.0)
Toxic Hepatopathy	0	2 (1.0)	8** (1.3)	30** (1.2)	45** (1.8)	53** (3.5)	16**▲▲ (1.3)
Cholangiofibrosis	1 (1.0)	1 (1.0)	2 (1.5)	1 (1.0)	11** (2.5)	31** (2.4)	1▲▲ (2.0)
Hepatocellular Adenoma (includes multiple)[c]							
Overall rate[d]	0/53 (0%)	0/54 (0%)	0/53 (0%)	0/53 (0%)	1/53 (2%)	13/53 (25%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	0.0%	0.0%	2.6%	29.9%	5.2%
Terminal rate[f]	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	7/21 (33%)	0/21 (0%)
First incidence (days)	—[i]	—	—	—	720	642	702
Poly-3 test[g]	P<0.001	—[j]	—	—	P=0.497	P<0.001	P=0.233
Poly-3 test[h]							P=0.004N
Hepatocholangioma[k]	0	0	0	0	0	2	0
Cholangioma[k]	0	0	0	0	0	0	1
Cholangiocarcinoma, Multiple	0	0	0	0	1	17**	1▲▲
Cholangiocarcinoma (includes multiple)[k]							
Overall rate	0/53 (0%)	0/54 (0%)	0/53 (0%)	1/53 (2%)	4/53 (8%)	25/53 (47%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	0.0%	2.9%	10.3%	54.9%	5.2%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	4/22 (18%)	13/21 (62%)	1/21 (5%)
First incidence (days)	—	—	—	529	731 (T)	610	729
Poly-3 test	P<0.001	—	—	P=0.474	P=0.057	P<0.001	P=0.232
Poly-3 test							P<0.001N

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
▲ Significantly different (P≤0.05) from the 100 ng/kg core study 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: 4/371, range 0%-4%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Pairwise comparison between the 100 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.
[k] Historical incidence: 0/371

Cholangiocarcinoma consisted of an irregular, relatively large, noncircumscribed lesion that replaced normal liver parenchyma (Plate 2). The lesion consisted of fibrous connective tissue stroma containing numerous atypical bile ducts, which frequently contained mucinous material and cellular debris. The epithelium forming the atypical bile ducts was often discontinuous, consisted of large atypical cells (Plate 3), and displayed degenerative changes. Mitotic figures and localized invasion of adjacent liver parenchyma were also observed.

Cholangiofibrosis appeared relatively small in size, was well demarcated, and did not show evidence of localized invasion (Plates 4 and 5).

Cholangioma was a well-demarcated mass consisting of multiple densely packed, irregular bile duct structures, some of which were moderately dilated, within a small amount of fibrous stroma. The bile ducts were composed of a single layer of densely packed, columnar, somewhat pleomorphic bile duct epithelial cells (Plates 6 and 7). Hepatocholangioma was composed of a mixture of proliferating hepatocellular and bile duct elements. Hepatocholangioma was a rather large, nodular mass with a distinct border that produced compression of surrounding normal parenchyma. The hepatocellular element appeared similar to that seen in hepatocellular adenoma and consisted of a rather uniform population of mildly to moderately pleomorphic hepatocytes that were generally normal sized or slightly larger than normal and were arranged in abnormal hepatic cords. Intermixed with the proliferating hepatocytes were numerous small to large biliary structures, surrounded by small amounts of dense fibrous tissue stroma, that appeared similar to the biliary structures seen within a cholangioma. The smaller biliary structures resembled proliferating small bile ducts while the large structures were generally irregular and sometimes moderately to markedly dilated. Some of the large structures became confluent, producing highly irregular cystic biliary structures that were incompletely separated by short septae projecting into the lumen. Some of the ductular lumens contained homogeneous, lightly eosinophilic material, but most were empty. The biliary structures were composed of a single layer of flattened to cuboidal to low columnar, somewhat pleomorphic, but otherwise relatively normal-appearing, bile duct epithelial cells.

At 2 years, the incidences of hepatocyte hypertrophy, multinucleated hepatocytes, eosinophilic focus, inflammation, pigmentation, diffuse fatty change, and toxic hepatopathy were significantly greater in all dosed groups administered 10 ng/kg or greater than in the vehicle controls, and the severity of these lesions generally increased with increasing dose in the core study groups (Tables 15, A5b, and A5c). Dose-related increased incidences of necrosis, oval cell hyperplasia, and bile duct hyperplasia occurred in core study rats administered 22 ng/kg or greater. The incidences of nodular hyperplasia and portal fibrosis in the 46 and 100 ng/kg core study groups and of bile duct cyst in the 100 ng/kg core study group were significantly increased. The incidences of nonneoplastic liver lesions were generally significantly decreased in the 100 ng/kg stop-exposure group compared to the 100 ng/kg core study group.

Hepatocyte hypertrophy was characterized by hepatocytes that were enlarged with increased amounts of eosinophilic cytoplasm. Minimal hypertrophy affected periportal hepatocytes, and as severity increased, hepatocytes in other areas of the hepatic lobule were also affected. The hypertrophy usually was not confined to periportal hepatocytes, and therefore, the general diagnosis of hepatocyte hypertrophy was used. Multinucleated hepatocytes were characterized by scattered hepatocytes that were enlarged and contained multiple (more than two and often four to six) nuclei. The presence of binucleated hepatocytes was not sufficient to make this diagnosis (Plate 8).

Eosinophilic, mixed, basophilic, and clear cell foci appeared similar and were characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic focus was composed of cells with eosinophilic cytoplasm. Mixed cell focus was composed of a mixture of cells with different staining properties, generally a mixture of eosinophilic cells and cells with clear cytoplasm (clear cells). To be classified as an eosinophilic focus, at least 80% of the cells within the focus had to be eosinophilic cells. Otherwise, the focus was classified as a mixed cell focus. Basophilic focus consisted of hepatocytes with basophilic cytoplasm, occasionally with basophilic linear (tigroid) intracytoplasmic aggregates. Clear cell focus was composed of cells having clear cytoplasm. If two or more foci of a given type were present in a liver, it was diagnosed as multiple.

The treatment-related foci were of the eosinophilic and mixed cell type and often differed somewhat from those in control animals. Foci in controls consisted of hepatocytes that were generally somewhat larger than normal but appeared otherwise normal and were arranged in a relatively normal lobular pattern. The hepatic cords at the periphery of these foci generally merged imperceptibly with the surrounding normal liver, resulting in an indistinct border and little or no compression of the adjacent liver parenchyma. In contrast, foci in treated animals generally had a more definite border, the cords within the focus often were not smoothly continuous with those in the surrounding parenchyma, and the foci consisted of cells that were often prominently enlarged with abundant eosinophilic or clear vacuolated cytoplasm. If more than 20% of the cells were vacuolated, the focus was classified as the mixed cell type; otherwise it was classified as an eosinophilic focus. In addition, some larger foci caused variable degrees of compression of the surrounding hepatic parenchyma. The cells were arranged in a relatively normal lobular pattern, and foci sometimes contained large blood vessels and/or portal areas. The presence of proliferating bile ducts or oval cells was not considered characteristic of a focus. If proliferating bile ducts were present, this was considered indicative of nodular hyperplasia, described below.

Inflammation was generally minimal to mild, 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. 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. Diffuse fatty change was generally a minor change consisting of discrete clear vacuoles (consistent with lipid) in the cytoplasm of hepatocytes scattered diffusely throughout the liver. Necrosis consisted of scattered necrotic areas of hepatic parenchyma that were often randomly distributed but occasionally, in more severe cases, were distributed more diffusely. Oval cell hyperplasia consisted of small ovoid cells with basophilic cytoplasm and a round to ovoid nucleus that were arranged in single or double rows and located predominantly in the portal areas. Bile duct hyperplasia consisted of increased numbers of portal bile ducts. Bile duct cyst was characterized by either single or multiple dilated bile ducts that were lined by attenuated epithelium (Plate 9). Portal fibrosis consisted of fibrous connective tissue accumulation that extended between adjacent portal areas.

Nodular hyperplasia was characterized by few to numerous, small to large nodular foci generally composed of hepatocytes that were considerably larger than normal hepatocytes (hepatocyte hypertrophy) sometimes mixed with areas of increased numbers of small hepatocytes (hepatocyte hyperplasia) (Plate 10). Areas of nodular hyperplasia sometimes blended with the surrounding parenchyma, although they often had a distinct border. Large focal to multifocal areas of nodular hyperplasia were sometimes seen that caused compression of surrounding tissue and/or bulging of the capsular surface. The cells within nodular hyperplasia generally were very large, larger than cells seen within adenomas and usually larger than cells seen within foci, with abundant eosinophilic cytoplasm and often with 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. 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 completely obscure the normal architecture. The presence of hypertrophic vacuolated hepatocytes together with proliferating biliary epithelium was considered to be useful in differentiating nodular hyperplasia (Plate 11) from hepatocellular adenoma (Plate 1).

Toxic hepatopathy included all nonneoplastic liver changes under one overall term. The severity of the toxic hepatopathy was graded in order to give an 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 focal cellular alteration, multinucleated hepatocytes, cystic degeneration, fatty change, inflammation, necrosis, pigmentation, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, hepatocyte degeneration, hepatocyte hypertrophy, oval cell hyperplasia, and portal fibrosis. 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: The absolute lung weights of 10 ng/kg rats at 14 and 31 weeks and of 46 ng/kg rats at 14 weeks were significantly increased; relative lung weights of all dosed groups at 31 weeks, of 100 ng/kg rats at 53 weeks, and of 10 ng/kg rats at 14 weeks were significantly increased (Table C1).

At 2 years, the incidence of cystic keratinizing epithelioma was significantly increased in the 100 ng/kg core study group, and multiple epitheliomas occurred in two animals in the 100 ng/kg core study group (Tables 16, A1b, and A3a). There were no epitheliomas in the 100 ng/kg stop-exposure group, and cystic keratinizing epitheliomas have not occurred in the historical vehicle controls (Tables 16 and A4b). The epitheliomas ranged from relatively small to very large lesions that replaced much of the normal lung parenchyma. They 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 (Plates 12 and 13).

The incidences of bronchiolar metaplasia of the alveolar epithelium were significantly greater in all core study dosed groups and in the 100 ng/kg stop-exposure group than those in the vehicle control group; the incidence in the stop-exposure group was significantly less than that in the 100 ng/kg core study group (Tables 16, A5b, and A5c). Squamous metaplasia was present in the 46 and 100 ng/kg core study groups and in the 100 ng/kg stop-exposure group. Bronchiolar metaplasia of the alveolar epithelium consisted of replacement of the normal alveolar epithelium by cuboidal to columnar, sometimes ciliated cells and was often accompanied by abundant mucus production in the affected area (Plate 14). The lesion generally diffusely affected the epithelium located at the bronchiolar-alveolar junction and adjacent alveoli. The incidences of histiocytic infiltration were significantly increased in the 22, 46, and 100 ng/kg groups. Aggregates of large alveolar macrophages were sometimes present in areas of bronchiolar metaplasia. Bronchiolar metaplasia was differentiated from alveolar epithelial hyperplasia, which was seen only in the vehicle controls. In alveolar hyperplasia, the alveoli were lined by cuboidal epithelium and, unlike bronchiolar metaplasia in dosed animals, prominent mucus production was not observed, and very prominent inflammatory cell infiltrate, consisting of large aggregates of alveolar macrophages commonly mixed with focal aggregates of neutrophils, was usually associated with the affected areas.

Squamous metaplasia of the alveolar epithelium was generally a minor change consisting of one or more small, irregular foci of keratinizing stratified squamous epithelium that had replaced the normal alveolar epithelium (Plate 15).

Oral Mucosa: There was a positive trend in the incidence of gingival squamous cell carcinoma of the oral cavity, and the incidence in the 100 ng/kg group was significantly increased. In addition, the incidences in the 46 ng/kg and 100 ng/kg core study and stop-exposure groups exceeded the historical control range (Tables 17, A3a, A3b, and A4c). The incidences of gingival squamous hyperplasia were significantly increased in all dosed groups including the stop-exposure group (Tables 17, A5b, and A5c). Gingival squamous hyperplasia and squamous cell carcinoma, although reduced in incidences when compared to the 100 ng/kg core study group, were still present in the 100 ng/kg stop-exposure group. Squamous cell carcinoma occurred within the oral mucosa of the palate and was located adjacent to the incisor tooth in nasal section III. It was characterized by irregular cords and clusters of stratified squamous epithelial cells that invaded deep into the underlying connective tissue and often invaded the bone of the maxilla (Plate 16). Squamous hyperplasia was a focal lesion that occurred in the stratified squamous epithelium of the gingival oral mucosa adjacent to the molars in nasal section III. It consisted of varying degrees of thickening of the epithelium, often with the formation of epithelial rete pegs that extended a short distance into the underlying connective tissue. Ends of hair shafts and/or some degree of inflammation were often present in the areas of squamous hyperplasia, suggesting, at least in these cases, that the hyperplasia was secondary to the presence of the hair shafts and associated inflammation. It was unclear whether there was an association between squamous hyperplasia and squamous cell carcinoma.

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
Number Examined							
Microscopically	53	54	53	52	53	52	50
Alveolar Epithelium, Metaplasia, Bronchiolar[a]	2 (1.0)[b]	19** (1.6)	33** (1.5)	35** (1.4)	45** (1.7)	46** (2.0)	31**▲ (1.3)
Infiltration Cellular, Histiocyte	41 (1.9)	46 (1.8)	43 (1.8)	43* (1.6)	49* (1.8)	50* (2.0)	41 (2.0)
Metaplasia, Squamous	1 (1.0)	0	0	0	5 (2.2)	6 (2.2)	3 (1.3)
Alveolar Epithelium, Hyperplasia	12 (1.1)	0**	0**	0**	0**	0**	0**
Cystic Keratinizing Epithelioma, Multiple	0	0	0	0	0	2	0
Cystic Keratinizing Epithelioma (includes multiple)[c]							
Overall rate[d]	0/53 (0%)	0/54 (0%)	0/53 (0%)	0/52 (0%)	0/53 (0%)	9/52 (17%)	0/50 (0%)
Adjusted rate[e]	0.0%	0.0%	0.0%	0.0%	0.0%	21.1%	0.0%
Terminal rate[f]	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	4/21 (19%)	0/21 (0%)
First incidence (days)	—[i]	—	—	—	—	610	—
Poly-3 test[g]	P<0.001	—[j]	—	—	—	P=0.002	—
Poly-3 test[h]							P=0.003N

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
▲ Significantly different (P≤0.05) from the 100 ng/kg core study group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups: 0/370
[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; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Pairwise comparison between the 100 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
Number Necropsied	53	54	53	53	53	53	50
Gingival, Hyperplasia, Squamous[a]	1 (4.0)[b]	7* (1.4)	14** (1.6)	13** (1.6)	15** (2.2)	16** (2.3)	8* (1.9)
Squamous Cell Carcinoma[c]							
Overall rate[d]	1/53 (2%)	2/54 (4%)	1/53 (2%)	0/53 (0%)	4/53 (8%)	10/53 (19%)	5/50 (10%)
Adjusted rate[e]	2.5%	5.7%	2.6%	0.0%	10.2%	22.0%	12.4%
Terminal rate[f]	0/25 (0%)	1/21 (5%)	0/23 (0%)	0/19 (0%)	1/22 (5%)	2/21 (10%)	0/21 (0%)
First incidence (days)	366	647	578	—[i]	659	546	520
Poly-3 test[g]	P<0.001	P=0.449	P=0.755	P=0.533N	P=0.169	P=0.007	P=0.099
Poly-3 test[h]							P=0.193N

* 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: 4/371, range 0%-2%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.
[h] Pairwise comparison between the 100 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[i] Not applicable; no neoplasms in animal group

Uterus: The incidence of squamous cell carcinoma in the 46 ng/kg group at 2 years was significantly greater than that in the vehicle controls, and there were two squamous cell carcinomas in the 100 ng/kg stop-exposure group (Tables 18, A3a, and A1c). No squamous cell carcinomas have occurred in the historical vehicle controls (Tables 18 and A4d). Squamous cell carcinoma occurred on the endometrial surface, often caused dilatation of the uterus, and was characterized by irregular cords and clusters of atypical stratified squamous epithelial cells that invaded the underlying myometrium (Plates 17 and 18). One squamous cell papilloma was seen in each of the vehicle control and 10 ng/kg groups. At 2 years, the incidence of squamous metaplasia was significantly less in the 100 ng/kg core study group compared to vehicle controls and the 100 ng/kg stop-exposure group (Tables 18, A5b, and A5c). The significance of this finding is 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.

Pancreas: At 31 and 53 weeks, the incidences of acinar cytoplasmic vacuolization were increased in the 100 ng/kg group. Chronic active inflammation and

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
Number Examined							
Microscopically	52	53	53	53	53	53	50
Metaplasia, Squamous[a]	29 (2.0)[b]	31 (2.0)	28 (2.5)	23 (2.5)	32 (2.5)	17** (1.7)	33▲▲(2.1)
Squamous Cell Papilloma	1	0	1	0	0	0	0
Squamous Cell Carcinoma[c]							
Overall rate[d]	0/53 (0%)	0/54 (0%)	0/53 (0%)	0/53 (0%)	5/53 (9%)	0/53 (0%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	0.0%	0.0%	12.3%	0.0%	5.2%
Terminal rate[f]	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	0/21 (0%)	2/21 (10%)
First incidence (days)	—[i]	—	—	—	589	—	731 (T)
Poly-3 test[g]	P=0.337	—[j]	—	—	P=0.032	—	P=0.232
Poly-3 test[h]							P=0.225

** Significantly different (P≤0.01) from the vehicle control group by the Poly-3 test
▲▲ Significantly different (P≤0.01) from the 100 ng/kg core study group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups: 1/371, range 0%-2%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Pairwise comparison between the 100 ng/kg core and stop-exposure groups
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

acinar atrophy were present in 100 ng/kg rats at 14 and 53 weeks (Tables 19 and A5a).

At 2 years, one acinar adenoma and two acinar cell carcinomas were seen in the 100 ng/kg core study group (Tables 19 and A3a). The incidences of acinar cell carcinoma and adenoma or carcinoma (combined) exceeded the historical control range (Tables 19 and A4e). The incidences of acinar cytoplasmic vacuolization were significantly increased in the 46 and 100 ng/kg core study groups, and the incidences of chronic active inflammation, acinar atrophy, and arterial chronic active inflammation were significantly increased in the 100 ng/kg core study group. One acinar carcinoma was seen in a 100 ng/kg stop-exposure rat; the incidences of nonneoplastic lesions were not significantly different from the vehicle controls. The incidences of the nonneoplastic lesions in the stop-exposure group were less than those in the 100 ng/kg core study group, and the incidence of acinar cytoplasmic vacuolization in the stop-exposure group was significantly decreased compared to the 100 ng/kg core study group.

Adenoma of the acinar cells was characterized by a discrete mass 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.

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
14-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation, Chronic Active[a]	0	0	0	0	0	2 (1.5)[b]
Acinus, Atrophy	0	0	0	0	0	2 (1.5)
31-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Acinus, Vacuolization Cytoplasmic	0	0	0	0	0	5* (1.0)
53-Week Interim Evaluation						
Number Examined Microscopically	8	8	8	8	8	8
Acinus, Vacuolization Cytoplasmic	0	0	0	0	0	7** (1.0)
Inflammation, Chronic Active	0	0	0	0	0	2 (2.0)
Acinus Atrophy	0	0	0	0	0	2 (2.0)

TABLE 19
Incidences of Neoplastic and Nonneoplastic Lesions of the Pancreas in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
2-Year Study							
Number Examined							
Microscopically	51	54	52	53	52	51	49
Acinus, Vacuolization							
Cytoplasmic	1 (2.0)	0	0	1 (1.0)	15** (1.1)	42** (1.8)	0▲▲
Inflammation							
Chronic Active	0	0	2 (1.5)	2 (1.0)	3 (1.3)	6* (2.0)	4 (1.5)
Acinus, Atrophy	1 (1.0)	2 (1.5)	4 (1.5)	4 (1.5)	4 (1.5)	9* (2.2)	4 (1.8)
Artery, Inflammation,							
Chronic Active	0	1 (3.0)	1 (2.0)	2 (2.5)	2 (3.0)	7* (2.3)	2 (2.5)
Acinus, Adenoma[c]	0	0	0	0	0	1	0
Acinus, Carcinoma[d]	0	0	0	0	0	2	1
Adenoma or Carcinoma[c]							
Overall rate[e]	0/51 (0%)	0/54 (0%)	0/52 (0%)	0/53 (0%)	0/52 (0%)	3/51 (6%)	1/49 (2%)
Adjusted rate[f]	0.0%	0.0%	0.0%	0.0%	0.0%	7.3%	2.7%
Terminal rate[g]	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	2/21 (10%)	1/21 (5%)
First incidence (days)	—[j]	—[k]	—	—	—	641	731 (T)
Poly-3 test[h]	P<0.001	—	—	—	—	P=0.129	P=0.494
Poly-3 test[i]							P=0.345N

* 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
▲▲ Significantly different (P≤0.01) from the 100 ng/kg core study group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with Sprague-Dawley vehicle control groups: 1/366, range 0%-2%
[d] Historical incidence: 0/366
[e] Number of animals with neoplasm per number of animals with pancreas examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Pairwise comparison between the 100 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by **N**.
[j] Not applicable; no neoplasms in animal group
[k] Value of statistic cannot be computed.

Atrophy was a focal to multifocal to diffuse change consisting of a reduction in the amount of acinar tissue with an associated increase in stromal fibrous connective tissue. Chronic active inflammation was generally associated with atrophy and consisted of an infiltrate of mononuclear cells with occasional neutrophils within the stroma. Cytoplasmic 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. Arterial chronic active inflammation was a focal to multifocal change characterized by a thick mantle of macrophages, lymphocytes, and plasma cells around the arteries, with infiltration into the muscular layers of the artery. There was often fibrinoid necrosis of the vessel, and the tunica intima was frequently thickened. Endothelial cells were swollen or decreased in number (Plate 19). This inflammatory reaction often extended into the surrounding parenchyma.

Thymus: The thymus weights of 46 and 100 ng/kg rats were significantly decreased at 14 weeks (Table C1). At 14, 31, and 53 weeks, the incidences of atrophy were significantly increased in the 46 and 100 ng/kg groups, and the severity was increased in the 100 ng/kg group (Tables 20 and A5a). At 2 years, atrophy occurred in all groups including the vehicle controls (Tables 20, A5b, and A5c) and was significantly increased in the 22 ng/kg and greater groups. Severity was greatest in the 100 ng/kg core study group. The incidence and severity of atrophy in the 100 ng/kg stop-exposure group were greater than those in the vehicle controls, which suggested a treatment-related effect. Atrophy consisted to varying degrees of loss of lymphoid cells from the cortex resulting in reduction of cortical thickness.

Adrenal Gland: Treatment-related changes included increased incidences of cortical atrophy and hyperplasia in core study groups administered 10 ng/kg or greater. The cortical atrophy was significant in the 100 ng/kg group, while hyperplasia was significant in the 22, 46, and 100 ng/kg groups. The incidences of cytoplasmic vacuolization were increased in the 22 ng/kg or greater groups (Tables 21, A5b, and A5c). The incidences of these lesions in the 100 ng/kg stop-exposure group were less than those in the 100 ng/kg core study group, and the difference in the incidence of cortical atrophy was significant. Cortical cystic degeneration was seen in all groups, including controls, the incidence being higher in treated groups, and was significantly increased in the 10 and 22 ng/kg groups.

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

Heart: At 2 years, significantly increased incidences of minimal to mild cardiomyopathy were seen in rats administered 10 ng/kg or greater (Tables 21, A5b, and A5c). The incidence of cardiomyopathy was significantly lower in the 100 ng/kg stop-exposure group compared to the 100 ng/kg core study group but was significantly greater than that in the vehicle controls. 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 hypereosinophilic myofibers that lacked cross striations, infiltrates of mononuclear cells, separation of myofibers by myxomatous material (bluish material on H&E stain), and eventually replacement of myofibers by fibrous connective tissue. The severity was graded based upon the number and extent of foci of myocardial degeneration. Minimal cardiomyopathy consisted of a few scattered foci while mild cardiomyopathy consisted of a greater number of lesions more diffusely scattered within the myocardium.

Clitoral Gland and Ovary: The incidences of cystic dilatation of the clitoral gland ducts were significantly increased in 22 and 100 ng/kg core study rats at 2 years (Tables 21, A5b, and A5c); the incidence of this lesion

TABLE 20
Incidences of Nonneoplastic Lesions of the Thymus in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
14-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Atrophy[a]	91 (1.0)[b]	0	0	4* (1.3)	10** (1.6)	
31-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Atrophy	2 (2.0)	1 (1.0)	5 (1.6)	6 (1.8)	7* (2.0)	10** (3.1)
53-Week Interim Evaluation						
Number Examined Microscopically	8	8	7	8	8	8
Atrophy	4 (2.0)	2 (2.0)	3 (2.3)	7 (2.3)	8* (3.4)	8* (3.4)

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
2-Year Study							
Number Examined Microscopically	51	52	52	49	46	42	49
Atrophy	36 (2.6)	41 (2.7)	44 (3.0)	41* (3.1)	44** (3.6)	42** (3.9)	45** (3.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

was significantly lower in the 100 ng/kg stop-exposure group compared to the 100 ng/kg core study group. This lesion consisted of dilated ducts that were filled with keratin and lined by attenuated epithelium. The severity varied from minimal to marked and was graded depending upon the size of the dilated ducts. Minimal lesions consisted of ducts dilated to approximately 2 to 3 mm, and marked lesions consisted of ducts dilated to approximately 1 cm or more in diameter. The incidence of ovarian atrophy was significantly lower in the 100 ng/kg core study group than in vehicle controls, and the incidence in the 100 ng/kg stop-exposure group was significantly higher compared to the 100 ng/kg core study group. Atrophy was characterized by absence of ovarian structures, primarily corpora lutea, but also by lack of follicles in some cases.

Kidney: At 2 years, the incidences of nephropathy were significantly increased in the 100 ng/kg core study and stop-exposure groups (Tables 21, A5b, and A5c); the incidence of nephropathy was significantly lower in the stop-exposure group compared to the 100 ng/kg core study group. Increased incidences of transitional epithelial hyperplasia occurred in the 46 and 100 ng/kg core study groups.

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 previously. Minimal nephropathy was characterized by small numbers of scattered affected tubules, usually involving less than 10% of the renal tubules. However, marked nephropathy involved approximately 50% to 60% or more of the tubules.

TABLE 21
Incidences of Selected Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control		3 ng/kg		10 ng/kg		22 ng/kg		46 ng/kg		100 ng/kg		100 ng/kg (Stop-Exposure)	
Adrenal Cortex[a]	53		54		53		53		53		53		50	
Atrophy[b]	2	(2.5)[c]	0		4	(1.5)	5	(2.6)	5	(1.4)	27**	(2.4)	4▲▲	(1.8)
Degeneration, Cystic	11	(2.5)	15	(2.1)	21*	(2.1)	18*	(2.3)	17	(2.2)	17	(2.3)	17	(2.2)
Hyperplasia	16	(2.0)	16	(2.2)	18	(2.4)	25*	(2.3)	29**	(2.3)	30*	(2.5)	20	(2.6)
Vacuolization Cytoplasmic	11	(1.4)	7	(1.3)	12	(1.3)	21**	(1.6)	18	(1.7)	15	(2.1)	13	(1.3)
Heart	53		54		53		52		53		52		50	
Cardiomyopathy	10	(1.2)	12	(1.0)	22**	(1.0)	25**	(1.1)	32**	(1.1)	36**	(1.3)	22**▲	(1.4)
Mesentery														
Chronic Active Artery Inflammation	0		1	(3.0)	0		0		4	(2.3)	7	(2.7)	1	(3.0)
Clitoral Gland	50		52		53		52		51		53		49	
Duct, Cyst	34	(2.2)	37	(2.1)	41	(2.2)	42*	(2.0)	41	(2.2)	48**	(2.3)	35▲▲	(2.0)
Ovary	51		53		53		53		53		53		49	
Atrophy	49	(4.0)	46	(4.0)	50	(4.0)	44	(4.0)	50	(4.0)	31**	(3.9)	45▲▲	(4.0)
Kidney	53		54		53		53		53		53		50	
Nephropathy	34	(1.2)	26	(1.1)	32	(1.3)	36	(1.4)	39	(1.4)	52**	(2.2)	41*▲	(1.4)
Transitional Epithelium, Hyperplasia	3	(2.3)	6	(1.7)	8	(1.8)	8	(1.3)	11*	(2.0)	11*	(2.0)	5	(1.4)
Stomach, Forestomach	53		54		53		53		53		53		50	
Hyperplasia, Squamous	3	(1.3)	4	(1.5)	4	(1.8)	2	(2.0)	7	(2.1)	11*	(1.7)	5	(1.8)
Bone Marrow	53		54		53		53		53		53		50	
Hyperplasia	36	(3.0)	41	(3.2)	32	(3.2)	35	(3.1)	37	(2.9)	43*	(2.8)	36	(2.9)
Lymph Node, Mandibular	51		54		52		50		51		52		49	
Ectasia	0		0		0		0		1	(2.0)	6*	(2.7)	2	(2.0)

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
▲ Significantly different (P≤0.05) from the 100 ng/kg core study group by the Poly-3 test
▲▲ P≤0.01
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

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

Forestomach: The incidence of squamous hyperplasia was significantly increased in the 100 ng/kg core study group at 2 years (Tables 21, A5b, and A5c). Squamous hyperplasia of the forestomach epithelium was generally a minimal to mild, focal or occasionally multifocal change characterized by varying degrees of thickening of the stratified squamous epithelium up to approximately five times normal thickness in more severe cases. Sometimes the hyperplasia occurred around a focal ulcer, although most cases occurred without the presence of an apparent ulcer.

Bone Marrow: At 2 years, dose-related increased incidences of hyperplasia occurred in the 3 and 100 ng/kg groups, and the incidence was significantly increased in the 100 ng/kg group (Tables 21 and A5b). 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 used when the distal end of the femur section contained 20% to 60% marrow.

Mesentery: Compared to the vehicle controls, the incidences of chronic active inflammation were increased in the 100 ng/kg core study group and the 100 ng/kg stop-exposure group.

Mandibular Lymph Node: The incidence of ectasia was significantly increased in the 100 ng/kg core study group at 2 years (Tables 21, A5b, and A5c). Ectasia consisted of mild to moderate, focal to multifocal dilatation of medullary sinuses (lymphangiectasis). The significance of this lesion was unclear.

Thyroid Gland: Dose-dependent increases in the incidences of follicular cell hypertrophy of the thyroid gland were observed at 14, 31, and 53 weeks and at 2 years (Tables 22, A5a, A5b, and A5c). At 2 years, dose-related increased incidences of minimal follicular cell hypertrophy were noted in core study rats administered 22 ng/kg or greater, and the incidences were significantly increased in the 46 and 100 ng/kg groups. The incidence of this lesion was significantly lower in the 100 ng/kg stop-exposure group compared to the 100 ng/kg core study group. 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 sometimes containing small, clear, resorption vacuoles. Since some degree of this change can occur spontaneously, the severity grade of minimal was recorded when 50% to 60% of the follicles were involved, mild severity when 60% to 75% of the follicles were involved, moderate when 75% to 90% of the follicles were involved, and marked when over 90% of the follicles were involved. The incidence of thyroid gland C-cell adenoma was significantly less in the 100 ng/kg core study group than in the vehicle controls (Tables 22 and A1b).

Mammary and Pituitary Glands: At 2 years, the incidences of mammary gland fibroadenoma [vehicle control, 33/53; 3 ng/kg, 40/54; 10 ng/kg, 34/53; 22 ng/kg, 29/53; 46 ng/kg, 36/53; 100 ng/kg (core study), 24/53; 100 ng/kg (stop-exposure), 32/50] and of adenoma or carcinoma (combined) (6/53, 8/54, 5/53, 2/53, 2/53, 1/53, 1/50) were significantly decreased in the 100 ng/kg core study group (Tables A3a and A3b). The incidence of pituitary gland (pars distalis or unspecified site) adenoma was significantly decreased in the 100 ng/kg core study group at 2 years (25/53, 20/54, 26/52, 15/53, 20/53, 11/52, 19/50). The incidences of mammary gland fibroadenoma and pituitary gland adenoma in the 100 ng/kg stop-exposure group were significantly greater than those in the 100 ng/kg core study group but not significantly different than the vehicle controls.

GENETIC TOXICOLOGY

TCDD (0.1 to 1,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without hamster or rat liver metabolic activation enzymes (S9 fraction) (Table B1; Mortelmans *et al*., 1984). No induction of trifluorothymidine resistance was noted in L5178Y $tk^{+/-}$ mouse lymphoma cells treated with 0.0625 to 1 µg/mL TCDD

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
14-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Follicular Cell, Hypertrophy[a]	0	1 (1.0)[b]	3 (3.0)	4* (2.5)	4* (2.8)	9**(2.1)
31-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Follicular Cell, Hypertrophy	0	3 (1.7)	3 (1.7)	3 (1.3)	4* (1.3)	6**(1.8)
53-Week Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Follicular Cell, Hypertrophy	0	1 (1.0)	2 (1.0)	2 (1.5)	3 (1.0)	5* (1.6)

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
2-Year Study							
Number Examined Microscopically	52	54	53	51	53	52	49
Follicular Cell, Hypertrophy	3 (2.3)	4 (1.5)	4 (2.0)	7 (1.1)	10* (1.2)	17** (1.2)	6▲ (1.5)
C-cell Adenoma							
Overall rate[c]	20/52 (38%)	15/54 (28%)	17/53 (32%)	16/51 (31%)	13/53 (25%)	11/52 (21%)	13/49 (27%)
Adjusted rate[d]	46.7%	40.4%	43.1%	43.2%	31.6%	25.6%	33.7%
Terminal rate[e]	10/25 (40%)	10/21 (48%)	13/23 (57%)	8/19 (42%)	8/22 (36%)	6/21 (29%)	7/21 (33%)
First incidence (days)	474	462	626	338	486	638	584
Poly-3 test[f]	P=0.010N	P=0.366N	P=0.459N	P=0.464N	P=0.111N	P=0.030N	P=0.166N
Poly-3 test[g]							P=0.288

TABLE 22
Incidences of Neoplastic and Nonneoplastic Lesions of the Thyroid Gland in Female Rats in the 2-Year Gavage Study of TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop-Exposure)
C-cell Adenoma or Carcinoma							
Overall rate	21/52 (40%)	15/54 (28%)	17/53 (32%)	16/51 (31%)	14/53 (26%)	11/52 (21%)	14/49 (29%)
Adjusted rate	49.0%	40.4%	43.1%	43.2%	34.0%	25.6%	36.3%
Terminal rate	11/25 (44%)	10/21 (48%)	13/23 (57%)	8/19 (42%)	9/22 (41%)	6/21 (29%)	8/21 (38%)
First incidence (days)	474	462	626	338	486	638	584
Poly-3 test	P=0.008N	P=0.288N	P=0.374N	P=0.380N	P=0.115N	P=0.017N	P=0.173N
Poly-3 test							P=0.208

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)
** $P \leq 0.01$
▲ Significantly different ($P \leq 0.05$) from the 100 ng/kg core study group by the Poly-3 test
a Number of animals with lesion
b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
c 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; the stop-exposure group is excluded from the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by **N**.
g Pairwise comparison between the 100 ng/kg core and stop-exposure groups

with or without S9 (Table B2; McGregor *et al*., 1991). In tests for chromosomal damage in cultured Chinese hamster ovary cells, TCDD did not induce sister chromatid exchanges (SCEs) or chromosomal aberrations (Abs) when tested up to toxic levels (0.8 µg/mL) with or without S9 activation enzymes (Tables B3 and B4; Galloway *et al*., 1987). Without S9, TCDD induced significant increases in SCEs at concentrations of 0.6 to 0.8 µg/mL in two of five independent trials when a prolonged culture time was employed to compensate for TCDD-induced cell cycle delay. However, because the response was not consistently reproducible, the SCE test was concluded to be negative overall. In addition, there was a complication from the ethanol solvent in these SCE experiments. A comparison of the true negative control culture and an ethanol solvent control culture without TCDD indicated that ethanol alone, in the amount needed to dissolve TCDD, increased SCE frequencies. No induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* following treatment with 50, 250, or 500 ppm TCDD (Table B5; Zimmering *et al*., 1985). Finally, no induction of Abs was observed in bone marrow cells of male B6C3F_1 mice 17 or 36 hours after intraperitoneal injection of 250, 500, or 1,000 µg/kg TCDD (Table B6). In summary, no mutagenic activity was detected with TCDD in a variety of *in vitro* and *in vivo* short-term tests.

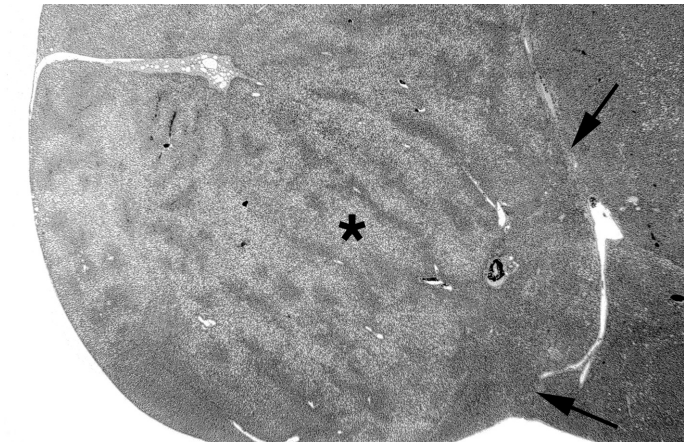


PLATE 1
Hepatocellular adenoma (asterisk) in the liver of a female rat administered 100 ng/kg TCDD by gavage for 2 years. The borders are distinct, and there is compression of the surrounding hepatic parenchyma (arrows). H&E; 2.5×

PLATE 2
Cholangiocarcinoma in the liver of a female rat administered 100 ng/kg TCDD by gavage for 2 years. In contrast to cholangiofibrosis, the cholangiocarcinoma is larger and widely involves the hepatic parenchyma. H&E; 2.5×

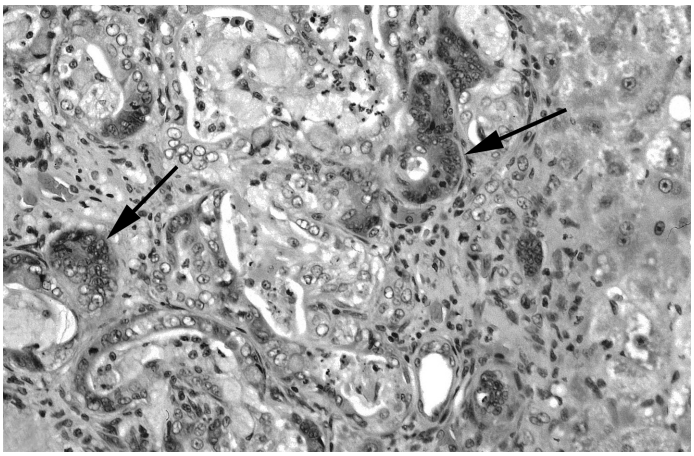


PLATE 3
Cholangiocarcinoma in the liver of a female rat administered 100 ng/kg TCDD by gavage for 2 years. Note the atypical, multilayered aspect of the neoplastic epithelium (arrows). H&E; 66×

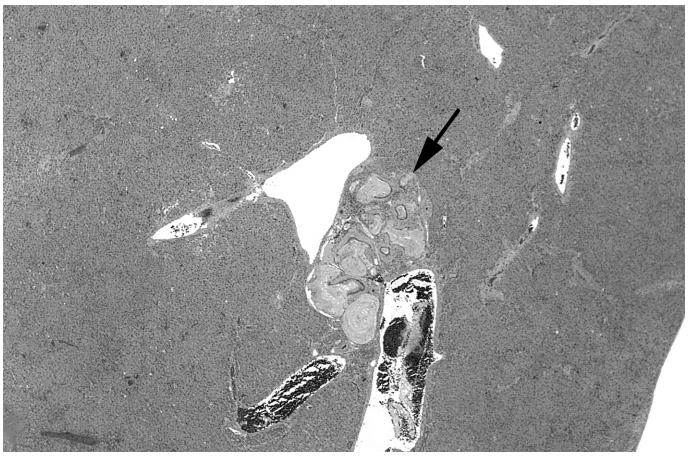


PLATE 4
Cholangiofibrosis (grade 3) in the liver of a female rat administered 100 ng/kg TCDD by gavage for 2 years. The lesion is small and well circumscribed (arrow). H&E; 5×

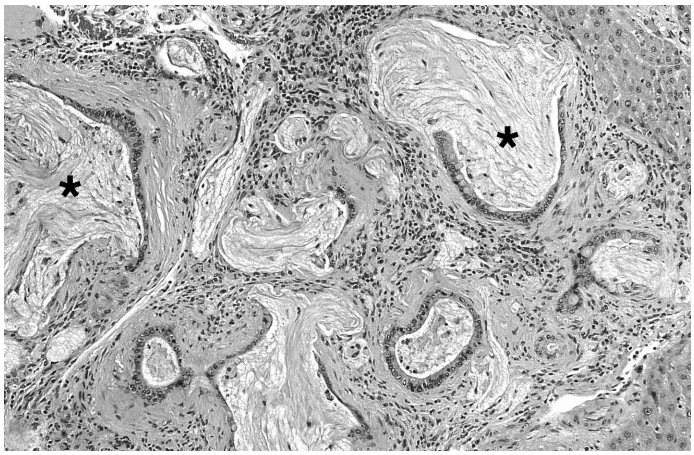


PLATE 5
Higher magnification of Plate 4. The proliferating biliary epithelium forming mucus-producing, crescent-shaped, glandular structures (asterisks) are surrounded by concentric layers of connective tissue. The glands are lined by a single layer of cells. H&E; 33×

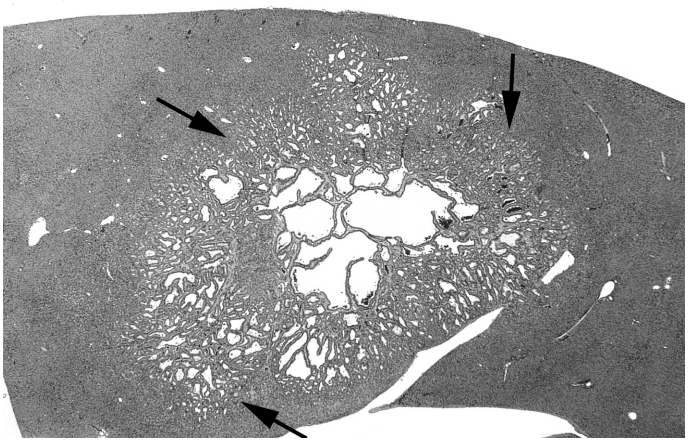


PLATE 6
Cholangioma in the liver of a female rat in the 100 ng/kg stop-exposure group. The lesion is well circumscribed, and the margins are indicated by arrows. H&E; 3.3×

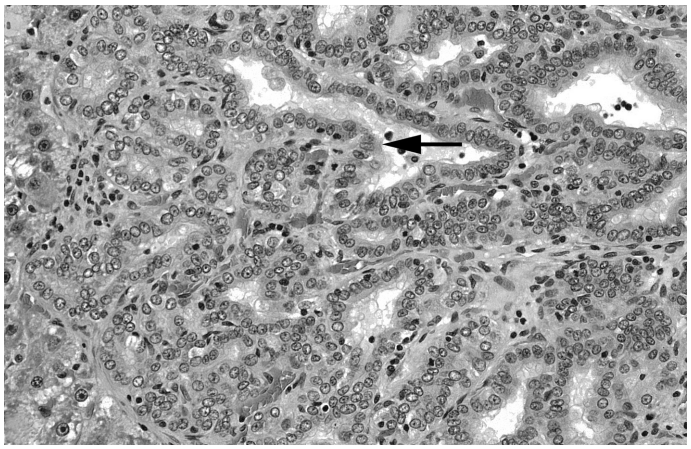


PLATE 7
Cholangioma in the liver of a female rat in the 100 ng/kg stop-exposure group. The mass consists of multiple often dilated bile ducts with little associated fibrous connective tissue stroma. The bile ducts are composed of a single layer of epithelium (arrow) that sometimes forms acinar or papillary structures. H&E; 66×

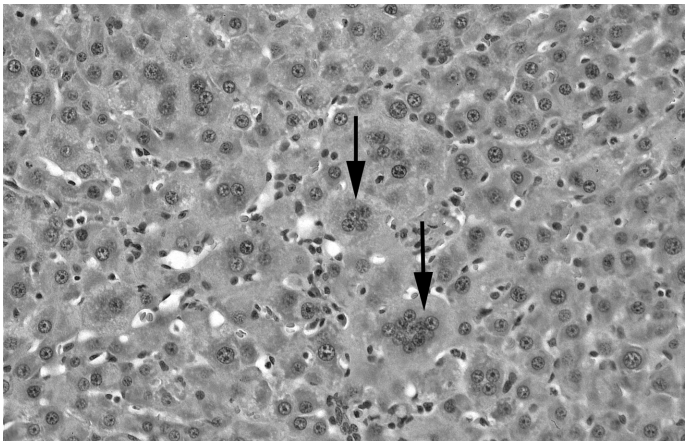


PLATE 8
Multinucleated hepatocytes (arrows) in the liver of a female rat administered 46 ng/kg TCDD by gavage for 2 years. H&E; 66×

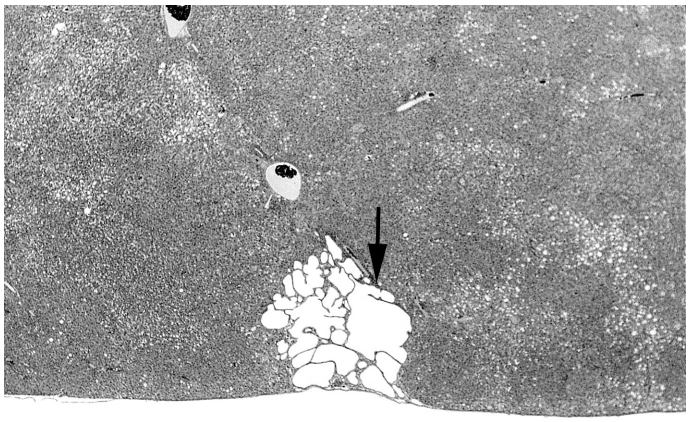


PLATE 9
Bile duct cyst in the liver of a female rat administered 100 ng/kg TCDD by gavage for 2 years. Multiple dilated bile ducts are lined by attenuated epithelium (arrow). H&E; 5×

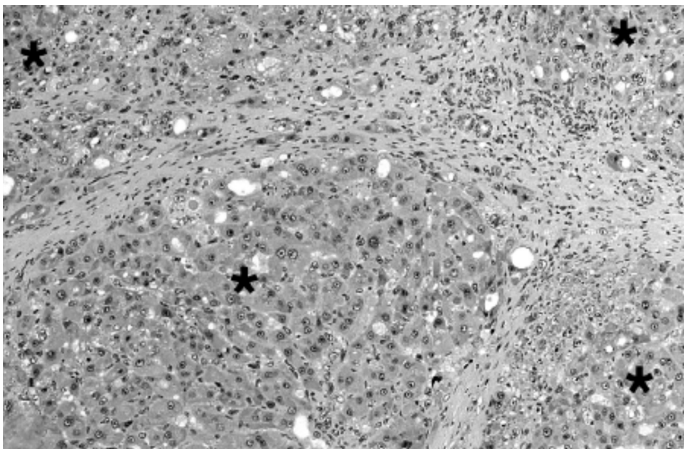


PLATE 10
Nodular hyperplasia in the liver of a female rat administered 100 ng/kg TCDD by gavage for 2 years. There are multiple nodular foci (asterisks) composed of hepatocytes that vary in size. H&E; 13.2×

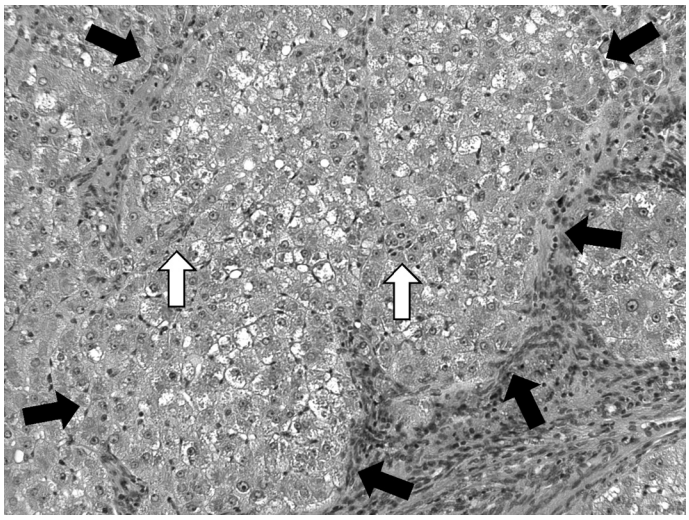


PLATE 11
Nodular hyperplasia in a female rat administered 100 ng/kg TCDD by gavage for 2 years. The nodule is circumscribed (dark arrows), slightly compressing the surrounding normal hepatic tissue, and there are occasional bile ducts (open arrows) within the nodule. H&E; 100×

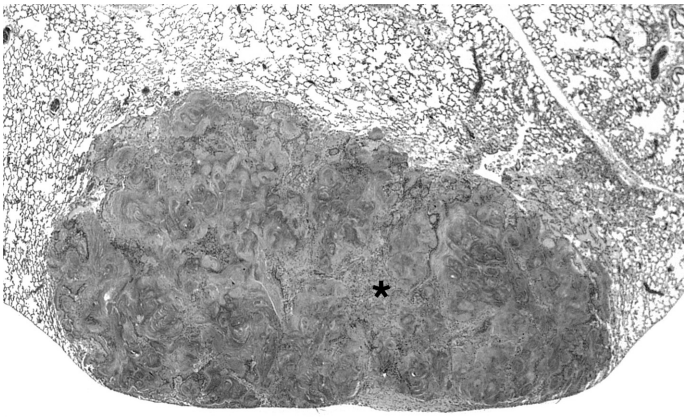


PLATE 12
Cystic keratinizing epithelioma in the lung of a female rat administered 100 ng/kg TCDD by gavage for 2 years. The single cystic structure consists of a highly irregular wall of keratinized stratified squamous epithelium and a center (asterisk) filled with keratin. H&E; 5×

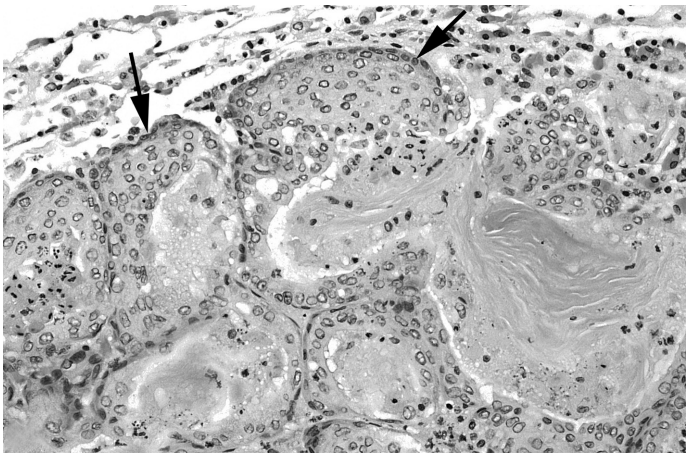


PLATE 13
Cystic keratinizing epithelioma in the lung of a female rat administered 100 ng/kg TCDD by gavage for 2 years. The outer portion of the lesion is composed of keratinized stratified squamous epithelium that grows by expansion into the adjacent lung without evidence of invasion (arrows). H&E; 66×

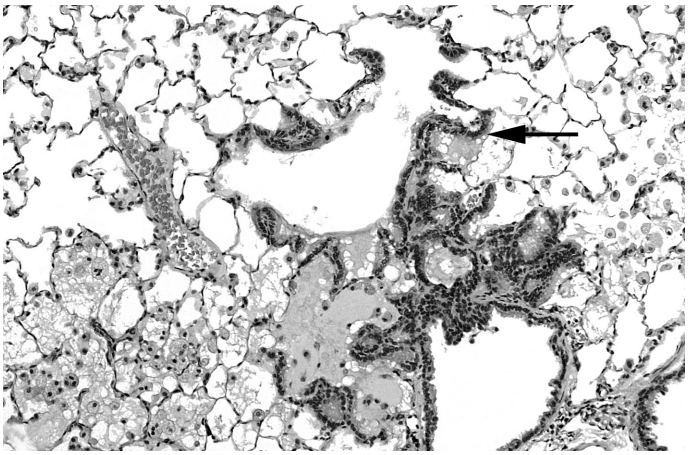


PLATE 14
Alveolar epithelial-bronchiolar metaplasia (grade 3) in the lung of a female rat administered 100 ng/kg TCDD by gavage for 2 years. The normal alveolar epithelium is replaced by cuboidal to columnar (arrow), ciliated epithelium intersparsed with dome-shaped multiciliated cells. H&E; 33×

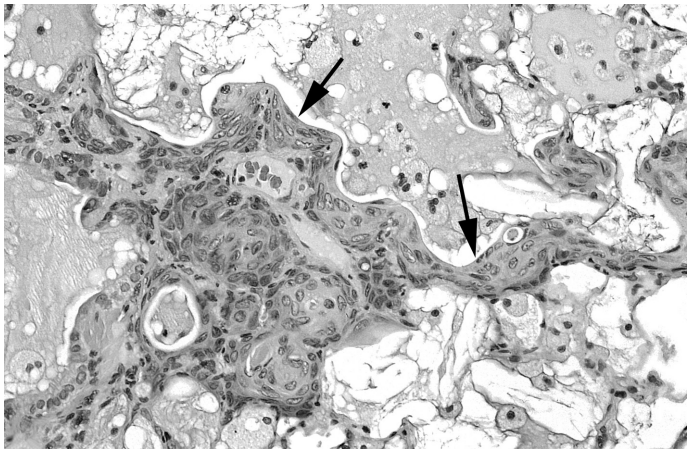


PLATE 15
Squamous metaplasia of the alveolar epithelium (grade 2) in the lung of a female rat administered 100 ng/kg TCDD by gavage for 2 years. Alveoli are lined with mature squamous cells (arrows). H&E; 66×

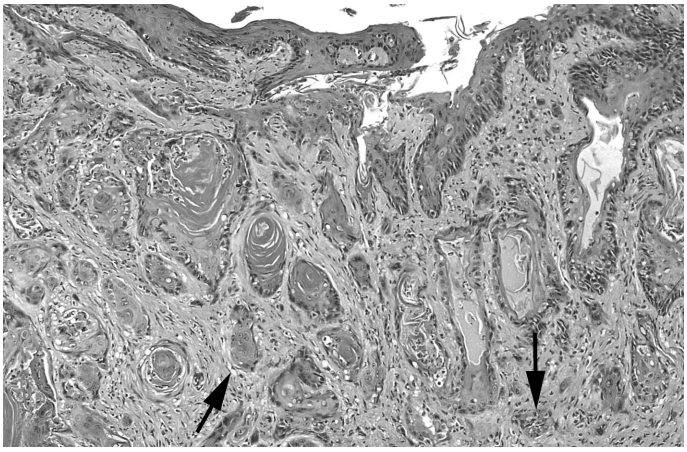


PLATE 16
Squamous cell carcinoma in the gingival epithelium of a female rat administered 100 ng/kg TCDD by gavage for 2 years. Nests of neoplastic squamous cells (arrows) are invading the adjacent stroma. H&E; 33×

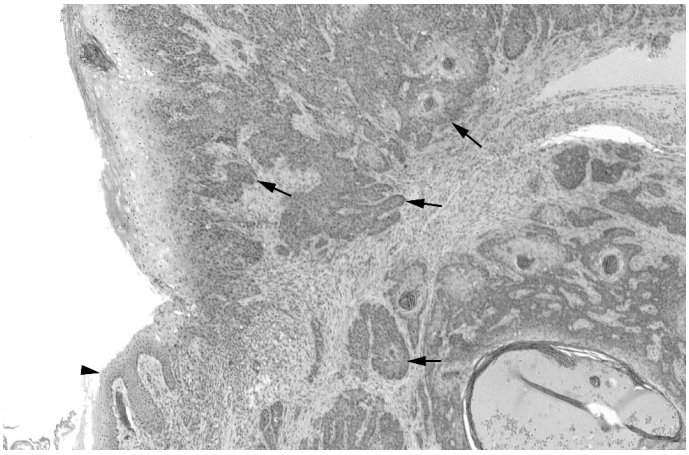


PLATE 17
Squamous cell carcinoma in the uterus of a female rat administered 46 ng/kg TCDD by gavage for 2 years. Note the invasion of cords and clusters of squamous cells (arrows) into the underlying myometrium. Arrowhead indicates the surface lining epithelium. H&E; 10×

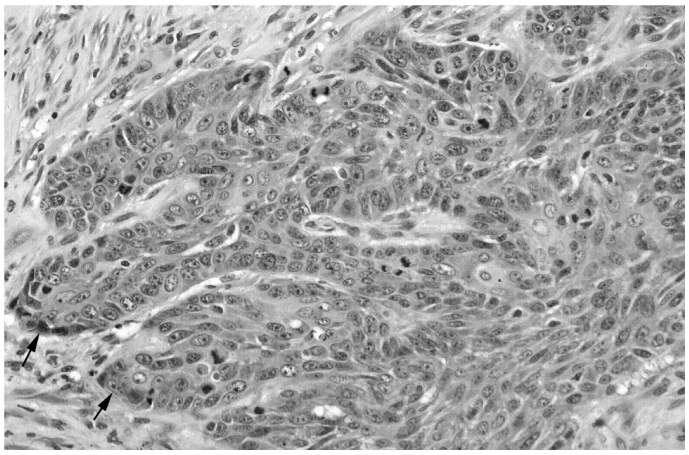


PLATE 18
Higher magnification of Plate 17. Note the invasion of cords and clusters of squamous cells (arrows) into the underlying myometrium. H&E; 66×

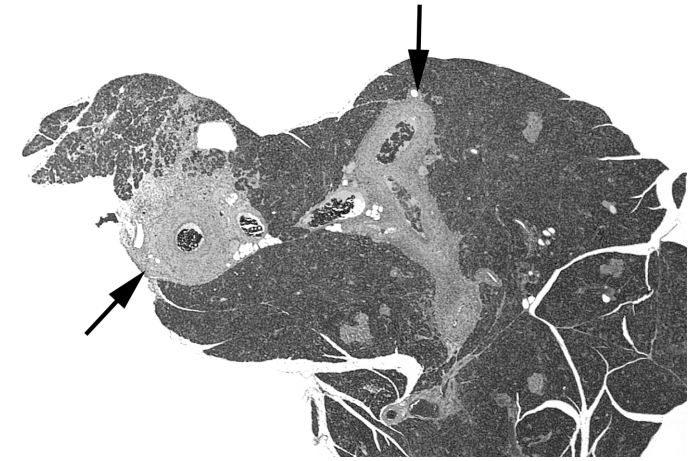


PLATE 19
Arterial inflammation (arrows) in the pancreas of a female rat administered 100 ng/kg TCDD by gavage for 2 years. H&E; 5×

DISCUSSION AND CONCLUSIONS

This 2-year study of the chronic toxicity and carcinogenicity of TCDD in female Harlan Sprague-Dawley rats is one in a series of studies carried out as part of a multistudy NTP initiative examining the relative chronic toxicity and carcinogenicity of dioxin-like compounds (DLCs) and structurally related polychlorinated biphenyls (PCBs) (see Overview section). While one of the primary aims of this dioxin toxic equivalency factor (TEF) evaluation was to analyze the comparative carcinogenicity of TCDD, PeCDF, and PCB126, in this Technical Report, only the results of the TCDD toxicology and carcinogenicity study are described. 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 by Toyoshiba *et al.* (2004) and Walker *et al.* (2005).

Dose selection for this study of TCDD was based on prior observations made in a 2-year carcinogenicity study of TCDD conducted by Dow Chemical Company (Kociba *et al*., 1978). This prior study in Spartan Sprague-Dawley rats used dosed feed corresponding to dosage levels of 1, 10, or 100 ng TCDD/kg body weight per day. Increases in liver tumors were observed at doses of 10 and 100 ng TCDD/kg body weight per day (Goodman and Sauer, 1992). For the current TCDD study, animals were treated by gavage with TCDD in corn oil:acetone (99:1) 5 days per week for up to 2 years at doses up to 100 ng/kg (3, 10, 22, 46, or 100 ng/kg). Two additional doses between 10 and 100 ng/kg were used to provide more information on the predicted dose response for induction of neoplasms.

In this study, there was no significant effect on survival as a result of TCDD exposure. Daily administration of TCDD at doses above 46 ng/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 duration of dosing. At 31 and 53 weeks, body weights of the 100 ng/kg group were 90% and 85% those of vehicle control group weights, respectively. Reduction in body weight gain is an often seen toxic response to DLCs. The reduction in body weight gain was treatment related and required daily administration. In the stop-exposure group, daily administration of compound ceased after 30 weeks of treatment and was followed by daily (5 days per week) gavage administration of corn oil:acetone (99:1) alone for the remainder of the study. Thereafter, the rate of body weight gain in these animals returned to normal and was similar to vehicle control animals; however, the terminal body weights were 92% of that observed in vehicle controls.

The principal findings of this study were increased incidences of benign and malignant neoplasms in several organs, with specific increased incidences of neoplasms in the lung (cystic keratinizing epithelioma), liver (cholangiocarcinoma and hepatocellular adenoma), and oral mucosa (gingival squamous cell carcinoma), following chronic administration of 100 ng/kg TCDD in corn oil:acetone (99:1) by gavage, five times per week for 2 years. In addition, there was an increase in the incidence of squamous cell carcinoma of the uterus in the 46 ng/kg group that was considered to be treatment related. A significant trend for increased incidence of pancreatic adenoma or carcinoma (combined) may have been related to treatment. Occurrences of hepatocholangioma and cholangioma of the liver may also have been related to TCDD treatment.

The principal nonneoplastic finding in this study was a significant increase in incidence and severity of hepatotoxicity. In addition, numerous organs exhibited increased incidences of nonneoplastic lesions, notably the lung, oral mucosa, pancreas, thymus, adrenal cortex, heart, clitoral gland, kidney, forestomach, mesentery, and thyroid gland.

Administration for 2 years led to significant accumulation of TCDD in liver and adipose tissue. The significant accumulation in adipose tissue is consistent with the lipophilic nature of this compound. Previous studies of TCDD indicate that the liver and adipose are the main depots for TCDD in rodents and, combined, contribute approximately 70% to 80% of the total body burden

within the animal (DeVito *et al*., 1995). As expected, the mean levels in the liver were higher than those in adipose on a wet weight basis. This is likely caused by the sequestration of TCDD in the liver as a result of binding to CYP1A2, which is also inducible by TCDD in the liver (Voorman and Aust, 1989; Diliberto *et al*., 1997). Because of high detection limits, levels of TCDD in lung and blood were undetectable in almost all animals examined. Prior studies with lower detection limits indicate that TCDD also accumulates in these tissues in Sprague-Dawley rats after chronic exposure to similar doses used in this study, albeit at much lower levels than those seen in liver and adipose (Kohn *et al*., 1993; Tritscher *et al*., 2000). The low detection limits in the present study as compared to detectable levels of TCDD in the liver and fat of controls in prior studies and also in the NTP study of the mixture of TCDD, PCB 126, and PeCDF (NTP, 2006b) were caused by the fact that a low-resolution gas chromatography/mass spectrometry system was used instead of a high resolution gas chromatography/mass spectrometry system.

Analysis of liver tissue levels of TCDD in this study indicated that 2-year administration at dose levels of 10 and 100 ng/kg resulted in mean liver levels of 2.2 and 9.3 ng/g tissue, respectively. The relationship between intake and liver levels was linear. By comparison, in the TCDD dosed feed study by Kociba *et al.* (1978), terminal liver TCDD levels were 5.1 and 24 ng/g at the 10 and 100 ng/kg doses, respectively, and over twofold higher than those seen in the present study.

The higher tissue levels of TCDD in the Kociba *et al.* (1978) study compared to the present study may be due to the different dosing regimens used. In the Kociba *et al.* (1978) study, animals were exposed to TCDD 7 days per week via dosed feed. In the present study, animals were treated 5 days per week by oral gavage. Modeling of the tissue concentrations produced by these two dosing scenarios using the pharmacokinetic model of TCDD indicated that the liver levels of TCDD in the dosed feed study would be higher than those by oral gavage (data not shown) over the course of the study, as was observed.

In the current study, cessation of daily treatment with TCDD in the stop-exposure group led to a decline in levels of TCDD in all tissues examined. At the end of the study, the mean level of TCDD in the liver of the stop-exposure animals was 0.7 ng/g (five of 10 animals had undetectable levels below 50 pg/g) compared to 9.3 ng/g in animals exposed for the full 2 years at the same administered dose. This level was similar to that observed at the end of the study in the 3 ng/kg group (mean liver level of 0.7 ng/g). Therefore, interpretation of the pathology results in the stop-exposure animals has to be made in light of the fact that exposure *per se* does not end on cessation of daily administration of compounds. TCDD levels do decline significantly over the remainder of the study, but because of the lipophilic nature of the compound, animals are still continually exposed throughout the course of the study. Using the pharmacokinetic model for TCDD, the predicted areas under the curve (AUC) for liver TCDD for the 22 and 46 ng/kg groups were 0.30×10^7 and 0.70×10^7 pg days/g, respectively. By comparison, the AUC at 2 years for the stop-exposure group was predicted to be 0.52×10^7 pg days/g. This 2-year AUC seen in the stop-exposure group is comparable to that predicted using a daily administered dose of 36 ng/kg per day. In addition, exposure to DLCs occurs as a result of exposure to low levels of these compounds that are present in rodent feed. Previous studies have shown that TCDD can be detected in control animals at low levels (Vanden Heuvel *et al*., 1994; Walker *et al.*, 1998). These concentrations are attributed to the ingestion of very low levels of polyhalogenated aromatic hydrocarbons present in rodent chow. TCDD in the liver of control animals in this study was below the limits of detection (50 pg/g) except in a single animal at the 53-week time point. All experimental treatments in the TEF studies were made in addition to a background of exposure to DLCs that are normally present in feed, and the control group exposure is not zero. Control levels of TCDD at 2 years in the TEF mixture study (NTP, 2006b) in liver and fat were 7.7 and 12.6 pg/g wet weight, respectively. While the level of TCDD in the feed used in the present study was not analyzed, subsequent analysis of polyhalogenated aromatic hydrocarbons in the NTP 2000 feed indicated that TCDD was below the limits of detection (mean level of quantitation of 0.0596 pg/g feed) (Table E5). However, predicted background intake is at least 100- to 1,000-fold lower than the lowest experimental doses used; therefore, the additional contribution of this background exposure rate to the observed neoplastic and nonneoplastic responses is likely to be negligible.

Increases in CYP1A1 and CYP1A2 are characteristic responses to DLCs and are directly linked to binding and activation of the aryl hydrocarbon receptor (AhR) (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, dose-dependent increases in CYP1A1- and CYP1A2-associated enzyme activity as a result of TCDD administration were observed at all time points. The finding that the liver was a target following TCDD administration was expected. It was also expected that administration of this compound would lead to increases in these specific responses. While not discussed in this report, data on increased expression of CYP1A1 and CYP1A2 from the present study together with data from the other studies of DLCs conducted as part of the TEF evaluation have been used for a quantitative 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 have demonstrated that the liver is a principal target organ site for the action of these compounds (Poland and Knutson, 1982; Kociba *et al.*, 1978; NTP, 1982a). In the present study, the principal hepatic neoplasms observed were cholangiocarcinoma and hepatocellular adenoma. Two cases of hepatocholangioma were seen only in the 100 ng/kg core study group, and a single case of cholangioma occurred in the stop-exposure group.

One of the principal findings of the study was the extensive hepatotoxicity induced by TCDD treatment. The incidence and pattern of hepatic toxicity exhibited a clear dose and duration dependence and preceded neoplastic effects on 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 exposure. Hepatic toxicity was characterized by foci of cellular alterations, multinucleated hepatocytes, diffuse fatty change, inflammation, necrosis, pigmentation, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, hepatocyte hypertrophy, oval cell hyperplasia, and portal fibrosis. A comprehensive term of toxic hepatopathy was also used, reflecting the overall severity grade of the nonneoplastic lesions. This term allowed 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 in the liver at the 14-week interim evaluation were restricted to hepatocyte hypertrophy, multinucleated hepatocytes, and diffuse fatty change. At 31 weeks, there was an increased incidence and severity of hepatocyte hypertrophy and increased incidence of fatty change; a broader spectrum of effects were observed including toxic hepatopathy, multinucleated hepatocytes, increased severity of inflammation, foci of cellular alterations, and pigmentation deposits. These changes continued to be observed at 53 weeks together with bile duct hyperplasia. At the end of the 2-year study, there were additional toxic changes including increased bile duct cysts, necrosis, cholangiofibrosis, inflammation, portal fibrosis, oval cell hyperplasia, and nodular hyperplasia. At the 10 ng/kg dose, where there were no observed liver neoplasms, significant increases in the incidence of nonneoplastic effects and toxic hepatopathy were seen, including foci of cellular alterations, diffuse fatty change, hepatocyte hypertrophy, multinucleated hepatocytes, inflammation, and pigmentation.

The increases in the incidence and severity of hepatotoxicity and increased 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). However, in this study, the most significant effect was on the incidence of cholangiocarcinoma. Previous studies of DLCs and PCBs have only rarely seen these cholangial lesions, despite the data showing that bile ducts are targets for DLCs. In an initiation-promotion study, cholangiocarcinoma was seen in 1/14 diethylnitrosamine-initiated female rats exposed to 100 ng TCDD/kg per day for 60 weeks (Walker *et al.*, 2000). In the 2-year bioassay of Aroclor 1254, there were no observed cholangiocarcinomas (Mayes *et al.*, 1998). In addition, there was no increased incidence of cholangioarcinoma in the TCDD feed study by Kociba *et al.* (1978). It is not clear why there was such a marked difference between the present study and these previous studies of TCDD and related DLCs with respect to the increased incidence of cholangiocarcinoma.

The incidence of hepatocellular adenoma in the 100 ng/kg core study group in the present study (13/53, 25%) was less than that seen in the Kociba *et al.* (1978) dosed feed study where the incidences of hepatocellular adenomas were 2/86, 9/50, and 14/45 (31%) 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/kg per day; the incidence of neoplastic nodules or hepatocellular carcinoma was 14/49 (29%). There was no significant effect in male rats. In the NCI (1980) study of a mixture of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin in Osborne-Mendel rats, the incidences of neoplastic nodules or hepatocellular carcinoma (combined) were 5/75 (7%), 10/50 (20%), 12/50 (24%), and 30/50 (60%) at doses of 0, 1,250, 2,500, or 5,000 ng/kg per week, respectively. Given the TEF of 0.1 for hexachlorodioxins (Van den Berg *et al.*, 1998), these weekly doses are over a similar range of total toxic equivalents (TEQs) as used in the present gavage study.

There are several explanations for the lower incidence of hepatocellular adenoma relative to the earlier TCDD studies. The terminal liver levels of TCDD in the present study are over twofold lower than those observed in the Kociba *et al.* (1978) dosed feed study, most likely because of the different dosing regimens used. Consequently, while the administered dose was the same between the two studies, the internal dose to the liver over the course of the study was likely lower than in the Kociba *et al.* (1978) study; therefore, the lower response may simply be a dose-response issue. In addition, the Kociba study used the Spartan substrain of Sprague-Dawley rats compared to the Harlan Sprague-Dawley rat strain used in the present study. Therefore, the lower incidence may in part be due to substrain differences in susceptibility.

Another aspect to consider with the liver neoplasms is that pathology nomenclature has changed. The Kociba *et al.* (1978) study indicated a 47% incidence of "hepatocellular hyperplastic nodules" in the 100 ng TCDD/kg group compared to a 9% incidence in control animals. Subsequent to that study, there was an evolution of nomenclature for hepatocellular proliferative lesions, and the slides from that study were reevaluated. In that reevaluation, neoplastic lesions were classified as adenoma or carcinoma. Using the newer nomenclature, the incidence of hepatocellular adenoma was 31% at the highest dose of 100 ng TCDD/kg (Goodman and Sauer, 1992). It is clear from the pathologic 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 (Goodman and Sauer, 1992), in the NCI (1980) TCDD study, and in the NTP (1982a) TCDD studies.

The spectrum of hepatocellular proliferative lesions observed in the present study is not common in NTP studies, and there is a lack of biological information relative to the progression and behavior of these lesions. These lesions generally occurred on a background of toxic hepatopathy, the components of which are listed above and described in the Results section. It is generally accepted that, in the rat, hepatocellular adenoma and hepatocellular carcinoma represent a morphological and biological continuum (Narama *et al.,* 2003; Hailey *et al.,* 2005). Foci of cellular alteration are often part of that continuum but not always. In high dose animals, proper categorization of the lesions was further complicated by the presence of the toxic hepatopathy. While the biological behavior of hepatocellular lesions within this study and other studies conducted as part of the dioxin TEF evaluation 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 dosed animals generally differed from the typical foci seen in vehicle controls. Foci seen in vehicle controls were usually smaller, lacked discrete borders and blended with the surrounding parenchyma, produced little or no compression, and consisted of cells that were normal-sized or slightly smaller or larger than normal. In contrast, foci in the livers of dosed animals generally had discrete borders, produced some compression of the adjacent parenchyma, and consisted of large, hypertrophic, often vacuolated cells. The significantly increased incidences of hypertrophy 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; 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. In the dioxin TEF evaluation studies, nodular hyperplasia varied in size but generally appeared morphologically similar whether in a high dose animal with severe toxic hepatopathy or in a lower dose animal where the toxic hepatopathy was minimal to nonexistent. Nodular hyperplasia was seen most commonly in the higher dose groups in which prominent toxic changes were present. However, a lesser degree of nodular hyperplasia was sometimes seen in lower dose animals in which the only evidence of liver pathology may have been hepatocellular hypertrophy.

Morphologically, a hyperplastic nodule associated with regeneration cannot be distinguished from a hyperplastic nodule of another pathogenesis. The morphological alterations suggest that regeneration is a significant contributor to the proliferative response in animals with toxic hepatopathy. However, this does not explain 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 interim 14-, 31-, and 53-week 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 the poorly demarcated foci of hypertrophic cells grew with continued dosing, giving rise to lesions diagnosed as foci, and that 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 presence of proliferating bile duct epithelium was an important feature, useful in differentiating adenoma from nodular hyperplasia.

The increased incidence of 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 these seven studies. These neoplasms are characterized by glandular structures lined by a single layer of well-differentiated epithelium (benign lesions) or single or multiple layers of epithelial cells that have malignant characteristics (e.g., high nuclear to cytoplasmic ratio, pleomorphism and anisokaryosis, and an increased mitotic rate).

In the present study, cholangiocarcinoma was diagnosed, and while it differed morphologically from spontaneous cholangiocarcinoma, it was similar to 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, 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 incidences of lesions similar to those observed in the present study (Maronpot *et al.*, 1991). 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 the present study, it appears that the cholangiocarcinomas were slowly 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 present study.

There was a single occurrence of cholangioma in the 100 ng/kg stop-exposure group, and two hepatocholangiomas occurred in the 100 ng/kg group. Both neoplasm types appear to be rare and did not occur in 371 vehicle control animals from this study or the other six dioxin TEF evaluation studies (NTP, 2006a,b,c,d,e,f). Hepatocholangiomas were mixed neoplasms with areas of hepatocytes that appeared identical to hepatocellular adenoma and areas of ductular structures lined by biliary epithelium that appeared identical to cholangioma. The pluripotent nature of these neoplasms was demonstrated by occasional ductular structures lined by cells resembling both hepatocytes and biliary epithelium. In contrast to the cholangiofibrosis and cholangiocarcinomas, a scirrhous response was not present within these neoplasms. While the histogenesis of hepatocholangioma is not clear, there was evidence of proliferation of hepatocytes, biliary epithelium, and oval cells within these studies. Therefore, the occurrence of these rare hepatocholangiomas in the 100 ng/kg group and the single incidence of cholangioma in the 100 ng/kg stop-exposure group may have been related to administration of TCDD.

The mechanism underlying the increase in incidence of cholangiocarcinoma is likely to be multifactorial. There was clearly 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 a direct effect on the biliary cells themselves. Tritscher *et al.* (1995) showed a high degree of staining for TGF alpha in bile duct cells after TCDD administration in female rats. The observed bile duct proliferation may represent a process of excessive and long-term repair following specific damage to hepatocytes, leading to the death of hepatocytes and perhaps also of the bile duct epithelium. The proliferative response may be a reparative response of proliferating hepatocytes, bile duct cells, and scarring tissue (cholangiofibrosis). The inflammation observed can produce oxidative stress that may also result in promotion of DNA damage. Consequently, the oxidative stress may be only a secondary phenomenon of the ongoing response to the toxic hepatopathy. In addition, there may be a direct stimulatory effect on the oval cells themselves. This is supported by the observed increases in oval cell hyperplasia. The oval cells may differentiate into hepatocytes or biliary cells. This may explain why both hepatocellular proliferative and biliary lesions are associated with exposure.

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

Numerous studies have shown that TCDD is a potent promoter of altered hepatic foci in initiation-promotion models of hepatocarcinogenesis (Pitot *et al*., 1980; Maronpot *et al*., 1993). 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 via the AhR may be caused by an increase in net growth rate of initiated cells because of selective growth advantage or decreased rate of cell death via suppression of apoptosis. In the current study with TCDD and in previous tumor promotion studies of 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 caused by alterations in intercellular communication, which have been observed in the livers of rats exposed to DLCs (Baker *et al*., 1995; Warngard *et al*., 1996; Bager *et al*., 1997). While TCDD is not a direct acting genotoxic agent, there are data indicating that persistent AhR active compounds may be indirectly genotoxic. This may contribute to an increase in the number of cells within the liver capable of undergoing promotion (Moolgavkar *et al*., 1996; Portier *et al*., 1996). It is hypothesized that the indirect genotoxicity may be via AhR-dependent induction of CYP1 family cytochromes P450 that leads to induction of oxidative stress caused by either inefficient electron transfer during P450 metabolism (Park *et al*., 1996) or the production of redox active estradiol metabolites as a result of CYP1 mediated estrogen metabolism (Lucier *et al*., 1991; Kohn *et al*., 1993). Studies have shown induction of oxidative stress and DNA damage by high dose acute exposure to TCDD (Stohs *et al*., 1990). The induction of lipid peroxidation and single stranded DNA breaks was also observed in tissues from the present TCDD study (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 of TCDD, an increase in the incidence of cystic keratinizing epithelioma (CKE) of the lung was observed at the highest dose of 100 ng/kg. Histopathologically, these lesions varied in size and number and appeared as cystic structures consisting of a highly irregular wall of highly keratinized stratified squamous epithelium, with a center filled with keratin. These lesions were absent in vehicle control and low dose animals.

In the 2-year feed study 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 evaluation of tissues 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 represent the same lesions. At the time of the Kociba *et al.* (1978) evaluation, CKE was not a consistently used diagnostic term. Diagnostic criteria for classification of CKE as a lesion distinct from squamous cell carcinoma were later developed at a workshop held in the mid 1990s (Boorman *et al.,* 1996).

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

In addition to an increase in the incidence of CKE, there was a significant increase in the incidences of bronchiolar metaplasia of the alveolar epithelium at all doses at 2 years including the stop-exposure group. These findings are consistent with prior observations of an increase in the incidences of alveolar-bronchiolar metaplasia following exposure to TCDD within the framework of a two stage initiation-promotion model in Sprague-Dawley rat lung (Tritscher *et al*., 2000). Of note was the significant reduction in the incidences of alveolar epithelial hyperplasia in all TCDD treated groups, with no incidences reported in the TCDD treated groups. Dose-related increased incidences and severities of squamous metaplasia and alveolar histiocytic infiltration were also noted in the 46 and 100 ng/kg groups.

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 damage to the type I cells, is a proliferation of the type II cells. This is often diagnosed as alveolar epithelial hyperplasia. In the present study, alveolar epithelial hyperplasia was observed only in vehicle control animals, and TCDD 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. Scattered throughout the ciliated cells were dome-shaped nonciliated cells, consistent with Clara cells. Clara cells are normally found in the lining of the bronchioles but not alveoli or alveolar ducts. Histochemical analyses of mucin and glutathione *S*-transferase pi in lung tissue from the dioxin TEF evaluation studies indicate that this appears to be similar to bronchiolar epithelium and is distinct from alveolar epithelial hyperplasia (Brix *et al*., 2004). It is not clear, however, if this lesion represents a destruction of type I alveolar epithelial cells with replacement by bronchiolar type epithelium (bronchiolar metaplasia) or an extension of bronchiolar epithelium from the terminal bronchiole (bronchiolar hyperplasia).

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

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

Gingival squamous cell carcinoma of the oral mucosa occurred in all dose groups except the 22 ng/kg group. The incidence of this lesion was significantly elevated in the 100 ng/kg core study group. In the TCDD feed study by Kociba *et al.* (1978), there were increases in the incidences of stratified squamous cell carcinoma of the hard palate/nasal turbinates in both male and female rats. The location of the squamous cell carcinomas in the present study was adjacent to the molars and invaded the hard palate/nasal turbinate areas, suggesting that the lesions seen in this and the Kociba *et al.* (1978) studies are similar. In addition to the squamous effects in the oral mucosa and lung, there were also increased incidences of forestomach squamous hyperplasia in the 46 and 100 ng/kg core study groups. All these squamous lesions may be related to the alteration in retinoid homeostasis that is known to be induced by TCDD.

In recent years, there has been an increasing awareness of the sensitivity of the oral cavity to the effects of DLCs. In two PCB/PCDF human poisoning episodes, one of the toxic responses observed in humans was early tooth eruption (Grassman *et al*., 1998). More recent studies have shown that TCDD can accelerate incisor tooth eruption and delay molar eruption. Proliferation of the periodontal squamous epithelium has been seen in juvenile mink exposed to TCDD (Render *et al*., 2001) but not in juvenile Long Evans 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 caused by a disruption in epidermal growth factor receptor-mediated signaling (Partanen *et al*., 1998) as has been shown for other developmental effects of TCDD such as a cleft palate (Abbott *et al*., 2003).

In the present study, there were increased incidences of adrenal cortical atrophy in the 100 ng/kg core study group and of cortical hyperplasia in the 22, 46, and 100 ng/kg groups. The incidence of cytoplasmic vacuolization was significantly increased in the 22 ng/kg group. Cortical atrophy 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 caused by other unknown mechanisms.

In the present study, there was a trend for increased incidence of pancreatic neoplasms associated with TCDD exposure. Majeed (1997) showed that acinar pancreatic carcinoma is a rare tumor seen in the pancreas of female Sprague-Dawley CD rats and has a spontaneous background rate of 0.02%. These data suggest that increased incidences of pancreatic neoplasms may have been related to TCDD treatment. By comparison, in the 2-year feed study by Kociba *et al.* (1978), there was a significant reduction in the incidence of acinar adenoma of the pancreas in male rats relative to the high spontaneous incidence seen in male rats in that study.

Dose-related increases in the incidences of acinar atrophy were seen in all treated groups, and the incidence was significantly elevated in the 100 ng/kg core study group. The incidences of acinar chronic active inflammation, acinar cytoplasmic vacuolization, and chronic arterial inflammation in the 100 ng/kg core study group and the incidence of acinar cytoplasmic vacuolization in the 46 ng/kg group were all significantly greater than those in the vehicle controls at 2 years.

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 present TCDD study, intestinal CCK is reduced by TCDD exposure. Down-regulation of CCK is likely caused by a general endocrine effect, resulting from the reduction in body weight gain following exposure to TCDD. CCK is an important regulator of pancreatic growth and function (Baldwin, 1995; Varga *et al*., 1998). Previous studies have shown that increased incidences of apoptosis and pancreatic acinar atrophy occurred in Otsuka Long-Evans Tokushima Fatty rats that lack the CCK-A receptor (Jimi *et al*., 1997). In addition, antagonism of CCK action can lead to reduced pancreatic growth (Ohlsson *et al*., 1995). The relationship among acinar atrophy, cytoplasmic vacuolization, and development of pancreatic neoplasms is unclear because these lesions were also observed in the PCB 126 study (NTP, 2006a), which was conducted as part of the dioxin TEF evaluation, yet there were no increases in the incidences of pancreatic neoplasms in the that study.

In the present study, the incidences of nephropathy were significantly greater in the 100 ng/kg core study and stop-exposure groups than in the vehicle controls. Dose-related increases in the incidences of hyperplasia of the transitional epithelium were noted in all treated groups and were significantly elevated in the 46 and 100 ng/kg core study groups. The significance of this difference was unclear; it did not appear to correlate with the increased severity of nephropathy because the animals with hyperplasia often had minimal nephropathy. While TCDD is known to induce biochemical responses in the kidney (Walker *et al*., 1995), the kidney historically has not been a target for TCDD-induced neoplasia.

Administration of TCDD to female Harlan Sprague-Dawley rats in this study significantly increased the incidences of cardiomyopathy in all dose groups administered 10 ng/kg or greater. 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 (Jokinen *et al*., 2003). The microscopic appearance of cardiomyopathy was the same in both the vehicle control and treated animals and was typical of that described for spontaneous lesions. This finding may suggest that exposure to the chemical increased the occurrence of the spontaneous change. The heart is a target for TCDD and related DLCs in both rodents and humans (Peterson *et al*., 1993; Flesch-Janys *et al*., 1995; Walker and Catron, 2000; Heid *et al*., 2001). In the 2-year feed study in which Sprague-Dawley rats were administered up to 100 ng/kg per day of TCDD , Kociba *et al.* (1978) also reported an increase of myocardial degenerative change above background levels in females only.

In this study, the incidence of thymic atrophy was significantly elevated at 46 and 100 ng/kg at 14, 31, and 53 weeks and at 22 ng/kg or greater at the end of the 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 also 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 this 2-year study, there was a significantly lower adjusted incidence of thyroid gland C-cell adenomas in the 100 ng/kg core study group. Similarly, there was a lower incidence of C-cell adenomas in male rats exposed to TCDD in feed (Kociba *et al*., 1978). The reduced incidence of C-cell adenomas may be related to the reduced body weight gain seen in the 100 ng/kg group.

In the present study, there were no significant increases in the incidences of thyroid gland follicular cell neoplasms, although there were increases in the incidences of follicular cell hypertrophy in the 46 and 100 ng/kg core study groups. By comparison, in the NTP 2-year gavage study of TCDD in Osborne-Mendel rats, there were significant increases in the incidences of thyroid gland follicular cell adenomas (NTP, 1982a) in male rats and a nonsignificant increase in female rats.

Alteration in thyroid hormone homeostasis by TCDD is well established (Van Birgelen *et al*., 1994, 1995a; Sewall *et al*., 1995). Analyses of thyroid hormones in the present study confirmed the alterations in thyroid hormone homeostasis by TCDD. The disruption of thyroid hormone homeostasis by DLCs is believed to be caused by the increase in thyroxine (T_4) glucuronidation as a result of increased expression of UDP-glucuronosyltransferases. This leads to a reduction in circulating T_4, decreased negative feedback inhibition of the thyroid gland, and overexpression of thyroid stimulating hormone (TSH) (Curran and DeGroot, 1991). Kohn *et al.* (1996) developed a mathematical model of the effects of TCDD on UDP-glucuronosyltransferase 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, significant alterations in T_4 and TSH were observed at the early time points, but a significant increase in TSH levels was seen only at 14 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 neoplasia.

In this study, there was a significantly lower adjusted incidence of mammary gland neoplasms following TCDD administration at 100 ng/kg per day. Fibroadenoma is a spontaneous lesion in female Sprague-Dawley rats and occurred at a high incidence (71%) in vehicle control animals. Mammary gland carcinoma was also seen in vehicle control rats at a lower incidence (10%). In the 100 ng/kg core study group, the incidences of fibroadenoma and carcinoma were 51% and 0%, respectively. In addition, there was a significantly lower incidence of spontaneous pituitary gland adenoma in the 100 ng/kg core study group (25%) than in the vehicle control group (58%).

The lower incidences of mammary gland and pituitary gland neoplasms in treated rodents are believed to be related to a general endocrine effect as a result of reductions in body weight gain associated with treatment. An association between reduced body weight gain and lower incidences of mammary gland and pituitary gland tumors has been observed in many NTP studies of F344/N rats (Seilkop, 1995). Significantly lower incidences of mammary gland and pituitary gland tumors were also observed in animals exposed to 100 ng/kg in the 2-year feed study by Kociba *et al*., (1978).

Reductions in insulin-like growth factor 1 (IGF-1) may underlie the inhibitory effect of reduced body weight gain on tumor development. 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, which regulates gallbladder contraction, pancreatic secretion, stomach emptying, and intestinal motility; CCK can also inhibit food intake. In an analysis of intestinal tissue obtained from the current study, Lee *et al*. (2000) showed lower levels of intestinal CCK and induction of insulin-like growth factor binding protein 3 (IGFBP3) by TCDD in this NTP study. Alterations in CCK processing enzymes by TCDD were also observed in cultured intestinal cells, suggesting a direct effect of TCDD on intestinal cells. The authors hypothesized that changes in CCK may be caused by alterations in processing enzymes and lower IGF-1 levels as a result of alterations in IGFBP3.

In the present TCDD study, there was a significantly increased incidence of squamous cell carcinoma of the uterus in animals administered 46 ng/kg. There were also two animals with squamous cell carcinomas in the stop-exposure group. The observed neoplasms were considered to be related to TCDD treatment because of the observed increased incidences of squamous neoplasms seen in other tissues in this study. There were no neoplasms in the 100 ng/kg core study group, which may be because of a suppressive effect of reduced body weight gain seen at the highest dose. Within this context, there was a significant reduction in the incidence of uterine squamous metaplasia in the 100 ng/kg core study group. By comparison, there was no effect on uterine squamous cell carcinoma in the TCDD dosed-feed study by Kociba *et al.* (1978). However, there was a significantly lower incidence of benign tumors of the uterus in the 100 ng/kg group.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity** of TCDD in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma and hepatocellular adenoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. The increased incidence of squamous cell carcinoma of the uterus was also considered to be related to TCDD administration. The marginally increased incidences of pancreatic acinar neoplasms and occurrences of hepatocholangioma and cholangioma of the liver may have been related to TCDD administration.

TCDD administration caused increased incidences of nonneoplastic lesions of the liver, lung, oral mucosa, pancreas, thymus, adrenal cortex, heart, clitoral gland, kidney, forestomach, mesentery, 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 Report Review Subcommittee comments and 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 TCDD

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Disposition Summary						
Animals initially in study	28	28	28	28	28	28
14-Week interim evaluation	10	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8	8
Animals examined microscopically	28	28	28	28	28	28
14-Week Interim Evaluation						
Genital System						
Uterus	(10)	(1)				(10)
Deciduoma benign		1 (100%)				
Integumentary System						
Mammary gland	(10)					(10)
Carcinoma	1 (10%)					
Systems Examined at 14 Weeks with No Neoplasms Observed						
Alimentary System						
Cardiovascular System						
Endocrine System						
General Body System						
Hematopoietic System						
Musculoskeletal System						
Nervous System						
Respiratory System						
Special Senses System						
Urinary System						
31-Week Interim Evaluation						
Integumentary System						
Mammary gland	(10)				(1)	(10)
Fibroadenoma					1 (100%)	
Skin	(1)					
Sarcoma	1 (100%)					

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Systems Examined at 31 Weeks with No Neoplasms Observed						
Alimentary System						
Cardiovascular System						
Endocrine System						
General Body System						
Genital System						
Hematopoietic System						
Musculoskeletal System						
Nervous System						
Respiratory System						
Special Senses System						
Urinary System						
53-Week Interim Evaluation						
Endocrine System						
Adrenal medulla	(8)		(1)			(8)
Pheochromocytoma benign			1 (100%)			
Pituitary gland	(8)		(1)			(7)
Adenoma			1 (100%)			
Genital System						
Uterus	(8)			(1)		(8)
Polyp stromal	1 (13%)					1 (13%)
Integumentary System						
Mammary gland	(8)	(2)	(2)			(8)
Fibroadenoma		2 (100%)	1 (50%)			
Fibroadenoma, multiple			1 (50%)			
Systems Examined at 53 Weeks with No Neoplasms Observed						
Alimentary System						
Cardiovascular System						
General Body System						
Hematopoietic System						
Musculoskeletal System						
Nervous System						
Respiratory System						
Special Senses System						
Urinary System						

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Neoplasm Summary						
Total animals with primary neoplasms[c]						
14-Week interim evaluation	1	1				
31-Week interim evaluation	1				1	
53-Week interim evaluation	1	2	3			1
Total primary neoplasms						
14-Week interim evaluation	1	1				
31-Week interim evaluation	1				1	
53-Week interim evaluation	1	2	4			1
Total animals with benign neoplasms						
14-Week interim evaluation		1				
31-Week interim evaluation					1	
53-Week interim evaluation	1	2	3			1
Total benign neoplasms						
14-Week interim evaluation		1				
31-Week interim evaluation					1	
53-Week interim evaluation	1	2	4			1
Total animals with malignant neoplasms						
14-Week interim evaluation	1					
31-Week interim evaluation	1					
Total malignant neoplasms						
14-Week interim evaluation	1					
31-Week interim evaluation	1					

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

TABLE A1b
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCDD[a]

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Disposition Summary						
Animals initially in study	53	54	53	53	53	53
Early deaths						
Accidental deaths			5			
Moribund	19	27	15	19	17	17
Natural deaths	9	6	10	15	14	15
Survivors						
Terminal sacrifice	25	21	23	19	22	21
Animals examined microscopically	53	54	53	53	53	53
Alimentary System						
Intestine large, rectum	(52)	(54)	(53)	(52)	(53)	(53)
Carcinoma		1 (2%)				
Intestine small, jejunum	(51)	(54)	(53)	(53)	(53)	(52)
Leiomyoma					1 (2%)	
Liver	(53)	(54)	(53)	(53)	(53)	(53)
Carcinoma, metastatic, pancreas						2 (4%)
Carcinoma, metastatic, uterus					2 (4%)	
Cholangiocarcinoma				1 (2%)	3 (6%)	8 (15%)
Cholangiocarcinoma, multiple					1 (2%)	17 (32%)
Hepatocellular adenoma					1 (2%)	10 (19%)
Hepatocellular adenoma, multiple						3 (6%)
Hepatocholangioma						2 (4%)
Mesentery	(2)	(2)		(1)	(6)	(8)
Carcinoma, metastatic, uterus					1 (17%)	
Schwannoma malignant	1 (50%)					
Oral mucosa	(2)	(9)	(16)	(16)	(22)	(29)
Gingival, squamous cell carcinoma	1 (50%)	2 (22%)	1 (6%)		4 (18%)	10 (34%)
Pancreas	(51)	(54)	(52)	(53)	(52)	(51)
Carcinoma, metastatic, uterus					1 (2%)	
Acinus, adenoma						1 (2%)
Acinus, carcinoma						2 (4%)
Stomach, forestomach	(53)	(54)	(53)	(53)	(53)	(53)
Squamous cell carcinoma	1 (2%)				1 (2%)	
Squamous cell papilloma					1 (2%)	1 (2%)
Stomach, glandular	(53)	(54)	(53)	(53)	(53)	(53)
Cardiovascular System						
Heart	(53)	(54)	(53)	(52)	(53)	(52)
Schwannoma malignant		1 (2%)				
Endocrine System						
Adrenal cortex	(53)	(54)	(53)	(53)	(53)	(53)
Adenoma	1 (2%)		2 (4%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma				1 (2%)		
Pheochromocytoma benign						1 (2%)
Capsule, sarcoma, metastatic, skeletal muscle			1 (2%)			
Adrenal medulla	(53)	(54)	(53)	(53)	(53)	(53)
Pheochromocytoma malignant						1 (2%)
Pheochromocytoma benign	3 (6%)	2 (4%)	2 (4%)	3 (6%)	3 (6%)	3 (6%)

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Endocrine System (continued)						
Islets, pancreatic	(51)	(54)	(53)	(53)	(52)	(52)
Adenoma	1 (2%)		2 (4%)			
Carcinoma	1 (2%)					
Parathyroid gland	(46)	(47)	(47)	(44)	(45)	(45)
Adenoma				1 (2%)		
Pituitary gland	(53)	(54)	(52)	(53)	(53)	(52)
Adenoma	4 (8%)					1 (2%)
Meningioma malignant, metastatic, brain				1 (2%)		
Pars distalis, adenoma	18 (34%)	20 (37%)	26 (50%)	15 (28%)	20 (38%)	10 (19%)
Pars distalis, adenoma, multiple	3 (6%)					
Pars intermedia, adenoma	2 (4%)					
Thyroid gland	(52)	(54)	(53)	(51)	(53)	(52)
Bilateral, C-cell, adenoma	3 (6%)	3 (6%)	2 (4%)	2 (4%)	1 (2%)	3 (6%)
C-cell, adenoma	17 (33%)	12 (22%)	15 (28%)	14 (27%)	12 (23%)	8 (15%)
C-cell, carcinoma	1 (2%)	1 (2%)			2 (4%)	
Follicular cell, adenoma	1 (2%)					
Follicular cell, carcinoma					1 (2%)	
General Body System						
None						
Genital System						
Clitoral gland	(50)	(52)	(53)	(52)	(51)	(53)
Ovary	(51)	(53)	(53)	(53)	(53)	(53)
Granulosa cell tumor malignant					1 (2%)	
Thecoma benign					1 (2%)	
Periovarian tissue, schwannoma malignant, metastatic, uterus	1 (2%)					
Uterus	(52)	(53)	(53)	(53)	(53)	(53)
Adenoma			1 (2%)			
Carcinoma		1 (2%)			2 (4%)	1 (2%)
Carcinoma, multiple				1 (2%)		
Hemangiosarcoma					1 (2%)	
Leiomyoma	1 (2%)					
Polyp stromal	9 (17%)	7 (13%)	4 (8%)	8 (15%)	9 (17%)	7 (13%)
Polyp stromal, multiple	2 (4%)			3 (6%)	1 (2%)	1 (2%)
Squamous cell carcinoma					4 (8%)	
Squamous cell carcinoma, multiple					1 (2%)	
Squamous cell papilloma	1 (2%)		1 (2%)			
Cervix, carcinoma		1 (2%)				
Cervix, schwannoma malignant	1 (2%)					
Vagina		(1)	(1)	(3)	(3)	(1)
Schwannoma malignant					1 (33%)	1 (100%)
Schwannoma malignant, metastatic, skin				1 (33%)		
Squamous cell carcinoma					1 (33%)	
Hematopoietic System						
Bone marrow	(53)	(54)	(53)	(53)	(53)	(53)
Lymph node	(2)	(6)	(3)	(5)	(6)	(9)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (50%)					
Lumbar, carcinoma, metastatic, uterus					1 (17%)	
Lymph node, mandibular	(51)	(54)	(52)	(50)	(51)	(52)

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Hematopoietic System (continued)						
Lymph node, mesenteric	(52)	(53)	(53)	(53)	(53)	(51)
Spleen	(51)	(54)	(53)	(53)	(52)	(52)
Hemangiosarcoma					1 (2%)	
Schwannoma malignant, metastatic, uterus	1 (2%)					
Thymus	(51)	(52)	(52)	(49)	(46)	(42)
Carcinoma, metastatic, lung		1 (2%)				
Histiocytic sarcoma, metastatic, lung			1 (2%)			
Integumentary System						
Mammary gland	(53)	(54)	(53)	(53)	(53)	(53)
Adenoma	2 (4%)		1 (2%)			1 (2%)
Carcinoma	3 (6%)	7 (13%)	4 (8%)	2 (4%)	2 (4%)	
Carcinoma, multiple	1 (2%)	1 (2%)				
Fibroadenoma	21 (40%)	26 (48%)	17 (32%)	17 (32%)	24 (45%)	20 (38%)
Fibroadenoma, multiple	12 (23%)	14 (26%)	17 (32%)	12 (23%)	12 (23%)	4 (8%)
Skin	(53)	(54)	(53)	(53)	(53)	(53)
Basal cell carcinoma	1 (2%)		1 (2%)			
Fibroma	1 (2%)		2 (4%)			1 (2%)
Fibrosarcoma				1 (2%)		
Keratoacanthoma				1 (2%)		
Lipoma		1 (2%)				
Myxosarcoma			1 (2%)			
Schwannoma malignant	1 (2%)			2 (4%)		1 (2%)
Musculoskeletal System						
Bone	(53)	(54)	(53)	(53)	(53)	(53)
Cranium, schwannoma malignant, metastatic, skin				1 (2%)		
Periosteum, fibrosarcoma				1 (2%)		
Skeletal muscle			(1)	(2)		
Fibrous histiocytoma				1 (50%)		
Sarcoma			1 (100%)			
Nervous System						
Brain	(53)	(54)	(53)	(53)	(53)	(53)
Glioma malignant			1 (2%)			
Granular cell tumor malignant			1 (2%)			
Respiratory System						
Lung	(53)	(54)	(53)	(52)	(53)	(52)
Alveolar/bronchiolar adenoma, multiple					1 (2%)	
Alveolar/bronchiolar carcinoma		1 (2%)				
Carcinoma, metastatic, mammary gland		1 (2%)				
Carcinoma, metastatic, pancreas						1 (2%)
Carcinoma, metastatic, thyroid gland	1 (2%)					
Cystic keratinizing epithelioma						7 (13%)
Cystic keratinizing epithelioma, multiple						2 (4%)
Nephroblastoma, metastatic, kidney		1 (2%)				
Sarcoma, metastatic, skeletal muscle			1 (2%)			
Mediastinum, sarcoma			1 (2%)			1 (2%)
Nose	(53)	(54)	(53)	(53)	(53)	(53)
Squamous cell carcinoma, metastatic, oral mucosa						1 (2%)

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Special Senses System						
Ear	(2)		(2)			(2)
Pinna, neural crest tumor	1 (50%)					
Harderian gland	(53)	(54)	(53)	(52)	(53)	(53)
Squamous cell carcinoma, metastatic, oral mucosa						3 (6%)
Zymbal's gland	(1)					
Carcinoma	1 (100%)					
Urinary System						
Kidney	(53)	(54)	(53)	(53)	(53)	(53)
Nephroblastoma		2 (4%)	1 (2%)			
Schwannoma malignant, metastatic, uterus	1 (2%)					
Stromal nephroma				1 (2%)		
Urinary bladder	(52)	(53)	(53)	(52)	(53)	(53)
Polyp		1 (2%)				
Schwannoma malignant, metastatic, uterus	1 (2%)					
Squamous cell carcinoma, metastatic, uterus					1 (2%)	
Transitional epithelium, carcinoma					1 (2%)	
Systemic Lesions						
Multiple organs[b]	(53)	(54)	(53)	(53)	(53)	(53)
Leukemia granulocytic			1 (2%)			
Lymphoma malignant		1 (2%)	1 (2%)		1 (2%)	2 (4%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	47	54	48	44	50	50
Total primary neoplasms	116	105	106	89	116	131
Total animals with benign neoplasms	45	49	45	40	46	42
Total benign neoplasms	102	86	92	78	88	87
Total animals with malignant neoplasms	13	19	13	11	24	34
Total malignant neoplasms	13	19	14	11	28	44
Total animals with metastatic neoplasms	2	3	2	2	3	6
Total metastatic neoplasms	6	3	3	3	6	7
Total animals with uncertain neoplasms - benign or malignant	1					
Total uncertain neoplasms	1					

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

TABLE A1c
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)[a]

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Disposition Summary			
Animals initially in study	53	53	50
Early deaths			
Moribund	19	17	16
Natural deaths	9	15	13
Survivors			
Terminal sacrifice	25	21	21
Animals examined microscopically	53	53	50
Alimentary System			
Intestine large, rectum	(52)	(53)	(50)
Fibrous histiocytoma, metastatic, skin			1 (2%)
Intestine small, jejunum	(51)	(52)	(50)
Schwannoma malignant, metastatic, heart			1 (2%)
Liver	(53)	(53)	(50)
Carcinoma, metastatic, pancreas		2 (4%)	
Cholangiocarcinoma		8 (15%)	1 (2%)
Cholangiocarcinoma, multiple		17 (32%)	1 (2%)
Cholangioma			1 (2%)
Fibrous histiocytoma, metastatic, skin			1 (2%)
Hepatocellular adenoma		10 (19%)	2 (4%)
Hepatocellular adenoma, multiple		3 (6%)	
Hepatocholangioma		2 (4%)	
Schwannoma malignant, metastatic, heart			1 (2%)
Mesentery	(2)	(8)	(1)
Schwannoma malignant	1 (50%)		
Oral mucosa	(2)	(29)	(11)
Gingival, squamous cell carcinoma	1 (50%)	10 (34%)	5 (45%)
Pancreas	(51)	(51)	(49)
Acinus, adenoma		1 (2%)	
Acinus, carcinoma		2 (4%)	1 (2%)
Stomach, forestomach	(53)	(53)	(50)
Squamous cell carcinoma	1 (2%)		
Squamous cell papilloma		1 (2%)	
Stomach, glandular	(53)	(53)	(50)
Tongue			(1)
Squamous cell carcinoma			1 (100%)
Cardiovascular System			
Blood vessel	(53)	(53)	(49)
Aorta, fibrous histiocytoma, metastatic, skin			1 (2%)
Heart	(53)	(52)	(50)
Carcinoma, metastatic, mammary gland			1 (2%)
Schwannoma malignant			3 (6%)
Endocrine System			
Adrenal cortex	(53)	(53)	(50)
Adenoma	1 (2%)	1 (2%)	
Pheochromocytoma benign		1 (2%)	
Capsule, schwannoma malignant, metastatic, heart			1 (2%)

TABLE A1c
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Endocrine System (continued)			
Adrenal medulla	(53)	(53)	(50)
Pheochromocytoma complex			1 (2%)
Pheochromocytoma malignant		1 (2%)	
Pheochromocytoma benign	3 (6%)	3 (6%)	1 (2%)
Islets, pancreatic	(51)	(52)	(49)
Adenoma	1 (2%)		
Carcinoma	1 (2%)		
Parathyroid gland	(46)	(45)	(44)
Pituitary gland	(53)	(52)	(50)
Adenoma	4 (8%)	1 (2%)	
Pars distalis, adenoma	18 (34%)	10 (19%)	19 (38%)
Pars distalis, adenoma, multiple	3 (6%)		
Pars intermedia, adenoma	2 (4%)		
Thyroid gland	(52)	(52)	(49)
Bilateral, C-cell, adenoma	3 (6%)	3 (6%)	2 (4%)
C-cell, adenoma	17 (33%)	8 (15%)	11 (22%)
C-cell, carcinoma	1 (2%)		1 (2%)
Follicular cell, adenoma	1 (2%)		
General Body System			
None			
Genital System			
Clitoral gland	(50)	(53)	(49)
Ovary	(51)	(53)	(49)
Periovarian tissue, schwannoma malignant, metastatic, uterus	1 (2%)		
Carcinoma, metastatic, uterus			1 (2%)
Uterus	(52)	(53)	(50)
Carcinoma		1 (2%)	2 (4%)
Leiomyoma	1 (2%)		
Polyp stromal	9 (17%)	7 (13%)	3 (6%)
Polyp stromal, multiple	2 (4%)	1 (2%)	1 (2%)
Squamous cell carcinoma			2 (4%)
Squamous cell papilloma	1 (2%)		
Cervix, schwannoma malignant	1 (2%)		
Vagina		(1)	
Schwannoma malignant		1 (100%)	
Hematopoietic System			
Bone marrow	(53)	(53)	(50)
Fibrous histiocytoma, metastatic, skin			1 (2%)
Lymph node	(2)	(9)	(5)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (50%)		
Lymph node, mandibular	(51)	(52)	(49)
Lymph node, mesenteric	(52)	(51)	(49)
Fibrous histiocytoma, metastatic, skin			1 (2%)

TABLE A1c
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Hematopoietic System (continued)			
Spleen	(51)	(52)	(49)
Fibrous histiocytoma, metastatic, skin			1 (2%)
Hemangiosarcoma			1 (2%)
Schwannoma malignant, metastatic, heart			1 (2%)
Schwannoma malignant, metastatic, uterus	1 (2%)		
Thymus	(51)	(42)	(49)
Fibrous histiocytoma, metastatic, skin			1 (2%)
Thymoma malignant			1 (2%)
Integumentary System			
Mammary gland	(53)	(53)	(50)
Adenolipoma			1 (2%)
Adenoma	2 (4%)	1 (2%)	
Carcinoma	3 (6%)		1 (2%)
Carcinoma, multiple	1 (2%)		
Fibroadenoma	21 (40%)	20 (38%)	19 (38%)
Fibroadenoma, multiple	12 (23%)	4 (8%)	13 (26%)
Skin	(53)	(53)	(50)
Basal cell carcinoma	1 (2%)		
Fibroma	1 (2%)	1 (2%)	1 (2%)
Fibrous histiocytoma			1 (2%)
Liposarcoma			1 (2%)
Schwannoma malignant	1 (2%)	1 (2%)	
Musculoskeletal System			
Bone	(53)	(53)	(50)
Squamous cell carcinoma, metastatic, tongue			1 (2%)
Skeletal muscle			(1)
Fibrous histiocytoma, metastatic, skin			1 (100%)
Nervous System			
Brain	(53)	(53)	(50)
Respiratory System			
Lung	(53)	(52)	(50)
Carcinoma, metastatic, mammary gland			1 (2%)
Carcinoma, metastatic, pancreas		1 (2%)	
Carcinoma, metastatic, thyroid gland	1 (2%)		
Cystic keratinizing epithelioma		7 (13%)	
Cystic keratinizing epithelioma, multiple		2 (4%)	
Fibrous histiocytoma, metastatic, skin			1 (2%)
Mediastinum, sarcoma		1 (2%)	
Schwannoma malignant, metastatic, heart			1 (2%)
Nose	(53)	(53)	(50)
Squamous cell carcinoma, metastatic, oral mucosa		1 (2%)	

TABLE A1c
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Special Senses System			
Ear	(2)	(2)	
Pinna, neural crest tumor	1 (50%)		
Harderian gland	(53)	(53)	(50)
Squamous cell carcinoma, metastatic, oral mucosa		3 (6%)	
Zymbal's gland	(1)		
Carcinoma	1 (100%)		
Urinary System			
Kidney	(53)	(53)	(50)
Fibrous histiocytoma, metastatic, skin			1 (2%)
Schwannoma malignant, metastatic, uterus	1 (2%)		
Urinary bladder	(52)	(53)	(50)
Schwannoma malignant, metastatic, uterus	1 (2%)		
Systemic Lesions			
Multiple organs[b]	(53)	(53)	(50)
Adenolipoma			1 (2%)
Lymphoma malignant		2 (4%)	1 (2%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	47	50	46
Total primary neoplasms	116	131	98
Total animals with benign neoplasms	45	42	41
Total benign neoplasms	102	87	74
Total animals with malignant neoplasms	13	34	19
Total malignant neoplasms	13	44	24
Total animals with metastatic neoplasms	2	6	5
Total metastatic neoplasms	6	7	19
Total animals with uncertain neoplasms - benign or malignant	1		
Total uncertain neoplasms	1		

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

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

Number of Days on Study	1 6 9	1 8 5	3 5 1	3 6 1	3 6 6	4 6 9	4 7 4	4 8 7	4 9 2	5 1 2	5 2 6	5 3 3	5 4 8	6 1 0	6 3 1	6 4 5	6 4 6	6 4 6	6 4 7	6 4 8	6 6 6	6 6 8	6 7 5	6 9 4	6 9 7	7 0 1	7 1 7	7 2 2
Carcass ID Number	0 8 6	0 2 5	0 2 1	0 2 4	0 8 2	0 4 2	0 8 4	0 0 7	0 3 5	0 6 3	0 1 3	0 0 6	0 8 3	0 7 3	0 6 1	0 2 3	0 7 5	0 8 7	0 4 1	0 3 9	0 0 4	0 8 1	0 0 9	0 2 2	0 4 3	0 7 1	0 6 4	0 3 4
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+																			+								
Schwannoma malignant	X																											
Oral mucosa					+																							
Gingival, squamous cell carcinoma					X																							
Pancreas	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																X												
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth		+													+	+				+	+		+			+		
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																												
Islets, pancreatic	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Carcinoma																X												
Parathyroid gland	+	M	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X	X			X									
Pars distalis, adenoma							X			X							X	X							X	X		X
Pars distalis, adenoma, multiple																												
Pars intermedia, adenoma																											X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																					X	X						
C-cell, adenoma							X		X		X	X				X							X	X	X			
C-cell, carcinoma																												
Follicular cell, adenoma																												

+: 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 TCDD: Vehicle Control

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 0 2	0 0 3	0 0 4	0 3 6	0 3 7	0 3 8	0 6 2	0 9 2	0 9 3	0 9 8	0 1 0	0 1 1	0 3 1	0 4 0	0 4 4	0 8 8	0 9 1	0 1 1	0 1 5	0 1 2	0 4 7	0 4 8	0 4 9	0 9 6	0 9 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Mesentery																										2
Schwannoma malignant																										1
Oral mucosa									+																	2
Gingival, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Squamous cell carcinoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth			+	+	+			+	+		+	+		+			+									16
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma											X															1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign												X		X								X				3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Adenoma	X																									1
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																					X					4
Pars distalis, adenoma		X	X		X				X	X	X	X					X			X				X	X	18
Pars distalis, adenoma, multiple													X		X			X								3
Pars intermedia, adenoma					X																					2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Bilateral, C-cell, adenoma										X																3
C-cell, adenoma			X		X						X			X		X		X	X		X				X	17
C-cell, carcinoma															X											1
Follicular cell, adenoma																			X							1

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

Number of Days on Study	1 6 9	1 8 5	3 5 1	3 6 1	3 6 6	4 6 9	4 7 4	4 8 7	4 9 2	5 1 2	5 2 6	5 3 3	5 4 8	6 1 0	6 3 1	6 4 5	6 4 6	6 4 6	6 4 7	6 4 8	6 6 6	6 6 8	6 7 5	6 9 4	6 9 7	7 0 1	7 1 7	7 2 2
Carcass ID Number	0 8 6	0 2 5	0 2 1	0 2 4	0 8 2	0 4 2	0 8 4	0 0 7	0 3 5	0 6 3	0 1 3	0 0 6	0 8 3	0 7 3	0 6 1	0 2 3	0 7 5	0 8 7	0 4 1	0 3 9	0 0 4	0 8 1	0 0 9	0 2 2	0 4 3	0 7 1	0 6 4	0 3 4
General Body System																												
None																												
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	+
Ovary	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Periovarian tissue, schwannoma malignant, metastatic, uterus												X															
Oviduct																										+	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																X											
Polyp stromal																X								X	X		
Polyp stromal, multiple																											
Squamous cell papilloma																											
Cervix, schwannoma malignant												X															
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																										+	
Deep cervical, carcinoma, metastatic, thyroid gland																											
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, uterus												X															
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X												
Carcinoma						X																					
Carcinoma, multiple																								X			
Fibroadenoma		X		X		X	X									X		X	X				X	X		X	
Fibroadenoma, multiple									X		X						X				X	X		X			X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																			X								
Fibroma													X														
Schwannoma malignant																									X		
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 0 0 2	0 0 2 3	0 0 1 4	0 0 3 6	0 0 3 7	0 0 3 8	0 0 6 2	0 0 9 2	0 0 9 3	0 0 9 8	0 1 0	0 1 1	0 1 1	0 3 1	0 4 0	0 4 4	0 8 8	0 9 1	0 0 1 1	0 0 5	0 1 2	0 4 7	0 4 8	0 4 9	0 9 6	0 9 7	Total Tissues/ Tumors
General Body System																											
None																											
Genital System																											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Periovarian tissue, schwannoma malignant, metastatic, uterus																											1
Oviduct			+																								2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Leiomyoma																											1
Polyp stromal				X								X		X					X	X			X				9
Polyp stromal, multiple										X														X			2
Squamous cell papilloma												X															1
Cervix, schwannoma malignant																											1
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																+											2
Deep cervical, carcinoma, metastatic, thyroid gland																X											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Schwannoma malignant, metastatic, uterus																											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma			X																								2
Carcinoma											X													X			3
Carcinoma, multiple																											1
Fibroadenoma		X			X			X	X						X	X				X	X		X	X		X	21
Fibroadenoma, multiple				X							X	X	X						X								12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Basal cell carcinoma																											1
Fibroma																											1
Schwannoma malignant																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, thyroid gland																X											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52

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

Number of Days on Study	1 6 9	1 8 5	3 5 1	3 6 1	3 6 6	4 6 9	4 7 4	4 8 7	4 9 2	5 1 2	5 2 6	5 3 3	5 4 8	6 1 0	6 3 1	6 4 5	6 4 6	6 4 6	6 4 7	6 4 8	6 6 6	6 6 8	6 7 5	6 9 4	7 0 1	7 1 7	7 2 2
Carcass ID Number	0 8 6	0 2 5	0 2 1	0 2 4	0 8 2	0 4 2	0 8 4	0 0 7	0 3 5	0 6 3	0 1 3	0 1 6	0 8 3	0 7 3	0 6 1	0 2 3	0 7 5	0 8 7	0 4 1	0 3 9	0 0 4	0 8 1	0 0 9	0 2 2	0 4 3	0 7 1	0 6 4	0 3 4
Special Senses System																											
Ear																									+	+	
Pinna, neural crest tumor																									X		
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland																											
Carcinoma																											
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, uterus												X															
Ureter																											
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, uterus												X															
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 0 2	0 0 3	0 0 4	0 3 6	0 3 7	0 3 8	0 6 2	0 9 2	0 9 3	0 9 8	0 1 0	0 1 1	0 3 1	0 4 0	0 4 4	0 8 8	0 9 1	0 0 1	0 0 5	0 1 2	0 4 7	0 4 8	0 4 9	0 9 6	0 9 7	Total Tissues/ Tumors
Special Senses System																									
Ear																									2
Pinna, neural crest tumor																									1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Zymbal's gland		+																							1
Carcinoma		X																							1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Schwannoma malignant, metastatic, uterus																									1
Ureter											+														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Schwannoma malignant, metastatic, uterus																									1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 3 ng/kg

Number of Days on Study	200	241	264	264	341	361	394	448	449	462	485	490	492	526	548	556	556	556	556	557	557	578	578	604	604	607	613	620	647
Carcass ID Number	165	114	128	141	210	242	117	115	106	181	555	122	129	208	103	202	255	113	184	154	163	116	179	182	183	177	164	168	110
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																													
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+																											+	
Oral mucosa								+	+												+							+
Gingival, squamous cell carcinoma																													X
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth									+							+				+			+			+	+	+	
Cardiovascular System																													
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant									X																				
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																													
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																								X	X		X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																													
C-cell, adenoma										X			X			X								X					
C-cell, carcinoma																								X					
General Body System																													
None																													
Genital System																													
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Oviduct																													
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Carcinoma																													
Polyp stromal														X								X							
Cervix, carcinoma																													
Vagina													+																

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 3 ng/kg

Number of Days on Study	6 4 8	6 8 1	7 1 8	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	1 0 9	1 1 8	1 6 2	1 2 6	1 0 4	1 2 1	1 2 4	1 3 1	1 3 4	1 5 1	1 6 1	1 7 0	1 7 6	1 9 8	1 1 1	1 2 5	1 5 3	1 0 7	1 2 2	1 2 3	1 3 0	1 3 3	1 4 4	1 4 5	1 9 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Carcinoma				X																						1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Mesentery																										2
Oral mucosa					+								+					+	+				+			9
Gingival, squamous cell carcinoma													X													2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Tooth						+				+		+	+			+		+	+	+			+			16
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Schwannoma malignant																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Pheochromocytoma benign															X				X							2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	M	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Pars distalis, adenoma					X	X	X	X	X	X		X	X		X	X			X	X	X	X	X		X	20
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Bilateral, C-cell, adenoma									X		X										X					3
C-cell, adenoma			X			X	X								X		X	X		X			X			12
C-cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Oviduct				+																						1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma														X												1
Polyp stromal					X				X			X						X							X	7
Cervix,carcinoma											X															1
Vagina																										1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 3 ng/kg

Number of Days on Study	200	241	264	264	341	361	394	448	449	462	485	490	492	526	548	556	556	556	556	557	557	578	578	604	604	607	613	620	647
Carcass ID Number	16 5	11 4	12 8	14 1	12 0	14 2	11 7	11 5	10 6	18 1	15 5	11 2	12 9	20 8	10 3	00 2	00 5	11 3	18 4	15 4	16 3	11 6	17 9	18 2	18 3	17 7	16 4	16 8	11 0
Hematopoietic System																													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+		+			+				+	+										+						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Carcinoma, metastatic, lung										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	X		X		
Fibroadenoma, multiple				X							X		X				X			X			X						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																													
Musculoskeletal System																													
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																													
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																													
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma										X																			
Carcinoma, metastatic, mammary gland					X																								
Nephroblastoma, metastatic, kidney			X																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																													
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																													
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma	X		X																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Polyp																													
Systemic Lesions																													
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant												X																	

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 3 ng/kg

Number of Days on Study	6 4 8	6 8 1	7 1 8	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	1 0 9	1 1 8	1 6 2	1 2 6	1 0 4	1 2 1	1 2 4	1 3 1	1 3 4	1 5 1	1 6 1	1 7 0	1 7 6	1 9 8	1 1 1	1 2 5	1 5 3	1 0 7	1 2 2	1 2 3	1 3 0	1 3 3	1 4 4	1 4 5	1 9 7	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymph node																										6
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Carcinoma					X	X										X					X					7
Carcinoma, multiple			X																							1
Fibroadenoma	X	X							X	X	X				X	X		X		X			X			26
Fibroadenoma, multiple						X	X						X	X			X				X	X		X		14
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lipoma							X																			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, mammary gland																										1
Nephroblastoma, metastatic, kidney																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Urniary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Nephroblastoma																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Polyp	X																									1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	54
Lymphoma malignant																										1

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

Number of Days on Study	0 4 7	0 5 4	1 7 7	2 3 6	2 4 0	3 4 4	3 4 8	4 2 9	5 5 0	5 7 8	5 7 8	5 7 8	5 7 8	5 7 8	6 0 4	6 1 3	6 1 4	6 2 6	6 3 5	6 4 5	6 5 0	6 5 9	6 5 9	6 7 6	6 7 9	7 1 7	7 1 8	7 2 0
Carcass ID Number	2 0 5	2 5 8	2 2 0	2 9 0	2 8 6	2 3 5	2 7 4	2 2 4	2 2 5	2 0 1	2 4 4	2 4 6	2 6 4	2 8 2	2 6 3	2 4 2	2 2 3	2 6 0	2 9 6	2 0 4	2 1 1	2 7 0	2 9 7	2 1 7	2 3 1	2 8 9	2 5 9	2 4 1
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oral mucosa					+			+		+	+	+					+					+	+		+			
Gingival, squamous cell carcinoma												X																
Pancreas	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth								+	+		+	+					+						+				+	
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Capsule, sarcoma, metastatic, skeletal muscle								X																				
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																												
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Parathyroid gland	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pars distalis, adenoma									X			X							X		X	X		X	X	X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																												
C-cell, adenoma																		X						X			X	X
General Body System																												
None																												
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oviduct																												
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Polyp stromal																						X						
Squamous cell papilloma																												
Vagina	+																											

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

Number of Days on Study	7 2 4	7 2 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 8 7	2 1 8	2 0 3	2 1 3	2 1 6	2 2 1	2 3 4	2 4 5	2 4 9	2 6 6	2 6 8	2 7 2	2 2 2	2 3 3	2 4 7	2 5 6	2 5 7	2 6 1	2 6 2	2 7 3	2 0 2	2 1 2	2 1 5	2 8 4	2 8 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Oral mucosa		+						+				+		+			+				+	+				16
Gingival, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth							+	+							+						+	+				12
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma						X										X										2
Capsule, sarcoma, metastatic, skeletal muscle																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign				X								X														2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																					X	X				2
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Pars distalis, adenoma	X	X	X	X	X	X	X						X	X	X	X	X	X				X	X	X	X	26
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma						X						X														2
C-cell, adenoma					X		X	X		X	X		X		X		X			X				X	X	15
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Oviduct																		+					+			2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma								X																		1
Polyp stromal	X							X						X												4
Squamous cell papilloma										X																1
Vagina	+																									1

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

Number of Days on Study	0 4 7	0 5 4	1 7 7	2 3 6	2 4 0	3 4 4	3 4 8	4 2 9	5 5 0	5 7 8	5 7 8	5 7 8	5 7 8	5 7 8	6 0 4	6 1 3	6 1 4	6 2 6	6 3 5	6 4 5	6 5 0	6 5 9	6 5 9	6 7 6	6 7 9	7 1 7	7 1 8	7 2 0
Carcass ID Number	2 0 5	2 5 8	2 2 0	2 9 0	2 8 6	2 3 5	2 7 4	2 2 4	2 2 5	2 0 1	2 4 4	2 4 6	2 6 4	2 8 2	2 6 3	2 4 2	2 2 3	2 6 0	2 9 6	2 0 4	2 1 1	2 7 0	2 9 7	2 1 7	2 3 1	2 8 9	2 5 9	2 4 1
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node										+								+										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Histiocytic sarcoma, metastatic, lung																												
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																												
Carcinoma																							X			X		
Fibroadenoma				X		X			X		X			X	X	X	X					X	X			X		X
Fibroadenoma, multiple										X			X						X					X	X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																							X					
Fibroma												X								X								
Myxosarcoma																X												
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle								+																				
Sarcoma								X																				
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant																	X											
Granular cell tumor malignant																												
Peripheral nerve																	+											
Spinal cord																	+											
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle								X																				
Mediastinum, sarcoma																												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																												
Ear																									+			
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	7 2 4	7 2 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 8 7	2 1 8	2 0 3	2 1 3	2 1 6	2 2 1	2 3 4	2 4 5	2 4 9	2 6 6	2 6 8	2 7 2	2 2 2	2 3 3	2 4 7	2 5 6	2 5 7	2 6 1	2 6 2	2 7 3	2 0 2	2 1 2	2 1 5	2 8 4	2 8 8	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node		+																								3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Histiocytic sarcoma, metastatic, lung							X																			1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																						X				1
Carcinoma		X													X											4
Fibroadenoma		X		X												X	X							X		17
Fibroadenoma, multiple			X		X	X	X	X	X					X	X					X	X		X		X	17
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Basal cell carcinoma																										1
Fibroma																										2
Myxosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Skeletal muscle																										1
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Glioma malignant																										1
Granular cell tumor malignant												X														1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Sarcoma, metastatic, skeletal muscle																										1
Mediastinum, sarcoma							X																			1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																										
Ear											+															2
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

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

Number of Days on Study	047	054	177	236	240	344	448	429	550	577	778	778	778	778	604	613	114	626	635	645	650	659	679	717	778	720
Carcass ID Number	20505	25808	22200	29900	28606	23635	37744	22244	22205	20201	24446	24466	26464	28822	26363	24232	22363	26230	29696	20464	21411	27710	29977	21771	23119	28899	25941
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephroblastoma					X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia granulocytic															X												
Lymphoma malignant																		X									

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

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

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

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

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

Number of Days on Study	6 4 5	6 7 5	6 8 0	6 9 0	7 0 8	7 0 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	
Carcass ID Number	3 6 5	3 1 6	3 4 3	3 4 5	3 4 8	3 6 2	3 0 1	3 0 2	3 0 5	3 2 6	3 3 4	3 4 1	3 4 2	3 8 1	3 9 4	3 9 6	3 1 2	3 3 2	3 3 3	3 3 6	3 4 6	3 4 8	3 9 2	3 9 5	3 6 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cholangiocarcinoma																										1
Mesentery				+																						1
Oral mucosa		+	+		+									+					+							16
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth					+	+					+		+		+										+	13
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																							X			2
Carcinoma																		X								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign												X											X	X		3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	44
Adenoma		X																								1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Meningioma malignant, metastatic, brain																										1
Pars distalis, adenoma						X				X		X	X	X			X			X	X		X	X		15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Bilateral, C-cell, adenoma						X				X																2
C-cell, adenoma			X	X					X			X		X	X	X				X				X		14
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, multiple									X																	1
Polyp stromal										X		X						X			X					8
Polyp stromal, multiple														X		X										3
Vagina				+																					+	3
Schwannoma malignant, metastatic, skin																										1

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

Number of Days on Study	0 5 3	1 9 7	3 3 8	3 7 3	3 8 0	3 8 9	4 0 6	4 1 6	4 1 7	4 5 3	4 6 2	4 8 5	5 2 2	5 3 3	5 4 5	5 5 7	5 6 1	5 7 8	5 7 8	5 7 8	5 9 2	6 0 1	6 0 7	6 1 5	6 2 6	6 2 6	6 2 6	6 4 1
Carcass ID Number	3 3 9	3 4 9	3 0 7	3 1 7	3 1 1	3 3 0	3 1 8	3 3 7	3 1 4	3 1 0	3 6 1	3 0 6	3 8 4	3 9 7	3 0 9	3 3 5	3 8 3	3 5 0	3 6 3	3 6 7	3 4 0	3 8 0	3 2 9	3 0 3	3 1 9	3 2 0	3 3 1	3 9 8
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																			+							+		
Lymph node, mandibular	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma			X																									
Fibroadenoma										X		X							X		X		X				X	X
Fibroadenoma, multiple														X		X				X				X	X	X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																												
Keratoacanthoma																												
Schwannoma malignant					X					X																		
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, schwannoma malignant, metastatic, skin					X																							
Periosteum, fibrosarcoma													X															
Skeletal muscle											+											+						
Fibrous histiocytoma											X																	
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																												
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stromal nephroma																												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	6 4 5	6 7 5	6 8 0	6 9 0	7 0 8	7 0 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	
Carcass ID Number	3 6 5	3 1 6	3 4 3	3 4 5	3 4 8	3 6 2	3 0 1	3 0 2	3 0 5	3 2 6	3 3 4	3 4 1	3 4 2	3 8 1	3 9 4	3 9 6	3 1 2	3 3 2	3 3 3	3 3 6	3 4 6	3 4 8	3 9 2	3 9 5	3 6 4	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node	+			+	+																					5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma		X																								2
Fibroadenoma	X				X		X	X				X	X		X							X	X		X	17
Fibroadenoma, multiple			X			X				X							X	X		X						12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibrosarcoma																			X							1
Keratoacanthoma			X																							1
Schwannoma malignant																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Cranium, schwannoma malignant, Metastatic, skin																										1
Periosteum, fibrosarcoma																										1
Skeletal muscle																										2
Fibrous histiocytoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Stromal nephroma					X																					1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

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

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

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

Number of Days on Study	701	708	720	731	731	731	731	731	731	731	731	731	731	732	732	732	732	733	733	733	733	733	733	733	733	
Carcass ID Number	48 89	40 02	47 08	40 05	40 06	41 14	41 15	43 00	45 04	45 55	46 02	47 22	48 88	44 89	45 00	46 03	47 05	40 13	42 17	44 84	46 44	46 70	47 87	48 90	49 96	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Leiomyoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, uterus																										2
Cholangiocarcinoma											X				X					X						3
Cholangiocarcinoma, multiple				X																						1
Hepatocellular adenoma			X																							1
Mesentery						+			+														+	+		6
Carcinoma, metastatic, uterus																										1
Oral mucosa	+	+	+			+		+				+	+						+		+		+		+	22
Gingival, squamous cell carcinoma		X	X																						X	4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, uterus																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Squamous cell carcinoma	X																									1
Squamous cell papilloma		X																								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth	+	+				+		+					+			+	+				+					14
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma							X																			1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma benign		X				X												X								3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pars distalis, adenoma	X		X		X			X		X		X		X	X	X	X							X	X	20
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Bilateral, C-cell, adenoma																		X								1
C-cell, adenoma				X		X	X				X		X	X											X	12
C-cell, carcinoma							X			X																2
Follicular cell, carcinoma																										1
General Body System																										
None																										

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

Number of Days on Study	1 1 3 3 3 4 4 4 4 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 7 4 8 1 4 9 4 7 8 8 1 5 7 8 9 0 9 2 4 4 5 5 7 7 7 7 8 9 0 1 5 7 1 6 4 4 6 6 8 7 8 9 0 9 1 2 5 7 9 2 3 3 5 7 3 4 1
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 2 4 4 1 8 6 1 6 8 1 7 5 1 1 5 6 9 7 4 0 1 0 6 7 8 7 2 6 5 7 3 3 5 6 6 1 0 9 2 7 8 1 7 7 1 6 9 2 7 9 7 2 4 8 9
Genital System	
Clitoral gland	+ + + + + M + + + + + + + + + + + + + M + + + + + + + +
Ovary	+ +
Granulosa cell tumor malignant	
Thecoma benign	X
Oviduct	+
Uterus	+ +
Carcinoma	
Hemangiosarcoma	
Polyp stromal	X X X
Polyp stromal, multiple	X
Squamous cell carcinoma	X X X X
Squamous cell carcinoma, multiple	X
Vagina	+ +
Schwannoma malignant	X
Squamous cell carcinoma	
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ + + + + +
Lumbar, carcinoma, metastatic, uterus	X
Lymph node, mandibular	+ + + + + + + + + + + + M + + M + + + + + + + + + + + +
Lymph node, mesenteric	+ +
Spleen	+ + + + + + + + + + + + + + M + + + + + + + + + + + +
Hemangiosarcoma	
Thymus	+ + + + + M + + + + + + + + + + + + + + + + M + + + + M
Integumentary System	
Mammary gland	+ +
Carcinoma	X
Fibroadenoma	X X X X X X X X X X X X X X
Fibroadenoma, multiple	X X X X
Skin	+ +
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma, multiple	
Nose	+ +
Trachea	+ +
Special Senses System	
Eye	+ +
Harderian gland	+ +

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

Number of Days on Study	7 0 1	7 0 8	7 2 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	4 8 8 9	4 0 0 2	4 0 7 8	4 0 0 5	4 0 0 6	4 0 1 4	4 0 1 5	4 0 3 0	4 0 4 5	4 0 5 5	4 0 6 2	4 0 7 2	4 0 8 8	4 0 4 9	4 0 5 0	4 0 6 3	4 0 7 5	4 0 7 0 1	4 0 2 7	4 0 4 4 8	4 0 4 6 4	4 0 4 7 0	4 0 4 7 7	4 0 4 8 0	4 0 4 9 7	4 0 9 6	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Granulosa cell tumor malignant						X																				1
Thecoma benign																										1
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma						X							X													2
Hemangiosarcoma										X																1
Polyp stromal			X				X								X		X			X	X					9
Polyp stromal, multiple																										1
Squamous cell carcinoma																										4
Squamous cell carcinoma, multiple																										1
Vagina																					+					3
Schwannoma malignant																										1
Squamous cell carcinoma																					X					1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node																										6
Lumbar, carcinoma, metastatic, uterus																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Hemangiosarcoma																			X							1
Thymus	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma					X																					2
Fibroadenoma			X	X						X	X			X	X	X		X						X		24
Fibroadenoma, multiple		X			X		X						X						X		X	X	X			12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Alveolar/bronchiolar adenoma, multiple																				X						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

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

Number of Days on Study	1 4 1	1 8 5	3 1 7	3 4 1	3 9 6	4 4 4	4 7 4	4 8 6	4 8 6	5 1 8	5 5 7	5 7 8	5 8 9	5 9 0	5 9 9	6 2 1	6 4 2	6 4 5	6 5 7	6 5 9	6 7 2	6 7 3	6 7 3	6 7 5	6 7 7	6 8 3	6 9 4	7 0 1
Carcass ID Number	4 2 6	4 4 5	4 4 7	4 1 3	4 8 3	4 6 5	4 1 6	4 6 6	4 8 1	4 1 0	4 7 9	4 5 2	4 1 7	4 1 8	4 5 1	4 6 7	4 9 7	4 7 1	4 4 6	4 0 9	4 1 2	4 0 7	4 6 9	4 7 7	4 8 2	4 7 4	4 2 8	4 1 9
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, uterus															X												
Transitional epithelium, carcinoma													X														
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant										X																	

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

Number of Days on Study	701	708	720	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	48909	40272	47808	40055	40066	41144	41155	43300	45054	45552	46222	47728	48849	44909	45030	46335	47505	40101	40217	40448	40464	40787	48907	40900	40966		Total Tissues/ Tumors
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Squamous cell carcinoma, metastatic, uterus																											1
Transitional epithelium, carcinoma																											1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		53
Lymphoma malignant																											1

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

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

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

Number of Days on Study	7 1 7	7 2 1	7 2 2	7 2 3	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	5 9 7	5 1 8	6 3 3	6 1 2	5 1 3	5 1 6	5 8 1	5 8 3	6 0 8	6 1 3	6 1 5	6 1 7	6 3 1	6 3 2	5 0 6	5 8 7	5 9 6	6 0 1	6 1 8	5 1 5	5 2 0	6 0 3	6 3 5	6 3 7	6 4 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma, metastatic, pancreas																		X								2
Cholangiocarcinoma		X						X			X						X				X					8
Cholangiocarcinoma, multiple				X									X	X	X			X		X		X	X	X	X	17
Hepatocellular adenoma	X										X		X										X	X	X	10
Hepatocellular adenoma, multiple				X				X								X										3
Hepatocholangioma																						X				2
Mesentery										+					+					+						8
Oral mucosa	+					+	+			+	+	+		+	+				+			+				29
Gingival, squamous cell carcinoma														X					X							10
Pancreas	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Acinus, adenoma																	X									1
Acinus, carcinoma																		X								2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Squamous cell papilloma											X															1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Tooth		+				+			+			+			+	+		+		+		+				18
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Heart	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																										1
Pheochromocytoma benign																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Pheochromocytoma malignant																										1
Pheochromocytoma benign			X																							3
Islets, pancreatic	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Parathyroid gland	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Adenoma												X														1
Pars distalis, adenoma	X		X						X						X						X					10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Bilateral, C-cell, adenoma																						X				3
C-cell, adenoma										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 TCDD: 100 ng/kg

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

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

Number of Days on Study	7 1 7	7 2 1	7 2 2	7 2 3	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	5 9 7	5 1 8	6 3 3	6 1 2	5 1 3	5 1 6	5 8 1	5 8 3	6 0 8	6 1 3	6 1 5	6 1 7	6 3 1	6 3 2	5 0 6	5 8 7	5 9 6	6 0 1	6 1 8	5 1 5	5 2 0	6 0 3	6 3 5	6 3 7	6 4 1	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Oviduct													+													1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Carcinoma																										1
Polyp stromal			X						X										X			X				7
Polyp stromal, multiple																										1
Vagina																										1
Schwannoma malignant																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Lymph node								+										+				+				9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	51
Spleen	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	M	+	+	+	+	42
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Adenoma																								X		1
Fibroadenoma			X			X	X		X	X		X			X		X			X			X	X	20
Fibroadenoma, multiple								X			X															4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Fibroma	X																									1
Schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Respiratory System																										
Lung	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	52
Carcinoma, metastatic, pancreas																		X								1
Cystic keratinizing epithelioma																							X	X	X	7
Cystic keratinizing epithelioma, multiple																	X									2
Mediastinum, sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53
Squamous cell carcinoma, metastatic, oral mucosa																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	53

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

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

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

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

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 100 ng/kg (Stop-Exposure)

Number of Days on Study	2 1 5	3 4 5	3 9 4	4 1 0	4 7 4	4 8 4	5 2 0	5 3 7	5 7 8	5 7 8	5 7 8	5 8 4	6 0 4	6 1 4	6 2 9	6 4 1	6 4 3	6 4 5	6 4 5	6 5 3	6 5 6	6 7 3	7 0 2	7 0 2	7 1 8
Carcass ID Number	5 4 6	5 6 6	5 4 7	5 3 7	5 4 2	5 3 2	5 6 1	5 5 5	5 2 9	5 7 0	5 7 4	5 6 4	5 6 8	5 5 0	5 4 0	5 7 2	5 3 6	5 4 3	5 6 3	5 3 0	5 7 3	5 3 1	5 2 7	5 5 7	5 5 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin			X																						
Intestine large, cecum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, heart																								X	
Intestine small, ileum	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																									
Cholangiocarcinoma, multiple																									
Cholangioma																									
Fibrous histiocytoma, metastatic, skin			X																						
Hepatocellular adenoma																							X		
Schwannoma malignant, metastatic, heart																								X	
Mesentery	+																								
Oral mucosa		+					+	+				+					+				+		+	+	+
Gingival, squamous cell carcinoma							X	X				X									X		X		
Pancreas	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, carcinoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																							+		
Squamous cell carcinoma																							X		
Tooth		+				+	+				+	+					+						+		
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aorta, fibrous histiocytoma, metastatic, skin			X																						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland								X																	
Schwannoma malignant						X																		X	
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, schwannoma malignant, metastatic, heart																								X	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma complex																									
Pheochromocytoma benign																								X	
Islets, pancreatic	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma												X		X		X					X		X	X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma												X				X							X		X
C-cell, carcinoma																									

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 100 ng/kg (Stop-Exposure)

Number of Days on Study	7 2 4	7 2 6	7 2 6	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	5 6 0	5 3 3	5 4 1	5 5 9	5 2 8	5 3 4	5 3 5	5 3 8	5 5 3	5 5 4	5 5 6	5 6 2	5 6 5	5 6 9	5 3 9	5 4 4	5 4 5	5 4 9	5 5 8	5 5 7	5 2 6	5 4 8	5 5 1	5 6 7	5 7 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant, metastatic, heart																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma				X																						1
Cholangiocarcinoma, multiple																			X							1
Cholangioma																					X					1
Fibrous histiocytoma, metastatic, skin																										1
Hepatocellular adenoma		X																								2
Schwannoma malignant, metastatic, heart																										1
Mesentery																										1
Oral mucosa													+								+					11
Gingival, squamous cell carcinoma																										5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, carcinoma																									X	1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell carcinoma																										1
Tooth						+			+				+	+							+					12
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Aorta, fibrous histiocytoma, metastatic, skin																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																										1
Schwannoma malignant												X														3
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, schwannoma malignant, metastatic, heart																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma complex					X																					1
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X	X			X			X		X	X			X			X	X				X	X	19
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, C-cell, adenoma					X						X															2
C-cell, adenoma	X		X					X				X				X						X			X	11
C-cell, carcinoma						X																				1

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 100 ng/kg (Stop-Exposure)

Number of Days on Study	215	345	394	410	447	448	520	537	578	577	578	584	604	614	629	641	644	645	645	653	655	663	670	702	722	718
Carcass ID Number	546	566	547	537	542	532	561	555	529	570	574	566	568	550	540	572	626	543	546	530	573	531	577	552	557	552
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																										
Oviduct																	+									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																										
Polyp stromal																										
Polyp stromal, multiple																										
Squamous cell carcinoma																										
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin			X																							
Lymph node					+						+	+	+												+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin			X																							
Spleen	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin			X																							
Hemangiosarcoma																										
Schwannoma malignant, metastatic, heart																									X	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	
Fibrous histiocytoma, metastatic, skin			X																							
Thymoma malignant																										
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenolipoma									X																	
Carcinoma								X																		
Fibroadenoma		X		X	X				X	X	X						X			X						
Fibroadenoma, multiple													X			X		X	X			X	X		X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																										
Fibrous histiocytoma			X																							
Liposarcoma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, tongue																							X			
Skeletal muscle			+																							
Fibrous histiocytoma, metastatic, skin			X																							
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 100 ng/kg (Stop-Exposure)

Number of Days on Study	7 2 4	7 2 6	7 2 6	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	5 6 0	5 3 3	5 4 1	5 5 9	5 2 8	5 3 4	5 3 5	5 3 8	5 5 3	5 5 4	5 5 6	5 6 2	6 6 5	6 3 9	5 3 9	5 4 4	5 4 5	5 4 9	5 5 8	5 7 5	5 2 6	5 4 8	5 5 1	5 6 7	5 7 1	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, metastatic, uterus						X																				1
Oviduct																		+								2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma						X											X									2
Polyp stromal				X																	X			X		3
Polyp stromal, multiple																	X									1
Squamous cell carcinoma						X														X						2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Lymph node																										5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma, metastatic, skin																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma, metastatic, skin																										1
Hemangiosarcoma				X																						1
Schwannoma malignant, metastatic, heart																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma, metastatic, skin																										1
Thymoma malignant									X																	1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenolipoma																										1
Carcinoma																										1
Fibroadenoma	X	X		X			X		X			X		X			X	X				X		X		19
Fibroadenoma, multiple			X					X			X								X	X	X					13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma																									X	1
Fibrous histiocytoma																										1
Liposarcoma																		X								1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, tongue																										1
Skeletal muscle																										1
Fibrous histiocytoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 100 ng/kg (Stop-Exposure)

Number of Days on Study	2 1 5	3 4 5	3 9 4	4 1 0	4 7 4	4 8 4	5 2 0	5 3 7	5 7 8	5 7 8	5 7 8	5 8 4	6 0 4	6 1 4	6 2 9	6 4 1	6 4 3	6 4 5	6 4 5	6 5 3	6 5 6	6 7 3	7 0 2	7 0 2	7 1 8
Carcass ID Number	5 4 6	5 6 6	5 4 7	5 3 7	5 4 2	5 3 2	5 6 1	5 5 5	5 2 9	5 7 0	5 7 4	5 6 4	5 6 8	5 5 0	5 4 0	5 7 2	5 3 6	5 4 3	5 6 3	5 3 0	5 7 3	5 3 1	5 2 7	5 5 7	5 5 2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland								X																	
Fibrous histiocytoma, metastatic, skin			X																						
Schwannoma malignant, metastatic, heart																								X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin			X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenolipoma									X																
Lymphoma malignant		X																							

TABLE A2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of TCDD: 100 ng/kg (Stop-Exposure)

Number of Days on Study	724	726	726	729	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	733	733	733	733	733	
Carcass ID Number	56 0	53 3	54 1	55 9	52 8	53 4	53 5	53 8	55 3	55 4	55 6	56 2	56 5	56 9	53 9	54 4	54 5	54 9	55 8	57 5	52 6	54 8	55 1	56 7	57 1	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																										1
Fibrous histiocytoma, metastatic, skin																										1
Schwannoma malignant, metastatic, heart																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenolipoma																										1
Lymphoma malignant																										1

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Adrenal Medulla: Benign Pheochromocytoma						
Overall rate[a]	3/53 (6%)	2/54 (4%)	2/53 (4%)	3/53 (6%)	3/53 (6%)	3/53 (6%)e
Adjusted rate[b]	7.6%	5.8%	5.2%	8.8%	7.7%	7.0%
Terminal rate[c]	3/25 (12%)	2/21 (10%)	2/23 (9%)	3/19 (16%)	2/22 (9%)	0/21 (0%)
First incidence (days)	731 (T)	731 (T)	731 (T)	731 (T)	708	642
Poly-3 test[d]	P=0.542	P=0.561N	P=0.509N	P=0.594	P=0.658	P=0.622N
Liver: Cholangiocarcinoma						
Overall rate	0/53 (0%)	0/54 (0%)	0/53 (0%)	1/53 (2%)	4/53 (8%)	25/53 (47%)
Adjusted rate	0.0%	0.0%	0.0%	2.9%	10.3%	54.9%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	4/22 (18%)	13/21 (62%)
First incidence (days)	—[f]	—	—	592	731 (T)	610
Poly-3 test	P<0.001	—[g]	—	P=0.474	P=0.057	P<0.001
Liver: Hepatocellular Adenoma						
Overall rate	0/53 (0%)	0/54 (0%)	0/53 (0%)	0/53 (0%)	1/53 (2%)	13/53 (25%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	2.6%	29.9%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	7/21 (33%)
First incidence (days)	—	—	—	—	720	642
Poly-3 test	P<0.001	—	—	—	P=0.497	P<0.001
Lung: Cystic Keratinizing Epithelioma						
Overall rate	0/53 (0%)	0/54 (0%)	0/53 (0%)	0/52 (0%)	0/53 (0%)	9/52 (17%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	21.1%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	4/21 (19%)
First incidence (days)	—	—	—	—	—	610
Poly-3 test	P<0.001	—	—	—	—	P=0.002
Mammary Gland: Fibroadenoma						
Overall rate	33/53 (62%)	40/54 (74%)	34/53 (64%)	29/53 (55%)	36/53 (68%)	24/53 (45%)
Adjusted rate	71.0%	81.7%	74.2%	70.3%	74.5%	51.0%
Terminal rate	16/25 (64%)	16/21 (76%)	16/23 (70%)	12/19 (63%)	15/22 (68%)	12/21 (57%)
First incidence (days)	185	241	236	453	141	396
Poly-3 test	P<0.001N	P=0.147	P=0.452	P=0.570N	P=0.435	P=0.031N
Mammary Gland: Fibroadenoma or Adenoma						
Overall rate	35/53 (66%)	40/54 (74%)	35/53 (66%)	29/53 (55%)	36/53 (68%)	24/53 (45%)
Adjusted rate	74.7%	81.7%	76.4%	70.3%	74.5%	51.0%
Terminal rate	17/25 (68%)	16/21 (76%)	17/23 (74%)	12/19 (63%)	15/22 (68%)	12/21 (57%)
First incidence (days)	185	241	236	453	141	396
Poly-3 test	P<0.001N	P=0.269	P=0.522	P=0.407N	P=0.589N	P=0.010N
Mammary Gland: Carcinoma						
Overall rate	4/53 (8%)	8/54 (15%)	4/53 (8%)	2/53 (4%)	2/53 (4%)	0/53 (0%)
Adjusted rate	9.9%	21.6%	10.2%	5.7%	5.1%	0.0%
Terminal rate	2/25 (8%)	4/21 (19%)	1/23 (4%)	0/19 (0%)	1/22 (5%)	0/21 (0%)
First incidence (days)	469	241	659	338	673	—
Poly-3 test	P=0.004N	P=0.132	P=0.626	P=0.401N	P=0.350N	P=0.053N
Mammary Gland: Adenoma or Carcinoma						
Overall rate	6/53 (11%)	8/54 (15%)	5/53 (9%)	2/53 (4%)	2/53 (4%)	1/53 (2%)
Adjusted rate	14.7%	21.6%	12.8%	5.7%	5.1%	2.4%
Terminal rate	3/25 (12%)	4/21 (19%)	2/23 (9%)	0/19 (0%)	1/22 (5%)	1/21 (5%)
First incidence (days)	469	241	659	338	673	731 (T)
Poly-3 test	P=0.005N	P=0.310	P=0.530N	P=0.182N	P=0.143N	P=0.048N

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma						
Overall rate	35/53 (66%)	43/54 (80%)	37/53 (70%)	31/53 (58%)	37/53 (70%)	24/53 (45%)
Adjusted rate	74.7%	86.2%	80.2%	73.2%	76.2%	51.0%
Terminal rate	17/25 (68%)	17/21 (81%)	17/23 (74%)	12/19 (63%)	15/22 (68%)	12/21 (57%)
First incidence (days)	185	241	236	338	141	396
Poly-3 test	P<0.001N	P=0.107	P=0.343	P=0.533N	P=0.526	P=0.010N
Oral Mucosa: Squamous Cell Carcinoma						
Overall rate	1/53 (2%)	2/54 (4%)	1/53 (2%)	0/53 (0%)	4/53 (8%)	10/53 (19%)
Adjusted rate	2.5%	5.7%	2.6%	0.0%	10.2%	22.0%
Terminal rate	0/25 (0%)	1/21 (5%)	0/23 (0%)	0/19 (0%)	1/22 (5%)	2/21 (10%)
First incidence (days)	366	647	578	—	659	546
Poly-3 test	P<0.001	P=0.449	P=0.755	P=0.533N	P=0.169	P=0.007
Pancreas: Adenoma or Carcinoma						
Overall rate	0/51 (0%)	0/54 (0%)	0/52 (0%)	0/53 (0%)	0/52 (0%)	3/51 (6%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	7.3%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	2/21 (10%)
First incidence (days)	—	—	—	—	—	641
Poly-3 test	P<0.001	—	—	—	—	P=0.129
Pituitary Gland (Pars Distalis): Adenoma						
Overall rate	25/53 (47%)	20/54 (37%)	26/52 (50%)	15/53 (28%)	20/53 (38%)	11/52 (21%)
Adjusted rate	58.4%	55.3%	64.2%	41.2%	47.3%	25.0%
Terminal rate	15/25 (60%)	16/21 (76%)	15/23 (65%)	9/19 (47%)	10/22 (46%)	4/21 (19%)
First incidence (days)	474	604	550	545	444	604
Poly-3 test	P<0.001N	P=0.478N	P=0.369	P=0.087N	P=0.202N	P<0.001N
Skin: Fibroma, Myxosarcoma, or Fibrosarcoma						
Overall rate	1/53 (2%)	0/54 (0%)	3/53 (6%)	1/53 (2%)	0/53 (0%)	1/53 (2%)
Adjusted rate	2.5%	0.0%	7.5%	2.9%	0.0%	2.3%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	1/19 (5%)	0/22 (0%)	0/21 (0%)
First incidence (days)	548	—	578	731 (T)	—	717
Poly-3 test	P=0.439N	P=0.529N	P=0.303	P=0.724	P=0.506N	P=0.747N
Thyroid Gland (C-cell): Adenoma						
Overall rate	20/52 (38%)	15/54 (28%)	17/53 (32%)	16/51 (31%)	13/53 (25%)	11/52 (21%)
Adjusted rate	46.7%	40.4%	43.1%	43.2%	31.6%	25.6%
Terminal rate	10/25 (40%)	10/21 (48%)	13/23 (57%)	8/19 (42%)	8/22 (36%)	6/21 (29%)
First incidence (days)	474	462	626	338	486	638
Poly-3 test	P=0.010N	P=0.366N	P=0.459N	P=0.464N	P=0.111N	P=0.030N
Thyroid Gland (C-cell): Adenoma or Carcinoma						
Overall rate	21/52 (40%)	15/54 (28%)	17/53 (32%)	16/51 (31%)	14/53 (26%)	11/52 (21%)
Adjusted rate	49.0%	40.4%	43.1%	43.2%	34.0%	25.6%
Terminal rate	11/25 (44%)	10/21 (48%)	13/23 (57%)	8/19 (42%)	9/22 (41%)	6/21 (29%)
First incidence (days)	474	462	626	338	486	638
Poly-3 test	P=0.008N	P=0.288N	P=0.374N	P=0.380N	P=0.115N	P=0.017N
Uterus: Stromal Polyp						
Overall rate	11/53 (21%)	7/53 (13%)	4/53 (8%)	11/53 (21%)	10/53 (19%)	8/53 (15%)
Adjusted rate	27.4%	19.6%	10.3%	30.3%	24.7%	18.2%
Terminal rate	8/25 (32%)	5/21 (24%)	2/23 (9%)	6/19 (32%)	5/22 (23%)	3/21 (14%)
First incidence (days)	645	526	659	557	590	598
Poly-3 test	P=0.404N	P=0.296N	P=0.045N	P=0.490	P=0.492N	P=0.223N

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Uterus: Squamous Cell Carcinoma						
Overall rate	0/53 (0%)	0/53 (0%)	0/53 (0%)	0/53 (0%)	5/53 (9%)	0/53 (0%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	12.3%	0.0%
Terminal rate	0/25 (0%)	0/21 (0%)	0/23 (0%)	0/19 (0%)	0/22 (0%)	0/21 (0%)
First incidence (days)	—	—	—	—	589	—
Poly-3 test	P=0.337	—	—	—	P=0.032	—
All Organs: Benign Neoplasms						
Overall rate	45/53 (85%)	49/54 (91%)	45/53 (85%)	40/53 (75%)	46/53 (87%)	42/53 (79%)
Adjusted rate	92.2%	97.1%	95.6%	90.2%	92.9%	84.7%
Terminal rate	23/25 (92%)	21/21 (100%)	22/23 (96%)	17/19 (90%)	21/22 (96%)	18/21 (86%)
First incidence (days)	185	241	236	338	141	396
Poly-3 test	P=0.010N	P=0.232	P=0.383	P=0.511N	P=0.610	P=0.173N
All Organs: Malignant Neoplasms						
Overall rate	13/53 (25%)	19/54 (35%)	13/53 (25%)	11/53 (21%)	24/53 (45%)	34/53 (64%)
Adjusted rate	29.6%	45.3%	30.3%	28.3%	56.5%	69.9%
Terminal rate	4/25 (16%)	7/21 (33%)	3/23 (13%)	3/19 (16%)	12/22 (55%)	14/21 (67%)
First incidence (days)	169	200	240	338	518	546
Poly-3 test	P<0.001	P=0.093	P=0.566	P=0.547N	P=0.008	P<0.001
All Organs: Benign or Malignant Neoplasms						
Overall rate	47/53 (89%)	54/54 (100%)	48/53 (91%)	44/53 (83%)	50/53 (94%)	50/53 (94%)
Adjusted rate	92.7%	100.0%	97.7%	94.4%	97.9%	96.6%
Terminal rate	23/25 (92%)	21/21 (100%)	22/23 (96%)	18/19 (95%)	21/22 (96%)	20/21 (95%)
First incidence (days)	169	200	236	338	141	396
Poly-3 test	P=0.560	P=0.046	P=0.223	P=0.535	P=0.205	P=0.317

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

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the Stop-Exposure Evaluation of TCDD

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	3/53 (6%)	3/53 (6%)	1/50 (2%)
Adjusted rate[b]	7.6%	7.0%	2.6%
Terminal rate[c]	3/25 (12%)	0/21 (0%)	0/21 (0%)
First incidence (days)	731 (T)	642	702
Poly-3 test[d]		P=0.622N	P=0.319N
Poly-3 test[e]			P=0.361N
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rate	3/53 (6%)	3/53 (6%)	2/50 (4%)
Adjusted rate	7.6%	7.0%	5.2%
Terminal rate	3/25 (12%)	0/21 (0%)	1/21 (5%)
First incidence (days)	731 (T)	642	702
Poly-3 test		P=0.622N	P=0.518N
Poly-3 test			P=0.567N
Heart: Schwannoma Malignant			
Overall rate	0/53 (0%)	0/52 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	7.7%
Terminal rate	0/25 (0%)	0/21 (0%)	1/21 (5%)
First incidence (days)	—[f]	—[g]	484
Poly-3 test			P=0.116
Poly-3 test			P=0.110
Liver: Cholangiocarcinoma			
Overall rate	0/53 (0%)	25/53 (47%)	2/50 (4%)
Adjusted rate	0.0%	54.9%	5.2%
Terminal rate	0/25 (0%)	13/21 (62%)	1/21 (5%)
First incidence (days)	—	610	729
Poly-3 test		P<0.001	P=0.232
Poly-3 test			P<0.001N
Liver: Hepatocellular Adenoma			
Overall rate	0/53 (0%)	13/53 (25%)	2/50 (4%)
Adjusted rate	0.0%	29.9%	5.2%
Terminal rate	0/25 (0%)	7/21 (33%)	0/21 (0%)
First incidence (days)	—	642	702
Poly-3 test		P<0.001	P=0.233
Poly-3 test			P=0.004N
Lung: Cystic Keratinizing Epithelioma			
Overall rate	0/53 (0%)	9/52 (17%)	0/50 (0%)
Adjusted rate	0.0%	21.1%	0.0%
Terminal rate	0/25 (0%)	4/21 (19%)	0/21 (0%)
First incidence (days)	—	610	—
Poly-3 test		P=0.002	—
Poly-3 test			P=0.003N
Mammary Gland: Fibroadenoma			
Overall rate	33/53 (62%)	24/53 (45%)	32/50 (64%)
Adjusted rate	71.0%	51.0%	71.8%
Terminal rate	16/25 (64%)	12/21 (57%)	13/21 (62%)
First incidence (days)	185	396	345
Poly-3 test		P=0.031N	P=0.562
Poly-3 test			P=0.027

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the Stop-Exposure Evaluation of TCDD

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Mammary Gland: Fibroadenoma or Adenoma			
Overall rate	35/53 (66%)	24/53 (45%)	32/50 (64%)
Adjusted rate	74.7%	51.0%	71.8%
Terminal rate	17/25 (68%)	12/21 (57%)	13/21 (62%)
First incidence (days)	185	396	345
Poly-3 test		P=0.010N	P=0.470N
Poly-3 test			P=0.027
Mammary Gland: Carcinoma			
Overall rate	4/53 (8%)	0/53 (0%)	1/50 (2%)
Adjusted rate	9.9%	0.0%	2.6%
Terminal rate	2/25 (8%)	0/21 (0%)	0/21 (0%)
First incidence (days)	469	—	537
Poly-3 test		P=0.053N	P=0.192N
Poly-3 test			P=0.481
Mammary Gland: Adenoma or Carcinoma			
Overall rate	6/53 (11%)	1/53 (2%)	1/50 (2%)
Adjusted rate	14.7%	2.4%	2.6%
Terminal rate	3/25 (12%)	1/21 (5%)	0/21 (0%)
First incidence (days)	469	731 (T)	537
Poly-3 test		P=0.048N	P=0.064N
Poly-3 test			P=0.740
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma			
Overall rate	35/53 (66%)	24/53 (45%)	33/50 (66%)
Adjusted rate	74.7%	51.0%	73.0%
Terminal rate	17/25 (68%)	12/21 (57%)	13/21 (62%)
First incidence (days)	185	396	345
Poly-3 test		P=0.010N	P=0.525N
Poly-3 test			P=0.019
Oral Mucosa: Squamous Cell Carcinoma			
Overall rate	1/53 (2%)	10/53 (19%)	5/50 (10%)
Adjusted rate	2.5%	22.0%	12.4%
Terminal rate	0/25 (0%)	2/21 (10%)	0/21 (0%)
First incidence (days)	366	546	520
Poly-3 test		P=0.007	P=0.099
Poly-3 test			P=0.193N
Pancreas: Adenoma or Carcinoma			
Overall rate	0/51 (0%)	3/51 (6%)	1/49 (2%)
Adjusted rate	0.0%	7.3%	2.7%
Terminal rate	0/25 (0%)	2/21 (10%)	1/21 (5%)
First incidence (days)	—	641	731 (T)
Poly-3 test		P=0.129	P=0.494
Poly-3 test			P=0.345N
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma			
Overall rate	25/53 (47%)	11/52 (21%)	19/50 (38%)
Adjusted rate	58.4%	25.0%	47.5%
Terminal rate	15/25 (60%)	4/21 (19%)	9/21 (43%)
First incidence (days)	474	604	584
Poly-3 test		P<0.001N	P=0.213N
Poly-3 test			P=0.023

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the Stop-Exposure Evaluation of TCDD

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Thyroid Gland (C-cell): Adenoma			
Overall rate	20/52 (38%)	11/52 (21%)	13/49 (27%)
Adjusted rate	46.7%	25.6%	33.7%
Terminal rate	10/25 (40%)	6/21 (29%)	7/21 (33%)
First incidence (days)	474	638	584
Poly-3 test		P=0.030N	P=0.166N
Poly-3 test			P=0.288
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	21/52 (40%)	11/52 (21%)	14/49 (29%)
Adjusted rate	49.0%	25.6%	36.3%
Terminal rate	11/25 (44%)	6/21 (29%)	8/21 (38%)
First incidence (days)	474	638	584
Poly-3 test		P=0.017N	P=0.173N
Poly-3 test			P=0.208
Uterus: Squamous Cell Carcinoma			
Overall rate	0/53 (0%)	0/53 (0%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	5.2%
Terminal rate	0/25 (0%)	0/21 (0%)	2/21 (10%)
First incidence (days)	—	—	731 (T)
Poly-3 test		—	P=0.232
Poly-3 test			P=0.225
Uterus: Stromal Polyp			
Overall rate	11/53 (21%)	8/53 (15%)	4/50 (8%)
Adjusted rate	27.4%	18.2%	10.5%
Terminal rate	8/25 (32%)	3/21 (14%)	3/21 (14%)
First incidence (days)	645	598	729
Poly-3 test		P=0.223N	P=0.050N
Poly-3 test			P=0.259N
All Organs: Benign Neoplasms			
Overall rate	45/53 (85%)	42/53 (79%)	41/50 (82%)
Adjusted rate	92.2%	84.7%	89.4%
Terminal rate	23/25 (92%)	18/21 (86%)	18/21 (86%)
First incidence (days)	185	396	345
Poly-3 test		P=0.173N	P=0.455N
Poly-3 test			P=0.343
All Organs: Malignant Neoplasms			
Overall rate	13/53 (25%)	34/53 (64%)	19/50 (38%)
Adjusted rate	29.6%	69.9%	44.3%
Terminal rate	4/25 (16%)	14/21 (67%)	9/21 (43%)
First incidence (days)	169	546	345
Poly-3 test		P<0.001	P=0.110
Poly-3 test			P=0.008N

TABLE A3b
Statistical Analysis of Primary Neoplasms in Female Rats in the Stop-Exposure Evaluation of TCDD

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
All Organs: Benign or Malignant Neoplasms			
Overall rate	47/53 (89%)	50/53 (94%)	46/50 (92%)
Adjusted rate	92.7%	96.6%	94.5%
Terminal rate	23/25 (92%)	20/21 (95%)	19/21 (91%)
First incidence (days)	169	396	345
Poly-3 test		P=0.317	P=0.522
Poly-3 test			P=0.501N

(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, pancreas, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by N.
[e] Pairwise comparison between the 100 ng/kg core and stop-exposure groups. A lower incidence in the stop-exposure group is indicated by N.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

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

Study	Incidence in Controls			
	Cholangioma	Hepatocholangioma	Hepatocellular Adenoma	Cholangiocarcinoma
Historical Incidence				
PCB 126	0/53	0/53	1/53	0/53
PCB 126/PCB 118 Mixture	0/53	0/53	2/53	0/53
PCB 126/PCB 153 Binary Mixture	0/53	0/53	0/53	0/53
PCB 153	0/53	0/53	0/53	0/53
PeCDF	0/53	0/53	1/53	0/53
TCDD	0/53	0/53	0/53	0/53
TEF Mixture	0/53	0/53	0/53	0/53
Overall Historical Incidence				
Total (%)	0/371	0/371	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
PCB 126/PCB 118 Mixture	0/53
PCB 126/PCB 153 Binary Mixture	0/53
PCB 153	0/52
PeCDF	0/53
TCDD	0/53
TEF Mixture	0/53
Overall Historical Incidence	
Total	0/370

[a] Data as of February 24, 2005

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

Study	Incidence in Controls
Historical Incidence	
PCB 126	0/53
PCB 126/PCB 118 Mixture	1/53
PCB 126/PCB 153 Binary Mixture	0/53
PCB 153	0/53
PeCDF	1/53
TCDD	1/53
TEF Mixture	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 A4d
Historical Incidence of Squamous Cell Carcinoma in the Uterus of Vehicle Control Female Sprague-Dawley Rats[a]

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

[a] Data as of February 24, 2005

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

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

[a] Data as of February 24, 2005

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Disposition Summary						
Animals initially in study	28	28	28	28	28	28
14-Week interim evaluation	10	10	10	10	10	10
31-Week interim evaluation	10	10	10	10	10	10
53-Week interim evaluation	8	8	8	8	8	8
Animals examined microscopically	28	28	28	28	28	28
14-Week Interim Evaluation						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fatty change, diffuse						2 (20%)
Inflammation	10 (100%)	10 (100%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)
Mixed cell focus		2 (20%)		1 (10%)	1 (10%)	2 (20%)
Pigmentation	1 (10%)					1 (10%)
Hepatocyte, hypertrophy	1 (10%)	1 (10%)	4 (40%)	7 (70%)	10 (100%)	10 (100%)
Hepatocyte, multinucleated						3 (30%)
Pancreas	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active						2 (20%)
Acinus, atrophy						2 (20%)
Endocrine System						
Adrenal cortex	(10)	(10)	(10)	(10)	(10)	(10)
Hypertrophy	1 (10%)	2 (20%)	2 (20%)	1 (10%)	1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy		1 (10%)	3 (30%)	4 (40%)	4 (40%)	9 (90%)
Genital System						
Ovary	(10)					(10)
Atrophy	2 (20%)					1 (10%)
Uterus	(10)	(1)				(10)
Metaplasia, squamous	1 (10%)					
Endometrium, hyperplasia, cystic	5 (50%)					4 (40%)
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	10 (100%)					10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy		1 (10%)			4 (40%)	10 (100%)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic					1 (10%)	

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

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Systems Examined at 14 Weeks with No Lesions Observed						
Cardiovascular System						
General Body System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						
Urinary System						
31-Week Interim Evaluation						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Angiectasis		1 (10%)			1 (10%)	
Fatty change, diffuse						6 (60%)
Hepatodiaphragmatic nodule		1 (10%)				
Inflammation	9 (90%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Mitotic alteration	1 (10%)					
Mixed cell focus	1 (10%)	3 (30%)	3 (30%)	2 (20%)	3 (30%)	3 (30%)
Mixed cell focus, multiple	2 (20%)	4 (40%)	1 (10%)		1 (10%)	7 (70%)
Necrosis						1 (10%)
Pigmentation		1 (10%)	8 (80%)	9 (90%)	10 (100%)	10 (100%)
Toxic hepatopathy						10 (100%)
Bile duct, hyperplasia						1 (10%)
Hepatocyte, hypertrophy		2 (20%)	3 (30%)	6 (60%)	9 (90%)	10 (100%)
Hepatocyte, multinucleated					5 (50%)	9 (90%)
Oval cell, hyperplasia						1 (10%)
Pancreas	(10)	(10)	(10)	(10)	(10)	(10)
Acinus, atrophy	2 (20%)		1 (10%)		1 (10%)	
Acinus, vacuolization cytoplasmic						5 (50%)
Endocrine System						
Adrenal cortex	(10)	(10)	(10)	(10)	(10)	(10)
Degeneration, cystic				1 (10%)	1 (10%)	
Hyperplasia				1 (10%)		
Hypertrophy		2 (20%)	3 (30%)	3 (30%)	3 (30%)	3 (30%)
Necrosis				1 (10%)		
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Follicular cell, hypertrophy		3 (30%)	3 (30%)	3 (30%)	4 (40%)	6 (60%)
Genital System						
Ovary	(10)					(10)
Atrophy	9 (90%)					7 (70%)
Uterus	(10)	(1)				(10)
Inflammation, suppurative		1 (100%)				
Metaplasia, squamous	8 (80%)					4 (40%)
Endometrium, hyperplasia, cystic	1 (10%)	1 (100%)				2 (20%)
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	10 (100%)					10 (100%)

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Hematopoietic System (continued)						
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy	2 (20%)	1 (10%)	5 (50%)	6 (60%)	7 (70%)	10 (100%)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	2 (20%)	3 (30%)	1 (10%)	1 (10%)		1 (10%)
Systems Examined at 31 Weeks with No Lesions Observed						
Cardiovascular System						
General Body System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						
Urinary System						
53-Week Interim Evaluation						
Alimentary System						
Liver	(8)	(8)	(8)	(8)	(8)	(8)
Basophilic focus			1 (13%)			2 (25%)
Basophilic focus, multiple		1 (13%)				1 (13%)
Cholangiofibrosis					1 (13%)	1 (13%)
Clear cell focus	2 (25%)					
Fatty change, diffuse					2 (25%)	6 (75%)
Hepatodiaphragmatic nodule				1 (13%)		
Inflammation	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Mixed cell focus	2 (25%)	1 (13%)		2 (25%)	1 (13%)	
Mixed cell focus, multiple		4 (50%)		4 (50%)	6 (75%)	8 (100%)
Pigmentation			5 (63%)	8 (100%)	8 (100%)	8 (100%)
Toxic hepatopathy			1 (13%)		6 (75%)	8 (100%)
Bile duct, cyst			1 (13%)			
Bile duct, fibrosis					1 (13%)	2 (25%)
Bile duct, hyperplasia			1 (13%)	2 (25%)	3 (38%)	8 (100%)
Hepatocyte, hypertrophy		4 (50%)	7 (88%)	8 (100%)	8 (100%)	8 (100%)
Hepatocyte, multinucleated					2 (25%)	8 (100%)
Pancreas	(8)	(8)	(8)	(8)	(8)	(8)
Inflammation, chronic active						2 (25%)
Acinus, atrophy						2 (25%)
Acinus, vacuolization cytoplasmic						7 (88%)
Endocrine System						
Adrenal cortex	(8)	(8)	(8)	(8)	(8)	(8)
Degeneration, cystic	1 (13%)	1 (13%)	1 (13%)	2 (25%)		1 (13%)
Hyperplasia	1 (13%)				3 (38%)	2 (25%)
Hypertrophy	5 (63%)	3 (38%)	4 (50%)	4 (50%)	5 (63%)	7 (88%)
Vacuolization cytoplasmic		1 (13%)				
Pituitary gland	(8)		(1)			(7)
Cyst	1 (13%)					

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Endocrine System (continued)						
Thyroid gland	(8)	(8)	(8)	(8)	(8)	(8)
Follicular cell, hypertrophy		1 (13%)	2 (25%)	2 (25%)	3 (38%)	5 (63%)
Genital System						
Ovary	(8)					(7)
Atrophy	8 (100%)					5 (71%)
Uterus	(8)			(1)		(8)
Metaplasia, squamous	7 (88%)					4 (50%)
Cervix, cyst, squamous	1 (13%)					
Endometrium, hyperplasia, cystic	5 (63%)			1 (100%)		2 (25%)
Hematopoietic System						
Spleen	(8)					(8)
Hyperplasia, focal, lymphoid	1 (13%)					
Pigmentation	8 (100%)					8 (100%)
Thymus	(8)	(8)	(7)	(8)	(8)	(8)
Atrophy	4 (50%)	2 (25%)	3 (43%)	7 (88%)	8 (100%)	8 (100%)
Integumentary System						
Mammary gland	(8)	(2)	(2)			(8)
Cyst	3 (38%)					
Respiratory System						
Lung	(8)	(8)	(8)	(8)	(8)	(8)
Infiltration cellular, histiocyte	3 (38%)	1 (13%)	2 (25%)	1 (13%)	4 (50%)	3 (38%)
Urinary System						
Kidney		(2)				(1)
Cyst		1 (50%)				
Inflammation, chronic active		1 (50%)				
Nephropathy						1 (100%)
Pelvis, dilatation		1 (50%)				1 (100%)
Ureter		(1)				(1)
Mineralization						1 (100%)
Transitional epithelium, hyperplasia		1 (100%)				1 (100%)
Urinary bladder		(1)				
Inflammation		1 (100%)				
Transitional epithelium, hyperplasia		1 (100%)				

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

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Disposition Summary						
Animals initially in study	53	54	53	53	53	53
Early deaths						
Accidental deaths			5			
Moribund	19	27	15	19	17	17
Natural deaths	9	6	10	15	14	15
Survivors						
Terminal sacrifice	25	21	23	19	22	21
Animals examined microscopically	53	54	53	53	53	53
Alimentary System						
Esophagus	(52)	(54)	(53)	(52)	(53)	(53)
Hemorrhage			1 (2%)			
Perforation			2 (4%)			
Muscularis, inflammation	1 (2%)	2 (4%)	1 (2%)	1 (2%)	2 (4%)	
Periesophageal tissue, inflammation			3 (6%)			
Intestine large, colon	(52)	(53)	(53)	(53)	(53)	(51)
Parasite metazoan	1 (2%)	2 (4%)	1 (2%)			2 (4%)
Intestine large, rectum	(52)	(54)	(53)	(52)	(53)	(53)
Parasite metazoan	6 (12%)	4 (7%)			2 (4%)	1 (2%)
Intestine small, duodenum	(53)	(54)	(53)	(52)	(53)	(53)
Cyst						1 (2%)
Ulcer		1 (2%)				
Epithelium, hyperplasia			1 (2%)			
Liver	(53)	(54)	(53)	(53)	(53)	(53)
Angiectasis	2 (4%)	1 (2%)	1 (2%)		3 (6%)	5 (9%)
Basophilic focus	8 (15%)	6 (11%)	8 (15%)	7 (13%)	3 (6%)	3 (6%)
Basophilic focus, multiple	4 (8%)	3 (6%)	5 (9%)	1 (2%)	2 (4%)	5 (9%)
Cholangiofibrosis	1 (2%)	1 (2%)	2 (4%)	1 (2%)	11 (21%)	31 (58%)
Clear cell focus	4 (8%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Clear cell focus, multiple	1 (2%)			2 (4%)	1 (2%)	1 (2%)
Congestion	2 (4%)					2 (4%)
Cytoplasmic alteration						2 (4%)
Degeneration, cystic				2 (4%)		4 (8%)
Eosinophilic focus	8 (15%)	6 (11%)	7 (13%)	10 (19%)	5 (9%)	2 (4%)
Eosinophilic focus, multiple	3 (6%)	8 (15%)	14 (26%)	17 (32%)	22 (42%)	42 (79%)
Fatty change, diffuse		2 (4%)	12 (23%)	17 (32%)	30 (57%)	48 (91%)
Fatty change, focal	2 (4%)	6 (11%)	4 (8%)	4 (8%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	2 (4%)	4 (8%)	2 (4%)	2 (4%)
Hemorrhage					1 (2%)	
Hepatodiaphragmatic nodule		2 (4%)		1 (2%)	2 (4%)	
Hyperplasia, nodular				3 (6%)	7 (13%)	36 (68%)
Inflammation	33 (62%)	46 (85%)	47 (89%)	50 (94%)	52 (98%)	49 (92%)
Mitotic alteration				1 (2%)	1 (2%)	
Mixed cell focus	4 (8%)	8 (15%)	8 (15%)		2 (4%)	4 (8%)
Mixed cell focus, multiple	26 (49%)	11 (20%)	21 (40%)	23 (43%)	28 (53%)	17 (32%)
Necrosis	1 (2%)	4 (7%)	4 (8%)	8 (15%)	10 (19%)	17 (32%)
Pigmentation	4 (8%)	9 (17%)	34 (64%)	48 (91%)	52 (98%)	53 (100%)
Thrombosis						1 (2%)
Toxic hepatopathy		2 (4%)	8 (15%)	30 (57%)	45 (85%)	53 (100%)
Artery, inflammation, chronic active						2 (4%)

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

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Alimentary System (continued)						
Liver (continued)	(53)	(54)	(53)	(53)	(53)	(53)
Bile duct, cyst	3 (6%)	1 (2%)	2 (4%)	2 (4%)		21 (40%)
Bile duct, fibrosis		2 (4%)		1 (2%)	3 (6%)	4 (8%)
Bile duct, hyperplasia	5 (9%)	4 (7%)	7 (13%)	22 (42%)	40 (75%)	53 (100%)
Centrilobular, degeneration	2 (4%)	2 (4%)		4 (8%)	3 (6%)	5 (9%)
Hepatocyte, hypertrophy		19 (35%)	19 (36%)	42 (79%)	41 (77%)	52 (98%)
Hepatocyte, multinucleated			16 (30%)	26 (49%)	36 (68%)	51 (96%)
Hepatocyte, centrilobular, atrophy					1 (2%)	
Oval cell, hyperplasia		4 (7%)	3 (6%)	20 (38%)	38 (72%)	53 (100%)
Portal, fibrosis					5 (9%)	27 (51%)
Mesentery	(2)	(2)		(1)	(6)	(8)
Metaplasia, osseous	1 (50%)					
Artery, inflammation, chronic active		1 (50%)			4 (67%)	7 (88%)
Fat, necrosis		1 (50%)		1 (100%)	1 (17%)	1 (13%)
Vein, thrombosis	1 (50%)					
Oral mucosa	(2)	(9)	(16)	(16)	(22)	(29)
Gingival, hyperplasia, squamous	1 (50%)	7 (78%)	14 (88%)	13 (81%)	15 (68%)	16 (55%)
Pancreas	(51)	(54)	(52)	(53)	(52)	(51)
Hemorrhage						1 (2%)
Inflammation, acute				1 (2%)		
Inflammation, chronic active			2 (4%)	2 (4%)	3 (6%)	6 (12%)
Necrosis				1 (2%)		1 (2%)
Acinus, atrophy	1 (2%)	2 (4%)	4 (8%)	4 (8%)	4 (8%)	9 (18%)
Acinus, hyperplasia	2 (4%)			1 (2%)	2 (4%)	
Acinus, vacuolization cytoplasmic	1 (2%)			1 (2%)	15 (29%)	42 (82%)
Artery, inflammation, chronic active		1 (2%)	1 (2%)	2 (4%)	2 (4%)	7 (14%)
Salivary glands	(51)	(54)	(52)	(50)	(51)	(52)
Atrophy				2 (4%)	1 (2%)	
Inflammation			2 (4%)	3 (6%)		1 (2%)
Mineralization	1 (2%)					
Stomach, forestomach	(53)	(54)	(53)	(53)	(53)	(53)
Cyst, squamous			1 (2%)			
Edema				1 (2%)		1 (2%)
Erosion			1 (2%)		1 (2%)	
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Hyperplasia, squamous	3 (6%)	4 (7%)	4 (8%)	2 (4%)	7 (13%)	11 (21%)
Inflammation	1 (2%)	1 (2%)	2 (4%)		2 (4%)	5 (9%)
Mineralization			1 (2%)			
Necrosis						1 (2%)
Ulcer	2 (4%)	1 (2%)		1 (2%)	1 (2%)	4 (8%)
Stomach, glandular	(53)	(54)	(53)	(53)	(53)	(53)
Diverticulum			1 (2%)			
Erosion	1 (2%)					
Metaplasia					1 (2%)	
Mineralization	3 (6%)			1 (2%)	3 (6%)	
Ulcer						2 (4%)
Tooth	(16)	(16)	(12)	(13)	(14)	(18)
Peridontal tissue, fibrosis	1 (6%)					
Peridontal tissue, inflammation	15 (94%)	16 (100%)	12 (100%)	13 (100%)	14 (100%)	18 (100%)
Cardiovascular System						
Blood vessel	(53)	(54)	(53)	(53)	(53)	(53)
Thrombosis			1 (2%)			
Aorta, mineralization	1 (2%)	1 (2%)			1 (2%)	

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Cardiovascular System (continued)						
Heart	(53)	(54)	(53)	(52)	(53)	(52)
Cardiomyopathy	10 (19%)	12 (22%)	22 (42%)	25 (48%)	32 (60%)	36 (69%)
Mineralization	1 (2%)				1 (2%)	
Artery, inflammation						1 (2%)
Artery, mineralization		1 (2%)				
Artery, thrombosis						1 (2%)
Endocrine System						
Adrenal cortex	(53)	(54)	(53)	(53)	(53)	(53)
Angiectasis	11 (21%)	21 (39%)	18 (34%)	17 (32%)	17 (32%)	11 (21%)
Atrophy	2 (4%)		4 (8%)	5 (9%)	5 (9%)	27 (51%)
Degeneration, cystic	11 (21%)	15 (28%)	21 (40%)	18 (34%)	17 (32%)	17 (32%)
Hyperplasia	16 (30%)	16 (30%)	18 (34%)	25 (47%)	29 (55%)	30 (57%)
Hypertrophy	41 (77%)	43 (80%)	46 (87%)	40 (75%)	45 (85%)	47 (89%)
Inflammation, chronic active	1 (2%)					
Inflammation, suppurative	1 (2%)					
Mineralization						1 (2%)
Necrosis		1 (2%)	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Thrombosis				1 (2%)		
Vacuolization cytoplasmic	11 (21%)	7 (13%)	12 (23%)	21 (40%)	18 (34%)	15 (28%)
Adrenal medulla	(53)	(54)	(53)	(53)	(53)	(53)
Angiectasis		1 (2%)	2 (4%)	1 (2%)		
Hyperplasia	19 (36%)	10 (19%)	18 (34%)	7 (13%)	10 (19%)	9 (17%)
Inflammation, suppurative				1 (2%)		
Islets, pancreatic	(51)	(54)	(53)	(53)	(52)	(52)
Hyperplasia			1 (2%)	1 (2%)		
Parathyroid gland	(46)	(47)	(47)	(44)	(45)	(45)
Fibrosis			1 (2%)			
Hyperplasia	1 (2%)					
Pituitary gland	(53)	(54)	(52)	(53)	(53)	(52)
Angiectasis	25 (47%)	18 (33%)	25 (48%)	20 (38%)	21 (40%)	9 (17%)
Atypia cellular	1 (2%)					
Cyst	1 (2%)	1 (2%)				1 (2%)
Cytoplasmic alteration	1 (2%)		1 (2%)	4 (8%)	3 (6%)	
Developmental malformation	1 (2%)					
Inflammation, chronic						1 (2%)
Vacuolization cytoplasmic		3 (6%)	1 (2%)	2 (4%)	2 (4%)	
Pars distalis, hyperplasia	19 (36%)	19 (35%)	16 (31%)	21 (40%)	26 (49%)	19 (37%)
Pars intermedia, hyperplasia	2 (4%)				1 (2%)	1 (2%)
Thyroid gland	(52)	(54)	(53)	(51)	(53)	(52)
Angiectasis		1 (2%)	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Cyst	1 (2%)			1 (2%)		1 (2%)
Inflammation, chronic active			1 (2%)			
Inflammation, suppurative			1 (2%)			
C-Cell, hyperplasia	19 (37%)	17 (31%)	22 (42%)	19 (37%)	16 (30%)	23 (44%)
Follicular cell, hyperplasia	2 (4%)	2 (4%)				
Follicular cell, hypertrophy	3 (6%)	4 (7%)	4 (8%)	7 (14%)	10 (19%)	17 (33%)
General Body System						
None						

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Genital System						
Clitoral gland	(50)	(52)	(53)	(52)	(51)	(53)
Hyperplasia, basal cell	1 (2%)		1 (2%)			
Inflammation	41 (82%)	40 (77%)	35 (66%)	34 (65%)	28 (55%)	26 (49%)
Duct, cyst	34 (68%)	37 (71%)	41 (77%)	42 (81%)	41 (80%)	48 (91%)
Ovary	(51)	(53)	(53)	(53)	(53)	(53)
Atrophy	49 (96%)	46 (87%)	50 (94%)	44 (83%)	50 (94%)	31 (58%)
Congestion				1 (2%)		
Cyst	14 (27%)	16 (30%)	13 (25%)	15 (28%)	14 (26%)	13 (25%)
Fibrosis			1 (2%)		1 (2%)	
Inflammation, chronic active				1 (2%)		1 (2%)
Inflammation, suppurative				1 (2%)	1 (2%)	
Artery, inflammation, chronic active						1 (2%)
Corpus luteum, cyst		1 (2%)				
Periovarian tissue, inflammation, suppurative				1 (2%)		
Oviduct	(2)	(1)	(2)		(1)	(1)
Inflammation	2 (100%)	1 (100%)	1 (50%)			1 (100%)
Necrosis					1 (100%)	
Epithelium, hyperplasia			1 (50%)			
Uterus	(52)	(53)	(53)	(53)	(53)	(53)
Adenomyosis					1 (2%)	
Cyst						1 (2%)
Hemorrhage	1 (2%)					1 (2%)
Inflammation, chronic active		2 (4%)	3 (6%)		3 (6%)	1 (2%)
Inflammation, suppurative	7 (13%)	6 (11%)	12 (23%)	9 (17%)	11 (21%)	5 (9%)
Metaplasia, squamous	29 (56%)	31 (58%)	28 (53%)	23 (43%)	32 (60%)	17 (32%)
Necrosis				1 (2%)		
Ulcer			1 (2%)			
Cervix, hyperplasia, stromal		1 (2%)	1 (2%)	1 (2%)	1 (2%)	
Cervix, inflammation, suppurative					1 (2%)	
Endometrium, adenomyosis			1 (2%)			
Endometrium, hyperplasia, cystic	30 (58%)	33 (62%)	35 (66%)	27 (51%)	31 (58%)	19 (36%)
Epithelium, hyperplasia				1 (2%)		
Serosa, inflammation, chronic active			1 (2%)			
Vagina		(1)	(1)	(3)	(3)	(1)
Cyst			1 (100%)		1 (33%)	
Hyperplasia, stromal		1 (100%)				
Inflammation				2 (67%)		
Necrosis				1 (33%)		
Hematopoietic System						
Bone marrow	(53)	(54)	(53)	(53)	(53)	(53)
Atrophy						1 (2%)
Hyperplasia	36 (68%)	41 (76%)	32 (60%)	35 (66%)	37 (70%)	43 (81%)
Lymph node	(2)	(6)	(3)	(5)	(6)	(9)
Deep cervical, hemorrhage				1 (20%)		
Inguinal, ectasia					1 (17%)	
Lumbar, ectasia	1 (50%)	2 (33%)	1 (33%)	2 (40%)		
Lumbar, hemorrhage				1 (20%)		
Lumbar, hyperplasia, plasma cell		2 (33%)		1 (20%)		
Lumbar, pigmentation				1 (20%)		
Mediastinal, congestion					1 (17%)	
Mediastinal, ectasia					1 (17%)	3 (33%)
Mediastinal, hemorrhage		1 (17%)		1 (20%)		5 (56%)
Mediastinal, hyperplasia					1 (17%)	
Mediastinal, hyperplasia, histiocytic			1 (33%)		1 (17%)	1 (11%)

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Hematopoietic System (continued)						
Bone marrow (continued)	(53)	(54)	(53)	(53)	(53)	(53)
Mediastinal, hyperplasia, lymphoid		1 (17%)				
Mediastinal, hyperplasia, plasma cell				1 (20%)		
Mediastinal, pigmentation			1 (33%)			1 (11%)
Renal, ectasia		1 (17%)				
Renal, hyperplasia, histiocytic		1 (17%)				
Lymph node, mandibular	(51)	(54)	(52)	(50)	(51)	(52)
Congestion	1 (2%)					
Ectasia					1 (2%)	6 (12%)
Hyperplasia, lymphoid		3 (6%)	3 (6%)			2 (4%)
Hyperplasia, plasma cell	25 (49%)	31 (57%)	23 (44%)	22 (44%)	15 (29%)	16 (31%)
Inflammation, suppurative						1 (2%)
Lymph node, mesenteric	(52)	(53)	(53)	(53)	(53)	(51)
Atrophy		1 (2%)		1 (2%)		
Ectasia						1 (2%)
Hemorrhage					1 (2%)	
Hyperplasia, histiocytic	1 (2%)				1 (2%)	2 (4%)
Hyperplasia, plasma cell					1 (2%)	
Spleen	(51)	(54)	(53)	(53)	(52)	(52)
Hematopoietic cell proliferation	46 (90%)	50 (93%)	38 (72%)	42 (79%)	44 (85%)	43 (83%)
Hyperplasia, lymphoid	1 (2%)					4 (8%)
Necrosis			1 (2%)			
Pigmentation	45 (88%)	49 (91%)	49 (92%)	51 (96%)	49 (94%)	47 (90%)
Lymphoid follicle, atrophy	4 (8%)		1 (2%)		1 (2%)	1 (2%)
Thymus	(51)	(52)	(52)	(49)	(46)	(42)
Atrophy	36 (71%)	41 (79%)	44 (85%)	41 (84%)	44 (96%)	42 (100%)
Cyst					1 (2%)	1 (2%)
Hemorrhage	1 (2%)		1 (2%)			
Inflammation, chronic active					1 (2%)	
Integumentary System						
Mammary gland	(53)	(54)	(53)	(53)	(53)	(53)
Cyst	4 (8%)	3 (6%)		1 (2%)	1 (2%)	1 (2%)
Hyperplasia	25 (47%)	21 (39%)	24 (45%)	22 (42%)	18 (34%)	16 (30%)
Inflammation, chronic active					1 (2%)	
Inflammation, granulomatous	1 (2%)	1 (2%)	1 (2%)			1 (2%)
Skin	(53)	(54)	(53)	(53)	(53)	(53)
Cyst epithelial inclusion	1 (2%)					2 (4%)
Hemorrhage			1 (2%)			
Hyperplasia, squamous			1 (2%)		1 (2%)	
Inflammation, chronic active			2 (4%)			1 (2%)
Necrosis			1 (2%)			
Ulcer			1 (2%)		1 (2%)	
Epidermis, inflammation						1 (2%)
Musculoskeletal System						
Bone	(53)	(54)	(53)	(53)	(53)	(53)
Fracture						1 (2%)
Maxilla, inflammation, focal, suppurative		1 (2%)				
Skeletal muscle			(1)	(2)		
Hemorrhage				1 (50%)		
Inflammation, chronic active				1 (50%)		

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Nervous System						
Brain	(53)	(54)	(53)	(53)	(53)	(53)
Hemorrhage			1 (2%)			
Hydrocephalus		1 (2%)	1 (2%)			
Inflammation, suppurative					1 (2%)	
Mineralization		1 (2%)	1 (2%)		2 (4%)	2 (4%)
Vacuolization cytoplasmic, focal			1 (2%)			
Artery, thrombosis					1 (2%)	
Cerebellum, developmental malformation	1 (2%)					
Cerebellum, necrosis			1 (2%)			
Glial cell, hyperplasia		1 (2%)				1 (2%)
Spinal cord			(1)			
Gliosis			1 (100%)			
Respiratory System						
Lung	(53)	(54)	(53)	(52)	(53)	(52)
Congestion			2 (4%)			
Edema			2 (4%)		1 (2%)	
Hemorrhage		1 (2%)				2 (4%)
Infiltration cellular, histiocyte	41 (77%)	46 (85%)	43 (81%)	43 (83%)	49 (92%)	50 (96%)
Inflammation, chronic active						1 (2%)
Inflammation, granulomatous	2 (4%)	1 (2%)	2 (4%)	2 (4%)		
Inflammation, suppurative	1 (2%)			2 (4%)		
Metaplasia, squamous	1 (2%)				5 (9%)	6 (12%)
Mineralization	1 (2%)				1 (2%)	1 (2%)
Necrosis				1 (2%)		1 (2%)
Pigmentation		1 (2%)				
Alveolar epithelium, hyperplasia	12 (23%)					
Alveolar epithelium, metaplasia, bronchiolar	2 (4%)	19 (35%)	33 (62%)	35 (67%)	45 (85%)	46 (88%)
Bronchiole, dilatation			1 (2%)			
Serosa, inflammation, focal, suppurative				1 (2%)		
Nose	(53)	(54)	(53)	(53)	(53)	(53)
Cyst, squamous					1 (2%)	
Inflammation	2 (4%)			3 (6%)	5 (9%)	4 (8%)
Goblet cell, septum, hyperplasia					1 (2%)	
Nasolacrimal duct, inflammation	2 (4%)					
Nasolacrimal duct, respiratory epithelium, hyperplasia					1 (2%)	
Nasopharyngeal duct, inflammation				1 (2%)		1 (2%)
Nasopharyngeal duct, necrosis				1 (2%)		
Olfactory epithelium, degeneration					1 (2%)	
Olfactory epithelium, inflammation					1 (2%)	1 (2%)
Respiratory epithelium, cyst					2 (4%)	
Respiratory epithelium, hyperplasia				1 (2%)	2 (4%)	
Septum, hyperplasia, squamous						1 (2%)
Septum, inflammation						2 (4%)
Squamous epithelium, inflammation, suppurative	1 (2%)					
Turbinate, hyperplasia, squamous						1 (2%)
Turbinate, septum, inflammation			1 (2%)	1 (2%)		2 (4%)
Turbinate, respiratory epithelium, hyperplasia			1 (2%)	1 (2%)		

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

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
Special Senses System						
Eye	(53)	(54)	(53)	(52)	(53)	(53)
Degeneration	1 (2%)					
Hemorrhage	1 (2%)					
Anterior chamber, inflammation, suppurative						1 (2%)
Cornea, inflammation, suppurative			1 (2%)			1 (2%)
Lens, degeneration					1 (2%)	
Retina, atrophy	5 (9%)	5 (9%)	5 (9%)	3 (6%)	4 (8%)	6 (11%)
Retina, degeneration					1 (2%)	
Harderian gland	(53)	(54)	(53)	(52)	(53)	(53)
Atrophy, focal	1 (2%)					
Inflammation	12 (23%)	3 (6%)	2 (4%)	2 (4%)	8 (15%)	6 (11%)
Urinary System						
Kidney	(53)	(54)	(53)	(53)	(53)	(53)
Calculus gross observation						1 (2%)
Calculus microscopic observation only	1 (2%)	1 (2%)	1 (2%)	3 (6%)	2 (4%)	
Casts protein	3 (6%)	5 (9%)	2 (4%)		1 (2%)	1 (2%)
Cyst	2 (4%)				1 (2%)	1 (2%)
Fibrosis		1 (2%)				
Infarct			1 (2%)			
Inflammation, chronic active	5 (9%)	1 (2%)		1 (2%)	1 (2%)	
Inflammation, suppurative			2 (4%)	1 (2%)	1 (2%)	2 (4%)
Mineralization	40 (75%)	39 (72%)	30 (57%)	32 (60%)	42 (79%)	42 (79%)
Necrosis				1 (2%)	1 (2%)	
Nephropathy	34 (64%)	26 (48%)	32 (60%)	36 (68%)	39 (74%)	52 (98%)
Papilla, necrosis			1 (2%)			
Pelvis, dilatation	2 (4%)	1 (2%)	1 (2%)		2 (4%)	
Pelvis, inflammation	2 (4%)	1 (2%)	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Renal tubule, degeneration						1 (2%)
Transitional epithelium, hyperplasia	3 (6%)	6 (11%)	8 (15%)	8 (15%)	11 (21%)	11 (21%)
Ureter	(1)					
Inflammation	1 (100%)					
Transitional epithelium, hyperplasia	1 (100%)					
Urinary bladder	(52)	(53)	(53)	(52)	(53)	(53)
Calculus microscopic observation only		1 (2%)				
Hemorrhage		1 (2%)				
Inflammation	6 (12%)	10 (19%)	4 (8%)		8 (15%)	3 (6%)
Metaplasia, squamous						1 (2%)
Transitional epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)		6 (11%)	2 (4%)

TABLE A5c
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)[a]

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Disposition Summary			
Animals initially in study	53	53	50
Early deaths			
Moribund	19	17	16
Natural deaths	9	15	13
Survivors			
Terminal sacrifice	25	21	21
Animals examined microscopically	53	53	50
Alimentary System			
Esophagus	(52)	(53)	(50)
Muscularis, inflammation	1 (2%)		
Intestine large, colon	(52)	(51)	(49)
Parasite metazoan	1 (2%)	2 (4%)	1 (2%)
Intestine large, rectum	(52)	(53)	(50)
Parasite metazoan	6 (12%)	1 (2%)	5 (10%)
Intestine small, jejunum	(51)	(52)	(50)
Inflammation, chronic			1 (2%)
Intestine small, duodenum	(53)	(53)	(49)
Cyst		1 (2%)	
Liver	(53)	(53)	(50)
Angiectasis	2 (4%)	5 (9%)	4 (8%)
Atypia cellular			1 (2%)
Basophilic focus	8 (15%)	3 (6%)	7 (14%)
Basophilic focus, multiple	4 (8%)	5 (9%)	9 (18%)
Cholangiofibrosis	1 (2%)	31 (58%)	1 (2%)
Clear cell focus	4 (8%)	1 (2%)	6 (12%)
Clear cell focus, multiple	1 (2%)	1 (2%)	2 (4%)
Congestion	2 (4%)	2 (4%)	
Cytoplasmic alteration		2 (4%)	1 (2%)
Degeneration, cystic		4 (8%)	
Eosinophilic focus	8 (15%)	2 (4%)	6 (12%)
Eosinophilic focus, multiple	3 (6%)	42 (79%)	21 (42%)
Fatty change, diffuse		48 (91%)	10 (20%)
Fatty change, focal	2 (4%)	2 (4%)	8 (16%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	2 (4%)
Hepatodiaphragmatic nodule			1 (2%)
Hyperplasia, nodular		36 (68%)	
Inflammation	33 (62%)	49 (92%)	43 (86%)
Mixed cell focus	4 (8%)	4 (8%)	1 (2%)
Mixed cell focus, multiple	26 (49%)	17 (32%)	28 (56%)
Necrosis	1 (2%)	17 (32%)	8 (16%)
Pigmentation	4 (8%)	53 (100%)	45 (90%)
Thrombosis		1 (2%)	
Toxic hepatopathy		53 (100%)	16 (32%)
Vacuolization cytoplasmic			1 (2%)
Artery, inflammation, chronic active		2 (4%)	
Bile duct, cyst	3 (6%)	21 (40%)	6 (12%)
Bile duct, fibrosis		4 (8%)	5 (10%)
Bile duct, hyperplasia	5 (9%)	53 (100%)	7 (14%)
Centrilobular, degeneration	2 (4%)	5 (9%)	
Hepatocyte, hypertrophy		52 (98%)	22 (44%)

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

TABLE A5c
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Alimentary System (continued)			
Liver (continued)	(53)	(53)	(50)
Hepatocyte, multinucleated		51 (96%)	32 (64%)
Oval cell, hyperplasia		53 (100%)	1 (2%)
Portal, fibrosis		27 (51%)	1 (2%)
Serosa, inflammation, chronic active			1 (2%)
Mesentery	(2)	(8)	(1)
Metaplasia, osseous	1 (50%)		
Artery, inflammation, chronic active		7 (88%)	1 (100%)
Fat, necrosis		1 (13%)	
Vein, thrombosis	1 (50%)		
Oral mucosa	(2)	(29)	(11)
Gingival, hyperplasia, squamous	1 (50%)	16 (55%)	8 (73%)
Pancreas	(51)	(51)	(49)
Angiectasis			1 (2%)
Basophilic focus			1 (2%)
Hemorrhage		1 (2%)	
Inflammation, chronic active		6 (12%)	4 (8%)
Necrosis		1 (2%)	
Acinus, atrophy	1 (2%)	9 (18%)	4 (8%)
Acinus, hyperplasia	2 (4%)		
Acinus, vacuolization cytoplasmic	1 (2%)	42 (82%)	
Artery, inflammation, chronic active		7 (14%)	2 (4%)
Salivary glands	(51)	(52)	(49)
Atrophy			2 (4%)
Fibrosis			1 (2%)
Inflammation		1 (2%)	1 (2%)
Mineralization	1 (2%)		
Stomach, forestomach	(53)	(53)	(50)
Edema		1 (2%)	
Hyperkeratosis	1 (2%)	3 (6%)	1 (2%)
Hyperplasia, squamous	3 (6%)	11 (21%)	5 (10%)
Inflammation	1 (2%)	5 (9%)	4 (8%)
Necrosis		1 (2%)	
Ulcer	2 (4%)	4 (8%)	4 (8%)
Stomach, glandular	(53)	(53)	(50)
Erosion	1 (2%)		1 (2%)
Mineralization	3 (6%)		
Ulcer		2 (4%)	
Tongue			(1)
Inflammation, chronic active			1 (100%)
Tooth	(16)	(18)	(12)
Peridontal tissue, fibrosis	1 (6%)		
Peridontal tissue, inflammation	15 (94%)	18 (100%)	12 (100%)
Cardiovascular System			
Blood vessel	(53)	(53)	(49)
Aorta, mineralization	1 (2%)		
Heart	(53)	(52)	(50)
Cardiomyopathy	10 (19%)	36 (69%)	22 (44%)
Mineralization	1 (2%)		
Inflammation			1 (2%)
Atrium, thrombosis			2 (4%)
Epicardium, inflammation, chronic active			1 (2%)
Artery, inflammation		1 (2%)	
Artery, thrombosis		1 (2%)	

TABLE A5c
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Endocrine System			
Adrenal cortex	(53)	(53)	(50)
Angiectasis	11 (21%)	11 (21%)	15 (30%)
Atrophy	2 (4%)	27 (51%)	4 (8%)
Degeneration, cystic	11 (21%)	17 (32%)	17 (34%)
Hyperplasia	16 (30%)	30 (57%)	20 (40%)
Hypertrophy	41 (77%)	47 (89%)	46 (92%)
Inflammation, chronic active	1 (2%)		
Inflammation, suppurative	1 (2%)		
Mineralization		1 (2%)	
Necrosis		1 (2%)	3 (6%)
Vacuolization cytoplasmic	11 (21%)	15 (28%)	13 (26%)
Adrenal medulla	(53)	(53)	(50)
Hyperplasia	19 (36%)	9 (17%)	15 (30%)
Islets, pancreatic	(51)	(52)	(49)
Hyperplasia			1 (2%)
Parathyroid gland	(46)	(45)	(44)
Hyperplasia	1 (2%)		
Pituitary gland	(53)	(52)	(50)
Angiectasis	25 (47%)	9 (17%)	15 (30%)
Atypia cellular	1 (2%)		
Cyst	1 (2%)	1 (2%)	
Cytoplasmic alteration	1 (2%)		1 (2%)
Developmental malformation	1 (2%)		
Inflammation, chronic		1 (2%)	
Vacuolization cytoplasmic			3 (6%)
Pars distalis, hyperplasia	19 (36%)	19 (37%)	18 (36%)
Pars intermedia, hyperplasia	2 (4%)	1 (2%)	
Thyroid gland	(52)	(52)	(49)
Angiectasis		1 (2%)	1 (2%)
Cyst	1 (2%)	1 (2%)	
C-Cell, hyperplasia	19 (37%)	23 (44%)	15 (31%)
Follicular cell, hyperplasia	2 (4%)		
Follicular cell, hypertrophy	3 (6%)	17 (33%)	6 (12%)
General Body System			
None			
Genital System			
Clitoral gland	(50)	(53)	(49)
Atrophy			1 (2%)
Hyperplasia, basal cell	1 (2%)		1 (2%)
Hyperplasia, squamous			1 (2%)
Inflammation	41 (82%)	26 (49%)	35 (71%)
Duct, cyst	34 (68%)	48 (91%)	35 (71%)
Ovary	(51)	(53)	(49)
Atrophy	49 (96%)	31 (58%)	45 (92%)
Cyst	14 (27%)	13 (25%)	16 (33%)
Inflammation, chronic active		1 (2%)	
Inflammation, granulomatous			1 (2%)
Artery, inflammation, chronic active		1 (2%)	
Oviduct	(2)	(1)	(2)
Inflammation	2 (100%)	1 (100%)	2 (100%)

TABLE A5c
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Genital System (continued)			
Uterus	(52)	(53)	(50)
Cyst		1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	
Inflammation, chronic active		1 (2%)	1 (2%)
Inflammation, suppurative	7 (13%)	5 (9%)	9 (18%)
Metaplasia, squamous	29 (56%)	17 (32%)	33 (66%)
Cervix, inflammation, chronic active			1 (2%)
Endometrium, hyperplasia, cystic	30 (58%)	19 (36%)	32 (64%)
Hematopoietic System			
Bone marrow	(53)	(53)	(50)
Atrophy		1 (2%)	1 (2%)
Hyperplasia	36 (68%)	43 (81%)	36 (72%)
Myelofibrosis			1 (2%)
Lymph node	(2)	(9)	(5)
Lumbar, ectasia	1 (50%)		2 (40%)
Lumbar, hyperplasia, plasma cell			2 (40%)
Mediastinal, ectasia		3 (33%)	
Mediastinal, hemorrhage		5 (56%)	1 (20%)
Mediastinal, hyperplasia, histiocytic		1 (11%)	
Mediastinal, hyperplasia, plasma cell			1 (20%)
Mediastinal, pigmentation		1 (11%)	
Lymph node, mandibular	(51)	(52)	(49)
Congestion	1 (2%)		
Ectasia		6 (12%)	2 (4%)
Hyperplasia, lymphoid		2 (4%)	2 (4%)
Hyperplasia, plasma cell	25 (49%)	16 (31%)	30 (61%)
Inflammation, suppurative		1 (2%)	
Lymph node, mesenteric	(52)	(51)	(49)
Ectasia		1 (2%)	
Hyperplasia, histiocytic	1 (2%)	2 (4%)	1 (2%)
Spleen	(51)	(52)	(49)
Hematopoietic cell proliferation	46 (90%)	43 (83%)	42 (86%)
Hyperplasia, lymphoid	1 (2%)	4 (8%)	1 (2%)
Pigmentation	45 (88%)	47 (90%)	42 (86%)
Lymphoid follicle, atrophy	4 (8%)	1 (2%)	1 (2%)
Red pulp, atrophy			1 (2%)
Thymus	(51)	(42)	(49)
Atrophy	36 (71%)	42 (100%)	45 (92%)
Cyst		1 (2%)	
Hemorrhage	1 (2%)		
Integumentary System			
Mammary gland	(53)	(53)	(50)
Cyst	4 (8%)	1 (2%)	4 (8%)
Hyperplasia	25 (47%)	16 (30%)	19 (38%)
Inflammation, granulomatous	1 (2%)	1 (2%)	
Skin	(53)	(53)	(50)
Cyst epithelial inclusion	1 (2%)	2 (4%)	
Inflammation, chronic active		1 (2%)	
Ulcer			1 (2%)
Epidermis, inflammation		1 (2%)	

TABLE A5c
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Musculoskeletal System			
Bone	(53)	(53)	(50)
Fracture		1 (2%)	
Nervous System			
Brain	(53)	(53)	(50)
Hemorrhage			1 (2%)
Inflammation, suppurative			1 (2%)
Mineralization		2 (4%)	
Cerebellum, developmental malformation	1 (2%)		
Glial cell, hyperplasia		1 (2%)	
Respiratory System			
Lung	(53)	(52)	(50)
Hemorrhage		2 (4%)	
Infiltration cellular, histiocyte	41 (77%)	50 (96%)	41 (82%)
Inflammation, chronic active		1 (2%)	
Inflammation, granulomatous	2 (4%)		
Inflammation, suppurative	1 (2%)		1 (2%)
Metaplasia, squamous	1 (2%)	6 (12%)	3 (6%)
Mineralization	1 (2%)	1 (2%)	
Necrosis		1 (2%)	
Alveolar epithelium, hyperplasia	12 (23%)		
Alveolar epithelium, metaplasia, bronchiolar	2 (4%)	46 (88%)	31 (62%)
Serosa, inflammation, suppurative			1 (2%)
Nose	(53)	(53)	(50)
Inflammation	2 (4%)	4 (8%)	1 (2%)
Goblet cell, respiratory epithelium, hyperplasia			1 (2%)
Nasolacrimal duct, inflammation	2 (4%)		1 (2%)
Nasopharyngeal duct, inflammation		1 (2%)	
Olfactory epithelium, degeneration			1 (2%)
Olfactory epithelium, inflammation		1 (2%)	1 (2%)
Olfactory epithelium, glands, hyperplasia			1 (2%)
Respiratory epithelium, cyst			1 (2%)
Respiratory epithelium, inflammation, suppurative			1 (2%)
Septum, hyperplasia, squamous		1 (2%)	
Septum, inflammation		2 (4%)	
Squamous epithelium, inflammation, suppurative	1 (2%)		
Turbinate, hyperplasia, squamous		1 (2%)	
Turbinate, septum, inflammation		2 (4%)	
Special Senses System			
Eye	(53)	(53)	(50)
Degeneration	1 (2%)		
Hemorrhage	1 (2%)		
Anterior chamber, inflammation, suppurative		1 (2%)	
Cornea, inflammation, suppurative		1 (2%)	
Retinal detachment			1 (2%)
Bilateral, cataract			1 (2%)
Retina, atrophy	5 (9%)	6 (11%)	6 (12%)
Retina, degeneration			1 (2%)

TABLE A5c
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCDD (Stop-Exposure)

	Vehicle Control	100 ng/kg	100 ng/kg (Stop-Exposure)
Special Senses System (continued)			
Harderian gland	(53)	(53)	(50)
Atrophy, focal	1 (2%)		
Hemorrhage			1 (2%)
Inflammation	12 (23%)	6 (11%)	9 (18%)
Urinary System			
Kidney	(53)	(53)	(50)
Accumulation, hyaline droplet			2 (4%)
Calculus gross observation		1 (2%)	
Calculus microscopic observation only	1 (2%)		
Casts protein	3 (6%)	1 (2%)	1 (2%)
Cyst	2 (4%)	1 (2%)	
Inflammation, chronic active	5 (9%)		
Inflammation, suppurative		2 (4%)	
Mineralization	40 (75%)	42 (79%)	42 (84%)
Nephropathy	34 (64%)	52 (98%)	41 (82%)
Papilla, transitional epithelium, hyperplasia			1 (2%)
Pelvis, dilatation	2 (4%)		1 (2%)
Pelvis, inflammation	2 (4%)	2 (4%)	
Renal tubule, degeneration		1 (2%)	
Transitional epithelium, hyperplasia	3 (6%)	11 (21%)	5 (10%)
Ureter	(1)		
Inflammation	1 (100%)		
Transitional epithelium, hyperplasia	1 (100%)		
Urinary bladder	(52)	(53)	(50)
Inflammation	6 (12%)	3 (6%)	2 (4%)
Metaplasia, squamous		1 (2%)	
Transitional epithelium, hyperplasia	1 (2%)	2 (4%)	

APPENDIX B
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1984). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of TCDD. The high dose was limited by toxicity. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by McGregor *et al.* (1991). TCDD was supplied as a coded aliquot by Radian Corporation. The high dose of 1 µg/mL was determined by toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring cells resistant to trifluorothymidine (TFT), subcultures were exposed to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day; to medium containing thymidine, hypoxanthine, and glycine for 1 day; and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with TCDD continued for 4 hours, at which time the medium plus TCDD was removed, and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells, and cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed without S9. Because a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced male F344 rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented by Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for TCDD to be considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a call of "questionable," and the absence of both a trend and peak response resulted in a "negative" call.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). TCDD was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of three doses of TCDD; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 25.5 hours with TCDD in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 25.5 hours, the medium containing TCDD was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with TCDD, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no TCDD. Incubation proceeded for an additional 25.5 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind, and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.,* 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with TCDD for 19.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with TCDD and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 8.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.,* 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assay for induction of sex-linked recessive lethal (SLRL) mutations was performed with adult flies as described by Zimmering *et al.* (1985). TCDD was supplied as a coded aliquot by Radian Corporation. TCDD was assayed in the SLRL test by injection into adult Canton-S wild-type males 24 to 72 hours old at the beginning of treatment.

To administer TCDD by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector that automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of TCDD at a level that would induce 30% mortality 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were treated with a solution of TCDD dissolved in peanut oil and allowed to recover for 24 hours. A concurrent peanut oil control group was also included. Treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings was treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls (Mason *et al.,* 1992) using a normal approximation to the binomial test (Margolin *et al.,* 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

MOUSE BONE MARROW CYTOGENETICS TEST PROTOCOL

A dose range-finding study was performed along with an analysis of published LD_{50} information to set the doses used in the study. The highest dose was 1,000 µg/kg. TCDD was tested for induction of Abs in mouse bone marrow by two different protocols. The first protocol used a standard harvest time of 17 hours, and the second protocol used a delayed harvest time of 36 hours.

Male $B6C3F_1$ mice (10 animals per dose group) were injected intraperitoneally with TCDD dissolved in corn oil. Solvent control animals received injections of corn oil only. The positive control was dimethylbenzanthracene. The mice were subcutaneously implanted with a BrdU tablet (McFee *et al.,* 1983) 18 hours before the scheduled harvest. (For the standard protocol, this required BrdU implantation to precede injection with TCDD by 1 hour.) The use of BrdU allowed selection of the appropriate cell population for scoring. (Abs induced by chemical administration are present in maximum number at the first metaphase following treatment; they decline in number during subsequent nuclear divisions due to cell death.) Two hours before sacrifice, the animals received an intraperitoneal injection of colchicine in saline. The animals were killed 17 or 36 hours after TCDD injection

(18 hours after BrdU dosing). One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). Cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained (with fluorescence-plus-Giemsa) and scored.

Fifty first-division metaphase cells were scored from up to eight animals per treatment group. Responses were evaluated as the percentage of aberrant metaphase cells, excluding gaps. The data were analyzed by a trend test (Margolin *et al.,* 1986).

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

Results

TCDD (0.1 to 1,000 µg/plate) was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without hamster or rat liver metabolic activation enzymes (S9 fraction) (Table B1; Mortelmans *et al.,* 1984). No induction of TFT resistance was noted in L5178Y $tk^{+/-}$ mouse lymphoma cells treated with 0.0625 to 1 µg/mL TCDD with or without S9 (Table B2; McGregor *et al.,* 1991). In tests for chromosomal damage in cultured CHO cells, TCDD did not induce SCEs or Abs when tested up to toxic levels (0.8 µg/mL) with or without S9 activation enzymes (Tables B3 and B4; Galloway *et al.,* 1987). Without S9, TCDD induced significant increases in SCEs at concentrations of 0.6 to 0.8 µg/mL, in two of five independent trials when a prolonged culture time was employed to compensate for TCDD-induced cell cycle delay. However, because the response was not consistently reproducible, the SCE test was concluded to be negative overall. In addition, there was a complication from the ethanol solvent in these SCE experiments. A comparison of a true negative control culture and an ethanol solvent control culture without TCDD indicated that ethanol alone, in the amount needed to dissolve TCDD, increased SCE frequencies. No induction of SLRL mutations was observed in germ cells of male *D. melanogaster* following treatment with 50, 250, or 500 ppm TCDD (Table B5; Zimmering *et al.,* 1985). Finally, no induction of Abs was observed in bone marrow cells of male $B6C3F_1$ mice 17 or 36 hours after intraperitoneal injection of 250, 500, or 1,000 µg/kg TCDD (Table B6). In summary, no mutagenic activity was detected with TCDD in a variety of *in vitro* and *in vivo* short-term tests.

TABLE B1
Mutagenicity of TCDD in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	119 ± 4.5	141 ± 10.1	98 ± 6.9	155 ± 12.8	86 ± 2.8	154 ± 9.0
	0.1	93 ± 10.0		96 ± 18.2		101 ± 8.7	
	1	83 ± 8.1		94 ± 12.5		96 ± 4.8	
	10	93 ± 5.5	170 ± 14.9	94 ± 4.7	117 ± 12.3	96 ± 8.6	188 ± 7.3
	33	85 ± 7.0	178 ± 8.6	78 ± 5.5	117 ± 7.5	84 ± 2.0	173 ± 2.5
	100	98 ± 5.5	162 ± 5.7	103 ± 7.3	111 ± 0.6	94 ± 3.4	195 ± 5.0
	333		176 ± 0.9		124 ± 12.4		155 ± 5.5
	1,000		149 ± 7.8		116 ± 9.4		177 ± 6.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		527 ± 7.4	374 ± 21.2	1,449 ± 87.8	1,527 ± 66.5	867 ± 18.7	853 ± 45.0
TA1535	0	16 ± 2.1	13 ± 1.3	12 ± 1.8	5 ± 1.0	15 ± 1.7	8 ± 1.0
	0.1	18 ± 2.2		6 ± 0.3		8 ± 0.6	
	1	20 ± 3.5		7 ± 0.6		13 ± 3.2	
	10	20 ± 2.9	15 ± 3.5	6 ± 1.2	8 ± 0.7	8 ± 0.7	11 ± 2.5
	33	17 ± 1.2	17 ± 0.6	8 ± 1.2	11 ± 1.2	7 ± 0.7	11 ± 3.7
	100	18 ± 2.3	17 ± 0.7	9 ± 2.0	8 ± 3.4	6 ± 0.9	8 ± 3.4
	333		18 ± 0.6		9 ± 3.2		7 ± 1.2
	1,000		18 ± 3.9		8 ± 2.3		8 ± 2.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		494 ± 14.6	308 ± 17.0	265 ± 12.7	242 ± 12.2	417 ± 26.1	121 ± 14.4
TA1537	0	15 ± 2.5	5 ± 1.3	19 ± 1.5	5 ± 0.0	15 ± 1.7	9 ± 1.7
	0.1	11 ± 2.3		23 ± 1.7		14 ± 1.2	
	1	11 ± 1.2		14 ± 3.0		16 ± 6.4	
	10	10 ± 1.8	5 ± 0.3	17 ± 1.8	5 ± 1.5	10 ± 2.3	7 ± 0.9
	33	13 ± 3.1	6 ± 0.0	18 ± 1.2	4 ± 0.6	11 ± 1.5	11 ± 1.0
	100	10 ± 2.5	7 ± 0.6	13 ± 2.4	6 ± 3.0	15 ± 1.5	4 ± 1.2
	333		5 ± 0.7		8 ± 0.3		8 ± 1.0
	1,000		7 ± 2.0		7 ± 1.5		7 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		147 ± 7.5	122 ± 11.1	383 ± 63.4	413 ± 22.8	441 ± 26.9	164 ± 4.2
TA98	0	21 ± 1.7	19 ± 3.5	33 ± 4.7	21 ± 5.2	46 ± 7.1	24 ± 2.1
	0.1	22 ± 2.5		27 ± 6.7		35 ± 4.8	
	1	18 ± 1.5		26 ± 0.9		26 ± 0.3	
	10	16 ± 0.0	22 ± 3.3	27 ± 1.8	23 ± 3.6	21 ± 2.2	27 ± 3.5
	33	20 ± 3.5	20 ± 4.6	29 ± 2.7	28 ± 2.0	22 ± 3.2	29 ± 2.7
	100	16 ± 1.9	18 ± 3.7	27 ± 2.7	24 ± 3.5	29 ± 4.0	26 ± 5.8
	333		18 ± 3.1		23 ± 2.2		21 ± 1.5
	1,000		18 ± 3.1		18 ± 2.8		26 ± 0.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		623 ± 85.1	725 ± 32.2	1,184 ± 18.8	1,204 ± 10.8	636 ± 80.3	384 ± 22.8

[a] Study was performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al.* (1984).
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE B2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by TCDD[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
–S9						
Trial 1						
Dimethylsulfoxide[c]		77	94	82	35	
		67	98	68	34	
		62	90	112	60	
		76	118	98	43	43
TCDD	0.0625	76	110	108	47	
		85	118	80	31	39
	0.125	64	101	72	38	
		64	112	56	29	33
	0.25	65	108	60	31	
		80	104	71	30	30
	0.5	71	105	61	29	
		76	98	58	26	27
	1	70	90	58	28	
		86	101	70	27	27
Methyl methanesulfonate[d]						
	15	46	45	176	129	
		47	34	208	148	
Trial 2						
Dimethylsulfoxide		86	114	48	19	
		76	98	44	19	
		72	99	79	36	
		89	88	60	23	24
TCDD	0.0625	80	110	49	20	
		75	119	53	23	22
	0.125	42	54	47	37	
		78	120	72	31	34
	0.25	63	83	81	43	
		80	176	56	23	33
	0.5	75	98	61	27	
		90	127	49	18	23
	1	98	88	98	33	
		97	115	70	24	29
Methyl methanesulfonate						
	15	60	43	217	122	
		51	41	190	124	23

TABLE B2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by TCDD

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
+S9						
Trial 1						
Dimethylsulfoxide		83	90	80	32	
		75	96	46	20	
		87	103	98	37	
		85	111	80	31	30
TCDD						
	0.0625	88	92	132	50	
		92	87	106	39	44
	0.125	86	86	123	47	
		81	79	119	49	48
	0.25	92	85	110	40	
		99	86	104	35	37
	0.5	83	90	105	42	
		102	101	90	30	36
	1	87	93	97	37	
		103	96	112	36	37
Methylcholanthrene[d]	2.5	78	73	337	145	
		93	73	373	134	19
Trial 2						
Dimethylsulfoxide		88	103	82	31	
	0	70	96	51	24	
		86	109	67	26	
		84	91	52	21	26
TCDD	0.0625	101	110	89	29	
		76	110	71	31	30
	0.125	88	117	79	30	
		82	123	64	26	28
	0.25	71	105	58	27	
		96	150	107	37	32
	0.5	92	114	100	36	
		75	107	81	36	36
	1	91	126	79	29	
		94	109	79	28	29
Methylcholanthrene	2.5	81	97	339	139	
		92	98	330	120	10

[a] Study was performed at Inveresk Research International. The detailed protocol and these data are presented by McGregor *et al.* (1991).
[b] Mutant fraction=mutant cells/10^6 clonable cells
[c] Solvent control
[d] Positive control

TABLE B3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by TCDD[a]

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosomes	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
–S9								
Trial 1								
Summary: Negative								
Ethanol[c]		50	1,031	422	0.41	8.44	25.5	
TCDD								
	0.04	50	1,059	432	0.41	8.64	25.5	–0.34
	0.133	50	1,067	454	0.43	9.08	25.5	3.95
	0.4	50	1,047	402	0.38	8.04	25.5	–6.20
					P=0.738[d]			
Mitomycin-C[e]	0.005	25	520	722	1.39	28.88	25.5	239.22
Trial 2								
Summary: Positive								
Ethanol		50	1,053	385	0.37	7.70	25.5	
TCDD								
	0.4	50	1,045	430	0.41	8.60	25.5	12.84
	0.6	50	1,033	429	0.42	8.58	25.5	13.59
	0.6	50	1,045	508	0.49	10.16	36.3[f]	32.96*
	0.8	50	1,045	563	0.54	11.26	36.3[f]	47.35*
					P=0.000			
Mitomycin-C	0.005	25	524	762	1.45	30.48	25.5	297.73
Trial 3								
Summary: Negative								
None		50	1,059	400	0.38	8.00	25.8	
Ethanol		50	1,052	527	0.50	10.54	32.5[f]	
TCDD								
	0.6	50	1,048	513	0.49	10.26	32.5[f]	–2.29
	0.7	50	1,059	537	0.51	10.74	32.5[f]	1.22
	0.8	50	1,045	597	0.57	11.94	32.5[f]	14.04
					P=0.012			
Mitomycin-C	0.005	25	517	705	1.36	28.20	25.8	172.21

TABLE B3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by TCDD

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosomes	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9								
Trial 1								
Summary: Negative								
Ethanol		50	1,038	625	0.60	12.50	25.5	
TCDD								
	0.04	50	1,034	580	0.56	11.60	25.5	–6.84
	0.133	50	1,045	552	0.53	11.04	25.5	–12.27
	0.4	50	1,042	582	0.56	11.64	25.5	–7.24
					P=0.944			
Cyclophosphamide[e]	1.5	25	523	897	1.72	35.88	25.5	184.85
Trial 2								
Summary: Negative								
Ethanol		50	1,043	534	0.51	10.68	25.5	
TCDD								
	0.4	50	1,037	525	0.51	10.50	25.5	–1.12
	0.6	50	1,035	569	0.55	11.38	25.5	7.38
	0.8	50	1,045	530	0.51	10.60	25.5	–0.94
					P=0.350			
Cyclophosphamide	1.5	25	526	834	1.59	33.36	25.5	209.69

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol and these data are presented by Galloway *et al.* (1987).
[b] SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[c] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[d] Solvent control
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[f] Positive control
Due to cell cycle delay, harvest time was extended to maximize the number of second-division metaphase cells available for analysis.

TABLE B4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by TCDD[a]

Compound	Dose (µg/mL)	Total Cells Scored	Total Aberrations: Number of Aberrations	Total Aberrations: Aberrations/ Cell	Total Aberrations: Cells with Aberrations (%)
–S9					
Harvest time: 21.5 hours					
Summary: Negative					
Ethanol[b]		100	0	0.00	0.0
		100	4	0.04	4.0
TCDD	0.6	100	2	0.02	2.0
	0.7	100	1	0.01	1.0
	0.8	100	2	0.02	2.0
					P=0.859[c]
Mitomycin-C[d]	0.062	50	14	0.28	22.0
			Complex Aberrations		
Ethanol		100	0	0.00	0.0
		100	2	0.02	2.0
TCDD	0.6	100	0	0.00	0.0
	0.7	100	1	0.01	1.0
	0.8	100	2	0.02	2.0
					P=0.424
Mitomycin-C	0.062	50	7	0.14	14.0
			Simple Aberrations		
Ethanol		100	0	0.00	0.0
		100	2	0.02	2.0
TCDD	0.6	100	1	0.01	1.0
	0.7	100	0	0.00	0.0
	0.8	100	0	0.00	0.0
					P=0.966
Mitomycin-C	0.062	50	7	0.14	10.0
			Other Aberrations		
Ethanol		100	0		0.0
		100	0		0.0
TCDD	0.6	100	1		1.0
	0.7	100	0		0.0
	0.8	100	0		0.0
Mitomycin-C	0.062	50	0		0.0

TABLE B4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by TCDD

Compound	Dose (µg/mL)	Total Cells Scored	Total Aberrations: Number of Aberrations	Total Aberrations: Aberrations/ Cell	Total Aberrations: Cells with Aberrations (%)
+S9					
Harvest time: 10.5 hours					
Summary: Negative					
Ethanol		100	5	0.05	5.0
		100	3	0.03	3.0
TCDD	0.4	100	5	0.05	5.0
	0.6	100	0	0.00	0.0
	0.8	100	6	0.06	5.0
					P=0.505
Cyclophosphamide[d]	50	50	13	0.26	22.0
			Complex Aberrations		
Ethanol		100	3	0.03	3.0
		100	1	0.01	1.0
TCDD	0.4	100	1	0.01	1.0
	0.6	100	0	0.00	0.0
	0.8	100	1	0.01	1.0
					P=0.625
Cyclophosphamide	50	50	6	0.12	10.0
			Simple Aberrations		
Ethanol		100	0	0.00	0.0
		100	1	0.01	1.0
TCDD	0.4	100	4	0.04	4.0
	0.6	100	0	0.00	0.0
	0.8	100	3	0.03	2.0
					P=0.605
Cyclophosphamide	50	50	7	0.14	12.0
			Other Aberrations		
Ethanol		100	2		2.0
		100	1		1.0
TCDD	0.4	100	0		0.0
	0.6	100	0		0.0
	0.8	100	2		2.0
Cyclophosphamide	50	50	0		0.0

[a] Study was performed at Litton Bionetics, Inc. The detailed protocol and these data are presented by Galloway *et al.* (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE B5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by TCDD[a]

Route of Exposure	Dose (ppm)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[b]
Injection	50	1	1/5,345	2/2,845	2/1,328	5/9,518 (0.05%)
	0[c]		3/3,592	0/2,258	1/1,371	4/7,221 (0.06%)
	250	27	0/283	0/43	0/0	0/326 (0.00%)
	0		0/227	0/124	0/82	0/433 (0.00%)
	500	35	0/606	0/54	0/107	0/767 (0.00%)
	0		2/1,762	0/457	0/200	2/2,419 (0.08%)
						P=0.502[d]

[a] Study was performed at Bowling Green State University. The detailed protocol and these data are presented by Zimmering *et al.* (1985).
[b] The mean mutant frequency from 518 negative control experiments is 0.074% (Mason *et al.*, 1992).
[c] Total number of lethal mutations/number of X chromosomes tested for three mating trials
[d] Data were corrected for the occurrence of spontaneous clusters.
Significance of total number of lethal mutations/total number of X chromosomes tested by a normal approximation to the binomial test (Margolin *et al*., 1983).

TABLE B6
Induction of Chromosomal Aberrations in Bone Marrow Cells of Male Mice Treated with TCDD by Intraperitoneal Injection[a]

Compound	Dose	Number of Mice with Erythrocytes Scored	Aberrations/Cell[b]	P Value[c]
Trial 1 (17 hours)				
Corn oil[d]		8	4.75 ± 0.84	
TCDD (µg/kg)				
	250	8	8.50 ± 1.12	0.0165
	500	7	10.00 ± 1.57	0.0028
	1,000	8	7.75 ± 1.44	0.0398
			P=0.094[e]	
Dimethylbenzanthracene (mg/kg)[f]				
	100	8	13.00 ± 0.85	0.0000
Trial 2 (17 hours)				
Corn oil		8	1.25 ± 0.53	
TCDD (µg/kg)				
	250	8	2.75 ± 0.65	0.0649
	500	8	2.50 ± 0.73	0.0962
	1,000	8	2.25 ± 0.70	0.1404
			P=0.253	
Dimethylbenzanthracene (mg/kg)				
	100	8	8.25 ± 1.67	0.0000
Trial 1 (36 hours)				
Corn oil		8	2.25 ± 0.88	
TCDD (µg/kg)				
	250	8	1.75 ± 0.70	0.6962
	500	8	0.50 ± 0.33	0.9832
	1,000	8	1.25 ± 0.37	0.8596
			P=0.906	
Dimethylbenzanthracene (mg/kg)				
	50	8	28.25 ± 2.19	0.0000

[a] Study was performed at Oak Ridge Associated Universities. The detailed protocol is presented by McFee *et al*. (1983).
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control. Dosed group values are significant at P≤0.008; positive control values are significant at P≤0.05 (Margolin *et al.,* 1986)
[d] Vehicle control
[e] Significance tested by the one-tailed trend test; significant at P≤0.025 (Margolin *et al.*, 1986)
[f] Positive control

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

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats at the 14-, 31-, and 53-Week Interim Evaluations in the 2-Year Gavage Study of TCDD[a]

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Necropsy body wt						
Week 14	270 ± 5	266 ± 5	283 ± 7	280 ± 9	269 ± 6	256 ± 6
Week 31	307 ± 7	300 ± 7	298 ± 5	290 ± 7	294 ± 7	274 ± 6**
Week 53	338 ± 6	333 ± 6	343 ± 16	322 ± 12	301 ± 8*	288 ± 10**
L. Kidney						
Week 14						
Absolute	0.761 ± 0.016	0.800 ± 0.015	0.790 ± 0.028	0.764 ± 0.020	0.758 ± 0.027	0.753 ± 0.019
Relative	2.823 ± 0.046	3.014 ± 0.052	2.793 ± 0.065	2.734 ± 0.052	2.811 ± 0.060	2.944 ± 0.051
Week 31						
Absolute	0.862 ± 0.031	0.906 ± 0.019	0.874 ± 0.020	0.854 ± 0.033	0.842 ± 0.021	0.819 ± 0.021
Relative	2.802 ± 0.068	3.030 ± 0.066*	2.939 ± 0.048	2.939 ± 0.070	2.866 ± 0.039	2.988 ± 0.044
Week 53						
Absolute	1.004 ± 0.021	0.997 ± 0.030	1.025 ± 0.043	0.983 ± 0.038	0.952 ± 0.021	0.931 ± 0.027
Relative	2.971 ± 0.070	2.991 ± 0.076	3.003 ± 0.100	3.052 ± 0.016	3.173 ± 0.066	3.248 ± 0.083*
Liver						
Week 14						
Absolute	7.936 ± 0.304	9.261 ± 0.302*	10.277 ± 0.399**	9.806 ± 0.514**	10.179 ± 0.296**	10.315 ± 0.361**
Relative	29.344 ± 0.64	34.836 ± 0.858**	36.240 ± 0.738**	34.856 ± 0.837**	37.766 ± 0.465**	40.273 ± 0.979**
Week 31						
Absolute	9.188 ± 0.277[b]	10.543 ± 0.345**	10.840 ± 0.296**	10.639 ± 0.357**	11.561 ± 0.386**	10.979 ± 0.238**
Relative	30.170 ± 0.415[b]	35.131 ± 0.541**	36.411 ± 0.713**	36.619 ± 0.557**	39.265 ± 0.633**	40.117 ± 0.641**
Week 53						
Absolute	10.760 ± 0.296	10.975 ± 0.313	12.117 ± 1.060	12.268 ± 0.583	12.346 ± 0.428	12.910 ± 0.450*
Relative	31.815 ± 0.742	32.932 ± 0.769	34.906 ± 1.570*	38.030 ± 0.801**	41.051 ± 0.724**	44.929 ± 0.861**
Lung						
Week 14						
Absolute	1.643 ± 0.040	1.861 ± 0.072	1.995 ± 0.084**	1.789 ± 0.046	1.877 ± 0.072*	1.747 ± 0.040
Relative	6.092 ± 0.111	7.014 ± 0.276	7.107 ± 0.384*	6.409 ± 0.147	7.015 ± 0.351	6.849 ± 0.187
Week 31						
Absolute	1.893 ± 0.065	2.325 ± 0.125	2.378 ± 0.152*	1.940 ± 0.100	2.236 ± 0.149	2.309 ± 0.117
Relative	6.194 ± 0.278	7.789 ± 0.440*	7.998 ± 0.497*	6.674 ± 0.270*	7.584 ± 0.438*	8.415 ± 0.358**
Week 53						
Absolute	2.104 ± 0.146	1.943 ± 0.080	1.991 ± 0.088	2.057 ± 0.106	2.151 ± 0.188	2.451 ± 0.110
Relative	6.243 ± 0.469	5.851 ± 0.287	5.865 ± 0.326	6.397 ± 0.254	7.104 ± 0.483	8.526 ± 0.280**
Ovaries						
Week 14						
Absolute	0.054 ± 0.004	0.053 ± 0.002[b]	0.061 ± 0.003	0.059 ± 0.005	0.053 ± 0.003	0.054 ± 0.004
Relative	0.200 ± 0.015	0.204 ± 0.008[b]	0.217 ± 0.011	0.208 ± 0.015	0.196 ± 0.007	0.211 ± 0.016
Week 31						
Absolute	0.047 ± 0.003	0.055 ± 0.003	0.048 ± 0.003	0.050 ± 0.005	0.050 ± 0.003	0.044 ± 0.004
Relative	0.152 ± 0.010	0.184 ± 0.010	0.161 ± 0.007	0.172 ± 0.013	0.171 ± 0.010	0.161 ± 0.014
Week 53						
Absolute	0.049 ± 0.003	0.053 ± 0.003	0.053 ± 0.003	0.056 ± 0.003	0.049 ± 0.003	0.054 ± 0.006
Relative	0.144 ± 0.010	0.160 ± 0.008	0.155 ± 0.011	0.174 ± 0.009	0.164 ± 0.008	0.186 ± 0.015*

TABLE C1
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 TCDD

	Vehicle Control	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg
n						
Week 14	10	10	10	10	10	10
Week 31	10	10	10	10	10	10
Week 53	8	8	8	8	8	8
Necropsy body wt						
Week 14	270 ± 5	266 ± 5	283 ± 7	280 ± 9	269 ± 6	256 ± 6
Week 31	307 ± 7	300 ± 7	298 ± 5	290 ± 7	294 ± 7	274 ± 6**
Week 53	338 ± 6	333 ± 6	343 ± 16	322 ± 12	301 ± 8*	288 ± 10**
Spleen						
Week 14						
Absolute	0.538 ± 0.015[b]	0.558 ± 0.033	0.570 ± 0.029	0.565 ± 0.024	0.528 ± 0.022	0.467 ± 0.025
Relative	2.007 ± 0.046[b]	2.091 ± 0.098	2.013 ± 0.089	2.019 ± 0.063	1.960 ± 0.065	1.828 ± 0.089
Week 31						
Absolute	0.562 ± 0.026	0.563 ± 0.023	0.520 ± 0.017	0.511 ± 0.025	0.513 ± 0.021	0.434 ± 0.011**
Relative	1.825 ± 0.066	1.874 ± 0.047	1.748 ± 0.047	1.759 ± 0.067	1.747 ± 0.059	1.586 ± 0.033**
Week 53						
Absolute	0.544 ± 0.028	0.549 ± 0.024	0.508 ± 0.025	0.512 ± 0.021	0.480 ± 0.026	0.452 ± 0.023*
Relative	1.613 ± 0.091	1.645 ± 0.058	1.488 ± 0.060	1.599 ± 0.063	1.592 ± 0.060	1.578 ± 0.092
Thymus						
Week 14						
Absolute	0.209 ± 0.019	0.227 ± 0.012	0.215 ± 0.014	0.190 ± 0.013	0.168 ± 0.006*	0.125 ± 0.010**
Relative	0.772 ± 0.062	0.854 ± 0.044	0.756 ± 0.037	0.691 ± 0.061	0.626 ± 0.024*	0.489 ± 0.036**
Thyroid gland						
Week 14						
Absolute	0.029 ± 0.002	0.025 ± 0.002	0.028 ± 0.002	0.026 ± 0.001	0.026 ± 0.002	0.028 ± 0.001
Relative	0.108 ± 0.005	0.094 ± 0.009	0.100 ± 0.004	0.093 ± 0.003	0.098 ± 0.006	0.109 ± 0.004
Week 31						
Absolute	0.025 ± 0.002	0.021 ± 0.002	0.020 ± 0.001	0.021 ± 0.002	0.024 ± 0.002	0.021 ± 0.002
Relative	0.083 ± 0.007	0.071 ± 0.006	0.067 ± 0.004	0.072 ± 0.008	0.081 ± 0.007	0.076 ± 0.006
Week 53						
Absolute	0.039 ± 0.002	0.039 ± 0.004	0.033 ± 0.002	0.032 ± 0.002	0.031 ± 0.002*	0.031 ± 0.002*
Relative	0.117 ± 0.006	0.116 ± 0.012	0.097 ± 0.006	0.101 ± 0.005	0.105 ± 0.008	0.108 ± 0.006

* 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

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

TCDD

TCDD was obtained from IIT Research Institute (Chicago, IL) 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 (Research Triangle Park, NC), and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the TCDD study are on file at the National Institute of Environmental Health Sciences.

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 D1), 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 present in Figures D1, D2, and D3, 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 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.

Formulation Materials

USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) in five lots and was used with corn oil as the vehicle in the 2-year gavage study. The identity of each lot was confirmed by the study laboratory using infrared spectroscopy prior to its use. The purity of each lot was determined by GC (system E) 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

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. The 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 E. No degradation was observed during the course of the study.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving TCDD working stocks in acetone first and then diluting in corn oil (Spectrum Quality Products) such that the final concentration contained 1.0% acetone (Table D2). The dose formulations were stored at room temperature in amber glass bottles with minimal headspace, sealed with Teflon®-lined lids, for up to 35 days.

Homogeneity studies of 1.2 and 40 ng/mL dose formulations and simulated animal room stability studies of a 1.2 ng/mL dose formulation were performed by the study laboratory using GC/MS by system F. Homogeneity was confirmed, and stability was confirmed for 3 hours under simulated animal room conditions. An additional stability study of a 0.2 ng/mL dose fomulation was performed by the analytical laboratory using GC by system G. In this study, stability was confirmed for at least 35 days for the dose formulation stored in sealed glass containers at 5° C and room temperature (approximately 25° C). Gavagability was confirmed by the study laboratory for the 40 ng/mL formulation.

Periodic analyses of the dose formulations of TCDD were conducted by the study laboratory using GC/MS by system F. During the 2-year study, the dose formulations were analyzed at least every 3 months (Table D3). Of the dose formulations analyzed, 97% (56/58) were within 10% of the target concentrations; all were within 14% of target. Ninety percent (19/21) of the animal room samples were within 10% of the target concentrations; all were within 16% of target.

TABLE D1
Gas Chromatography Systems Used in the 2-Year Gavage Study of TCDD[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		Helium at 1.5 mL/minute	200° C to 300° C at 10° C/minute, then held for 8 minutes
Electron capture	Supelco PTE-5, 30 m × 0.32 mm, 1-µm film thickness (Supelco Inc., Bellefonte, PA)		
System E			
Flame ionization	20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcoport, 2.4 m × 2 mm (Supelco, Inc.)	Helium at 30 mL/minute	40° C for 4 minutes, then 10° C/minute to 170° C
System F			
Mass spectrometry, selected ion recording	J&W DB-5 MS, 15 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium, ultra high purity at 2 mL/minute	140° C for 1 minute, then 25° C/minute to 310° C
System G			
Mass spectrometry, selected ion recording	J&W DB-5 MS, 30 m × 0.32 mm, 0.25-µm film thickness (J&W Scientific)	Helium at 1.2 mL/minute	100° C to 300° C at 8° C/minute, then held for 10 minutes

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

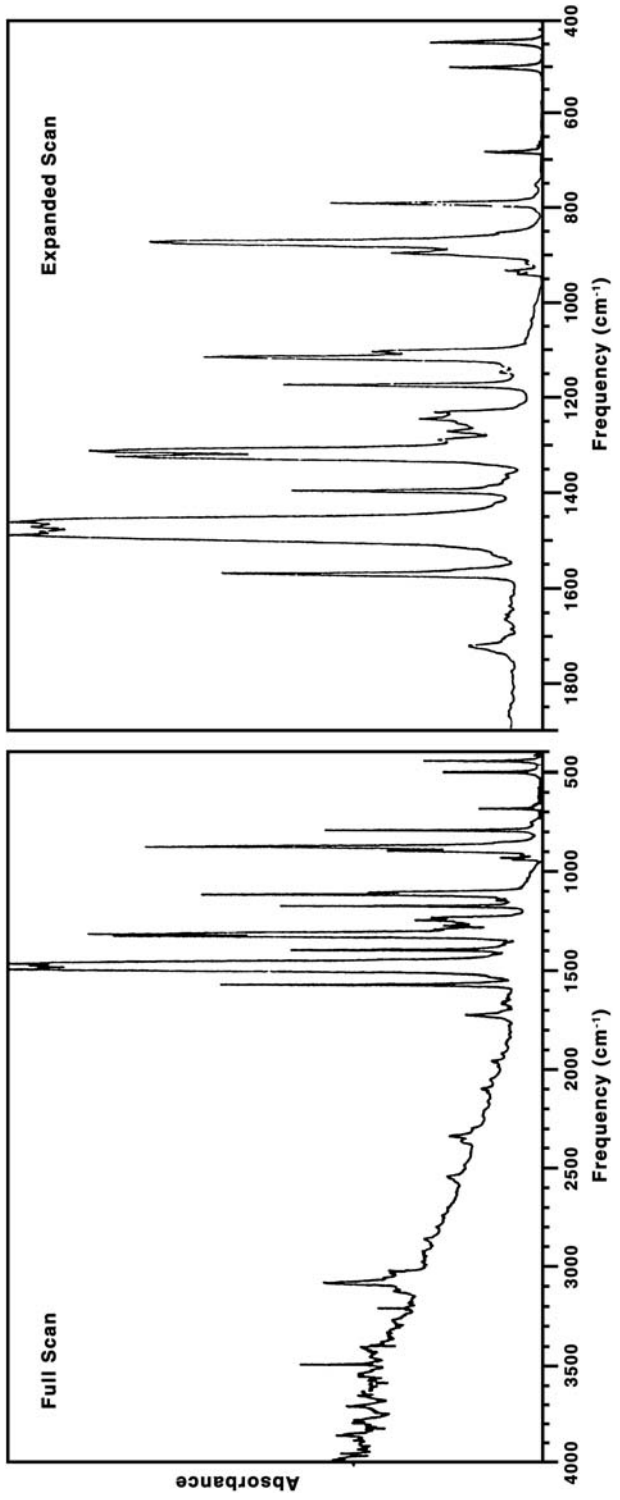

FIGURE D1
Infrared Spectrum of TCDD

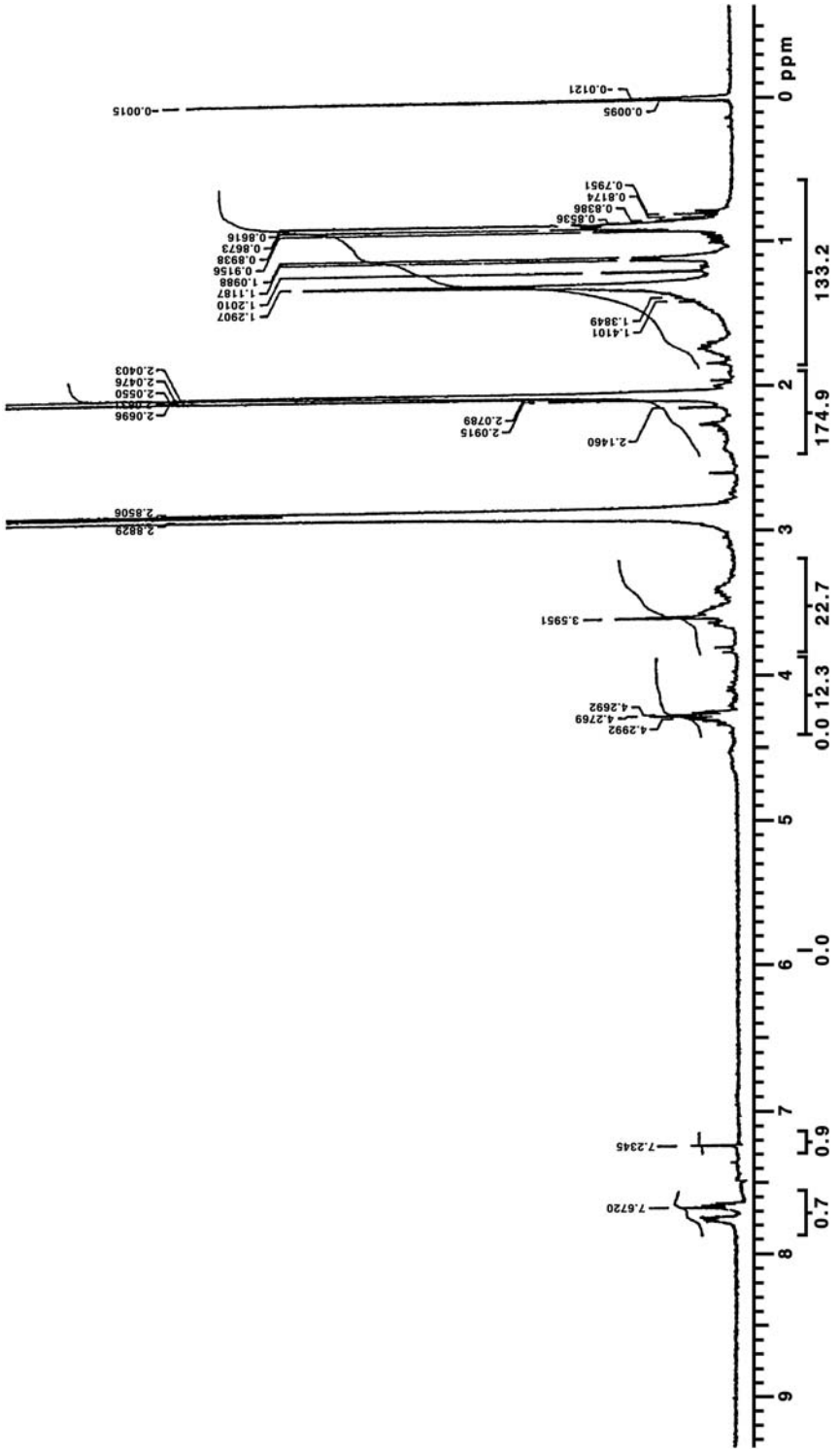

FIGURE D2
Proton Nuclear Magnetic Resonance Spectrum of TCDD

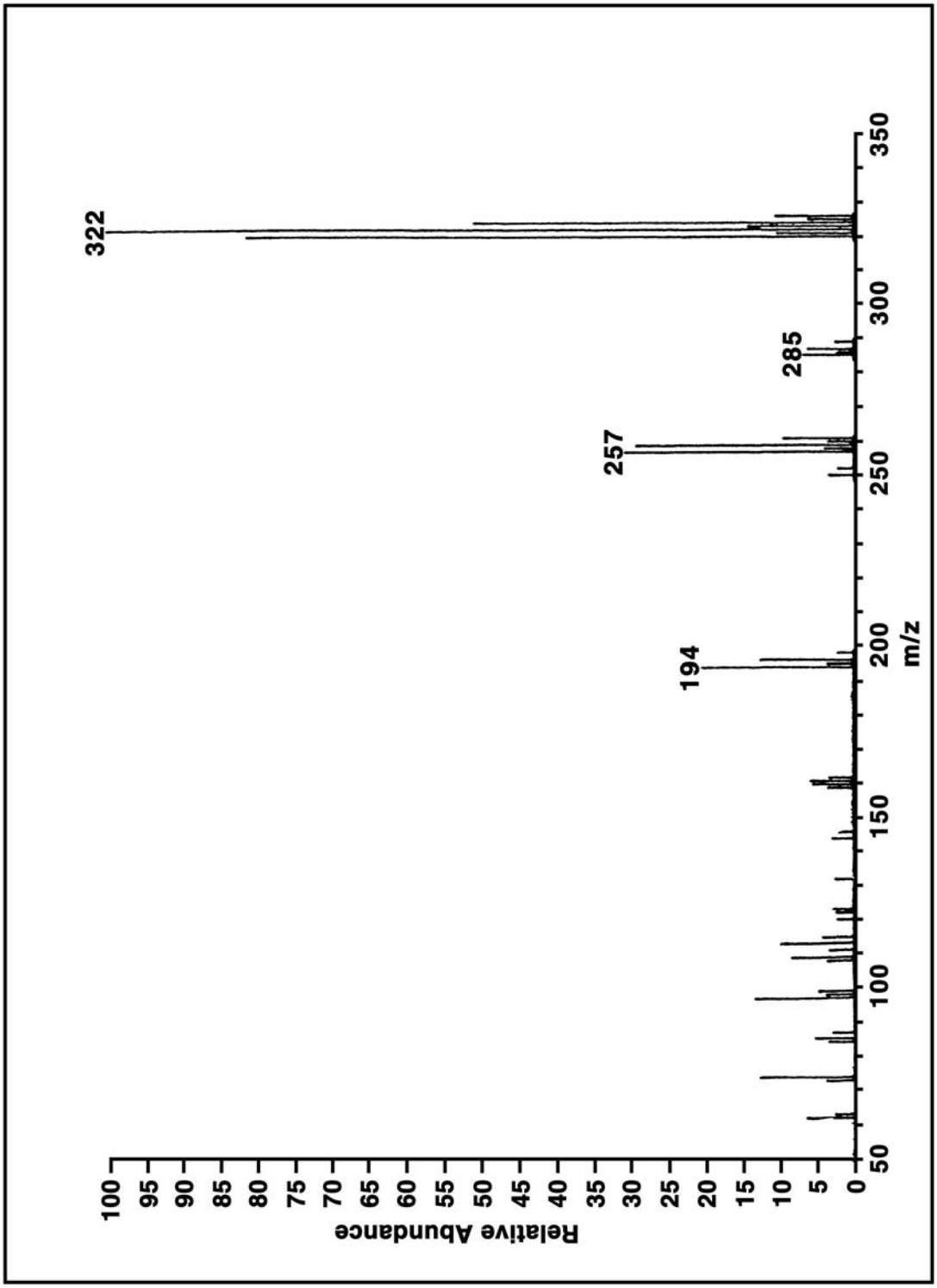

FIGURE D3
Direct Probe Mass Spectrum of TCDD

TABLE D2
Preparation and Storage of Dose Formulations in the 2-Year Gavage Study of TCDD

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

Dose formulations were prepared by filling a 2 L volumetric flask approximately half full with corn oil. 20 mL of acetone was added in three aliquots to the appropriate dose formulation working stock bottle; capped, vortexed, sonicated, and transferred to the volumetric flask after each rinse. The contents of the volumetric flask were diluted to volume with corn oil, capped, shaken vigorously, and stirred on a stirplate for 3 or 24 hours, with periodic inverting and shaking.

Chemical Lot Number
CR82-2-2

Maximum Storage Time
35 days

Storage Conditions
Working stocks of TCDD were stored in 15 mL amber glass vials, and sealed with Teflon®-lined lids at room temperature (approximately 25° C). Dose formulations were stored in 60, 120, or 250 mL amber glass screw-cap bottles with Teflon®-lined lids at room temperature (approximately 25° C).

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

TABLE D3
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of TCDD

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration[a] (ng/mL)	Difference from Target (%)
May 22, 1998	May 27, 1998	1.2	1.189	–1
		4	4.032	+1
		8.8	9.070	+3
		18.4	18.31	0
		40	40.90	+2
	July 2, 1998[b]	1.2	1.243	+4
		4	4.083	+2
		8.8	8.633	–2
		18.4	18.35	0
		40	40.97	+2
July 17, 1998	July 22, 1998	1.2	1.193	–1
		4	3.788	–5
		8.8	8.697	–1
		18.4	17.67	–4
		40	38.47	–4
		40	37.93	–5
October 9, 1998	October 13, 1998	1.2	1.113	–7
		4	3.836	–4
		8.8	8.646	–2
		18.4	16.66	–9
		40	37.89	–5
		40	40.16	–0
December 4, 1998	December 9-10, 1998	1.2	1.124	–6
		4	3.529[c]	–12
		8.8	8.202	–7
		18.4	15.88[c]	–14
		40	35.93	–10
		40	37.85	–5
	January 12-13, 1999[b]	1.2	1.127	–6
		4	3.722	–7
		8.8	8.110	–8
		18.4	16.85	–8
		40	36.50	–9
		40	38.31	–4
February 26, 1999	March 4, 1999	1.2	1.318	+10
		4	4.021	+1
		8.8	9.262	+5
		18.4	18.14	–1
		40	37.86	–5
April 23, 1999	April 28-29, 1999	1.2	1.120	–7
		4	3.816	–5
		8.8	8.218	–7
		18.4	17.19	–7
		40	36.47	–9

TABLE D3
Results of Analyses of Dose Formulations Administered to Female Rats in the 2-Year Gavage Study of TCDD

Date Prepared	Date Analyzed	Target Concentration (ng/mL)	Determined Concentration (ng/mL)	Difference from Target (%)
July 16, 1999	July 20, 1999	1.2	1.196	0
		4	3.827	–4
		8.8	8.832	0
		18.4	17.19	–7
		40	38.55	–4
	September 1, 1999[b]	1.2	1.179	–2
		4	3.740	–7
		8.8	8.531	–3
		18.4	17.14	–7
		40	38.57	–4
September 10, 1999	September 17, 1999	1.2	1.203	0
		4	3.746	–6
		8.8	8.807	0
		18.4	17.73	–4
		40	38.20	–5
December 6 and 10, 1999	December 17, 1999	1.2	1.174	–2
		4	3.609	–10
		8.8	8.555	–3
		18.4	16.89	–8
		40	37.12	–7
January 28, 2000	February 2, 2000	1.2	1.179 ± 0.015	–2
		4	3.780 ± 0.028	–6
		8.8	8.435 ± 0.101	–4
		18.4	17.08 ± 0.26	–7
		40	38.22 ± 0.41	–4
	March 7, 2000[b]	1.2	1.074 ± 0.023	–11
		4	3.701 ± 0.088	–7
		8.8	8.608 ± 0.180	–2
		18.4	15.40 ± 0.22	–16
		40	36.29 ± 1.06	–9
April 21, 2000	April 26, 2000	1.2	1.154 ± 0.015	–4
		4	3.779 ± 0.078	–6
		8.8	8.605 ± 0.102	–2
		18.4	16.78 ± 0.49	–9
		40	38.47 ± 0.46	–4

[a] Reported value is the average of duplicate analyses or the average ± standard deviation of quadruplicate analyses. Dosing volume=2.5 mL/kg; 1.2 ng/mL=3 ng/kg, 4 ng/mL=10 ng/kg, 8.8 ng/mL=22 ng/kg, 18.4 ng/mL=46 ng/kg, 40 ng/mL=100 ng/kg.
[b] Animal room samples
[c] Formulation was outside the acceptable range of ± 10% of target concentration, but was used at NTP's direction.

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

TABLE E1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

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

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

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.4 ± 0.42	12.7 – 14.5	24
Crude fat (% by weight)	8.1 ± 0.23	7.6 – 8.6	24
Crude fiber (% by weight)	9.1 ± 0.61	7.9 – 10.0	24
Ash (% by weight)	4.9 ± 0.17	4.7 – 5.4	24
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)	5,773 ± 940	4,220 – 7,790	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)[b]	7.8 ± 0.85	6.1 – 9.3	24
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 (%)	0.984 ± 0.039	0.903 – 1.060	24
Phosphorus (%)	0.554 ± 0.025	0.505 – 0.592	24
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 E4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.16 ± 0.080	0.10 – 0.37	24
Cadmium (ppm)	0.04 ± 0.007	0.04 – 0.07	24
Lead (ppm)	0.09 ± 0.053	0.05 – 0.25	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.19 ± 0.033	0.15 – 0.28	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	10.4 ± 2.07	9.04 – 16.8	24
Nitrite nitrogen (ppm)[c]	<0.61		24
BHA (ppm)[d]	<1.0		24
BHT (ppm)[d]	<1.0		24
Aerobic plate count (CFU/g)	<10		24
Coliform (MPN/g)	0		24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	4.6 ± 1.54	2.1 – 8.8	24
N-Nitrosodimethylamine (ppb)[e]	1.9 ± 0.93	1.0 – 5.1	24
N-Nitrosopyrrolidine (ppb)[e]	2.7 ± 0.97	1.0 – 5.6	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.126 ± 0.115	0.020 – 0.499	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.231 ± 0.218	0.020 – 0.826	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

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

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

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

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

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

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

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

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

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

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

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

APPENDIX F
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from randomly selected male and female rats during the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to Microbiological Associates, Inc./BioReliance Corp. (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

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

RESULTS

All serology tests were negative.

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

SINGLE-DOSE TOXICOKINETIC STUDY IN FEMALE SPRAGUE-DAWLEY RATS

INTRODUCTION

A single dose of TCDD was administered by gavage to Harlan Sprague-Dawley rats at 50 or 100 ng/kg. TCDD levels were determined in postdose blood, lung, liver and fat tissue samples at time points up to 100 days. The results were analyzed to establish basic toxicokinetic parameters. Because the dose volume used in the bioassay was 2.5 mL/kg rather than the 5.0 mL/kg used in most studies of TCDD, both volumes were used at the high dose to determine the effect of reducing the dose volume. Bioavailability was not determined in this study because no intravenous administration was conducted.

MATERIALS AND METHODS

TCDD was procured in one lot (970401R-AC) from AccuStandard, Inc. (New Haven, CT), and was characterized by nuclear magnetic resonance and mass spectrometry. It was found to be 94% pure with one impurity with a mass spectrum consistent with triphenylphosphine. Because it was a single administration study and the dose would be so low (about 6 ng triphenylphosphine/kg), it was decided that the material would be suitable for the study. Formulations for the study were prepared in corn oil at concentrations of either 2.5 or 5.0 mL/kg with 1% acetone as described in Appendix D.

Female rats (11 weeks of age at the start of the study) were used for the study. They were administered 50 or 100 ng/kg of formulation in a volume of 2.5 mL/kg or 100 ng/kg of formulation in a volume of 5.0 mL/kg. At 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 13, 16, and 24 hours and 2, 4, 6, 10, 15, 30, 45, 60, 75, 90, 105, 120, and 150 days, postdosing groups of five rats per time point were anesthetized with a CO_2/O_2 mixture and as much blood as possible was collected in EDTA tubes by cardiac puncture and stored at 5° C for analysis. At 8 and 24 hours and 2, 30, 60, 90, 120, and 150 days, the rats were euthanized with CO_2 after the blood was collected and liver, lung, and mesenteric fat were collected, weighed, and frozen at –20° C until analysis.

For analysis, 20 µL of a 500 ng/mL solution of $^{13}C_{12}$-TCDD (internal standard) was added to a 1 mL aliquot of blood or 0.5 g aliquot of lung or liver and 2 mL of 50% potassium hydroxide solution. The samples were placed in a 40° C water bath for 15 minutes and digested overnight on a horizonal shaker. The digested samples were extracted with 10 mL of acetone:hexane (80:20) by vortexing followed by centrifugation. Fat tissue (0.5 g) was extracted with 10 mL of hexane without saponification after addition of the internal standard. For all tissues, the extraction solvent was removed with a nitrogen stream. Each residue was dissolved in about 1 mL of hexane and eluted through a silica gel column. The eluates were reduced to about 200 µL and transferred to an autosampler. Samples were analyzed by gas chromatography/mass spectrometry using an HP 6890 gas chromatograph and an HP 5973 MSD mass spectrometer. A DB-5MS column, 30 m × 0.25 mm ID, 0.25-µm film thickness was used; the oven temperature started at 100° C, increased at 8° C/minute to 300° C, and was then held for 10 minutes. Mass spectra were collected in single ion monitoring mode at 70 eV, scanning m/z 334, 332 (TCDD), 322, and 320 (internal standard). Responses for TCDD were quantitated using least squares linear regression of a calibration curve generated from matched tissues from untreated Sprague-Dawley rats spiked with TCDD. Table G1 provides figures of merit from the validation of the methods used.

Noncompartmental modeling with WinNonlin® (Pharsight Corp., Apex, NC) was used to derive toxicokinetic parameters from concentration versus time data.

Results and Discussion

There were insufficient data for toxicokinetic modeling of blood and lung (Table G2). Measured blood concentrations of TCDD were generally below the limit of quantitation, suggesting rapid absorption and distribution to tissues. The limited number of timepoints limited the toxicokinetic analysis of the remaining tissues. Elimination half-lives and terminal elimination rate constants could not be estimated. While half-lives could not be calculated, it appears that TCDD is eliminated more rapidly from liver than from fat. There was no effect of dose volume on toxicokinetic parameters. Tissue concentration data are presented in Figures G1 to G4.

Toxicokinetic modeling of the data in this study was limited to simple noncompartmental analysis, primarily area under the concentration versus time curve (AUC), due to the limited data set. Doses to liver and fat as estimated by AUC/dose were approximately linear with administered dose. The AUCs for liver and fat were similar. In previously reported studies, tissue concentrations of ^{14}C-TCDD-derived radioactivity were also nearly equal in liver and fat 3 weeks after a single dose (Rose *et al.*, 1976). This same study reported three to five times as much TCDD in liver than in fat after daily dosing for 1, 3, or 7 weeks. In the present study, TCDD concentration in liver was maximal at day 1, but C_{max} in fat occurred 20 to 40 days later, implying redistribution to fat. Because the redistribution to fat was slow with respect to the dosing interval, TCDD concentrations would be expected to be higher in liver after multiple daily doses.

TABLE G1
Figures of Merit for Assays of TCDD in the Single Gavage Dose Toxicokinetic Study in Female Sprague-Dawley Rats

Tissue	Linearity[a]	Limit of Quantitation	Precision[b]	Accuracy[c]
Blood	> 0.99	200 pg/g	Within 15%	34% or less
Lung	> 0.99	200 pg/g	Within 15%	15% or less
Liver	> 0.99	200 pg/g	Within 15%	15% or less
Fat	> 0.99	120 pg/g	Within 15%	22% or less

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

TABLE G2
Toxicokinetic Parameter Estimates in Female Sprague-Dawley Rats after a Single Gavage Dose of TCDD

Parameter	Dose[a] (ng/kg)	Tissue Parameter Estimate	
		Liver	Fat
C_{max} (pg/g)	50	494	105
	100	943	483
	100[b]	1,350	397
T_{max} (days)	50	1	40
	100	1	20
	100[b]	1	1
AUC_{last} (days • pg/g)	50	8,600	5,300
	100	14,600	19,200
	100[b]	12,900	27,500
AUC_{last}/Dose (days • pg/g)/(ng/kg)	50	172	106
	100	146	192
	100[b]	129	275

[a] Dose volume=2.5 mL/kg except where otherwise indicated
[b] Dose volume=5.0 mL/kg

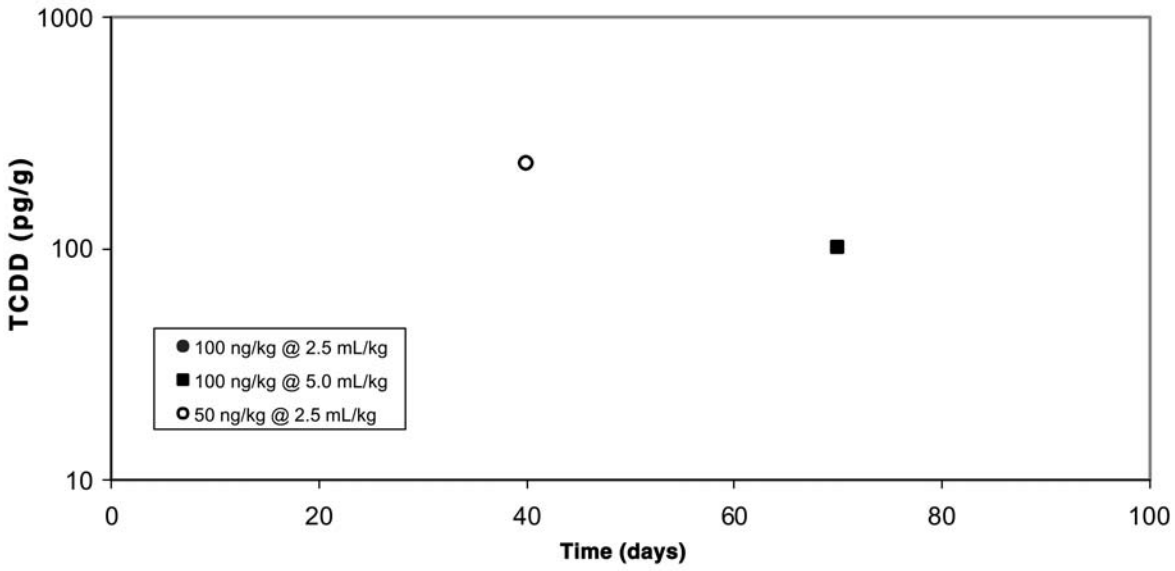

FIGURE G1
Lung Concentrations of TCDD in Female Sprague-Dawley Rats after a Single Gavage Dose of TCDD

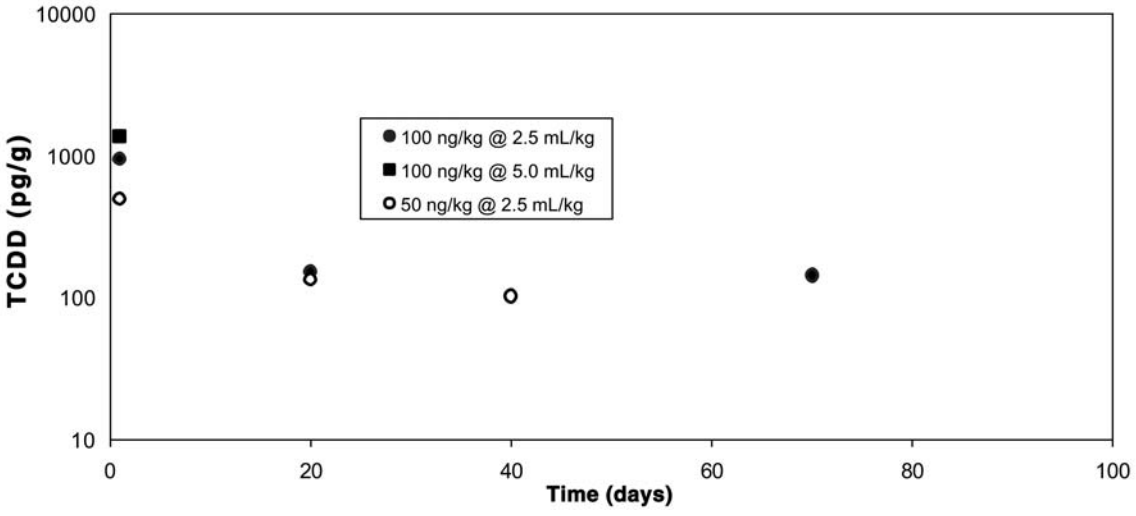

FIGURE G2
Liver Concentrations of TCDD in Female Sprague-Dawley Rats after a Single Gavage Dose of TCDD

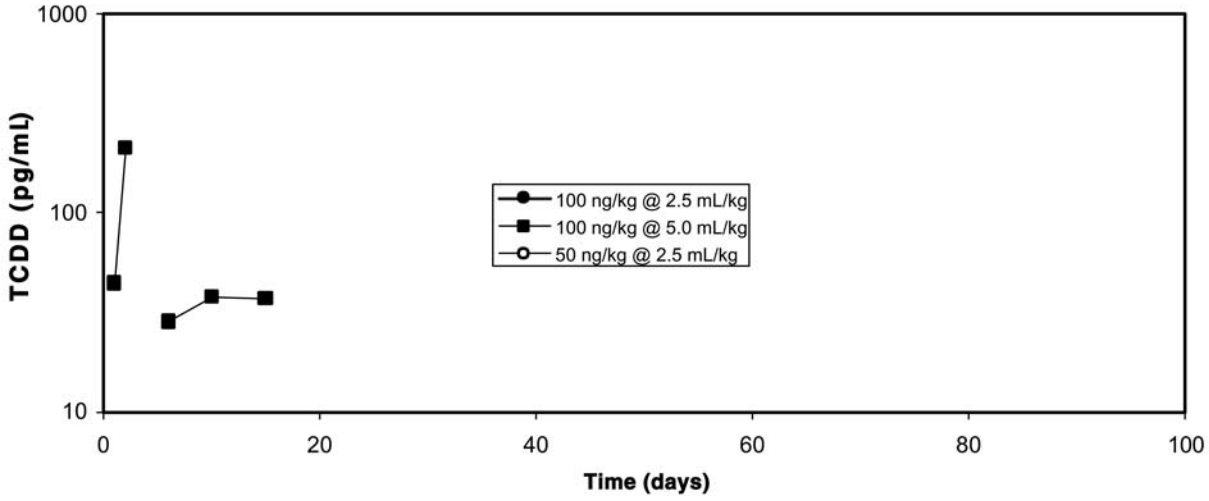

FIGURE G3
Blood Concentrations of TCDD in Female Sprague-Dawley Rats after a Single Gavage Dose of TCDD

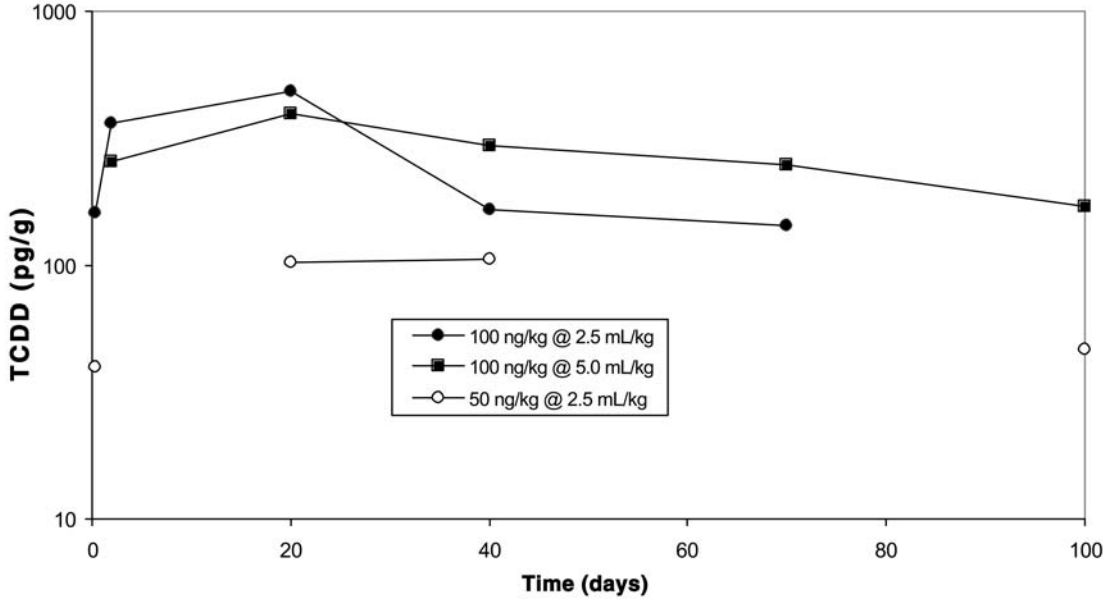

FIGURE G4
Fat Concentrations of TCDD in Female Sprague-Dawley Rats after a Single Gavage Dose of TCDD

APPENDIX H
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A goal for the physiologically based pharmacokinetic (PBPK) modeling of the disposition data from the dioxin toxic equivalency factor (TEF) evaluation studies is a general model for the tissue distribution of dioxin-like compounds (DLCs) and mixtures of compounds that interact with the aryl hydrocarbon receptor (AhR) in the Sprague-Dawley rat.

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

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

The disposition of a chemical within the body is governed by the absorption of an administered chemical and its distribution among tissues, metabolism, and elimination from the body (ADME). These processes for TCDD and related DLCs in part depend upon their physicochemical properties (e.g., tissue permeation and partition coefficients, kinetic constants, and biochemical parameters) and physiological parameters (e.g., organ volumes and blood flow rates).

A PBPK model for TCDD is a mathematical structure that describes the relationship between these factors and ADME. This model describes the pharmacokinetics of TCDD by a series of mass-balance differential equations in which the state variables represent the concentration of TCDD in anatomically distinct regions, "compartments" of the body. These tissue compartments are linked by a physiologically realistic pattern of blood perfusion and flow through the different tissue compartments.

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

MODEL DEVELOPMENT

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

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

There were three main steps to building a PBPK model for the dioxin TEF evaluation studies; conversion of Kohn's model, addition of a lung compartment, and addition of the study-specific body weights to the model. A copy of Kohn's model coded in the ScoP simulation was converted to both a Matlab model and a Simulink model. Simulations from the three models were compared to each other to confirm that the conversion to Matlab/Simulink was accurate. Next, a lung compartment was added to the Simulink model because the NTP data for the TEF studies include lung tissue concentrations. The lung compartment is diffusion-limited and includes the same equations used in the liver for the AhR, CYP1A1, and CYP1B1. The lung and liver compartments use the same gene expression parameters on a per liter basis. The final step was to include the rat body weights from each study rather than the body weight function from Kohn's model. Body weights were available weekly for the first 12 weeks of the studies and then monthly for the remainder of the studies. Interpolation of the mean body weights was used to estimate the body weight as a function of time.

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

Results and Discussion

The model predictions match the data over the dose range and across tissues. Kohn's model (Kohn *et al.,* 2001) makes accurate predictions of the new NTP data for liver and adipose (results not shown). The model predictions for lung and blood are correctly below the limit of detection. The parameter estimates for the lung partition coefficient and lung permeability are 4.57 and 0.85 respectively. The lung partition coefficient is very close to the liver value and the permeability value is between the liver and kidney permeability values. All of these tissues are well perfused and should have similar values. The accuracy of the predictions suggests that it was reasonable to use the parameters from the liver to describe the cytochrome P450 and AhR dynamics in the lung. Lung data with a lower limit of detection would be useful to better evaluate the model. Overall, the model succeeds in describing the data and can be used as a base model for modeling similar chemicals that bind to the AhR.

TABLE H1
TCDD Model Parameters

Parameter	Model Value	Unit
lt (lag time for induced expression)	0.2	day
Cardiac Output	14.7	L/hr per $kg^{0.7}$
$V_{Protein}$ (blood binding protein)	300	nmole/L per day
$Ki_{Protein}$ (inhibition of blood protein production)	0.0006	nM
$Kd_{Protein}$ (blood binding protein)	10	nM
K_{AhR}	0.27	nM
$K_{CYP1A2\ TCDD}$	30	nM
$V_{metabolism}$	9.12	nmole/L per day
$K_{metabolism}$	0.968	nM
$n_{metabolism}$	1.12	—
$k_{subchronic\ absorption}$	0.65	$kg^{0.75}$/day
$k_{absorption}$	4.8	$kg^{0.75}$/day
$k_{binding}$	1,000	/nmole per day
$k_{Ah\ degradation}$	2.16	/day
$k_{Ah\ TCDD\ degradation}$	5.15	/day
$k_{proteolysis}$	0.2727	/day
$k_{deadenylation}$	576	nt/day
$k_{mRNA\ degradation}$	13.4	/day
k_{urine}	5.36	/day
k_{bile}	3.81	/day
k_{feces}	1.152	/day
k_{lysis}	200	/day
$critical_{accumulation}$	0.6	nmole
$k_{recovery}$	0.01	/day
$critical_{concentration}$	2	nM

TABLE H2
Partition Coefficients and Permeability Coefficients

	Partition	Permeability
Fat	187.0	0.024
Muscle	4.48	0.171
Viscera	3.35	0.334
Liver	4.60	1.49
Kidney	3.35	0.559
Gastrointestinal tract	3.35	0.248
Lung	4.57[a]	0.85[a]

[a] These coefficients are estimated

TABLE H3
Gene Expression Parameters Given in the Kohn *et al.* (2001) Model

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

[a] From optimization

TABLE H4
Physiological Parameters

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

TABLE H5
Initial Conditions for Protein in Blood[a]

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

[a] Conditions are measured in nmoles.

TABLE H6
Initial Conditions for Cytochrome P450s and the Aryl Hydrocarbon Receptor (AhR)[a]

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

[a] Conditions are measured in nmoles.

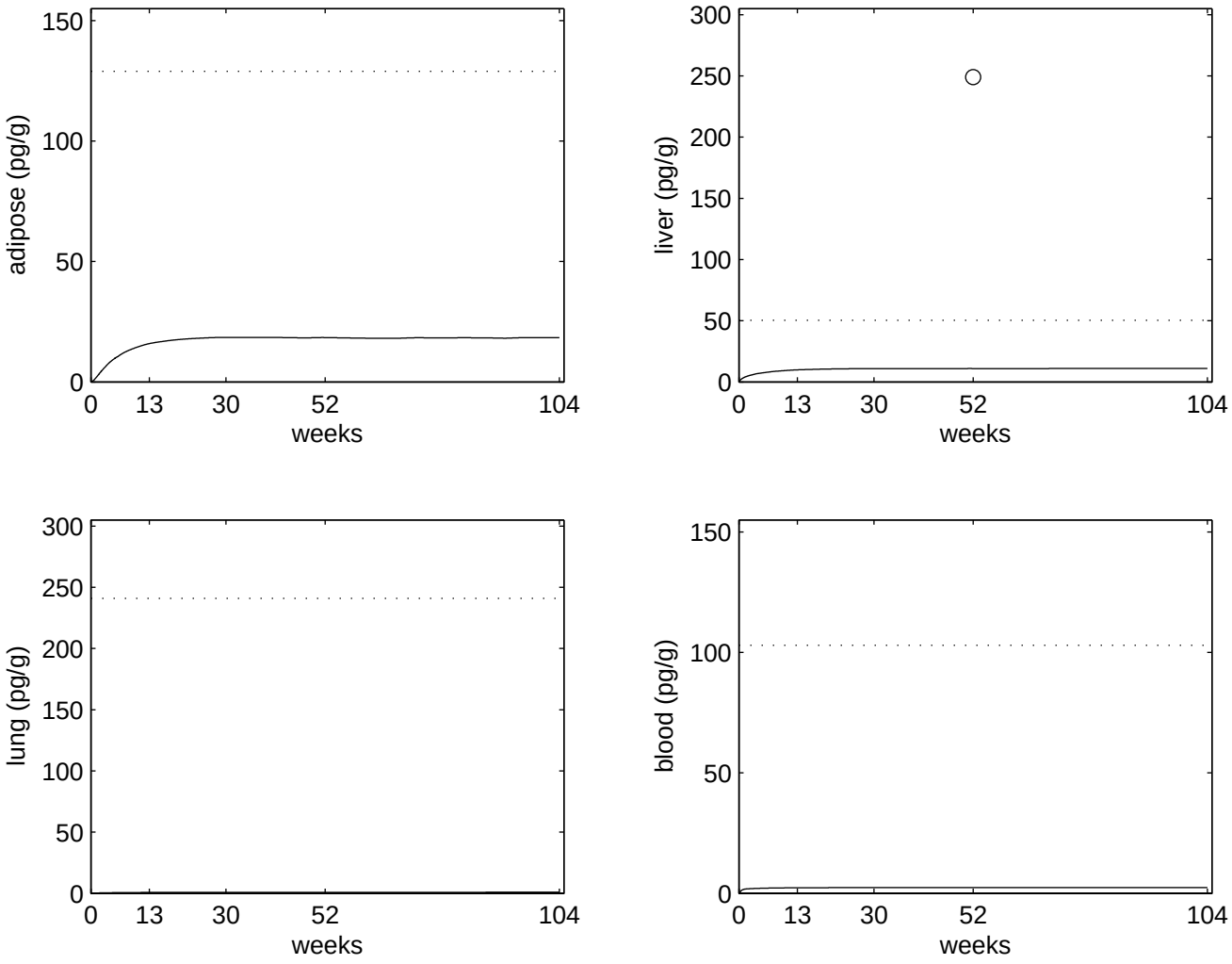

FIGURE H1
Model Predictions (–) and Individual Tissue Data (○) for the Vehicle Control Group in the 2-Year Study
The limit of detection is shown with a dotted line.

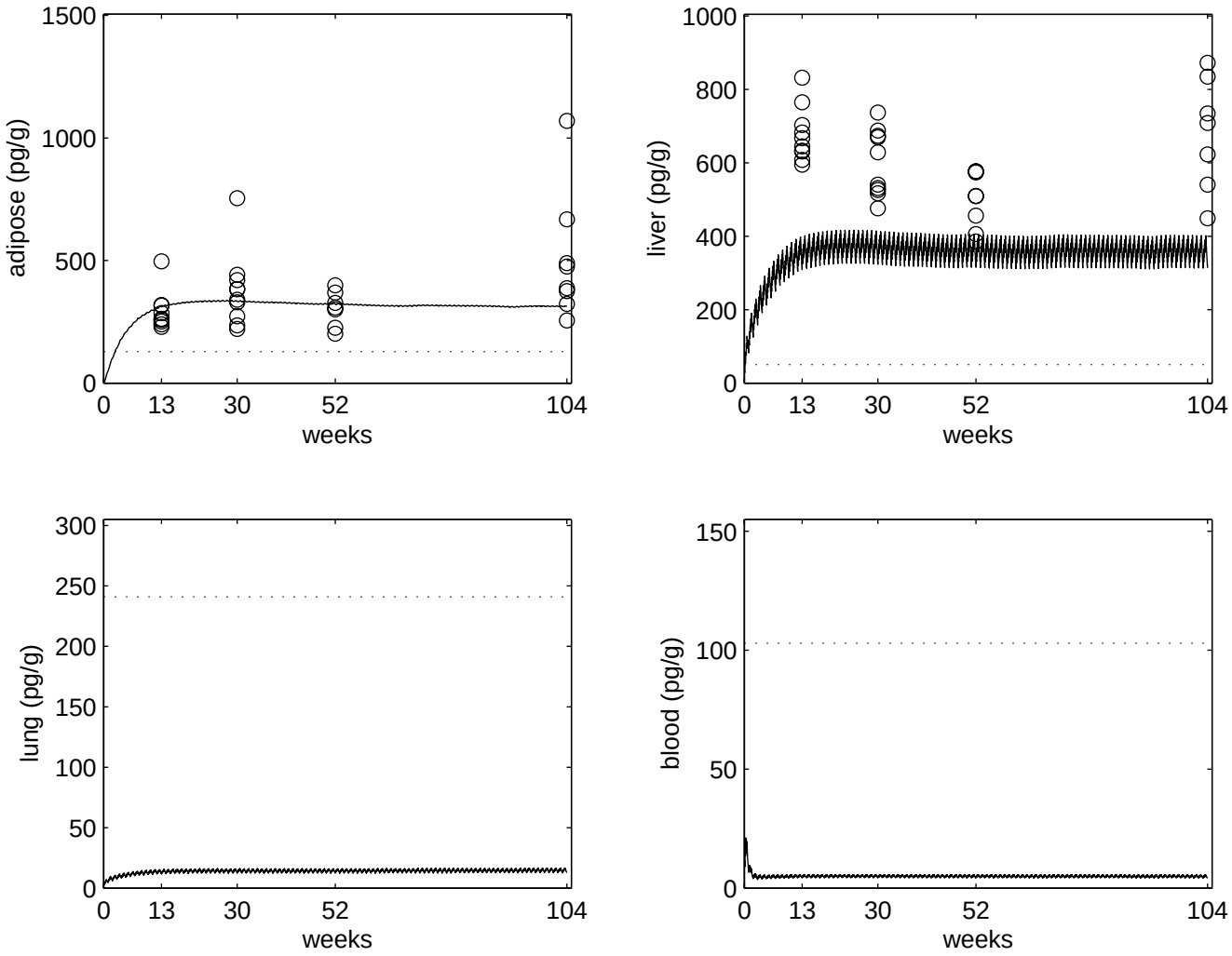

FIGURE H2
Model Predictions (–) and Individual Tissue Data (○) for the 3 ng/kg Group in the 2-Year Study
The limit of detection is shown with a dotted line.

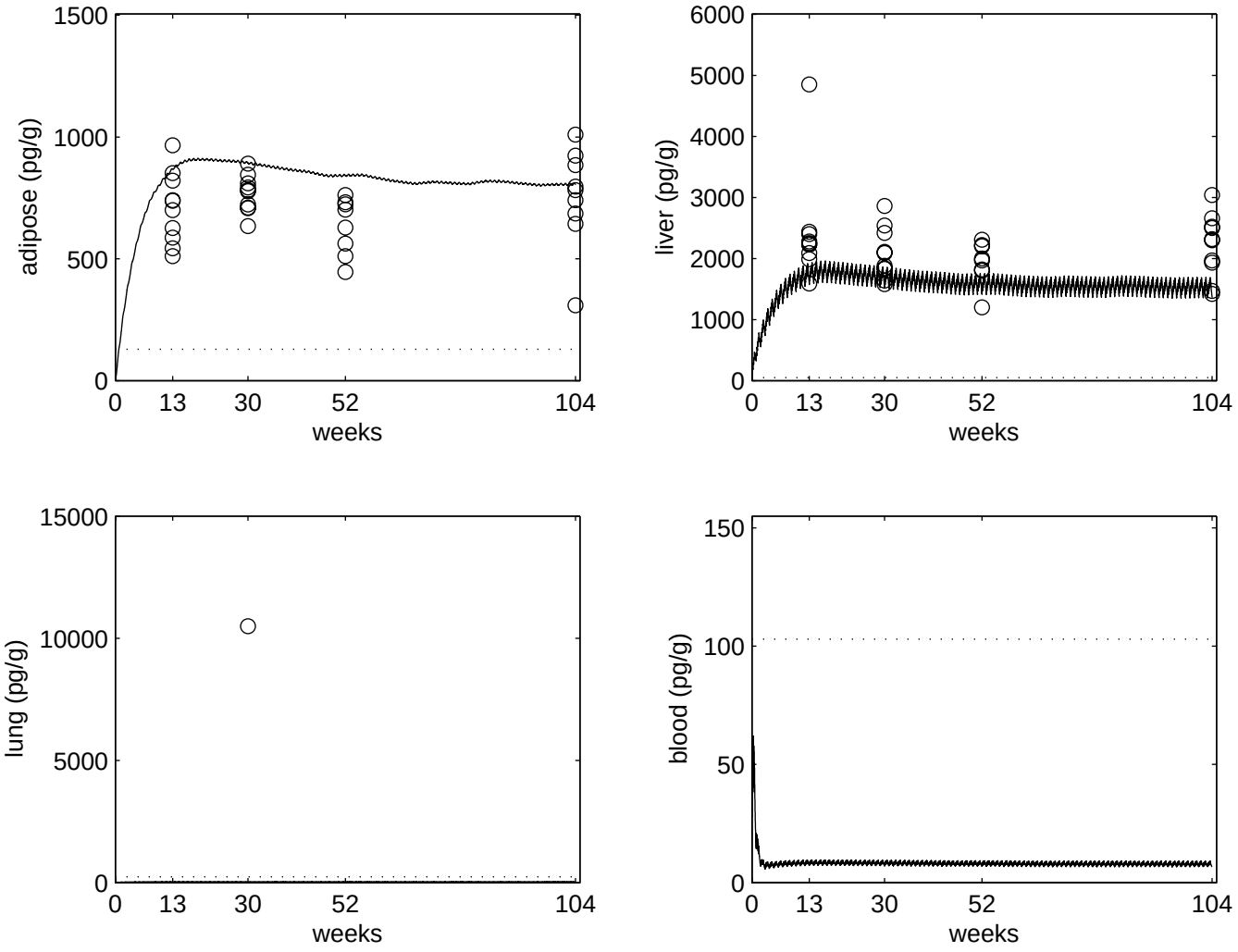

FIGURE H3
Model Predictions (–) and Individual Tissue Data (○) for the 10 ng/kg Group in the 2-Year Study
The limit of detection is shown with a dotted line.

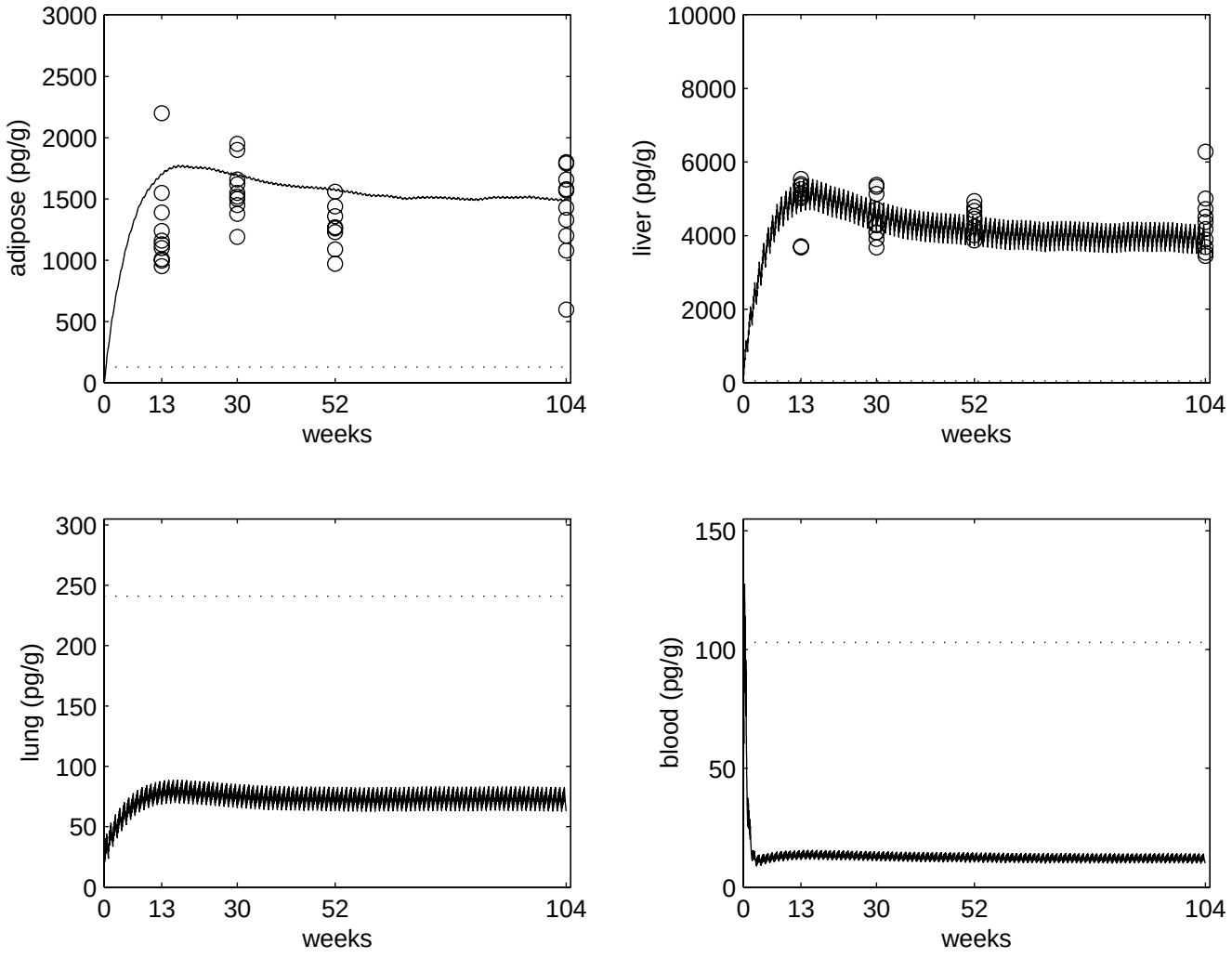

FIGURE H4
Model Predictions (–) and Individual Tissue Data (○) for the 22 ng/kg Group in the 2-Year Study
The limit of detection is shown with a dotted line.

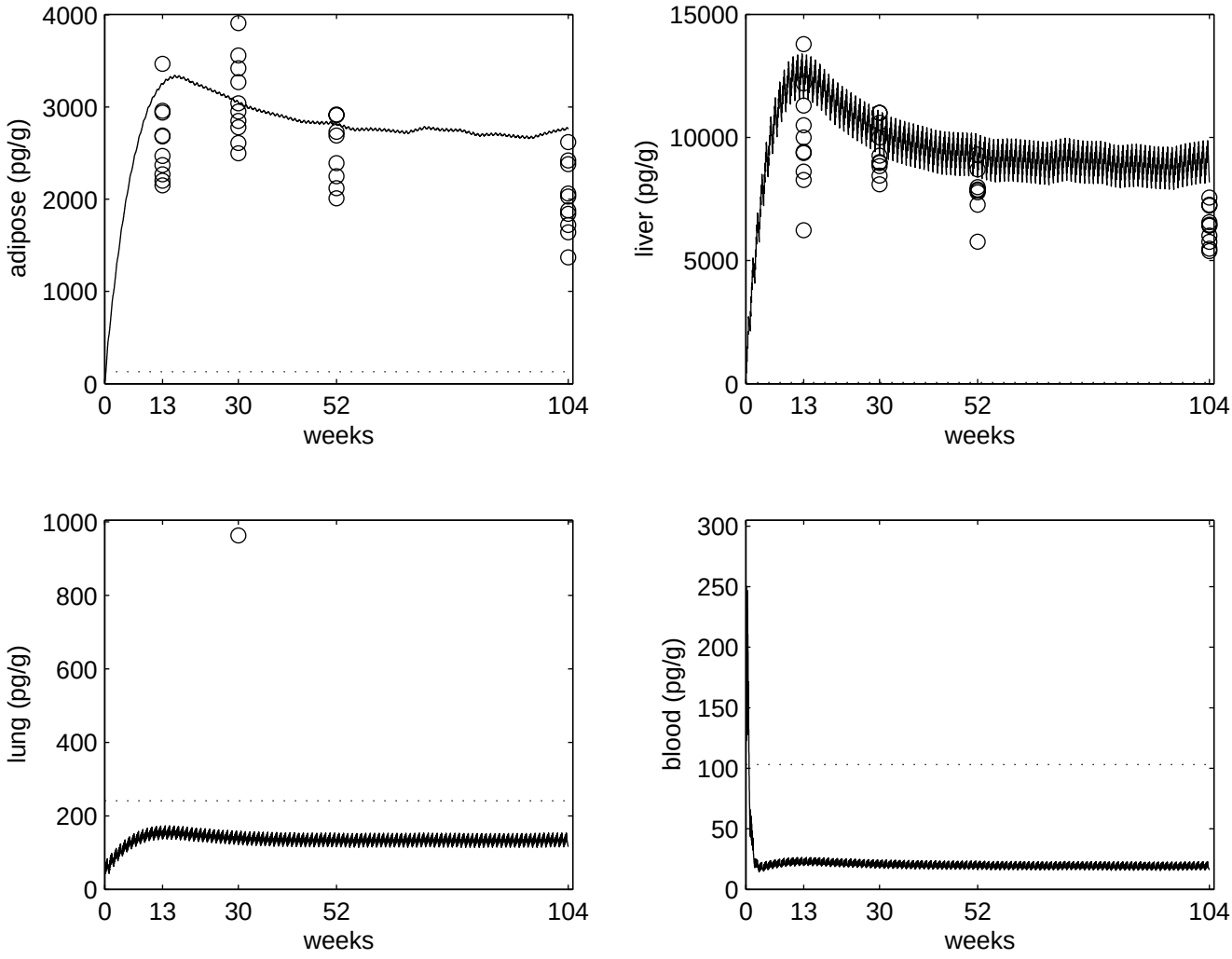

FIGURE H5
Model Predictions (–) and Individual Tissue Data (○) for the 46 ng/kg Group in the 2-Year Study
The limit of detection is shown with a dotted line.

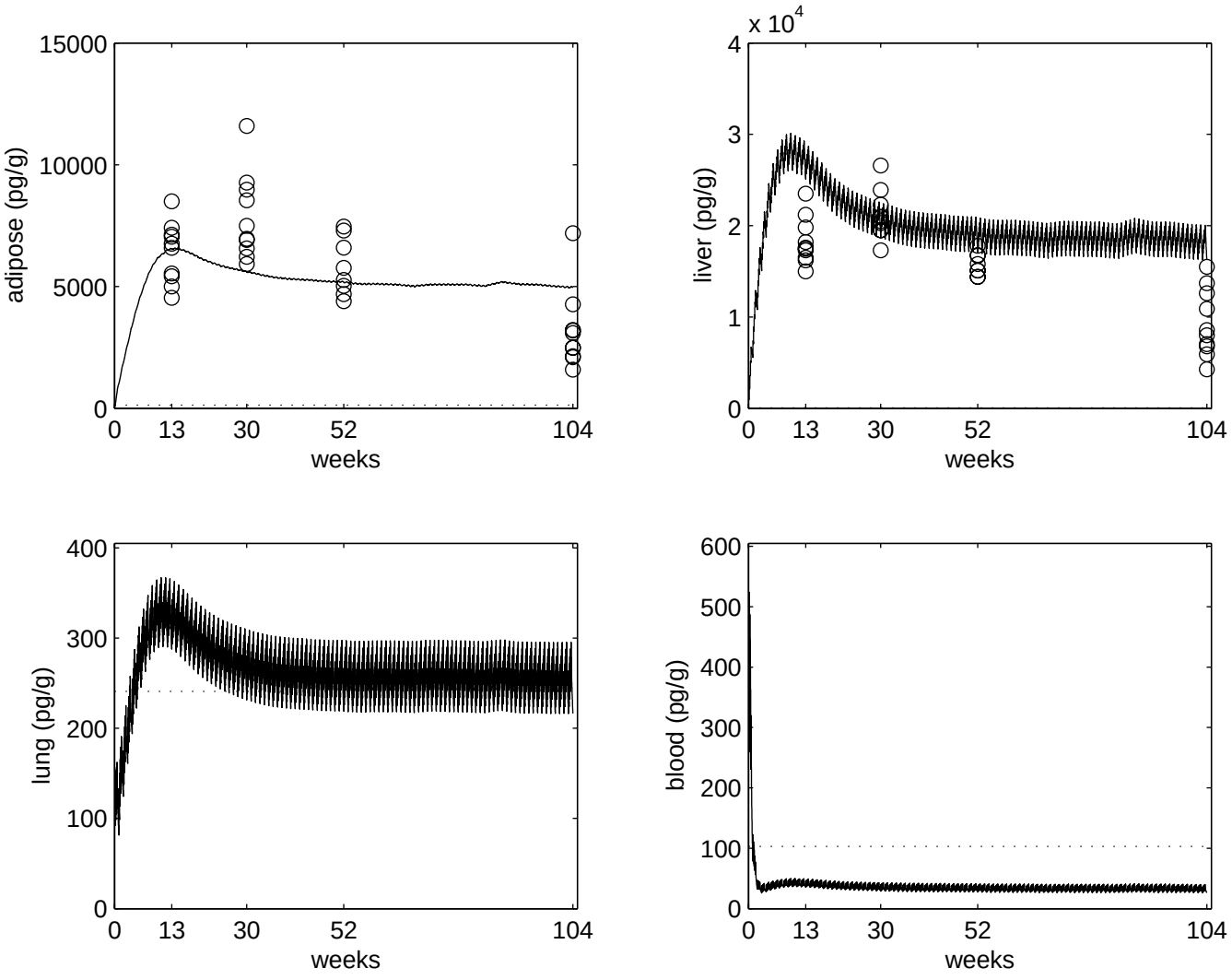

FIGURE H6
Model Predictions (–) and Individual Tissue Data (○) for the 100 ng/kg Group in the 2-Year Study
The limit of detection is shown with a dotted line.

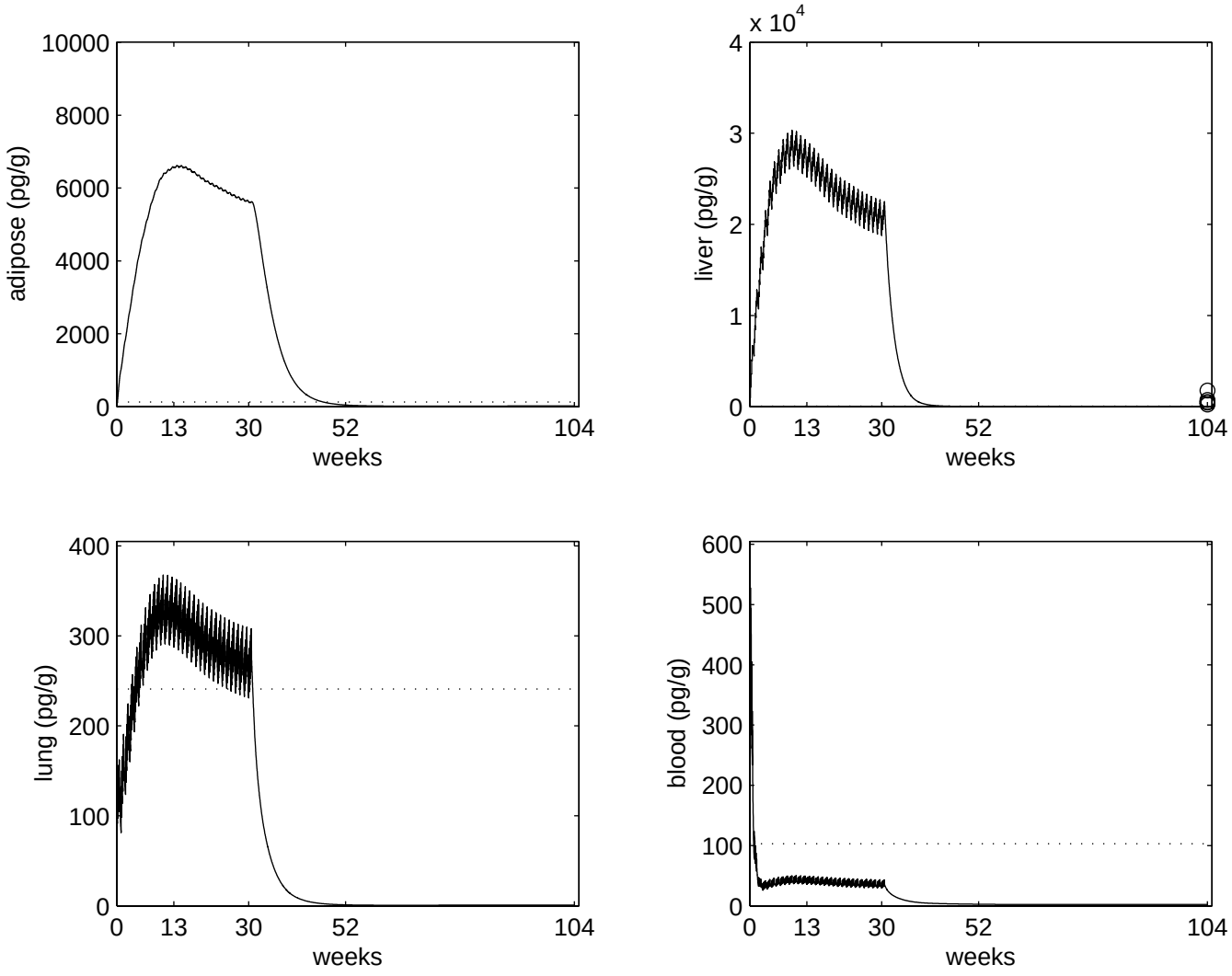

FIGURE H7
Model Predictions (–) and Individual Tissue Data (○) for the 100 ng/kg Stop-Exposure Group in the 2-Year Study
The limit of detection is shown with a dotted line.

APPENDIX I
ASSOCIATED PUBLICATIONS

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

Brix, A.E., Jokinen, M.P., Walker, N.J., Sells, D.M., and Nyska, A. (2004). Characterization of bronchiolar metaplasia of the alveolar epithelium in female Sprague-Dawley rats exposed to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126). *Toxicol. Pathol.* **32**, 333-337.

Brix, A.E., Nyska, A., Haseman, J.K., Sells, D.M., Jokinen, M.P., and Walker, N.J. (2005). Incidences of selected lesions in control female Harlan Sprague-Dawley rats from two-year studies performed by the National Toxicology Program. *Toxicol. Pathol.* **33**, 477-483.

Hailey, J.R., Walker, N.J., Sells, D.M., Brix, A.E., Jokinen, M.P., and Nyska, A. (2005). Classification of proliferative hepatocellular lesions in Harlan Sprague-Dawley rats chronically exposed to dioxin-like compounds. *Toxicol. Pathol.* **33**, 165-174.

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

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

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

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

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

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

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

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

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

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

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

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

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