NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats

Technical Report 598

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Foreword

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

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

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

NTP Technical Reports are indexed in the National Center for Biotechnology Information (NCBI) Bookshelf database and are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov). Toxicity data are available through NTP's Chemical Effects in Biological Systems (CEBS) database: https://www.niehs.nih.gov/research/resources/databases/index.cfm.

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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 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 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 on 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 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.

Peer Review

The members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats* on December 12, 2019, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members had 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.

Peer Reviewers

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Abstract

Perfluorooctanoic acid (PFOA) is a perfluorinated alkyl substance (PFAS) with widespread exposure in the environment and human population. Lifetime exposure to this chemical is likely, which includes in utero and postnatal development. Previously conducted chronic carcinogenicity studies of PFOA began exposure after these critical periods of development, so it is unknown whether the carcinogenic response is altered if exposure during gestation and lactation is included. The current PFOA chronic studies were designed to assess the contribution of combined gestational and lactational exposure (herein referred to as perinatal exposure) to the chronic toxicity and carcinogenicity of PFOA. The hypothesis tested was that including exposure during gestation and lactation (perinatal exposure) with postweaning exposure would change the PFOA carcinogenic response quantitatively (more neoplasms) or qualitatively (different neoplasm types) compared to postweaning exposure alone.

This hypothesis was tested using a design of exposing time-mated Sprague Dawley (Hsd:Sprague Dawley® SD®) rats to 0, 150, or 300 ppm PFOA during the perinatal period, after which the F_1 male rats were provided 150 or 300 ppm PFOA (i.e., perinatal/postweaning exposures of 0/0, 0/150, 150/150, 0/300, and 300/300 ppm) and the F_1 female rats were provided 300 or 1,000 ppm PFOA (i.e., 0/0, 0/300, 150/300, 0/300, and 300/1,000 ppm) during the postweaning period (n = 50/sex/dose). Female rats have a lower systemic exposure due to a faster PFOA elimination rate than males, so a higher feed exposure concentration was provided to female rats postweaning. An interim necropsy (n = 10/sex/group) at 16 weeks (19 weeks of age) was conducted.

Due to unanticipated toxicity in male rats observed at the 16-week interim time point, males were removed from the first study at week 21. A second study of males only was started that used lower postweaning feed concentrations. In this second study, the pregnant females were exposed to a single feed concentration of 300 ppm PFOA because this exposure was well tolerated.

Sixteen-week Interim Evaluation

In general, toxicity was observed in the liver, glandular stomach, kidney, and thyroid gland in males and in the liver, kidney, and thyroid gland in females at the 16-week interim evaluation. Body weights were lower in exposed groups of males and females compared to control groups as exposure concentrations increased. Plasma concentrations of PFOA were consistently higher in males compared to females and consistent between animals that were exposed to PFOA perinatally and postweaning versus postweaning exposure alone. Acyl-CoA oxidase activity in the liver was consistently elevated in males and females (males had higher activity than females) regardless of their exposure during the perinatal period.

Two-year Studies

Survival was unaffected by PFOA exposure, and there were exposure-related decreases in body weight compared to control groups in both male and female rats. Male rats had increased incidences of hepatocellular adenomas in the 0/40, 300/40, 0/80, and 300/80 ppm groups compared to the 0/0 ppm control group, and higher incidences of hepatocellular carcinomas were observed in the 300/80 ppm group compared to the 0/80 group. Increased pancreatic acinar cell adenomas and adenocarcinomas were observed in all postweaning exposed groups (20, 40, and 80 ppm) with or without perinatal exposure. Although not statistically significant, there were

occurrences in female rats of pancreatic acinar cell adenomas and adenocarcinomas in the 0/1,000 and 300/1,000 ppm female groups compared to the 0/0 ppm control group. Marginally higher numbers of hepatocellular carcinomas and uterine adenocarcinomas were also observed in the PFOA-exposed groups regardless of perinatal exposure. Nonneoplastic lesions were only observed in the liver and pancreas of male rats, whereas lesions were increased in the liver, kidney, forestomach, and thyroid gland of female rats.

In general, very few significant differences were observed between the responses of groups of animals exposed to PFOA postweaning only versus groups with both perinatal and postweaning exposures, and most of these differences were considered sporadic. The response to PFOA in female rats was generally less than that of male rats, which was consistent with the lower internal plasma concentrations of PFOA in female rats relative to male rats.

Conclusions

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity*[a] of PFOA in male Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas. The additional effect of combined perinatal and postweaning exposure was limited to a higher incidence of hepatocellular carcinomas in male rats compared to postweaning exposure alone.

There was *some evidence of carcinogenic activity* of PFOA in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure. The combined perinatal and postweaning exposure was not observed to change the neoplastic or nonneoplastic response compared to the postweaning exposure alone in female rats.

Exposure to PFOA resulted in increased incidences of nonneoplastic lesions in the liver and pancreas of male rats and in the liver, kidney, forestomach, and thyroid gland of female rats.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity.

Summary of the Two-year Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid with and without Perinatal Exposure

	Male Sprague Dawley Rats	Female Sprague Dawley Rats
Concentrations in Feed		
Postweaning	0/0, 0/20, 0/40, 0/80 ppm	0/0, 0/300, 0/1,000 ppm
Perinatal + Postweaning	300/0, 300/20, 300/40, 300/80 ppm	0/0, 150/300, 300/1,000 ppm
Survival Rates		
Postweaning	36/50, 42/50, 34/50, 36/50	23/50, 26/50, 23/50
Perinatal + Postweaning	34/50, 38/50, 38/50, 39/50	23/50, 32/50, 22/50
Body Weights		
Postweaning	0/80 ppm group: 82–90% of the 0/0 ppm control group weight after week 6	0/1,000 ppm group: 78–88% of the 0/0 ppm control group weight after week 2
Perinatal + Postweaning	300/80 ppm group: 83–90% of the 0/0 ppm control group weight after week 6	300/1,000 ppm group: 73–86% of the 0/0 ppm control group weight after week 2
Nonneoplastic Effects		
Postweaning	Liver: hepatocyte, cytoplasmic alteration (0/50, 12/50, 34/50, 46/50); hepatocyte, hypertrophy (0/50, 13/50, 34/50, 43/50); hepatocyte, single cell death (1/50, 1/50, 11/50, 24/50); necrosis (2/50, 17/50, 23/50, 20/50); pigment (0/50, 7/50, 15/50, 30/50) Pancreas: acinus, hyperplasia (18/50, 32/50, 37/50, 31/50)	Liver: hepatocyte, cytoplasmic alteration (0/50, 9/50, 49/49); hepatocyte, hypertrophy (0/50, 11/50, 48/49); hepatocyte, single cell death (0/50, 4/50, 29/49); necrosis (0/50, 1/50, 8/49); pigment (3/50, 5/50, 43/49); bile duct hyperplasia (16/50, 25/50, 22/49); hepatocyte, increased mitoses (2/50, 3/50, 4/49) Kidney: papilla, urothelium, hyperplasia (4/50, 21/50, 40/49); papilla, necrosis (0/50, 0/50, 12/49); renal tubule, mineral (5/50, 6/50, 16/49) Forestomach: ulcer (2/50, 2/50, 9/49); epithelium, hyperplasia (4/50, 5/50, 22/49); submucosa, inflammation, chronic active (3/50, 2/50, 16/49) Thyroid gland: follicular cell, hypertrophy (4/50, 8/50, 28/49)
Perinatal + Postweaning	Liver: hepatocyte, cytoplasmic alteration (0/50, 4/50, 29/50, 41/50); hepatocyte, hypertrophy (1/50, 4/50, 29/50, 42/50); hepatocyte, single cell death (1/50, 3/50, 5/50, 29/50); necrosis (1/50, 11/50, 14/50, 21/50); pigment (0/50, 4/50, 11/50, 26/50) Pancreas: acinus, hyperplasia (23/50, 27/50, 38/50, 33/50)	Liver: hepatocyte, cytoplasmic alteration (0/50, 17/50, 49/50); hepatocyte, hypertrophy (0/50, 16/50, 49/50); hepatocyte, single cell death (0/50, 5/50, 32/50); necrosis (0/50, 4/50, 5/50); pigment (3/50, 10/50, 40/50); bile duct hyperplasia (16/50, 27/50, 27/50); hepatocyte, increased mitoses (2/50, 5/50, 10/50)

	Male Sprague Dawley Rats	Female Sprague Dawley Rats
		Kidney: papilla, urothelium, hyperplasia (4/50, 8/50, 45/50); papilla, necrosis (0/50, 0/50, 22/50); renal tubule, mineral (5/50, 8/50, 8/50) Forestomach: ulcer (2/50, 1/50, 11/50); epithelium, hyperplasia (4/50, 3/50, 21/50); submucosa, inflammation, chronic active (3/50, 2/50, 18/50) Thyroid gland: follicular cell, hypertrophy (4/50, 9/50, 19/50)
Neoplastic Effects		
Liver	Postweaning: hepatocellular adenoma (0/50, 0/50, 7/50, 11/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 0/50); hepatocellular adenoma or carcinoma (0/50, 0/50, 7/50,11/50) Perinatal + Postweaning: hepatocellular adenoma (0/50, 1/50, 5/50, 10/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 4/50); hepatocellular adenoma or carcinoma (0/50, 1/50, 5/50, 12/50)	None
Pancreas	Postweaning: acinar cell adenoma (3/50, 28/50, 26/50, 32/50); acinar cell adenocarcinoma (0/50, 3/50, 1/50, 3/50), acinar cell adenoma or adenocarcinoma (3/50, 29/50, 26/50, 32/50) Perinatal + Postweaning: acinar cell adenoma (7/50, 18/50, 30/50, 30/50); acinar cell adenocarcinoma (0/50, 2/50, 1/50, 3/50), acinar cell adenoma or adenocarcinoma (7/50, 20/50, 30/50, 30/50)	Postweaning: acinar cell adenoma (0/50, 0/50, 1/49); acinar cell adenocarcinoma (0/50, 0/50, 1/49); acinar cell adenoma or adenocarcinoma (0/50, 0/50, 2/49) Perinatal + Postweaning: acinar cell adenoma (0/50, 0/50, 3/50); acinar cell adenocarcinoma (0/50, 0/50, 2/50); acinar cell adenoma or adenocarcinoma (0/50, 0/50, 5/50)
Equivocal Findings		
Liver	None	Postweaning: hepatocellular carcinoma (1/50, 1/50, 3/49) Perinatal + Postweaning: hepatocellular carcinoma (1/50, 0/50, 4/50)
Uterus	None	Postweaning: adenocarcinoma (1/50, 5/50, 8/50) Perinatal + Postweaning: adenocarcinoma (1/50, 3/50, 5/50)
Level of Evidence of Carcinogenic Activity	Clear evidence	Some evidence

Overview

The per/polyfluorinated alkyl substances (PFAS) class was nominated to the National Toxicology Program (NTP) by the U.S. Environmental Protection Agency for a variety of in vitro and in vivo toxicity assessments. These included evaluation of the toxicokinetics of seven PFAS chemicals after a single dose in rats; toxicity comparisons of seven PFAS chemicals in 28-day rat studies; and in vitro class evaluations of potential neurotoxicity, mitochondrial toxicity, and immunotoxicity, with a follow-up in vivo immunotoxicity study on perfluorodecanoic acid.

In addition to the above studies, an assessment of perinatal (gestational and lactational) perfluorooctanoic acid exposure on chronic toxicity and carcinogenicity was evaluated in rats. These studies are presented in this Technical Report.

As the PFAS class continues to expand with new uses and replacements, NTP continues to assess the potential toxicity of these chemicals through a variety of methods, including in silico, in vitro, and in vivo studies.

Introduction

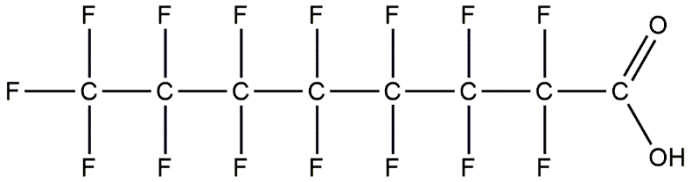


Figure 1. Perfluorooctanoic Acid (CAS No. 335-67-1; Chemical Formula $C_8HF_{15}O_2$; Molecular Weight: 414.07)

Chemical and Physical Properties

Perfluorooctanoic acid (PFOA) is a white powder at room temperature and, as a free acid, has a melting point of 54°C and a boiling point of 192°C. At 25°C, PFOA has a water solubility of 9,500 mg/L and a vapor pressure of 0.525 mm Hg[1]. PFOA has an estimated log K_{OW} of 4.81[2].

Production, Use, and Human Exposure

PFOA was widely used in the manufacturing of a variety of consumer products that included many nonstick applications, such as for clothing and cookware[3]. However, due to concerns about persistence and potential toxicity, PFOA was removed from commerce through a 2006 agreement between the U.S. Environmental Protection Agency (EPA) and U.S. manufacturers to phase it out of production by 2015[1]. Although PFOA is no longer produced in the United States, breakdown of other per/polyfluorinated alkyl substances (PFAS) may lead to continued PFOA exposure[4]. In addition, concentrations of PFAS can persist in the population for a long time due to a slow elimination rate in humans and persistence in the environment.

Many surveys have been conducted of PFOA exposure in humans with general findings of widespread but lower exposure concentrations in the general population, higher concentrations around communities in areas of manufacturing and use, and the highest concentrations in manufacturing plants. The U.S. National Health and Nutrition Examination Survey (NHANES) has been evaluating PFOA concentrations in the U.S. general population for nearly two decades. The geometric mean from the 1999–2000 evaluation was 5.21 μg/L serum, which dropped to 1.56 μg/L in the 2015–2016 assessment[5]. Near areas of manufacturing or use of PFOA, community concentrations were notably higher than were those in the general population. In members of the community around the Washington Works manufacturing plant in West Virginia, median concentrations were 28.2 μg/L, but concentrations were 147.8 μg/L for those working in the plant[6]. Prior to its discontinued use, mean serum concentrations for workers were as high as 1,030 to 1,090 μg/L in other plants[7].

Regulatory Status

As mentioned, PFOA is not currently used in commerce because EPA and U.S. manufacturers came to an agreement to phase out its production[1]. However, it is still present in many areas of the environment due to its persistence, most notably in drinking water. EPA established a PFOA drinking water health advisory of 70 parts per trillion (ppt) (70 ng/L) in 2016[8] based on decreased ossification in mice pups and accelerated male puberty[9]. The Agency for Toxic

Substances and Disease Registry (ATSDR) recently developed provisional PFOA minimal risk levels of 78 ppt (78 ng/L) for adults and 21 ppt (21 ng/L) for children[10] given research showing increased locomotor activity in adult mouse offspring[11] and altered mouse bone morphology[12]. Individual states have also developed PFOA drinking water values in accordance with their assessments; New Jersey developed a drinking water guidance of 14 ppt (14 ng/L) and Minnesota developed a Health Risk Limit of 35 ppt (35 ng/L)[13; 14]. Recently, the European Food Safety Authority developed tolerable weekly intakes of 6 ng/kg/week for PFOA based on human endpoints[15].

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Several studies have evaluated the absorption, distribution, metabolism, and excretion properties of PFOA. PFOA is generally well absorbed and the highest concentrations are typically observed in the plasma, serum, and liver, followed by the kidney[16]. PFOA is water soluble, and most of its elimination occurs via urine without metabolism. Very little to no accumulation has been observed in the brain[17]. A range of elimination rates have been reported and vary across species. In addition, differences in elimination occur between the sexes, most notably in rats. In rats, half-lives of 2 to 15 days have been reported in males depending on the dose[17-23]. In female rats, much faster half-lives have been observed in the order of 2 to 12 hours[17; 19; 21]. In mice, half-lives of 22 days in males and 16 days in females were estimated after a single dose[24]. In cynomolgus monkeys, a half-life of 14 to 42 days was estimated[25].

Reuptake via renal absorption has been proposed to explain the differential elimination of the PFAS class across species and sexes. The notable sex difference of PFOA elimination in rats is reduced by castration of males, which increases clearance of PFOA, with evidence suggesting changes in organic anion transporter (OAT) 2 and OAT3 as the mechanism[26]. This mechanism is assumed to apply to the other PFAS that display similar sex differences. In addition, protein binding may be another mechanism. Modeling of PFAS pharmacokinetics has included a saturable renal resorption function[24; 27-31].

Humans

The half-life of PFOA, determined by measuring blood concentrations in retired workers (n = 26), was estimated to be 3.8 years (arithmetic mean) and 3.5 years (geometric mean)[32]. A half-life average of 2.7 years was estimated from a community (n = 106) exposed to drinking water contaminated with PFOA[33]. A small study of ski wax technicians (n = 6), who use ski wax and wax-related products that contain PFOA, estimated a half-life average of 2.4 years[34].

Toxicity

Experimental Animals

Extensive literature exists on the toxicity of PFOA in a variety of species. A common target is the liver, with findings ranging from liver hypertrophy or necrosis to alterations in liver enzymes and clinical chemistry changes. Peroxisome proliferator-activated receptor alpha (PPARα) activation by PFAS in rodents is a mechanistic pathway that is frequently attributed to PFOA

exposure, and PPARα potency across the class varies with chain length[35-40]. Gene expression profiling of the liver also suggests that constitutive androstane receptor (CAR) induction is another factor in liver toxicity and enzyme alterations[41; 42], with additional studies showing PFOA increases liver PPARα and CAR/pregnane X receptor activation leading to increases in cytochrome P450 (Cyp) 2B1/2, Cyp3A1, and Cyp4A1 protein concentrations[43]. In male Crl:CD BR rats exposed to ammonium perfluorooctanoate (APFO) in feed (up to 100 parts per million [ppm]) for 13 weeks, toxicity findings included increased liver weight, hepatocellular hypertrophy, increased liver palmitoyl-CoA oxidase activity, and decreased serum cholesterol[44]. Exposure of cynomolgus monkeys to APFO for 6 months resulted in increases in triglycerides, decreases in bilirubin, increased liver weight, and decreases in thyroid hormones without histological lesions[45].

Humans

Numerous epidemiology studies have evaluated the relationship between health effects or outcomes and exposure to PFOA. These studies include both occupational and environmental exposure, including studies of individuals living in West Virginia's Mid-Ohio Valley that were exposed to high concentrations of PFOA in the drinking water from an industrial plant. An expert panel[46] and ATSDR[10] reviewed these studies and concluded that PFOA exposure was associated with liver damage[10], increases in serum lipid concentrations, especially cholesterol, pregnancy-induced hypertension and/or pre-eclampsia, immune effects, and thyroid diseases[10; 46].

Reproductive and Developmental Toxicity

Experimental Animals

PFOA has been evaluated in several rodent studies for developmental and reproductive toxicity. Gestational exposure to PFOA in mice resulted in developmental and reproductive toxicity ranging from whole litter loss to developmental delays[9; 47]. A two-generation study in Sprague Dawley rats observed a small reduction in pup survival during lactation, decreases in body weights, and delays in pubertal markers at 30 mg/kg, with changes in organ weights and body weights occurring at lower doses[48].

Humans

PFOA exposure has been proposed to be associated with reproductive and developmental effects in humans. Reproductive effects include irregular and long menstrual cycles and delayed onset of menarche in adolescent females. Both ATSDR[10] and the C8 panel reports[46] concluded that PFOA exposure was associated with pregnancy-induced hypertension[46] or pre-eclampsia[10]. Several meta-analyses[49; 50] have reported that PFOA is associated with lower infant birth weight; however, ATSDR noted that the decrease in birth weight is small and may not affect the infant's health. The interpretation of these findings is unclear as many studies are cross-sectional and the potential for reverse causality and potential bias exists. The association of low birth weight and PFOA exposure may be a case of reverse causality: lower birth weight leads to a lower glomerular filtration rate, resulting in higher concentrations of PFOA in maternal blood sampled later in pregnancy. A recent meta-analysis found that associations with decreased birth weight were mainly limited to studies that sampled maternal blood in the third trimester and found little evidence of an association in studies sampling blood early in pregnancy[51]. A systematic review

identified several human health categories of concern in children with respect to exposure to PFAS, including dyslipidemia, immunity (vaccine response and asthma), renal function, and age at menarche[52].

Immunotoxicity

Experimental Animals

Several studies have demonstrated the immunomodulatory effects of PFOA in mice. Across multiple strains, short-term exposure to PFOA resulted in atrophy of the spleen and thymus, reduction in splenic and thymic lymphocytes, and suppressed the T-cell dependent antibody response (TDAR). These effects occurred in the range of 9.6 to 33 mg/kg/day and were consistent across gavage, feed, and drinking water routes of exposure[53-56]. The most sensitive effects observed were reductions in TDAR and T-cell independent antibody responses at 3.75 and 1.88 mg/kg/day, respectively, in female C57Bl6 mice exposed via drinking water for 15 days[57; 58]. Two additional studies report an increased hypersensitivity response in BALB/c mice following PFOA exposure[59; 60].

Humans

Epidemiological studies provide evidence to suggest a link between PFOA exposure and immunomodulation. Elevated serum PFOA concentrations during development were associated with a reduced antibody response (postvaccination) in Norwegian and Faroe Island birth cohort studies[61-63] and in a cross-sectional study of adolescents using NHANES data[64]. Similar reductions in antibody concentrations were observed following administration of the influenza vaccine in adults in the Mid-Ohio Valley[65]. Additional immune-related outcomes associated with PFOA exposure include increased hypersensitivity, reduced resistance to infectious disease, and an increase in autoimmune disease. In addition, the National Toxicology Program (NTP)[66] conducted a systematic review of immune effects and concluded that PFOA is presumed to be an immune hazard based on evidence from human and animal studies. NTP concluded that there was a moderate level of evidence from studies in humans that PFOA suppresses antibody response, with evidence from developmental, childhood, and adult exposures.

Carcinogenicity

Experimental Animals

Two rodent studies have examined the carcinogenic activity of PFOA. The first study, conducted in the early 1980s with Sprague Dawley rats exposed to 0, 30, or 300 ppm PFOA, showed increased incidences of Leydig cell adenomas of the testis in males and female mammary gland fibroadenomas in the 300 ppm groups[67]. Further review of the mammary gland fibroadenomas from this study found that they were not in fact increased, as more were observed in the control group than initially reported[68]. A follow-up study in male Sprague Dawley rats exposed to 300 ppm PFOA reported increased incidences of hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell tumors[69]. Review of the pancreas from the first study found increased incidences of acinar cell hyperplasia and a single incidence of an acinar cell carcinoma, which was more consistent with the follow-up study[70]. A tumor promotion study in rainbow trout found the high dose of PFOA (1,800 ppm, or 50 mg/kg per day) increased the

combined incidence of liver tumors in aflatoxin-initiated fish, whereas PFOA alone did not induce liver tumors[71].

Humans

The carcinogenic activity of PFOA in humans was recently reviewed by the International Agency for Research on Cancer (IARC)[72]. Approximately a dozen epidemiological studies have evaluated the possible carcinogenic effects of PFOA in different exposure scenarios (e.g., occupational, community). IARC concluded that PFOA was possibly carcinogenic to humans (Group 2B) because the studies evaluated showed limited evidence in humans of associations with cancer of the testis and kidneys. The previously mentioned animal carcinogenicity studies also suggest limited evidence in animals.

Genetic Toxicity

The genetic toxicity of PFOA has been evaluated in bacterial mutagenicity assays, in vitro tests using human and rodent cells, and one animal study. Whereas bacterial mutagenicity assays using several *Salmonella typhimurium* test strains were uniformly negative, conflicting results were obtained for PFOA using in vitro tests with mammalian cells. In the single in vivo study, PFOA was reported to induce oxidative damage to DNA obtained from rat liver cells[73].

PFOA was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the absence or presence of exogenous rat liver metabolic enzymes (S9 mix) at concentrations up to 5 μmol/plate[74]. PFOA was also negative in *S. typhimurium* test strains TA98, TA100, TA102, and TA104 in the absence or presence of S9 mix when tested up to 500 μM[75].

Using the comet assay, significant, dose-dependent increases in DNA damage were observed in human lymphoblastoid TK6 cells exposed to concentrations of PFOA that ranged from 125 to 500 ppm for 2 hours[76]. Small increases in 8-hydroxy-2′-deoxyguanosine DNA adducts (per 10^5 dG) as detected using high-performance liquid chromatography (HPLC)-mass spectrometry (MS) were also reported using the same exposure protocol. In HepG2 cells, PFOA induced significant, dose-dependent increases in DNA damage in the comet assay, in 8-hydroxy-2′-deoxyguanosine as detected by immunostaining, and in micronuclei after 1, 3, or 24 hours of exposure, respectively; the same concentrations, ranging from 50 to 400 μM, were used for each assay[77]. Significant increases in DNA damage, as detected using the comet assay, were also observed in HepG2 cells exposed to PFOA at concentrations ranging from 0.2 to 20 μM for 24 hours[78]. PFOA was negative in the comet assay when HepG2 cells were evaluated after 1 or 24 hours of exposure to concentrations ranging from 5 to 400 μM, and was negative in the micronucleus assay when HepG2 cells were evaluated 24 hours after exposure using the same conditions; however, the percentage of DMSO, a known free radical scavenger that was used for these experiments, was relatively high at 2.5%[79]. There was no increase in DNA damage in the comet assay in Syrian hamster embryo cells after 5 or 24 hours of exposure to concentrations of PFOA of up to 300 μM[80]. Also, negative results were obtained in the comet assay when freshly isolated Wistar rat testicular cells were exposed to 100 or 300 μM PFOA for 24 hours[81].

One study examined the genotoxicity of PFOA in vivo. Using HPLC and an electrochemical detector (ECD) system, small increases in 8-hydroxy-2′-deoxyguanosine DNA adducts (per 10^5 dG) were detected in DNA obtained from the liver, but not the kidney, of male F344 rats

exposed to 0.02% PFOA in feed for 2 weeks, or at 3, 5, or 8 days after a single intraperitoneal injection of 100 mg/kg PFOA[73]. Increases in 8-hydroxy-2′-deoxyguanosine (per 10^5 dG) in DNA obtained from the liver or kidney were not observed at 1 day after injection. Considering that oxidative damage to DNA typically undergoes rapid repair, the small increases in 8-hydroxy-2′-deoxyguanosine that were detected by Takagi et al.[73] several days after injection may not necessarily have been due to exposure to PFOA.

In a review of unpublished genetic toxicity test data generated in industry-sponsored studies, PFOA tested as either an ammonium or sodium salt was found to be negative in bacterial mutagenicity assays, the Chinese hamster ovary HGPRT forward mutation assay, the chromosomal aberration assay when performed using Chinese hamster ovary cells or primary human lymphocytes, and in the mouse bone marrow micronucleus assay[82].

In genetic toxicity testing conducted by NTP, PFOA was negative in bacterial mutagenicity assays and in the micronucleus assay[83]. PFOA was negative in *Salmonella* tester strains TA100 and TA98 and *E. coli* WP2 *uvrA* in the presence or absence of 10% rat liver S9. There were no increases in micronucleated reticulocytes in the peripheral blood of male and female Hsd:Sprague Dawley® SD® rats exposed for 28 days via gavage, and no changes were noted in the percentage of immature erythrocytes in peripheral blood of either sex, suggesting that PFOA did not induce bone marrow toxicity.

Study Rationale

The PFAS class was nominated by EPA for toxicity evaluations. Concern that exposure to PFOA could lead to unexpected carcinogenic effects due to exposures during early life development was considered in the nomination. Previous carcinogenicity studies of PFOA[67; 69] began exposure after the critical periods of in utero and early postnatal development, so it is unknown whether the carcinogenic response is altered if exposure during gestation and lactation is included. NTP tested the hypothesis that including exposure during gestation and lactation (perinatal exposure) would change the PFOA carcinogenic response quantitatively (more neoplasms) or qualitatively (different neoplasm types). Feed exposure was selected as it is a common route of exposure in the human population.

Design of this study was based on previous NTP assessments of early developmental exposures influencing carcinogenic activity[84-86]. This approach uses a matrix-type design that evaluates different gestation/lactation doses. Comparisons were made between groups that received exposure during the perinatal and postweaning periods with groups exposed during the postweaning period only. Exposure concentrations were selected based on available literature and a 16-week interim necropsy was included to assess ongoing toxicity. Due to the identified sex differences in PFOA elimination rates, doses were increased in female rats after weaning to ensure systemic exposure. The male rat portion of this study was stopped and restarted due to observed toxicity occurring at the 16-week interim necropsy.

Materials and Methods

Procurement and Characterization of Perfluorooctanoic Acid

PFOA was obtained from Sigma-Aldrich (St Louis, MO) in a single lot (03427TH). Identity, purity, and stability analyses were conducted under the analytical chemistry laboratory and study laboratory at Battelle (Columbus, OH) (Appendix A). Reports on analyses performed in support of the PFOA studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

PFOA lot 03427TH is a white crystalline solid. The lot was identified using infrared (IR) spectroscopy, ^{19}F and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography (GC) with mass spectrometry (MS) detection. The IR and NMR spectra are in good agreement with the structure of PFOA. GC-MS detection confirmed that the primary peak was PFOA.

The purity of lot 03427TH was evaluated using GC with flame ionization detection (FID) and ion chromatography (IC) with conductivity detection. GC-FID analysis yielded a purity of 98.3% and four impurities with areas greater than or equal to 0.1% of the total peak area. IC with conductivity detection yielded a purity of 98.8% with three impurities with areas greater than 0.1% of the total peak area. GC with electron capture detection (ECD) showed that the impurities are likely fluorinated compounds. GC-MS detection showed that two of the impurities representing ~1% of the total area were isomers of PFOA; additional impurities were determined to be perfluorooctenoic acid (0.51%) and a structurally dissimilar fluorinated compound (0.11%). The purity of PFOA evaluated using differential scanning calorimetry (DSC) was 98.96%. Karl Fischer titration performed at Pervalere Life Science, Inc. (Whitesboro, NY) yielded a water content of 0.24%. The overall purity of lot 03427TH was determined to be greater than 98%.

To ensure stability, the test chemical was stored in amber glass bottles at ambient temperature (~25ºC). Periodic analyses of lot 03427TH of the test chemical were performed prior to and during the animal studies by the study laboratory using GC-ECD; no degradation of the test chemical was detected.

Preparation and Analysis of Dose Formulations

Prior to conducting studies, the homogeneity of PFOA (30 and 300 ppm) in NIH-07 and NTP-2000 formulations was confirmed by the analytical chemistry laboratory using GC-FID. Homogeneity of PFOA in NIH-07 (150 and 300 ppm PFOA) and NTP-2000 (20, 80, 150, 300, and 1,000 ppm PFOA) formulations was also confirmed by the study laboratory using GC-ECD.

Stability studies were performed by the analytical chemistry laboratory at 30 ppm PFOA in NIH-07 and NTP-2000 formulations up to 42 days. Formulations of NIH-07 were stable when stored refrigerated (~5ºC), whereas formulations of NTP-2000 were stable when stored refrigerated (~5ºC) or at room temperature (~25ºC).

Dosed feed formulations were prepared monthly (Table A-1) for both the 21-week and 2-year studies using two types of feed (NIH-07 and NTP-2000). Formulations were stored in plastic

bag-lined buckets up to 42 days. Formulations of NIH-07 were refrigerated (~5ºC), whereas formulations of NTP-2000 were stored at room temperature (~25ºC).

Analysis of preadministration and postadministration (animal room) dose formulations of PFOA was conducted every 1–3 months over the course of the study (Table A-3, Table A-4). All preadministration formulations in both the 21-week and 2-year studies were within 10% of the target concentration. All of the postadministration samples collected from feed buckets were within 10% of the target concentration. Of the few postadministration formulation samples collected from residual animal food bowls, one sample from the 21-week study, and three samples from the 2-year study were lower than 10% of the target concentration (11.5–13.4% below target).

Animal Source

Time-mated (F_0) female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Harlan, Inc. (Madison, WI, or Indianapolis, IN; now Envigo), for the 2-year studies.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the Battelle Columbus Operations (Columbus, OH) Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health (NIH) and National Toxicology Program (NTP) animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-year Studies

Exposure Concentration Selection Rationale

Initial design of these studies was based on previous NTP assessments of early developmental exposures influencing carcinogenic activity[84-86]. This approach uses a matrix-type design that evaluates different gestation/lactation doses. PFOA exposure concentrations were selected based on previous chronic studies[67; 69] and reproductive studies[48] in the literature, in addition to known sex differences in elimination rates[21]. Exposure concentrations of 150 or 300 ppm during gestation and lactation (these combined developmental periods are referred to as perinatal exposure in this report) were not expected to negatively affect litter size or survival, but still result in significant internal exposure.

Due to the known differences in kinetics between male and female rats, females were exposed to higher exposure concentrations (300 or 1,000 ppm) than were males (150 or 300 ppm) in the postweaning period of the first study (Table 1). An interim necropsy at 16 weeks (19 weeks of age) was included to assess toxicity and internal exposure in both males and females. A second study was started that focused entirely on males due to observed toxicity at the 16-week interim, and postweaning exposure concentrations were lowered (Table 2). Only a single perinatal exposure concentration was used (300 ppm) to compare to the unexposed rats during that developmental period because this exposure was well tolerated and postweaning comparisons (e.g., 0/80 versus 300/80) were the main objective of the study.

Table 1. Exposures during Perinatal and Postweaning Periods in Rats in the First Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

Perinatal Exposure	Postweaning Exposure			
Feed (ppm)	**0**	**150**	**300**	**1,000**
0	M/F	M	M/F	F
150	–	M	F	–
300	–	–	M	F

M = male; F = female.

Table 2. Exposures during Perinatal and Postweaning Periods in Male Rats in the Second Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

Perinatal Exposure	Postweaning Exposure			
Feed (ppm)	**0**	**20**	**40**	**80**
0	M	M	M	M
300	M	M	M	M

M = male.

Male and Female Rats (Study 1)

F_0 female rats were 12 weeks old upon receipt. Gestation day (GD) 1 was defined as the first day with evidence of mating; F_0 females were received on GD 2 and held for 4 days. F_0 females were randomly assigned to three exposure groups on GD 5; 103 to the 0 ppm group and 36 each to the 150 and 300 ppm groups. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM (Xybion Medical Systems Corporation, Cedar Knolls, NJ). Exposure of F_0 females to PFOA in feed began on GD 6.

F_0 females were quarantined for 36 days after receipt. Ten nonmated females received in the same shipment as the time-mated dams were designated for disease monitoring and were used for gross necropsies 2 days after arrival; samples were collected for serological analyses, and the animals were terminated, necropsied, and examined for the presence of disease or parasites. The health of the F_1 animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All results were negative.

F_0 females were housed individually during gestation and with their respective litters during lactation. F_0 females were weighed on GDs 5, 6, 9, 12, 15, 18, and 21 and on postnatal days (PNDs) 1 (with whole litter), 4, 7, 14, and 21. F_1 pups were weighed on PNDs 4, 7, 14, and 21. The day of parturition was considered PND 0. F_0 females that did not deliver were euthanized on GD 27 and the uteri were stained for implantations/resorptions. On PNDs 1 and 4, the numbers of litters and pups and sex ratios for each litter were recorded. On PND 4, litters were standardized to eight pups per litter (four males and four females where possible); litters with fewer than eight pups or two pups per sex were removed from the study. During gestation and lactation, feed consumption per cage was measured continuously over 3- or 4-day intervals.

On the day the last litter reached PND 18, litters were randomly selected and assigned to exposure groups for the 2-year study; F_1 rats from these litters were then randomly selected for use in the 2-year study. On the day the last litter reached PND 21, dams were removed from the cages, and the pups were weaned (PND 21–23). All F_1 exposure groups consisted of 60 male or 60 female rats with two pups/sex/litter assigned to postweaning exposure groups.

F_1 pups were housed up to two per cage (males) or four per cage (females). Feed and water were available ad libitum. Feed consumption was measured weekly for 13 weeks and then for a week-long period at 4-week intervals, except that feed consumption was also recorded the week following the 16-week interim evaluation. Cages were changed twice weekly and rotated every 2 weeks. Further details of animal maintenance are given in Table 3. Information on feed composition and contaminants is provided in Appendix B.

Male Rats (Study 2)

F_0 female rats were 11 to 13 weeks old upon receipt. GD 1 was defined as the first day with evidence of mating; F_0 females were received on GD 2 and held for 4 days. F_0 females were randomly assigned to two exposure groups on GD 5 with 147 going to each of the 0 and 300 ppm groups. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM. Exposure of F_0 females to PFOA in feed began on GD 6.

F_0 female rats were quarantined for 37 days after receipt. Ten nonmated females received in the same shipment as the time-mated dams were designated for disease monitoring and were used for gross necropsies 2 days after arrival; samples were collected for serological analyses, and the animals were terminated, necropsied, and examined for the presence of disease or parasites. The health of the F_1 animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All results were negative.

F_0 females were housed individually during gestation and with their respective litters during lactation. F_0 females were weighed on GDs 5, 6, 9, 12, 15, 18, and 21 and on PND 1 (with whole litter), 4, 7, 14, and 21. F_1 pups were weighed on PNDs 4, 7, 14, and 21. The day of parturition was considered PND 0. F_0 females that did not deliver were euthanized on GD 27 and the uteri were stained for implantations/resorptions. On PNDs 1 and 4, the numbers of litters and pups and sex ratios for each litter were recorded. On PND 4, litters were standardized to eight pups per litter (four males and four females where possible); litters with fewer than eight pups or two pups per sex were removed from the study. During gestation and lactation, feed consumption per cage was measured continuously over 3- or 4-day intervals.

Select litters were removed on GD 18 and PND 4 to quantify PFOA plasma and tissue concentrations. On GD 18, blood was collected from the retroorbital sinus of randomly selected dams (n = 5 per exposure group). Blood samples were collected into tubes containing ethylene diamine tetraacetic acid (EDTA), centrifuged, and the plasma harvested. Each dam's fetuses were collected, pooled by litter, and flash frozen in liquid nitrogen. On PND 4, dams with unacceptable litters from the 0 ppm (n = 5) and 300 ppm (n = 4) groups were selected for biological sampling. Plasma was collected in the same manner as for GD 18. Up to four pups were collected from each dam (two per sex when possible) and flash frozen in liquid nitrogen. All samples were stored frozen between −30°C and −15°C before shipment on dry ice to Battelle's Chemistry Technical Center (CTC) for analysis.

On the day the last litter reached PND 18, litters were randomly selected and assigned to exposure groups for the 2-year study and then F_1 males from these litters were randomly selected for use in the 2-year study. On the day the last litter reached PND 21, dams were removed from the cages, and the pups were weaned (PND 21–23). All F_1 exposure groups consisted of 60 males.

F_1 males were housed up to two per cage. Feed and water were available ad libitum. Feed consumption was measured weekly for 13 weeks and then for a week-long period at 4-week intervals, except that feed consumption was also recorded the week following the 16-week interim evaluation. Cages were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 3. Information on feed composition and contaminants is provided in Appendix B.

Clinical Examinations and Pathology (Studies 1 and 2)

During the 2-year studies, all animals in both studies were observed twice daily. Individual body weights of F_1 pups were recorded on day 1 (postweaning), weekly for the first 13 weeks, at 4-week intervals thereafter, and again prior to termination. Clinical observations were recorded at 4-week intervals beginning during study week 5 and again prior to termination. As an exception to the 4-week intervals, body weights and clinical observations were recorded 2 weeks after the week 16 interim evaluation and data were recorded again 3 weeks later, resulting in one 5-week and one 3-week interval.

Ten rats were selected from each exposure group for interim evaluation at 16 weeks. Body weights were collected, as were blood samples for clinical chemistry determinations and plasma PFOA concentrations. Rats were anesthetized with 70% CO_2 and 30% O_2, and blood was taken from the retroorbital plexus and placed into tubes containing EDTA for plasma or into serum separator tubes for clinical chemistry. Clinical chemistry parameters were analyzed using a Cobas c501 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). The clinical chemistry parameters that were measured are listed in Table 3. Plasma, approximately 1 mL, was collected in the morning and stored frozen between −30°C and −15°C until transferred to Battelle's CTC for PFOA concentration analysis. Two, 1 g sections of liver were collected from 5 males and 5 females (Study 1) and 10 males (Study 2) per exposure group. One sample was immediately homogenized, snap frozen in liquid nitrogen, stored between −80°C and −60°C, and analyzed for the determination of acyl-CoA oxidase activity. The second sample was flash frozen in liquid nitrogen, stored, and analyzed for determination of aromatase activity. Acyl-CoA oxidase activity was used as a marker of PPARα induction, as PFOA is expected to be an inducer. Liver homogenates were centrifuged, and the supernatant added to 96-well plates along with palmitoyl-CoA solution. Kinetic reactions were analyzed by measuring absorbance at 502 nm for 5 minutes. Liver aromatase activity was evaluated to provide insight into potential endocrine-related lesions such as Leydig cell hyperplasia and/or neoplasia. Microsomal suspensions were prepared from liver samples and were incubated with radiolabeled androst-4-ene-3,17-dione and β-NADPH solutions for 1 hour. Samples were centrifuged, the aqueous layer was mixed with scintillation cocktail, and radioactivity was counted using a liquid scintillation counter (Beckman Coulter, Brea, CA). Another section of liver, approximately 100 mg, was collected from 10 males and 10 females (Study 1) and 10 males (Study 2) and stored frozen between −30°C and −10°C until transferred to Battelle's CTC for PFOA concentration analysis. The parameters measured are listed in Table 3.

For determination of internal dose, plasma and liver homogenate samples were analyzed using validated analytical methods that included protein precipitation and liquid chromatography (LC) tandem mass spectrometry (MS/MS) as described in Dzierlenga et al.[17]. The concentration in plasma was expressed as ng/mL and in tissues as ng/g. Fetal and pup homogenate (Study 2) were analyzed using methods similar to those for liver analysis.

Complete necropsies were performed on all F_1 rats. At the 16-week interim evaluations, the right adrenal gland, heart, right kidney, liver, lung, pituitary gland, spleen, right testis, thymus, thyroid gland, and uterus (Study 1) 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 (except eyes and testes, which were initially fixed in Davidson's solution or modified Davidson's solution, respectively), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin (H&E) for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. In the original evaluation of the uterus, a transverse section through each uterine horn, approximately 0.5 cm cranial to the cervix, was collected for histopathology evaluation. For the extended evaluation of the uterus, all remaining cervical, vaginal, and uterine tissue remnants stored in 10% neutral buffered formalin were trimmed longitudinally, embedded, sectioned, and stained with H&E. Tissues examined microscopically are listed in Table 3. Complete microscopic examinations were performed on all F_1 females and interim males in Study 1, on F_1 males in the 0/0 and 300/80 ppm groups at the interim evaluation of Study 2, and on all F_1 males at the end of Study 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment (QA) laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a QA pathologist evaluated slides from all neoplasms and all potential target organs, which included kidney, liver, pancreas, pancreatic islets, stomach (forestomach and glandular), testes, thyroid gland, and uterus.

The QA report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the QA pathologist and other pathologists experienced in rodent toxicologic pathology. 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[87] and Boorman et al.[88]. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was based generally on the guidelines of McConnell et al.[89].

Table 3. Experimental Design and Materials and Methods in the Two-year Feed Studies of Perfluorooctanoic Acid

Study 1 (Male and Female)	Study 2 (Male)
Study Laboratory	
Battelle (Columbus, OH)	Same as Study 1
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®) rats	Same as Study 1
Animal Source	
Harlan, Inc. (Madison, WI), now Envigo	Harlan, Inc. (Indianapolis, IN), now Envigo
Time Held Before Studies	
4 days (F_0 females)	Same as Study 1
Average Age When Studies Began	
12 to 13 weeks (F_0 females)	12 to 14 weeks (F_0 females)
Date of First Exposure	
September 19, 2008 (F_0 females) October 27, 2008 (F_1 males) October 28, 2008 (F_1 females)	June 19, 2009 (F_0 females) July 27, 2009 (F_1 males)
Duration of Exposure	
GD 6 to PND 21 (F_0 females) 21 weeks (F_1 males—study stopped) 107 weeks (F_1 females)	GD 6 to PND 21 (F_0 females) 107 weeks (F_1 males)
Date of Last Exposure	
October 27, 2008 (F_0 females) March 18, 2009 (F_1 males) November 12, 2010 (F_1 females)	July 27, 2009 (F_0 females) August 12, 2011 (F_1 males)
Necropsy Dates	
Interim evaluation: February 12 (F_1 males) or 13 (F_1 females), 2009 Core: November 10 through 12, 2010 (F_1 females)	Interim evaluation: November 11, 2009 (F_1 males) Core: August 8 through 12, 2011 (F_1 males)
Average Age at Necropsy	
Interim evaluation: 19 weeks (F_1 males and females) Core: 24 weeks (F_1 males—euthanized early) Core: 110 to 111 weeks (F_1 females)	Interim evaluation: 19 weeks (F_1 males) Core: 109 to 110 weeks (F_1 males)
Size of Study Groups	
F_0 females: 103 (0 ppm group) or 36 (150 and 300 ppm groups) F_1 rats: 60 males and 60 females	F_0 females: 147 F_1 males: 60
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights	Same as Study 1

Study 1 (Male and Female)	Study 2 (Male)
Animals per Cell	
F_0 females: One (plus litter) F_1 rats: Two (males) or four (females)	F_0 females: One (plus litter) F_1 males: Two
Method of Animal Identification	
F_0 females: Tail marking with permanent pen F_1 rats: Tail tattoo	Same as Study 1
Diet	
NIH-07 meal feed (perinatal phase) or irradiated NTP-2000 wafer diet (chronic phase) (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed every 3 days	Same as Study 1
Water	
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available ad libitum	Same as Study 1
Cages	
Polycarbonate (Lab Products, Inc., Seaford, DE), changed and sanitized at least once weekly (during gestation) or twice weekly; rotated every 2 weeks after weaning	Same as Study 1
Bedding	
Irradiated Sani-Chips® hardwood chips (P. J. Murphy Forest Products Corp., Montville, NJ), changed once weekly (during gestation) or twice weekly	Same as Study 1
Rack Filters	
Spun bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as Study 1
Racks	
Stainless steel (Lab Products, Inc.), changed and rotated every 2 weeks	Same as Study 1
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Same as Study 1
Exposure Concentrations	
F_0 females: 0, 150, or 300 ppm F_1 males: 0/0, 0/150, 0/300, 150/150, or 300/300 ppm F_1 females: 0/0, 0/300, 0/1,000, 150/300, or 300/1,000 ppm	F_0 females: 0 or 300 ppm F_1 males: 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, or 300/80 ppm

Study 1 (Male and Female)	Study 2 (Male)
Type and Frequency of Observation	
F_0 females: Observed twice daily. Body weights were recorded on GDs 5, 6, 9, 12, 15, 18, and 21 and on PNDs 1 (with litters), 4, 7, 14, and 21. Feed consumption was measured from GD 6 to PND 21. F_1 rats: Observed twice daily. Clinical observations were recorded at 4-week intervals beginning during study week 5 and again prior to termination. Body weights were recorded on PNDs 4, 7, 14, and 21; weekly for 13 weeks, then every 4 weeks. Feed consumption was recorded weekly for 13 weeks, then for 1 week every 4 weeks. Body weights and clinical observations were also recorded 2 weeks after the interim evaluation and feed consumption was also recorded 1 week after the interim evaluation.	Same as Study 1
Method of Euthanasia	
Carbon dioxide	Same as Study 1
Necropsy	
Necropsies were performed on all F_1 rats. Organs weighed at the interim evaluation were right adrenal gland, heart, right kidney, liver, lung, pituitary gland, spleen, right testis, thymus, thyroid gland, and uterus.	Necropsies were performed on all F_1 rats. Organs weighed at the interim evaluation were right adrenal gland, heart, right kidney, liver, lung, pituitary gland, spleen, right testis, thymus, and thyroid gland.
Clinical Pathology	
At the 16-week interim, blood was collected from the retroorbital plexus of 10 interim male and female rats per exposure group for clinical chemistry analyses. ***Clinical chemistry:*** albumin, urea nitrogen, creatinine, creatine kinase, glucose, total protein, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, cholesterol, triglycerides, and bile acids	At week 16, blood was collected from the retroorbital plexus of the interim male rats for clinical chemistry analyses. ***Clinical chemistry:*** Same as Study 1
Histopathology	
Complete histopathology was performed on all F_1 female rats and male rats at the 16-week interim and on all remaining F_1 rats at the end of the study. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle (interim evaluation only) skin, spleen, stomach (forestomach and glandular), testis with epididymis (interim evaluation only), thymus, thyroid gland, trachea, urinary bladder, and uterus. An additional extended evaluation of the uterus was performed which included all remaining cervical, vaginal, and uterine tissue remnants.	Complete histopathology was performed on F_1 males in the 0/0 and 300/80 ppm groups at the interim evaluation and on all remaining F_1 males at the end of the study. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, esophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder. The liver was examined in the remaining exposure groups at the interim evaluation.

Study 1 (Male and Female)	Study 2 (Male)
Internal Dose Assessment	
At the interim evaluation, PFOA concentrations were determined in plasma from blood collected from the retroorbital plexus and in liver samples collected from 10 males and 10 females per exposure group.	Maternal plasma and fetal (pooled by litter) PFOA concentrations were measured at GD 18; maternal plasma and whole pup PFOA concentrations were measured at PND 4. PFOA plasma concentrations were also measured in 10 males per exposure group at the interim evaluation.
Liver Enzyme Concentrations	
Two, 1 g liver samples were collected from five males and five females per exposure group at the interim evaluation for determination of acyl-CoA oxidase and aromatase activity.	Same as Study 1, except from 10 males per exposure group

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[90] and is presented graphically. Animals surviving to the end of the observation period are treated as censored observations, as are animals dying from unnatural causes within the observation period. Animals dying from natural causes are included in analyses but are treated as uncensored observations. Dose-related trends and pairwise dose-related effects on survival are assessed using a Cox Proportional Hazards Model[91] with a random litter effect. All reported p values for the survival analyses are two-sided.

Calculation of Incidence

Incidences of neoplastic and nonneoplastic lesions are presented 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 incidence rates, the denominator for most neoplasms and all nonneoplastic lesions is the number of animals where the site was examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. These statistical analyses 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), used in tables presented in this Technical Report, accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal euthanasia.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

Statistical analyses of neoplasm and nonneoplastic lesion incidences considered two features of the data. Some animals did not survive the entire 2 years of the study, so survival differences between groups had to be taken into account. Also, up to two animals per sex were randomly

selected from each litter to participate in the study. The statistical analysis of lesion incidences used the Poly-3 test to account for survival differences, with a Rao-Scott adjustment for litter effects, as described below.

The Poly-k test[92-94] 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 euthanasia; if the animal died prior to terminal euthanasia 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 on the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time[92]. 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[92] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F$_1$ mice[95]. Bailer and Portier[92] 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[96]. Poly-3 tests used the continuity correction described by Nam[97].

Littermates tend to be more like each other than like fetuses/pups in other litters. Failure to account for correlation within litters leads to underestimates of variance in statistical tests, resulting in higher probabilities of Type I errors ("false positives"). Because up to two pups per sex per litter were present in the core study, the Poly-3 test was modified to accommodate litter effects using the Rao-Scott approach[98]. The Rao-Scott approach accounts for litter effects by estimating the ratio of the variance in the presence of litter effects to the variance in the absence of litter effects. This ratio is then used to adjust the sample size downward to yield the estimated variance in the presence of litter effects. The Rao-Scott approach was implemented in the Poly-3 test as recommended by Fung et al.[99], formula $\overline{\tau}_{RS2}$.

Tests of significance included pairwise comparisons of each dosed group with control groups and a test for an overall dose-related trend. Continuity-corrected Rao-Scott-adjusted 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 observed without litter structure (e.g., at the interim evaluation), Poly-3 tests that included the continuity correction, but without adjustment for potential litter effects, were used for trend and pairwise comparisons to the control group.

To evaluate incidence rates by litter, the proportions of litters affected by each lesion type were tested among groups. Cochran-Armitage trend tests and Fisher exact tests[100] were used to test for trends and pairwise differences from the control group, respectively.

Analysis of Continuous Variables

Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey[101], for small samples ($n < 20$), and Tukey's outer fences method[102], for large samples ($n \geq 20$), were examined by NTP personnel and implausible values were eliminated from the analysis. Organ and interim sacrifice body weight measurements, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[103] and Williams[104; 105]. For sets of pairwise comparisons across perinatal exposure groups at fixed or increasing postweaning exposures, t-tests were used with a Hommel procedure[106] to adjust for multiple comparisons.

Clinical chemistry, parent compound, liver enzyme data, litter sizes, pup survival, implantations, number of resorptions, and proportions of male pups per litter, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[107] (as modified by Williams[108]) and Dunn[109]. For all quantitative endpoints unaffected by litter structure, Jonckheere's test[110] was used to assess the significance of the dose-related trends and to determine, at the 0.01 level of significance, whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than would be a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). For sets of pairwise comparisons across perinatal exposure groups at fixed or increasing postweaning exposures, a Wilcoxon rank-sum test was used with a Hommel procedure[106] to adjust for multiple comparisons.

Postweaning body weights were measured on two pups per sex per litter in the 2-year study; more than two pups per sex per litter were possible in preweaning body weight measurements. The analyses of pup body weights and body weights adjusted for litter size (described below) of these animals took litter effects into account using a mixed model, where litters were the random effects. To adjust for multiple comparisons in these models, a Dunnett-Hsu adjustment was used[111]. Dam body weights during gestation, as well as dam body weights during lactation, were analyzed with the parametric multiple comparison procedures of Dunnett[103] and Williams[104; 105], depending on whether Jonckheere's test indicated the use of a trend-sensitive test. P values for these analyses are two-sided.

Analysis of Gestational and Fertility Indices

Dose-related trends in gestational and fertility indices were tested using Cochran-Armitage trend tests. Pairwise comparisons of each dosed group with the control group were conducted using the Fisher exact test. P values for these analyses are two-sided.

Body Weight Adjustments

Preweaning pup body weights were adjusted for live litter size as follows: A linear model was fit to body weights as a function of dose and litter size. The estimated coefficient of litter size was then used to adjust each pup body weight based on the difference between its litter size and the mean litter size. Prestandardization PND 4 body weights were adjusted for PND 1 litter size, and body weights measured between PND 4 poststandardization and PND 21 were adjusted for PND 4 poststandardization litter size. Following adjustment, body weights were analyzed with a linear mixed model with a random litter effect.

Testing Effects of Perinatal and Postweaning Exposures

Trend and pairwise testing were performed to assess effects of postweaning exposure at given concentrations of perinatal exposure, perinatal exposure effects at given concentrations of postweaning exposure, and combined effects of perinatal and postweaning exposures.

For the 16-week interim evaluation in males and females and the two-year study in females (Study 1), the following comparisons were performed:

- For the groups without perinatal exposure, trend and pairwise comparisons were made to the 0/0 ppm control group for postweaning exposures. For males, this involved the 0/0, 0/150, and 0/300 ppm groups. For females, this involved the 0/0, 0/300, and 0/1,000 ppm groups.
- Trend and pairwise comparisons were made to the 0/0 ppm control group at increasing concentrations of both the perinatal and postweaning exposures. For males, this involved the 0/0, 150/150, and 300/300 ppm groups. For females, this involved the 0/0, 150/300, and 300/1,000 ppm groups.
- For a given postweaning exposure, a series of pairwise comparisons were made between perinatal exposures. For males, this involved comparing the 0/150 to the 150/150 ppm group and the 0/300 to the 300/300 ppm group. For females, this involved comparing the 0/300 to the 150/300 ppm group and the 0/1,000 to the 300/1,000 ppm group.

For the 16-week interim evaluation in males and the two-year study in males (Study 2), the following comparisons were performed:

- For the groups without perinatal exposure, trend and pairwise comparisons were made to the 0/0 ppm control group for postweaning exposures. This involved the 0/0, 0/20, 0/40, and 0/80 ppm groups.
- For animals with a 300 ppm perinatal exposure, trend and pairwise comparisons were made to the 300/0 ppm group for postweaning exposures. This involved the 300/0, 300/20, 300/40, and 300/80 ppm groups.
- For a given postweaning exposure, a series of pairwise comparisons were made between perinatal exposures. This involved comparing the 0/0 to the 300/0 ppm group, 0/20 to the 300/20 ppm group, the 0/40 to the 300/40 ppm group, and the 0/80 to the 300/80 group.
- Pairwise comparisons were made of the 0/0 to the 300/20 ppm group, 0/0 to the 300/40 ppm group, and 0/0 to the 300/80 ppm group.

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 control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The

NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[112-114] including the concurrent control for comparison across multiple technical reports. Due to the sectioning of residual tissues for the uterus, the historical control for this organ consists of three studies at this time: indole-3-carbinol, 2-hydroxy-4-methoxybenzophenone, and this study. In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidence of neoplasms in control groups for all routes of administration are included for comparison, including the current study.

Quality Assurance Methods

The 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[115]. In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Results

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR598.

Study 1: Two-year Study in Females and 16-week Interim Evaluation in Males and Females

Perinatal Exposure

No exposure-related effects were observed on the pregnancy status, maternal survival, or number of dams that littered (Table 4). Maternal body weights and body weight gains of exposed dams during gestation were comparable to those of the control group (Table 5). During lactation, there were no consistent effects on maternal weight or maternal body weight gain across time points among exposed groups of dams (Table 6). Feed consumption (g/animal/day) during gestation was marginally (3% to 4%) lower in the 150 and 300 ppm groups compared to the control groups at select intervals during gestation (Table 7). Feed consumption was marginally lower (up to 4%) in the 300 ppm group from lactation days (LDs) 1 to 14, during which primarily the dam, and not the offspring, was consuming the feed (Table 7). Chemical consumption was 10.9 mg PFOA/kg body weight/day and 21.7 mg/kg/day for the 150 and 300 ppm groups during gestation, respectively. Chemical consumption was 23.3 and 45.2 mg/kg/day for the 150 and 300 ppm groups from LD 1–14, respectively; chemical consumption from LD 14–21 was not calculated due to the entire litter eating feed and an accurate assessment could not be made.

Table 4. Summary of Disposition during Perinatal Exposure and F_1 Allocation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 1)

	0 ppm	150 ppm	300 ppm
Time-mated Females	103	36	36
Nonpregnant Females	12	5	4
Pregnant Females Delivering	91	31	32
Pregnant Dams Not Delivering	0	0	0
Littered/Pregnant on GD 21	91/91 (100%)	31/31 (100%)	32/32 (100%)
Litters Removed (PND 4)[a]	5	1	0
Litters Poststandardization (PND 4)	86	30	32
Weaned Males/Females	180/180	60/60	60/60

[a]Removed due to insufficient size.

Table 5. Mean Body Weights and Body Weight Gains of F_0 Females during Gestation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 1)[a]

Gestation Day	0 ppm	150 ppm	300 ppm
Body Weight (g)			
6	240.02 ± 1.41 (91)	241.40 ± 1.83 (31)	240.10 ± 1.96 (32)
9	256.33 ± 1.37 (91)	255.53 ± 2.04 (31)	254.66 ± 1.98 (32)
12	271.57 ± 1.38 (91)	268.85 ± 1.96 (31)	269.44 ± 2.02 (32)
15	291.06 ± 1.53 (91)	288.32 ± 2.28 (31)	287.63 ± 2.26 (32)
18	331.58 ± 2.00 (91)	329.09 ± 2.81 (31)	328.88 ± 2.72 (32)
21	379.39 ± 2.92 (91)	375.53 ± 3.86 (31)	374.91 ± 3.33 (30)
Body Weight Change (g)			
6 to 9	16.32 ± 0.57* (91)	14.13 ± 0.73 (31)	14.56 ± 0.91 (32)
9 to 12	15.24 ± 0.44 (91)	13.31 ± 0.84* (31)	14.78 ± 0.59 (32)
12 to 15	19.49 ± 0.51* (91)	19.47 ± 0.95 (31)	18.19 ± 0.53 (32)
15 to 18	40.52 ± 0.80 (91)	40.77 ± 1.27 (31)	41.25 ± 1.01 (32)
18 to 21	47.81 ± 1.13* (91)	46.44 ± 1.39 (31)	44.83 ± 1.20 (30)
6 to 21	139.37 ± 2.48* (91)	134.13 ± 3.32 (31)	133.39 ± 2.79 (30)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$.
[a]Mean ± standard error (number of dams). Each exposed group was compared to the control group using Williams' test when a significant trend ($p \leq 0.01$) was present by Jonckheere's test or with Dunnett's test when no significant trend was present.

Table 6. Mean Body Weights and Body Weight Gains of F_0 Females during Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 1)[a]

Lactation Day	0 ppm	150 ppm	300 ppm
Body Weight (g)			
1	281.59 ± 1.60 (91)	279.45 ± 2.04 (31)	277.89 ± 2.47 (32)
4	298.08 ± 1.51* (86)	295.41 ± 2.13 (30)	289.97 ± 3.18* (32)
7	304.13 ± 1.48* (86)	298.58 ± 2.32 (30)	299.36 ± 2.53 (32)
14	321.16 ± 1.65 (86)	316.43 ± 2.21 (30)	316.98 ± 2.22 (32)
21	306.22 ± 1.52 (86)	306.24 ± 1.84 (30)	305.80 ± 2.69 (32)
Body Weight Change (g)			
1 to 4	15.75 ± 0.81 (86)	16.06 ± 1.20 (30)	12.07 ± 2.30 (32)
4 to 7	6.05 ± 0.84 (86)	3.17 ± 1.41 (30)	9.39 ± 1.90 (32)
7 to 14	17.03 ± 0.95 (86)	17.85 ± 1.24 (30)	17.63 ± 1.47 (32)
14 to 21	−14.94 ± 1.19* (86)	−10.19 ± 1.22* (30)	−11.18 ± 1.60 (32)
1 to 21	23.89 ± 1.14** (86)	26.89 ± 1.74 (30)	27.91 ± 2.07 (32)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Mean ± standard error (number of dams). Each exposed group was compared to the control using Williams' test when a significant ($p \leq 0.01$) trend was present by Jonckheere's test or with Dunnett's test when no significant trend was present.

Table 7. Feed Consumption by F_0 Females during Gestation and Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 1)[a]

Days	0 ppm	150 ppm	300 ppm
Gestation (g/animal/day)			
6 to 9	19.60 ± 0.18* (91)	19.25 ± 0.29 (31)	19.11 ± 0.26 (32)
9 to 12	20.14 ± 0.15* (91)	19.42 ± 0.30* (31)	19.55 ± 0.25 (32)
12 to 15	20.54 ± 0.14** (91)	19.93 ± 0.30** (31)	19.62 ± 0.27** (32)
15 to 18	22.11 ± 0.17 (91)	22.14 ± 0.28 (31)	22.22 ± 0.30 (32)
18 to 21	23.89 ± 0.20 (91)	23.68 ± 0.33 (31)	23.56 ± 0.34 (32)
6 to 21	21.26 ± 0.13* (91)	20.88 ± 0.25 (31)	20.81 ± 0.24 (32)
Lactation (g/animal/day)			
1 to 4	38.17 ± 0.60** (84)	38.47 ± 1.74 (29)	36.53 ± 1.86** (32)
4 to 7	40.94 ± 0.78** (85)	40.18 ± 0.77 (29)	36.92 ± 0.65** (32)
7 to 10	49.48 ± 0.67** (85)	45.97 ± 1.70* (30)	46.67 ± 0.64** (32)
10 to 14	58.71 ± 0.48 (86)	58.70 ± 0.82 (30)	57.80 ± 0.81 (32)
14 to 17	61.97 ± 0.72* (86)	58.53 ± 0.82** (30)	61.83 ± 1.23 (32)
17 to 21	70.10 ± 0.93 (86)	69.24 ± 1.41 (30)	69.29 ± 1.42 (32)
1 to 14	47.59 ± 0.35** (82)	47.10 ± 0.80 (28)	45.51 ± 0.77** (32)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Mean ± standard error in grams/animal/day (number of dams). Each exposed group was compared to the control group using Shirley's test when a significant ($p \leq 0.01$) trend was present by Jonckheere's test or with Dunn's test when no significant trend was present.

Total and live litter sizes and survival of the F_1 rats during lactation were not affected by exposure (Table 8). Male and female pup body weights on postnatal day (PND) 1 were 5% lower in the 300 ppm groups compared to the 0/0 ppm control group and 5% to 8% lower at PND 7, 14, and 21 (Table 9).

Table 8. Mean Litter Size and Survival Ratio of F_1 Rats during Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 1)[a]

Postnatal Day	0 ppm	150 ppm	300 ppm
Total Litter Size			
1	13.68 ± 0.34 (91)	13.26 ± 0.42 (31)	13.19 ± 0.42 (31)
Live Litter Size			
1	13.60 ± 0.34 (91)	13.26 ± 0.42 (31)	13.16 ± 0.42 (32)
4	13.42 ± 0.37 (91)	13.16 ± 0.42 (31)	13.13 ± 0.41 (32)
4 (Poststandardization)	8.00 ± 0.00 (86)	8.00 ± 0.00 (30)	8.00 ± 0.00 (32)
21	7.87 ± 0.06 (86)	7.83 ± 0.08 (30)	7.97 ± 0.03 (32)
Survival Ratio			
1 to 4[b]	0.985 ± 0.011 (90)	0.993 ± 0.004 (31)	0.998 ± 0.002 (32)
4 to 21[c]	0.984 ± 0.007 (86)	0.979 ± 0.011 (30)	0.996 ± 0.004 (32)

[a]Mean ± standard error (number of dams). Each exposed group was compared to the control group using Shirley's test when a significant ($p \leq 0.01$) trend was present by Jonckheere's test or with Dunn's test when no significant trend was present.
[b]Number of live pups on PND 4/number of live pups on PND 1.
[c]Number of live pups on PND 21/number of live pups on PND 4 poststandardization.

Table 9. Mean Body Weight of F_1 Rats during Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 1)

Postnatal Day	0 ppm	150 ppm	300 ppm
Male (g)			
1[a]	7.17 ± 0.07** (89)	7.10 ± 0.11 (31)	6.79 ± 0.11** (32)
4[b,c]	10.02 ± 0.11 (337/86)	10.13 ± 0.16 (120/30)	9.73 ± 0.19 (131/32)
7[c]	15.38 ± 0.18** (335/86)	15.42 ± 0.23 (119/30)	14.09 ± 0.34** (131/32)
14[c]	32.49 ± 0.28** (330/86)	32.11 ± 0.40 (119/30)	30.27 ± 0.51** (130/32)
21[c]	51.65 ± 0.52** (330/86)	50.61 ± 0.72 (119/30)	48.68 ± 0.95** (130/32)
Female (g)			
1[a]	6.87 ± 0.07** (90)	6.75 ± 0.10 (31)	6.51 ± 0.13** (32)
4[b,c]	9.56 ± 0.11 (351/86)	9.70 ± 0.17 (120/30)	9.45 ± 0.20 (125/32)
7[c]	14.66 ± 0.19** (351/86)	14.58 ± 0.26 (118/30)	13.66 ± 0.33** (125/32)
14[c]	31.12 ± 0.29** (348/86)	30.52 ± 0.42 (117/30)	29.40 ± 0.51** (125/32)
21[c]	48.68 ± 0.49** (347/86)	47.85 ± 0.76 (116/30)	46.22 ± 0.92* (125/32)
All Pups (g)			
1[a]	7.02 ± 0.06** (90)	6.91 ± 0.10 (31)	6.63 ± 0.11** (32)
4[b,c]	9.79 ± 0.10 (688/86)	9.91 ± 0.15 (240/30)	9.59 ± 0.19 (256/32)
7[c]	15.02 ± 0.17** (686/86)	15.00 ± 0.24 (237/30)	13.89 ± 0.33** (256/32)
14[c]	31.81 ± 0.26** (678/86)	31.32 ± 0.39 (236/30)	29.84 ± 0.49** (255/32)
21[c]	50.15 ± 0.47** (677/86)	49.27 ± 0.71 (235/30)	47.49 ± 0.91** (255/32)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Mean ± standard error (number of dams). Each exposed group was compared to the control group using Williams' test when a significant ($p \leq 0.01$) trend was present by Jonckheere's test or with Dunnett's test when no significant trend was present.
[b]PND 4 poststandardization.
[c]Mean ± standard error (number of pups/number of dams). Individual pup weights first adjusted for litter size on PND 4 poststandardization. Each exposed group was compared to the control group using a mixed model with a Dunnett-Hsu adjustment.

Sixteen-week Interim Evaluation in Males and Females (Study 1)

No early deaths occurred among rats designated for interim evaluation at 16 weeks (19 weeks of age). Due to the concern of overt toxicity in males, the male portion of the 2-year study was terminated at 21 weeks (24 weeks of age). There were no exposure-related clinical observations (CEBS, Study 1, E05). No other evaluation was performed on the males removed from study at 21 weeks.

Group mean feed (g/animal/day) and compound consumption (mg/kg/day) for male rats through 21 weeks are available in CEBS (Study 1, E08). Compared to the 0/0 ppm control group, mean feed consumption by exposed males was lower through week 13 of the study. Mean feed consumption (g/animal/day) by 0/150, 150/150, 0/300, and 300/300 ppm groups was 13%, 11%, 23%, and 19% less than the 0/0 ppm control group, respectively, at 13 weeks. This reduction in feed consumption may be due in part to the smaller size of the animals (described below) compared to the 0/0 ppm control group. This difference would be captured in feed consumption expressed in g/kg body weight/day, but these values were not calculated. Compound consumption for the 0/150 and 150/150 ppm groups through week 13 averaged 15.6 and

15.8 mg/kg/day, respectively, and for the 0/300 and 300/300 ppm groups, 31.7 and 32.1 mg/kg/day, respectively.

Mean feed consumption (g/animal/day) for F_1 females is available in CEBS (Study 1, E08). Feed consumption for females in the 0/300 and 150/300 ppm groups was within 10% of the 0/0 ppm control group throughout the study. Mean feed consumption for the 0/1,000 and 300/1,000 ppm female groups was reduced through the first 4 weeks postweaning. However, by the fifth week, mean feed consumption for these two groups was within 10% of the 0/0 ppm control group and remained so through the end of the study. Compound consumption averaged 29.6 mg/kg/day for the 0/300 and 150/300 ppm groups and 98.6 and 104.4 mg/kg/day for the 0/1,000 and 300/1,000 ppm groups, respectively.

Plasma PFOA concentrations in males were consistent between groups with and without exposure during the perinatal period and were within 10% of each other between the 0/150 and 150/150 ppm groups and between the 0/300 and 300/300 ppm groups (Table 10). Liver concentrations showed a similar pattern. Plasma concentrations in females showed a similar pattern to the males (e.g., minor differences between perinatal exposures and liver patterns); however, PFOA concentrations were much lower compared to males even though female exposure (mg/kg/day) was 2 to 3 times higher compared to males (Table 11). Plasma concentrations were approximately 12-fold lower in the 0/300 ppm female group compared to the 0/300 ppm male group.

Table 10. Summary of Plasma and Liver Concentration Data for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a,b]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**150 ppm**	**300 ppm**
n		10	10	10
Plasma Concentration (ng/mL)	0 ppm	BD[c]	193,000 ± 11,325	242,500 ± 12,731
	150 ppm	–	175,390 ± 14,956	–
	300 ppm	–	–	223,400 ± 8,422
Plasma Concentration (μM)	0 ppm	BD[c]	466.1 ± 27.4	585.7 ± 30.7
	150 ppm	–	423.6 ± 36.1	–
	300 ppm	–	–	539.5 ± 20.3
Liver Concentration (ng/g)	0 ppm	BD	157,400 ± 5,418	171,000 ± 7,578
	150 ppm	–	144,300 ± 5,752	–
	300 ppm	–	–	193,800 ± 9,704
Liver Concentration (μM)	0 ppm	BD	380.1 ± 13.1	413.0 ± 18.3
	150 ppm	–	348.5 ± 13.9	–
	300 ppm	–	–	468.0 ± 23.4
Liver/Plasma Ratio	0 ppm	BD	0.84 ± 0.05	0.73 ± 0.06
	150 ppm	–	0.86 ± 0.06	–
	300 ppm	–	–	0.88 ± 0.05

Pairwise comparisons across perinatal exposures (0/150 vs. 150/150 ppm and 0/300 vs. 300/300 ppm) did not show any statistically significant differences.
Values adjusted for molar concentration were calculated by dividing by the molecular weight of 414.06.
BD = below detection; group did not have over 20% of its values above the limit of quantification. In these cases, no statistical analyses were performed.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).
[c]N = 9; decrease in N in the 0/0 ppm control group is due to the exclusion of an implausible plasma concentration value.

Table 11. Summary of Plasma and Liver Concentration Data for Female Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a,b]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**300 ppm**	**1,000 ppm**
n		10	10	10
Plasma Concentration (ng/mL)	0 ppm	BD	20,420 ± 1,212	72,250 ± 4,351
	150 ppm	–	20,800 ± 1,043	–
	300 ppm	–	–	70,160 ± 6,895
Plasma Concentration (μM)	0 ppm	BD	49.3 ± 2.9	174.5 ± 10.5
	150 ppm	–	50.2 ± 2.5	–
	300 ppm	–	–	169.4 ± 16.7
Liver Concentration (ng/g)	0 ppm	BD	16,420 ± 787	69,040 ± 3,942
	150 ppm	–	16,660 ± 750	–
	300 ppm	–	–	67,840 ± 5,681
Liver Concentration (μM)	0 ppm	BD	39.7 ± 1.9	166.7 ± 9.5
	150 ppm	–	40.2 ± 1.8	–
	300 ppm	–	–	163.8 ± 13.7
Liver/Plasma Ratio	0 ppm	BD	0.82 ± 0.03	0.96 ± 0.04
	150 ppm	–	0.81 ± 0.03	–
	300 ppm	–	–	0.99 ± 0.05

Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.
Values adjusted for molar concentration were calculated by dividing by the molecular weight of 414.06.
BD = below detection; group did not have over 20% of its values above the limit of quantification. In these cases, no statistical analyses were performed.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Males in all exposure groups gained weight through the interim study evaluation at week 16 (Figure 2, Figure 3; CEBS, Study 1, E04). However, exposed males did not gain weight at the same rate as the 0/0 ppm control group; this reduced weight gain was related to exposure. Body weights of males in the 0/150 and 150/150 ppm groups evaluated at 16 weeks were 79% and 76% of the 0/0 ppm control group weight, respectively, and body weights of males in the 0/300 and 300/300 ppm groups were 55% of the 0/0 ppm control group weight.

Group mean body weights of females are reported in CEBS (Study 1, E04). Mean body weights for females in the 0/300 and 150/300 ppm groups were within 10% of the 0/0 ppm control group. Mean body weights for the 0/1,000 and 300/1,000 ppm female groups were approximately 10–15% less than the 0/0 ppm control group throughout most of the postweaning period. For the females evaluated at 16 weeks, mean body weights for the 0/1,000 and 300/1,000 ppm groups were 12% less than that of the 0/0 ppm control group.

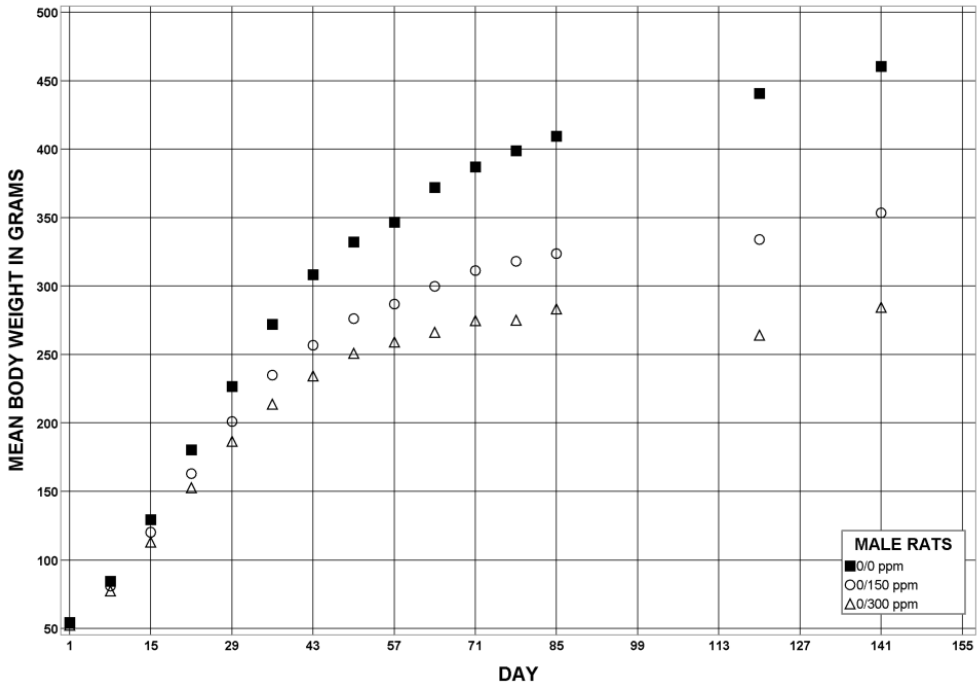


Figure 2. Growth Curves for Male Rats with Postweaning-only Exposure to 0/0, 0/150, or 0/300 ppm Perfluorooctanoic Acid in Feed for 21 Weeks

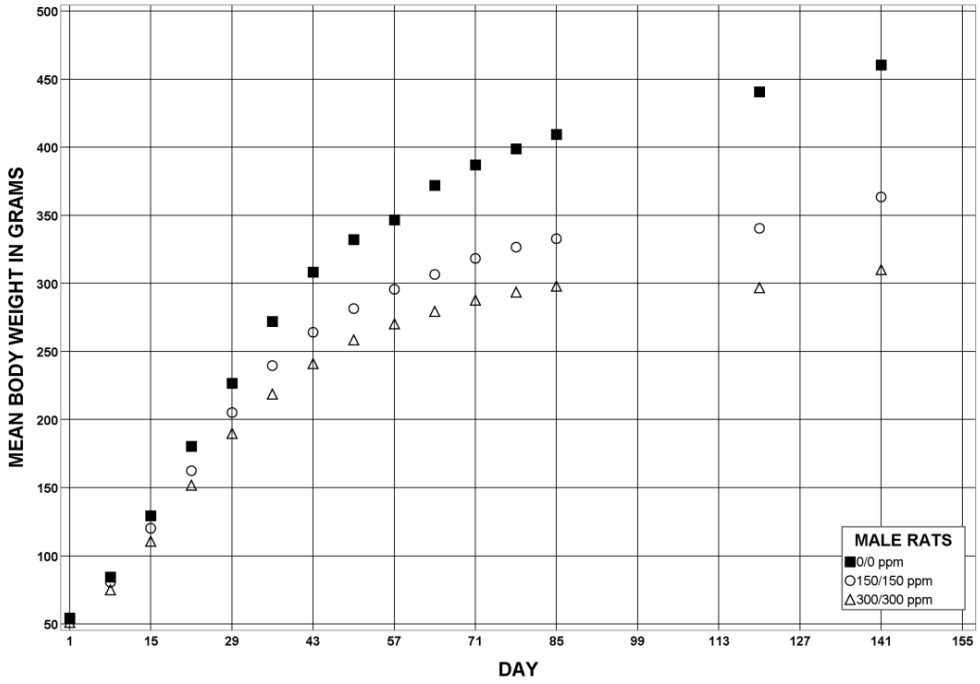


Figure 3. Growth Curves for Male Rats with Perinatal and Postweaning Exposure to 0/0, 150/150, or 300/300 ppm Perfluorooctanoic Acid in Feed for 21 Weeks

The group mean absolute and relative liver weights of all exposed groups of males and of 0/1,000 and 300/1,000 ppm female groups were significantly greater than those of the 0/0 ppm control group (Table 12, Table 13). Absolute liver weights were increased up to 42% (males) and 36% (females) of the 0/0 ppm control group. Histological correlates (hepatocyte hypertrophy, cytoplasmic alteration, and pigment) were present in all groups that had liver weight changes (Table 12, Table 13). Groups with both perinatal and postweaning exposure did not differ from groups with postweaning-only exposure.

Acyl-CoA oxidase enzyme activity, a marker of PPARα activity, was increased in all male and female PFOA-exposed groups. Activity increased in males in the 0/150 and 150/150 ppm groups by 10-fold and increased in the 0/300 and 300/300 ppm groups by 11- to 12-fold compared to the 0/0 ppm control group. The magnitude of the effect was smaller in females with a 1.4-fold increase in the mid-doses of 0/300 and 150/300 ppm groups and a 5.5- and 6.5-fold increase in the 300/1,000 and 0/1,000 ppm groups, respectively, compared to the 0/0 ppm control group. No changes were observed in liver aromatase activity in males or females (Table 12, Table 13).

Table 12. Summary of Hepatic Findings for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**150 ppm**	**300 ppm**
n		10	10	10
Necropsy Body Weight (g)[a,b]	0 ppm	425.9 ± 10.4**##	336.7 ± 9.1**	234.4 ± 20.4**
	150 ppm	–	325.3 ± 13.8**	–
	300 ppm	–	–	234.3 ± 16.2**
Liver Weight[a,b]				
Absolute (g)	0 ppm	16.52 ± 0.59	23.41 ± 0.51**	19.57 ± 0.98*
	150 ppm	–	21.44 ± 0.75**	–
	300 ppm	–	–	18.69 ± 0.59*
Relative (mg/g)	0 ppm	38.72 ± 0.62**##	69.90 ± 2.13**	87.37 ± 5.54**
	150 ppm	–	66.55 ± 2.62**	–
	300 ppm	–	–	82.07 ± 3.95**
Histological Findings[c]				
Liver[d]		10	10	10
Hepatocyte, Cytoplasmic Alteration[e]	0 ppm	0**##	10** (2.2)[f]	10** (2.6)
	150 ppm	–	10** (2.1)	–
	300 ppm	–	–	10** (2.8)
Hepatocyte, Hypertrophy	0 ppm	0**##	10** (2.1)	6** (2.5)
	150 ppm	–	10** (1.9)	–
	300 ppm	–	–	10**^ (1.6)
Hepatocyte, Single Cell Death	0 ppm	0**##	10** (1.3)	10** (1.0)
	150 ppm	–	9** (1.1)	–
	300 ppm	–	–	10** (1.0)
Necrosis	0 ppm	0#	6** (1.2)	2 (1.0)
	150 ppm	–	2 (1.5)	–
	300 ppm	–	–	4* (1.8)
Pigment	0 ppm	0**##	8** (1.1)	10** (2.0)
	150 ppm	–	9** (1.2)	–
	300 ppm	–	–	10** (1.9)
n		5	5	5
Acyl-CoA Oxidase Activity (nmol/min/mg)[a,b]	0 ppm	2.212 ± 0.100**#	21.160 ± 1.741**	24.360 ± 0.698**
	150 ppm	–	21.360 ± 2.133**	–
	300 ppm	–	–	25.340 ± 1.810**
Aromatase Activity (pmol/mg/min)[a,b]	0 ppm	6.972 ± 1.212	14.292 ± 2.424	5.588 ± 2.135
	150 ppm	–	11.602 ± 2.118	–
	300 ppm	–	–	11.896 ± 2.696

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
#Statistically significant trend at $p \leq 0.05$ when comparing across the 0/0, 150/150, and 300/300 ppm groups; ##$p \leq 0.01$.
^Statistically significant at $p \leq 0.05$ when comparing 0/300 vs. 300/300 ppm.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests.
[c]Statistical analysis performed by the Poly-3 test.
[d]Number of animals examined microscopically.
[e]Number of animals with lesion.
[f]Average severity grade of lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Table 13. Summary of Hepatic Findings for Female Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**300 ppm**	**1,000 ppm**
n		10	10	10
Necropsy Body Weight (g)[a,b]	0 ppm	255.2 ± 4.5**##	248.9 ± 8.5	223.7 ± 3.9**
	150 ppm	–	242.3 ± 7.1	–
	300 ppm	–	–	223.4 ± 8.4**
Liver Weight[a,b]				
Absolute (g)	0 ppm	9.35 ± 0.40**##	9.78 ± 0.32	12.70 ± 0.36**
	150 ppm	–	9.36 ± 0.23	–
	300 ppm	–	–	12.71 ± 0.56**
Relative (mg/g)	0 ppm	36.55 ± 1.08**##	39.34 ± 0.61	56.78 ± 1.33**
	150 ppm	–	38.70 ± 0.70	–
	300 ppm	–	–	56.86 ± 1.19**
Histological Findings[c]				
Liver[d]		10	10	10
Hepatocyte, Cytoplasmic Alteration[e]	0 ppm	0**##	0	10** (1.3)[f]
	150 ppm	–	0	–
	300 ppm	–	–	10** (2.0)
Hepatocyte, Hypertrophy	0 ppm	0**##	0	10** (1.4)
	150 ppm	–	0	–
	300 ppm	–	–	10** (2.0)
Hepatocyte, Single Cell Death	0 ppm	0	0	1 (1.0)
	150 ppm	–	0	–
	300 ppm	–	–	0
Necrosis	0 ppm	0	0	2 (2.5)
	150 ppm	–	0	–
	300 ppm	–	–	0
Pigment	0 ppm	0##	0	2 (1.0)
	150 ppm	–	0	–
	300 ppm	–	–	6** (1.0)

	Perinatal Exposure	Postweaning Exposure		
		0 ppm	**300 ppm**	**1,000 ppm**
n		5	5	5
Acyl-CoA Oxidase Activity (nmol/min/mg)[a,b]	0 ppm	2.366 ± 0.106**[##]	3.402 ± 0.300**	15.360 ± 0.375**
	150 ppm	–	3.300 ± 0.213**	–
	300 ppm	–	–	12.980 ± 0.712**
Aromatase Activity (pmol/mg/min)[a,b]	0 ppm	1.590 ± 0.404	1.762 ± 0.302	1.710 ± 0.433
	150 ppm	–	2.672 ± 0.881	–
	300 ppm	–	–	2.616 ± 0.572

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[#]Statistically significant trend at $p \leq 0.05$ when comparing across the 0/0, 150/300, and 300/1,000 ppm groups; [##]$p \leq 0.01$.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests.
[c]Statistical analysis performed by the Poly-3 test.
[d]Number of animals examined microscopically.
[e]Number of animals with lesion.
[f]Average severity grade of lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

The mean absolute and relative spleen and absolute thymus weights of all exposed male groups were significantly less than those of the 0/0 ppm control group (Table 14; CEBS, Study 1, PA06). The decrease in absolute spleen and thymus weights was up to 54% and 49%, respectively, compared to the 0/0 ppm control group; however, there were no correlated histological findings.

Statistically significant differences between exposed and 0/0 ppm control group males in absolute and relative heart, lung, right kidney, testis, thyroid gland, pituitary gland, and adrenal gland mean weights were considered related to the significantly decreased body weights, and there were no correlated histological findings (CEBS, Study 1, PA06; P03; P10; P18).

Significantly decreased mean absolute right kidney and lung weights of the 0/1,000 and 300/1,000 ppm female groups, mean absolute heart weight of the 0/1,000 ppm female group, and mean absolute pituitary gland weight of the 300/1,000 ppm female group were also considered related to reduced body weights.

Table 14. Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a,b]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**150 ppm**	**300 ppm**
n		10	10	10
Necropsy Body Weight (g)	0 ppm	425.9 ± 10.4**##	336.7 ± 9.1**	234.4 ± 20.4**
	150 ppm	–	325.3 ± 13.8**	–
	300 ppm	–	–	234.3 ± 16.2**
Spleen Weight				
Absolute (g)	0 ppm	0.805 ± 0.031**##	0.551 ± 0.022**	0.373 ± 0.038**
	150 ppm	–	0.543 ± 0.036**	–
	300 ppm	–	–	0.395 ± 0.038**
Relative (mg/g)	0 ppm	1.89 ± 0.05**#	1.63 ± 0.03**	1.58 ± 0.06**
	150 ppm	–	1.66 ± 0.07*	–
	300 ppm	–	–	1.66 ± 0.07*
Thymus Weight				
Absolute (g)	0 ppm	0.369 ± 0.015**##	0.277 ± 0.017**	0.187 ± 0.027**
	150 ppm	–	0.284 ± 0.014**	–
	300 ppm	–	–	0.208 ± 0.026**
Relative (mg/g)	0 ppm	0.87 ± 0.04	0.83 ± 0.05	0.77 ± 0.06
	150 ppm	–	0.88 ± 0.04	–
	300 ppm	–	–	0.86 ± 0.07

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/150 vs. 150/150 ppm and 0/300 vs. 300/300 ppm) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
#Statistically significant trend at $p \leq 0.05$ when comparing across the 0/0, 150/150, and 300/300 ppm groups; ##$p \leq 0.01$.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Select clinical chemistry data are presented in Table 15 (males) and Table 16 (females). Urea nitrogen concentrations were significantly increased in all exposed male groups; this was most likely due to a decrease in water consumption (i.e., mild dehydration), which is supported by the observed decreases in feed consumption. Triglyceride concentrations were significantly decreased in all exposed male groups.

Globulin concentrations were significantly decreased in all exposed groups of males and females. In exposed male groups, the degree of decrease in the globulin concentration resulted in a significant decrease in total protein concentration. Albumin concentrations were significantly increased in the 0/150 and 150/150 ppm male groups and in the 0/1,000, and 300/1,000 ppm female groups compared to the 0/0 ppm control group. The combination of these protein changes resulted in significant increases in the albumin:globulin ratios in all exposed groups.

With and without perinatal exposure, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and sorbitol dehydrogenase (SDH) enzyme activities were significantly increased in all exposed male groups. In females, ALT activity was significantly increased in the 0/1,000 and 300/1,000 ppm groups, whereas SDH activity was increased in the 300/1,000 ppm group and ALP activity increased in the 300/1,000 ppm group compared to the 0/0 ppm control group. Additionally, bile acid concentrations were significantly increased in all exposed male groups with and without perinatal exposure.

Table 15. Select Clinical Chemistry Data for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a,b]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**150 ppm**	**300 ppm**
n		10	10	10
Urea Nitrogen (mg/dL)	0 ppm	15.3 ± 0.6**##	21.2 ± 0.7**	21.3 ± 0.8**
	150 ppm	–	20.6 ± 1.0**	–
	300 ppm	–	–	24.1 ± 1.0**
Total Protein (g/dL)	0 ppm	6.59 ± 0.07**##	6.17 ± 0.09**	5.18 ± 0.15**
	150 ppm	–	5.87 ± 0.13**	–
	300 ppm	–	–	5.35 ± 0.22**
Albumin (g/dL)	0 ppm	4.13 ± 0.04	4.69 ± 0.07**	4.00 ± 0.14
	150 ppm	–	4.48 ± 0.09*	–
	300 ppm	–	–	4.09 ± 0.16
Globulin (g/dL)	0 ppm	2.46 ± 0.06**##	1.48 ± 0.07**	1.18 ± 0.03**
	150 ppm	–	1.39 ± 0.06**	–
	300 ppm	–	–	1.26 ± 0.08**
A/G Ratio	0 ppm	1.69 ± 0.05**##	3.24 ± 0.18**	3.40 ± 0.12**
	150 ppm	–	3.27 ± 0.15**	–
	300 ppm	–	–	3.31 ± 0.16**
Triglycerides (mg/dL)	0 ppm	115.1 ± 6.2**##	61.5 ± 6.5**	52.4 ± 6.4**
	150 ppm	–	58.4 ± 3.6**	–
	300 ppm	–	–	52.3 ± 2.0**

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**150 ppm**	**300 ppm**
Alanine Aminotransferase (IU/L)	0 ppm	50.70 ± 1.80*##	71.20 ± 4.05**	66.70 ± 6.71*
	150 ppm	–	70.20 ± 3.09**	–
	300 ppm	–	–	65.10 ± 4.49**
Alkaline Phosphatase (IU/L)	0 ppm	174.1 ± 9.3**##	412.6 ± 38.1**	399.1 ± 27.0**
	150 ppm	–	398.7 ± 25.0**	–
	300 ppm	–	–	410.8 ± 33.1**
Sorbitol Dehydrogenase (IU/L)	0 ppm	8.2 ± 0.7**##	16.7 ± 1.8**	20.6 ± 7.7 **
	150 ppm	–	15.9 ± 1.3**	–
	300 ppm	–	–	16.3 ± 1.8**
Bile salt/acids (μmol/L)	0 ppm	25.4 ± 1.6**##	45.7 ± 3.6**	127.0 ± 26.5**
	150 ppm	–	46.2 ± 6.4**	–
	300 ppm	–	–	69.8 ± 15.2**

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/150 vs. 150/150 ppm and 0/300 vs. 300/300 ppm) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
#Statistically significant trend at $p \leq 0.05$ when comparing across the 0/0, 150/150, and 300/300 ppm groups; ##$p \leq 0.01$.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Table 16. Select Clinical Chemistry Data for Female Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a,b]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**300 ppm**	**1,000 ppm**
n		10	10	10
Total Protein (g/dL)	0 ppm	6.87 ± 0.04#	6.80 ± 0.10	7.09 ± 0.11
	150 ppm	–	6.70 ± 0.08	–
	300 ppm	–	–	7.19 ± 0.08*
Albumin (g/dL)	0 ppm	4.65 ± 0.05**##	4.85 ± 0.08	5.25 ± 0.09**
	150 ppm	–	4.74 ± 0.04	–
	300 ppm	–	–	5.33 ± 0.07**
Globulin (g/dL)	0 ppm	2.22 ± 0.04**##	1.95 ± 0.04**	1.84 ± 0.04**
	150 ppm	–	1.96 ± 0.07**	–
	300 ppm	–	–	1.86 ± 0.05**
A/G Ratio	0 ppm	2.10 ± 0.05**##	2.49 ± 0.06**	2.86 ± 0.05**
	150 ppm	–	2.44 ± 0.09**	–
	300 ppm	–	–	2.88 ± 0.08**

	Perinatal Exposure	Postweaning Exposure		
		0 ppm	300 ppm	1,000 ppm
Alanine Aminotransferase (IU/L)	0 ppm	43.70 ± 2.18**##	50.50 ± 3.08	56.00 ± 3.06**
	150 ppm	–	51.00 ± 2.75	–
	300 ppm	–	–	54.90 ± 2.07**
Alkaline Phosphatase (IU/L)	0 ppm	129.8 ± 9.0*	122.4 ± 7.8	166.3 ± 6.9*
	150 ppm	–	154.8 ± 11.3	–
	300 ppm	–	–	144.5 ± 11.6
Sorbitol Dehydrogenase (IU/L)	0 ppm	8.1 ± 0.6#	9.2 ± 0.9	10.3 ± 1.1
	150 ppm	–	9.8 ± 0.9	–
	300 ppm	–	–	10.9 ± 0.7*

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
#Statistically significant trend at $p \leq 0.05$ when comparing across the 0/0, 150/300, and 300/1,000 ppm groups; ##$p \leq 0.01$.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Data for nonneoplastic lesions in male and female rats at the 16-week interim evaluation are presented in CEBS (Study 1, P03; P10; P18).

The histopathological descriptions of the nonneoplastic and neoplastic data for the 16-week interim and 2-year studies in males and females discussed in this section are presented in the Histopathological Descriptions section following the presentation of the 2-year study in males.

Liver: Compared to the 0/0 ppm control group, the incidences of hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, hepatocyte single cell death, and pigment were significantly increased in all exposed male groups (Table 12). The incidences of hepatocyte necrosis were significantly increased in the 0/150 and 300/300 ppm groups; two incidences of necrosis also occurred in the 0/300 and 150/150 ppm groups. No differences between groups with perinatal and without perinatal exposures were observed.

In females, the incidences of hepatocyte hypertrophy and hepatocyte cytoplasmic alteration were significantly increased in the 0/1,000 and 300/1,000 ppm groups compared to the 0/0 ppm control group (Table 13). The incidence of pigment was significantly increased in the 300/1,000 ppm group relative to the 0/0 ppm control group.

Thyroid Gland: In males, the incidence of follicular cell hypertrophy was significantly increased in the 0/300 ppm group compared to the 00/0 ppm control group (Table 17). In females, the incidence of follicular cell hypertrophy was significantly increased in the 300/1,000 ppm group compared to the 0/0 ppm control (Table 18). No differences between groups with perinatal and without perinatal exposures were observed.

Kidney: In males, the incidences of renal tubule mineral were significantly increased in the 0/150, 0/300, and 300/300 ppm groups compared to the 0/0 ppm control group (Table 17).

In females, the incidences of papilla urothelium hyperplasia were significantly increased in the 0/1,000 and 300/1,000 ppm groups compared to the 0/0 ppm control (Table 18). The incidence of renal tubule mineral in the 0/1,000 ppm group was significantly greater than that of the 0/0 ppm control group. No differences between groups with perinatal and without perinatal exposures were observed.

Glandular Stomach: The incidences of chronic active inflammation of the submucosa were increased in all exposed male groups compared to the 0/0 ppm control group, but the increases were statistically significant only in the 0/300 ppm group (Table 17). No differences between groups with perinatal and without perinatal exposures were observed.

Table 17. Incidences of Select Nonneoplastic Lesions of the Thyroid Gland, Kidney, and Glandular Stomach in Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**150 ppm**	**300 ppm**
n		10[b]	10	10
Thyroid Gland				
Follicular Cell, Hypertrophy[c]	0 ppm	0**	2 (1.0)[d]	6** (1.0)
	150 ppm	–	0	–
	300 ppm	–	–	2 (1.0)
Kidney				
Renal Tubule, Mineral	0 ppm	0**[##]	4* (1.0)	5** (1.0)
	150 ppm	–	1 (1.0)	–
	300 ppm	–	–	6** (1.0)
Glandular Stomach				
Submucosa, Inflammation, Chronic Active	0 ppm	0**	2 (1.0)	5** (1.0)
	150 ppm	–	2 (1.0)	–
	300 ppm	–	–	2 (1.0)

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/150 vs. 150/150 ppm and 0/300 vs. 300/300 ppm) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[##]Statistically significant trend at $p \leq 0.01$ when comparing across the 0/0, 150/150, and 300/300 ppm groups.
[a]Statistical analysis performed by the Poly-3 test.
[b]Number of animals examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Table 18. Incidences of Select Nonneoplastic Lesions of the Thyroid Gland and Kidney in Female Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)[a]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**300 ppm**	**1,000 ppm**
n		10[b]	10	10
Thyroid Gland				
Follicular Cell, Hypertrophy[c]	0 ppm	0[##]	0	1 (1.0)[d]
	150 ppm	–	0	–
	300 ppm	–	–	4* (1.3)
Kidney				
Renal Tubule, Mineral	0 ppm	2** (1.0)	1 (1.0)	7* (1.0)
	150 ppm	–	2 (1.0)	–
	300 ppm	–	–	5 (1.2)
Papilla, Urothelium, Hyperplasia	0 ppm	0**[##]	0	4* (1.3)
	150 ppm	–	0	–
	300 ppm	–	–	4* (1.0)

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[##]Statistically significant trend at $p \leq 0.01$ when comparing across the 0/0, 150/300, and 300/1,000 ppm groups.
[a]Statistical analysis performed by the Poly-3 test.
[b]Number of animals examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Two-year Study in Females (Study 1)

Survival of exposed groups of females was similar to that of the 0/0 ppm control group (Table 19, Table 20; Figure 4). There were no exposure-related clinical observations (CEBS, Study 1, E05).

Table 19. Survival of Female Rats Following Postweaning-only Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

	0/0 ppm	0/300 ppm	0/1,000 ppm
Animals Initially in Study	50	50	50
Moribund	15	16	20
Natural Deaths	12	8	7
Animals Surviving to Study Termination	23	26	23
Percent Probability of Survival at End of Study[a]	46.0	56.0	46.0
Mean Survival (days)[b]	646.6	695.3	626.1
Survival Analysis[c]	p = 0.901	p = 0.126N	p = 0.777N

[a]Kaplan-Meier determinations.
[b]Mean of litter means of all deaths (uncensored, censored, and study termination).
[c]The result of the trend test is in the 0/0 ppm column, and the results of the pairwise comparisons with the 0/0 ppm control group are in the exposed group columns. Negative trends are indicated by N. Analyses were performed using a Cox Proportional Hazards Model with dam ID as a random effect.

Table 20. Survival of Female Rats Following Perinatal and Postweaning Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

	0/0 ppm	150/300 ppm	300/1,000 ppm
Animals Initially in Study	50	50	50
Moribund	15	11	17
Natural Deaths	12	7	11
Animals Surviving to Study Termination	23	32	22
Percent Probability of Survival at End of Study[a]	46.0	64.0	46.0
Mean Survival (days)[b]	646.6	681.1	660.0
Survival Analysis[c]	p = 0.831	p = 0.080N	p = 0.831N

[a]Kaplan-Meier determinations.
[b]Mean of litter means of all deaths (uncensored, censored, and study termination).
[c]The result of the trend test is in the 0/0 ppm column, and the results of the pairwise comparisons with the 0/0 ppm control group are in the exposed group columns. Negative trends are indicated by N. Analyses were performed using a Cox Proportional Hazards Model with dam ID as a random effect.

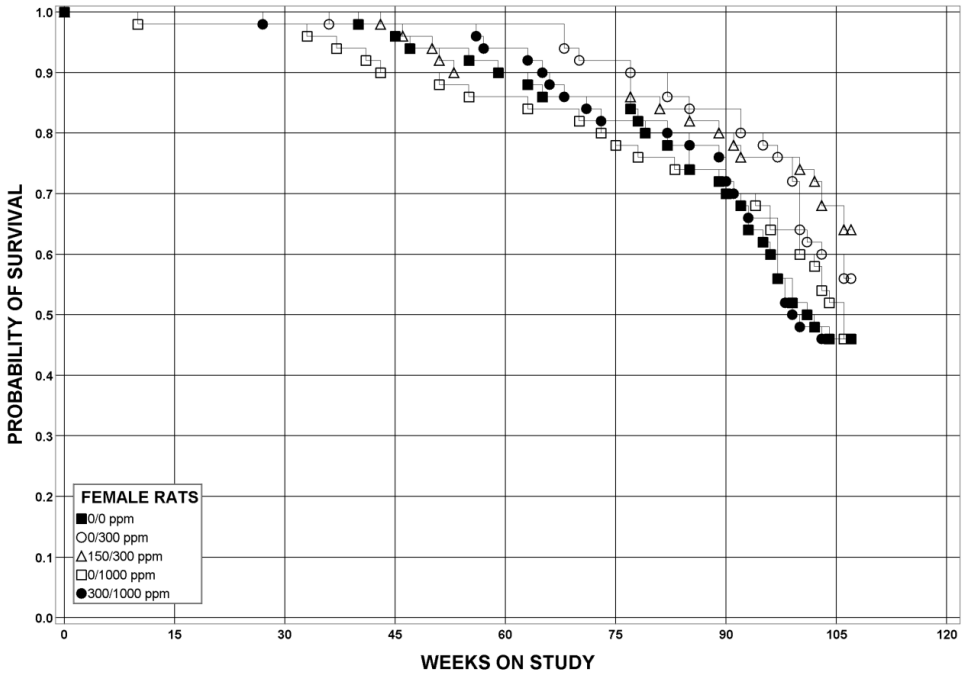


Figure 4. Kaplan-Meier Survival Curves for Female Rats Exposed to Perfluorooctanoic Acid in Feed for Two Years

Exposure-related decreases in group mean body weights were observed for exposed females throughout the study (Table 21, Table 22; Figure 5; Figure 6). At study termination, group mean body weights for the 0/1,000 and 300/1,000 ppm groups were lower (19% and 27%, respectively) than those of the 0/0 ppm control group.

Table 21. Mean Body Weights and Survival of Female Rats Following Postweaning-only Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

	0/0 ppm		0/300 ppm			0/1,000 ppm		
Day	Av. Wt. (g)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	55.4	32	53.3	96.2	29	53.3	96.3	31
8	82.3	32	77.7	94.4	29	67.8	82.4	31
15	111.6	32	109.3	97.9	29	89.4	80.1	31
22	146.3	32	143.7	98.3	29	118.0	80.7	31
29	168.8	32	167.9	99.4	29	141.5	83.8	31
36	188.5	32	185.4	98.3	29	159.1	84.4	31
43	202.2	32	201.3	99.5	29	174.4	86.2	31
50	213.2	32	209.3	98.2	29	185.9	87.2	31
57	224.4	32	219.7	97.9	29	194.9	86.9	31
64	231.4	32	226.1	97.7	29	201.0	86.8	31
71	236.8	32	231.4	97.7	29	208.7	88.1	31
78	241.2	32	237.3	98.3	29	213.6	88.5	31
85	245.7	32	241.8	98.4	29	216.0	87.9	31
120	256.6	32	255.2	99.4	29	227.3	88.6	31
141	269.1	32	263.2	97.8	29	238.4	88.6	31
169	279.3	32	273.2	97.8	29	243.5	87.2	31
197	287.1	32	279.8	97.4	29	249.2	86.8	31
225	291.9	32	285.1	97.7	29	255.2	87.4	30
253	299.6	32	291.0	97.1	29	261.3	87.2	29
281	302.9	32	296.3	97.8	29	261.1	86.2	29
309	313.0	32	302.0	96.5	29	268.5	85.8	28
337	317.3	31	305.6	96.3	29	268.5	84.6	28
365	324.2	31	313.2	96.6	29	275.1	84.9	28
393	331.2	30	320.2	96.7	29	276.0	83.3	28
421	339.7	29	329.0	96.9	29	279.6	82.3	28
449	339.8	29	332.9	98.0	29	276.6	81.4	28
477	346.4	29	336.7	97.2	29	286.8	82.8	28
505	356.3	29	342.2	96.0	29	291.1	81.7	28
533	364.0	28	347.3	95.4	29	300.6	82.6	28
561	366.0	27	353.5	96.6	29	296.0	80.9	27
589	369.8	26	351.5	95.1	27	301.1	81.4	27
617	372.9	26	351.9	94.4	27	300.6	80.6	27
645	376.8	24	356.7	94.7	27	300.1	79.7	26
673	383.1	23	359.9	94.0	27	300.2	78.4	25
701	378.2	21	375.6	99.3	23	305.1	80.7	23
729	383.9	19	369.3	96.2	21	303.5	79.0	20
EOS	**387.0**	**19**	**369.7**	**95.5**	**19**	**314.6**	**81.3**	**18**

EOS = end of study.

Table 22. Mean Body Weights and Survival of Female Rats Following Perinatal and Postweaning Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

	0/0 ppm		150/300 ppm			300/1,000 ppm		
Day	Av. Wt. (g)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	55.4	32	54.9	99.0	29	51.6	93.1	30
8	82.3	32	79.2	96.3	29	62.9	76.5	30
15	111.6	32	106.4	95.3	29	82.6	74.0	30
22	146.3	32	143.8	98.3	29	109.7	75.0	30
29	168.8	32	166.7	98.7	29	133.3	79.0	30
36	188.5	32	183.3	97.2	29	152.7	81.0	30
43	202.2	32	197.6	97.7	29	166.3	82.2	30
50	213.2	32	208.6	97.8	29	179.9	84.4	30
57	224.4	32	217.7	97.0	29	189.1	84.3	30
64	231.4	32	223.2	96.4	29	196.6	84.9	30
71	236.8	32	228.7	96.6	29	202.5	85.5	30
78	241.2	32	234.4	97.2	29	206.8	85.7	30
85	245.7	32	237.6	96.7	29	210.9	85.8	30
120	256.6	32	254.0	99.0	29	221.0	86.1	30
141	269.1	32	260.5	96.8	29	229.4	85.3	30
169	279.3	32	269.9	96.6	29	237.4	85.0	30
197	287.1	32	277.8	96.8	29	243.0	84.6	30
225	291.9	32	282.1	96.7	29	247.9	84.9	30
253	299.6	32	287.0	95.8	29	253.8	84.7	30
281	302.9	32	294.2	97.1	29	250.8	82.8	30
309	313.0	32	298.2	95.2	29	257.2	82.2	30
337	317.3	31	302.8	95.4	29	261.0	82.3	30
365	324.2	31	310.4	95.8	29	264.0	81.4	30
393	331.2	30	305.3	92.2	28	266.0	80.3	30
421	339.7	29	311.6	91.7	28	268.4	79.0	30
449	339.8	29	317.6	93.5	28	267.4	78.7	30
477	346.4	29	322.1	93.0	28	272.3	78.6	29
505	356.3	29	327.3	91.8	28	279.0	78.3	29
533	364.0	28	333.7	91.7	28	285.3	78.4	28
561	366.0	27	335.8	91.7	28	287.2	78.5	28
589	369.8	26	339.0	91.7	27	291.1	78.7	27
617	372.9	26	339.0	90.9	27	291.2	78.1	27
645	376.8	24	340.0	90.2	24	291.3	77.3	25
673	383.1	23	338.2	88.3	24	294.2	76.8	23
701	378.2	21	344.4	91.1	24	286.6	75.8	20
729	383.9	19	342.2	89.1	22	289.0	75.3	20
EOS	**387.0**	**19**	**346.5**	**89.5**	**22**	**283.7**	**73.3**	**19**

EOS = end of study.

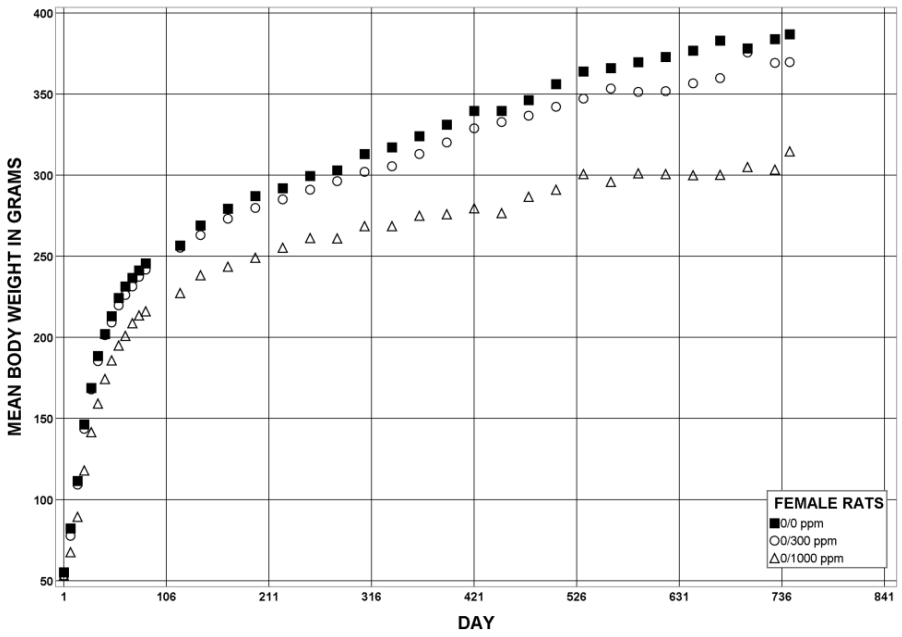


Figure 5. Growth Curves for Female Rats with Postweaning-only Exposure to 0/0, 0/300, or 0/1,000 ppm Perfluorooctanoic Acid in Feed for Two Years (Study 1)

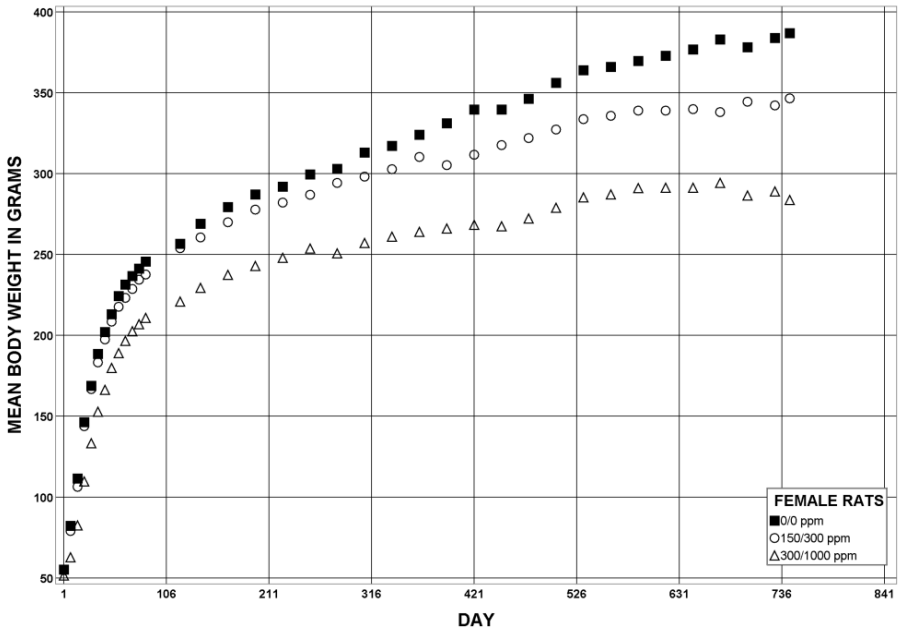


Figure 6. Growth Curves for Female Rats with Perinatal and Postweaning Exposure to 0/0, 150/300, or 300/1,000 ppm Perfluorooctanoic Acid in Feed for Two Years (Study 1)

Group mean feed consumption over the course of the study averaged 93%, 99%, 96%, and 88% of the 0/0 ppm control group for the 0/300, 150/300, 0/1,000, and 300/1,000 ppm groups, respectively (CEBS, Study 1, E08). After weaning, compound consumption for females in the 0/300 and 150/300 ppm groups averaged 18.2 and 18.4 mg/kg/day, respectively. Compound consumption averaged 63.4 and 63.5 mg/kg/day for the 0/1,000 and 300/1,000 ppm groups, respectively.

Pathology and Statistical Analyses

The morphologic features of the lesions discussed in this section are presented in the Histopathological Descriptions section following the Study 2 results.

Liver: Chronic exposure with and without perinatal exposure resulted in slight increases in the incidences of hepatocellular carcinoma in the 0/1,000 and 300/1,000 ppm groups, respectively; however, the increases were not significantly different from the incidence in the 0/0 ppm control group (Table 23). The occurrences of hepatocellular adenomas did not differ among the groups. The combined incidence of adenomas and carcinomas was marginally higher in the 1,000 ppm postweaning groups, which was primarily due to the higher incidence of carcinomas in these groups (Table 23). The one hepatocellular carcinoma present in the historical control is from this study. No differences between groups with perinatal and without perinatal exposures were observed.

Chronic exposure with and without perinatal exposure resulted in exposure concentration-related increases in the incidences of a spectrum of nonneoplastic hepatocellular lesions (Table 23; CEBS, Study 1, P10; P18). Compared to the incidences in the 0/0 ppm control group, the incidences of hepatocyte cytoplasmic alteration and hepatocyte hypertrophy were significantly increased in all exposed female groups in the 2-year study. In general, the severity of these lesions tended to be minimal to moderate and were more severe in groups receiving the highest exposure concentrations. Hepatocyte hypertrophy and cytoplasmic alteration were generally colocalized hepatocellular changes.

The incidences of hepatocyte single cell death were significantly increased in the 150/300, 0/1,000, and 300/1,000 ppm groups compared to the 0/0 ppm control group (Table 23; CEBS, Study 1, P10; P18).

The incidences of necrosis were increased in all exposed groups; however, only the increase in the 0/1,000 ppm group reached statistical significance compared to the 0/0 ppm control group (Table 23; CEBS, Study 1, P10; P18).

The incidences of pigment were significantly increased in the 150/300, 0/1,000, and 300/1,000 ppm groups compared to the 0/0 ppm control group (Table 23; CEBS, Study 1, P10; P18).

The incidences of bile duct hyperplasia were increased in all exposed groups compared to the 0/0 ppm control group; the increase was significant only in the 300/1,000 ppm group (Table 23; CEBS, Study 1, P10; P18).

The incidences of hepatocyte mitoses were significantly increased in the 300/1,000 ppm group compared to the 0/0 ppm control group (Table 23; CEBS, Study 1, P10; P18).

Table 23. Incidences of Neoplastic and Nonneoplastic Liver Lesions in Female Rats in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**300 ppm**	**1,000 ppm**
n		50[a]	50	50[b]
Hepatocyte, Cytoplasmic Alteration[c]	0 ppm	0**##	9** (1.4)[d]	49** (2.3)
	150 ppm	–	17** (1.1)	–
	300 ppm	–	–	49** (2.2)
Hepatocyte, Hypertrophy	0 ppm	0**##	11** (1.7)	48** (2.4)
	150 ppm	–	16** (1.6)	–
	300 ppm	–	–	49** (2.4)
Hepatocyte, Single Cell Death	0 ppm	0**##	4 (1.0)	29** (1.3)
	150 ppm	–	5* (1.0)	–
	300 ppm	–	–	32** (1.2)
Necrosis	0 ppm	0**	1 (1.0)	8* (1.5)
	150 ppm	–	4 (1.3)	–
	300 ppm	–	–	5 (2.4)
Pigment	0 ppm	3**## (1.3)	5 (1.4)	43** (1.7)
	150 ppm	–	10* (1.1)	–
	300 ppm	–	–	40** (1.8)
Bile Duct, Hyperplasia	0 ppm	16# (1.3)	25 (1.2)	22 (1.2)
	150 ppm	–	27 (1.1)	–
	300 ppm	–	–	27* (1.3)
Hepatocyte, Increased Mitoses	0 ppm	2# (1.0)	3 (1.0)	4 (1.5)
	150 ppm	–	5 (1.6)	–
	300 ppm	–	–	10* (1.3)
Hepatocellular Adenoma[e]				
Overall Rate[f]	0 ppm	2/50 (4%)	0/50 (0%)	1/49 (2%)
Litters Rate[g]		2/32 (6%)	0/29 (0%)	1/31 (3%)
Rao-Scott Adjusted Poly-3 Test[h,i]		p = 0.543N/0.322	p = 0.262N	p = 0.506N
Overall Rate	150 ppm		0/50 (0%)	
Litters Rate		–	0/29 (0%)	–
Rao-Scott Adjusted Poly-3 Test			p = 0.251N	
Overall Rate	300 ppm			3/50 (6%)
Litters Rate		–	–	3/30 (10%)
Rao-Scott Adjusted Poly-3 Test				p = 0.508
Hepatocellular Carcinoma[j]				
Overall Rate	0 ppm	1/50 (2%)	1/50 (2%)	3/49 (6%)
Litters Rate		1/32 (3%)	1/29 (3%)	3/31 (10%)
Rao-Scott Adjusted Poly-3 Test		p = 0.211/0.089	p = 0.709N	p = 0.318
Overall Rate	150 ppm		0/50 (0%)	
Litters Rate		–	0/29 (0%)	–
Rao-Scott Adjusted Poly-3 Test			p = 0.524N	
Overall Rate	300 ppm			4/50 (8%)
Litters Rate		–	–	4/30 (13%)
Rao-Scott Adjusted Poly-3 Test				p = 0.233

	Perinatal Exposure	Postweaning Exposure		
		0 ppm	300 ppm	1,000 ppm
Hepatocellular Adenoma or Carcinoma, (Combined)[k]				
Overall Rate	0 ppm	3/50 (6%)	1/50 (2%)	4/49 (8%)
Litters Rate		3/32 (9%)	1/29 (3%)	4/31 (13%)
Rao-Scott Adjusted Poly-3 Test		p = 0.332/0.093	p = 0.275N	p = 0.491
Overall Rate	150 ppm		0/50 (0%)	
Litters Rate		–	0/29 (0%)	–
Rao-Scott Adjusted Poly-3 Test			p = 0.126N	
Overall Rate	300 ppm			6/50 (12%)
Litters Rate		–	–	6/30 (20%)
Rao-Scott Adjusted Poly-3 Test				p = 0.269

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.
*Significantly different ($p \leq 0.05$) from the 0/0 ppm control group by the Rao-Scott test.; **$p \leq 0.01$.
[#]Statistically significant trend at $p \leq 0.05$ when comparing across the 0/0, 150/300, and 300/1,000 ppm groups; [##]$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]N = 49 for the 0/1,000 ppm group.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[e]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 14/340 (3.63% ± 2.59%); range: 0% to 8%.
[f]Number of animals with neoplasm per number of animals necropsied.
[g]Number of litters with animals with neoplasm per number of litters necropsied.
[h]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidences are the p values corresponding to pairwise comparisons between the respective control and that exposed group. The Rao-Scott test adjusts the Poly-3 test for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[i]Rao-Scott trend values presented as: p value (postweaning-only exposure groups)/p value (perinatal and postweaning exposure groups).
[j]Historical control incidence: 1/340 (0.33% ± 0.82%); range: 0% to 2%.
[k]Historical control incidence: 15/340 (3.96% ± 2.77%); range: 0% to 8%.

Pancreas: In the 300/1,000 ppm group, slight increases were observed in the incidences of acinar cell adenoma, acinar cell adenocarcinoma and adenoma or adenocarcinoma combined that did not reach statistical significance compared to the 0/0 ppm control group (Table 24). One duct adenocarcinoma occurred in the 300/1,000 ppm group. (See 2-year study in males for description of the pancreatic lesions.) There were occurrences of hyperplasia, but they were not statistically significant. No differences between groups with perinatal and without perinatal exposures were observed.

Table 24. Incidences of Neoplastic and Nonneoplastic Pancreas Lesions in Female Rats in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

	Perinatal Exposure	Postweaning Exposure		
		0 ppm	300 ppm	1,000 ppm
n		50[a]	50	50[b]
Acinus, Hyperplasia	0 ppm	0	1 (2.0)	1 (2.0)
	150 ppm	–	0	–
	300 ppm	–	–	1 (4.0)

	Perinatal Exposure	Postweaning Exposure: 0 ppm	Postweaning Exposure: 300 ppm	Postweaning Exposure: 1,000 ppm
Acinar Cell Adenoma[c]				
Overall Rate[d]	0 ppm	0/50 (0%)	0/50	1/49 (2%)
Litters Rate[e]		0/32 (0%)	0/25	1/31 (3%)
Rao-Scott Adjusted Poly-3 Test[f]		p = 0.084[g]	–[h]	(e)
Overall Rate	150 ppm		0/50 (0%)	
Litters Rate		–	0/29 (0%)	–
Rao-Scott Adjusted Poly-3 Test			(e)	
Overall Rate	300 ppm			3/50 (6%)
Litters Rate		–	–	3/30 (10%)
Rao-Scott Adjusted Poly-3 Test				p = 0.215
Acinar Cell Adenocarcinoma[i]				
Overall Rate	0 ppm	0/50 (0%)	0/50	1/49 (2%)
Litters Rate		0/32 (0%)	0/25	1/31 (3%)
Rao-Scott Adjusted Poly-3 Test		p = 0.178[g]	–	(e)
Overall Rate	150 ppm		0/50 (0%)	
Litters Rate		–	0/29 (0%)	–
Rao-Scott Adjusted Poly-3 Test			(e)	
Overall Rate	300 ppm			2/50 (4%)
Litters Rate		–	–	2/30 (7%)
Rao-Scott Adjusted Poly-3 Test				p = 0.340
Acinar Cell Adenoma or Adenocarcinoma, (Combined)[j]				
Overall Rate	0 ppm	0/50 (0%)	0/50 (0%)	2/49 (4%)
Litters Rate		0/32 (0%)	0/29 (0%)	2/31 (6%)
Rao-Scott Adjusted Poly-3 Test		p = 0.174/0.018[k]	(e)	p = 0.337
Overall Rate	150 ppm		0/50 (0%)	
Litters Rate		–	0/29 (0%)	–
Rao-Scott Adjusted Poly-3 Test			(e)	
Overall Rate	300 ppm			5/50 (10%)
Litters Rate		–	–	5/30 (17%)
Rao-Scott Adjusted Poly-3 Test				p = 0.086

Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.
[a]Number of animals with tissue examined microscopically.
[b]N = 49 for the 0/1,000 ppm group.
[c]Historical control incidence for all routes of 2-year studies: 0/340.
[d]Number of animals with neoplasm per number of animals necropsied.
[e]Number of litters with animals with neoplasm per number of litters necropsied.
[f]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidences are the p values corresponding to pairwise comparisons between the respective control and that exposed group. The Rao-Scott test adjusts the Poly-3 test for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[g]P value represents the trend for the 0/0, 150/300, and 300/1,000 ppm groups.
[h]Not applicable; no neoplasms in group.
[i]Historical control incidence: 0/340.
[j]Historical control incidence: 0/340.
[k]Rao-Scott trend values presented as: p value (postweaning-only exposure groups)/p value (perinatal and postweaning exposure groups).
(e) = value of statistic could not be computed.

Uterus: In the standard evaluation of the perinatal and/or postweaning exposures, increased incidences of adenocarcinomas of the uterus, compared to the 0/0 ppm control group, occurred in the 0/300, 0/1,000, 150/300, and 300/1,000 ppm groups (CEBS, Study 1, P05; P08).

In the extended evaluation, additional adenocarcinomas were diagnosed in the uteri of exposed groups (CEBS, Study 1, P05; P08). The incidences of adenocarcinoma were increased in the 0/300 and 0/1,000 ppm groups; the increase in the 0/1,000 ppm group was significant.

In the combined standard and extended evaluations, the incidences of adenocarcinoma were increased in the 0/300, 0/1,000, 150/300, and 300/1,000 ppm groups; however, the increase was significant only in the 0/1,000 ppm group (Table 25). No differences between groups with perinatal and without perinatal exposures were observed.

Table 25. Incidences of Neoplastic and Nonneoplastic Lesions in the Standard and Extended Evaluations of the Uterus in the Two-year Feed Study of Perfluorooctanoic Acid in Female Rats (Study 1)[a]

		Postweaning Exposure		
	Perinatal Exposure	**0 ppm**	**300 ppm**	**1,000 ppm**
n		50[b]	50	50[c]
Standard or Extended Evaluation (Combined)				
Hyperplasia, Atypical[d,e]	0 ppm	3/50 (2.0)	4/49 (2.0)	3/48 (2.7)
	150 ppm	–	7/50 (2.1)	–
	300 ppm	–	–	3/48 (4.0)
Adenoma[f]				
Overall Rate[g] Litters Rate[h] Rao-Scott Adjusted Poly-3 Test[i]	0 ppm	1/50 (2%) 1/32 (3%) p = 0.374N/0.513N[j]	1/50 (2%) 1/29 (3%) p = 0.712N	0/50 (0%) 0/31 (0%) p = 0.485N
Overall Rate Litters Rate Rao-Scott Adjusted Poly-3 Test	150 ppm	–	0/50 (0%) 0/29 (0%) p = 0.576N	–
Overall Rate Litters Rate Rao-Scott Adjusted Poly-3 Test	300 ppm	–	–	0/50 (0%) 0/30 (0%) p = 0.573N
Adenocarcinoma[k]				
Overall Rate Litters Rate Rao-Scott Adjusted Poly-3 Test	0 ppm	1/50 (2%) 1/32 (3%) p = 0.028/0.094	5/50 (10%) 5/29 (17%) p = 0.164	8/50 (16%) 7/31 (23%) p = 0.031
Overall Rate Litters Rate Rao-Scott Adjusted Poly-3 Test	150 ppm	–	3/50 (6%) 3/29 (10%) p = 0.345	–
Overall Rate Litters Rate Rao-Scott Adjusted Poly-3 Test	300 ppm	–	–	5/50 (10%) 5/30 (17%) p = 0.115

	Perinatal Exposure	Postweaning Exposure 0 ppm	Postweaning Exposure 300 ppm	Postweaning Exposure 1,000 ppm
Adenoma or Adenocarcinoma, (Combined)[l]				
Overall Rate	0 ppm	2/50 (4%)	5/50 (10%)	8/50 (16%)
Litters Rate		2/32 (6%)	5/29 (17%)	7/31 (23%)
Rao-Scott Adjusted Poly-3 Test		p = 0.052/0.169	p = 0.289	p = 0.065
Overall Rate	150 ppm	–	3/50 (6%)	–
Litters Rate			3/29 (10%)	
Rao-Scott Adjusted Poly-3 Test			p = 0.535	
Overall Rate	300 ppm	–	–	5/50 (10%)
Litters Rate				5/30 (17%)
Rao-Scott Adjusted Poly-3 Test				p = 0.220

Pairwise comparisons across perinatal exposures (0/300 vs. 150/300 ppm and 0/1,000 vs. 300/1,000 ppm) did not show any statistically significant differences.
[a]Data presented here are for the combined incidence in the standard and extended evaluations. Additional data for standard and extended evaluations alone can found in CEBS.
[b]Number of animals with tissue examined microscopically.
[c]N = 49 for the 0/1,000 ppm group.
[d]Number of animals with lesion.
[e]N = 49 for the 0/300 ppm group; N = 48 for the 0/1,000 and 300/1,000 ppm groups.
[f]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 1/150 (0.67% ± 1.15%); range: 0% to 2%.
[g]Number of animals with neoplasm per number of animals necropsied.
[h]Number of litters with animals with neoplasm per number of litters necropsied.
[i]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidences are the p values corresponding to pairwise comparisons between the respective control and that exposed group. The Rao-Scott test adjusts the Poly-3 test for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[j]Rao-Scott trend values presented as: p value (postweaning-only exposure groups)/p value (perinatal and postweaning exposure groups).
[k]Historical control incidence: 11/150 (7.33% ± 4.62%); range: 2% to 10%.
[l]Historical control incidence: 12/150 (8% ± 3.46%); range: 4% to 10%.

Kidney: Compared to the 0/0 ppm control group, the incidences of hyperplasia of the renal papillary epithelium were significantly increased in the 0/300, 0/1,000, and 300/1,000 ppm groups (Table 26). The incidences of papilla necrosis were significantly increased in the 0/1,000 and 300/1,000 ppm groups. The incidence of renal tubule mineral was significantly increased in the 0/1,000 ppm group. In general, the incidences of these kidney lesions increased with increasing exposure concentration. There were some statistical differences observed in groups with and without perinatal exposure. The incidence of hyperplasia of the renal papillary epithelium in the 150/300 ppm group was significantly decreased compared to the 0/300 ppm group. It is not clear if this was related to perinatal exposure as this only occurred with the 150 ppm perinatal exposure and not the 300 ppm perinatal exposure. In addition, there was a decrease of renal tubule mineral in the 300/1,000 ppm group compared to the 0/1,000 ppm group. Similarly, it is unclear if this is related to perinatal exposure.

Table 26. Incidences of Select Nonneoplastic Lesions in the Kidney, Stomach, and Thyroid Gland of Female Rats in the Two-year Feed Study of Perfluorooctanoic Acid (Study 1)

		Postweaning Exposure		
	Perinatal Exposure	0 ppm	300 ppm	1,000 ppm
n		50[a]	50	50[b]
Kidney				
Papilla, Urothelium, Hyperplasia[c]	0 ppm	4**## (1.0)[d]	21** (1.0)	40** (1.9)
	150 ppm	–	8^^ (1.0)	–
	300 ppm	–	–	45** (1.8)
Papilla, Necrosis	0 ppm	0**##	0	12** (2.3)
	150 ppm	–	0	–
	300 ppm	–	–	22** (2.1)
Renal Tubule, Mineral	0 ppm	5** (1.2)	6 (1.3)	16** (1.0)
	150 ppm	–	8 (1.0)	–
	300 ppm	–	–	8^ (1.5)
Forestomach				
Ulcer	0 ppm	2**## (1.5)	2 (1.5)	9* (1.6)
	150 ppm	–	1 (1.0)	–
	300 ppm	–	–	11* (2.1)
Epithelium, Hyperplasia	0 ppm	4**## (2.3)	5 (1.8)	22** (2.8)
	150 ppm	–	3 (2.3)	–
	300 ppm	–	–	21** (2.5)
Submucosa, Inflammation, Chronic Active	0 ppm	3**## (2.3)	2 (2.5)	16** (2.6)
	150 ppm	–	2 (2.0)	–
	300 ppm	–	–	18** (2.5)
Thyroid Gland				
Follicular Cell, Hypertrophy	0 ppm	4**## (2.3)	8 (2.1)	28** (2.0)
	150 ppm	–	9 (1.6)	–
	300 ppm	–	–	19** (1.7)

Statistical significance for a treatment group indicates a significant pairwise test compared to the 0/0 ppm control group.
Statistical significance for the 0/0 ppm control group indicates a significant trend test.
*Significantly different ($p \leq 0.05$) from the 0/0 ppm control by the Rao-Scott test; **$p \leq 0.01$.
##Statistically significant trend at $p \leq 0.01$ when comparing across the 0/0, 150/300, and 300/1,000 ppm groups.
^Statistically different ($p \leq 0.05$) when comparing the 0/300 vs. 150/300 ppm groups or 0/1,000 vs. 300/1,000 groups; ^^$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]N = 49 for the 0/1,000 ppm group.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Forestomach: The incidences of ulcer, epithelium hyperplasia, and chronic active inflammation of the submucosa in the 0/1,000 and 300/1,000 ppm groups were significantly greater than those in the 0/0 ppm control group (Table 26). Both exposed groups had a single case of a squamous cell papilloma (CEBS, Study 1, P05).

Thyroid Gland: The incidences of follicular cell hypertrophy in the 0/1,000 and 300/1,000 ppm groups were significantly greater than those in the 0/0 ppm control group (Table 26). No differences between groups with perinatal and without perinatal exposures were observed.

Study 2: Two-year Study in Males with 16-week Interim Evaluation

Perinatal Exposure

No exposure-related effects were observed on the pregnancy status, maternal survival, or number of dams that littered (Table 27). PFOA fetal and lactational transfer was assessed at GD 18 and PND 4, respectively (Table 28). Maternal plasma concentrations of the 300 ppm group were 75.1 μM on GD 18 and 74.2 μM on PND 4. Concentrations of PFOA from fetuses pooled by litter on GD 18 were 23 μM, indicating some maternal transfer with maternal plasma concentrations at 75 μM. On PND 4, concentrations from whole male and female pups were comparable at 11 μM and 10 μM, respectively, indicating some lactational transfer (Table 28). Concentrations of PFOA were below detection in the control group.

Table 27. Summary of Disposition during Perinatal Exposure and F_1 Allocation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 2)

	0 ppm	300 ppm
Time-mated Females	147	147
Pregnant Females	129	123
Nonpregnant Females	18	24
Biological Sample Analysis (GD 18)	6[a]	5
Pregnant Dams Not Delivering	4	6
Moribund (Dystocia)	1	0
Littered/Pregnant on GD 21	119/123 (97%)	112/118 (95%)
Litters Removed (PND 4)[b]	11	5
Litters Poststandardization (PND 4)	108	107
Weaned Males	240	240

[a]Includes one dam that was not pregnant.
[b]Removed due to insufficient size.

Table 28. Perfluorooctanoic Acid Concentrations in F_0 and F_1 Rats in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 2)[a]

	0 ppm	300 ppm
Gestation Day 18		
F_0 Plasma Concentration (ng/mL)	BD	31,080 ± 1,227 (5)
F_0 Plasma Concentration (μM)	BD	75.1 ± 3.0 (5)
F_1 Pooled Whole Fetus (ng/mL)	BD	9,374 ± 1,785 (5)
F_1 Pooled Whole Fetus (μM)	BD	23 ± 4 (5)

	0 ppm	300 ppm
Postnatal Day 4		
F_0 Plasma Concentration (ng/mL)	BD	30,725 ± 2,782 (4)
F_0 Plasma Concentration (μM)	BD	74.2 ± 6.74 (4)
F_1 Pooled Whole Male Pup (ng/mL)	BD	4,539 ± 320 (4)
F_1 Pooled Whole Male Pup (μM)	BD	11 ± 1 (4)
F_1 Pooled Whole Female Pup (ng/mL)	BD	4,132 ± 517 (3)
F_1 Pooled Whole Female Pup (μM)	BD	10 ± 1 (3)

BD = below detection.
[a]Mean ± standard error (number of dams, fetuses, or pups). No statistical analysis was performed.

Maternal body weights of the 300 ppm group during gestation were similar to those of the control group (Table 29). A slight decrease in body weight gain was observed at the first interval of GD 6 to 9, but overall weight gain from GD 6 to 21 was not affected by exposure (Table 29). During lactation, there was a marginal decrease (2% to 3%) in maternal weight compared to the control group and a decrease in body weight gain over this time period (Table 30).

Table 29. Mean Body Weights and Body Weight Gains of F_0 Females during Gestation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 2)[a]

Gestation Day	0 ppm	300 ppm
Body Weight (g)		
6	214.14 ± 1.11 (129)	213.74 ± 1.18 (123)
9	229.97 ± 1.33 (129)	227.52 ± 1.40 (123)
12	247.19 ± 1.20 (129)	245.27 ± 1.21 (123)
15	264.53 ± 1.34 (129)	262.78 ± 1.30 (123)
18	298.93 ± 1.97 (129)	299.40 ± 1.83 (123)
21	341.68 ± 2.88 (124)	341.25 ± 2.71 (118)
Body Weight Change (g)		
6 to 9	15.83 ± 0.68 (129)	13.77 ± 0.66* (123)
9 to 12	17.22 ± 0.57 (129)	17.75 ± 0.70 (123)
12 to 15	17.34 ± 0.51 (129)	17.51 ± 0.43 (123)
15 to 18	34.40 ± 0.89 (129)	36.62 ± 0.90 (123)
18 to 21	43.04 ± 1.07 (124)	42.24 ± 1.16 (118)
6 to 21	127.70 ± 2.49 (124)	127.68 ± 2.46 (118)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.
*Statistically significant at $p \leq 0.05$.
[a]Mean ± standard error (number of dams). The exposed group was compared to the control group using a t-test.

Table 30. Mean Body Weights and Body Weight Gains of F_0 Females during Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 2)[a]

Lactation Day	0 ppm	300 ppm
Body Weight (g)		
1	259.90 ± 1.35 (119)	257.71 ± 1.35 (112)
4	271.11 ± 1.41 (119)	267.13 ± 1.36* (112)
7	281.72 ± 1.37 (108)	276.49 ± 1.36** (107)
14	296.06 ± 1.64 (107)	290.23 ± 1.35** (107)
21	283.21 ± 1.60 (107)	274.49 ± 1.40** (107)
Body Weight Change (g)		
1 to 4	11.21 ± 0.60 (119)	9.42 ± 0.52* (112)
4 to 7	9.63 ± 0.65 (108)	9.72 ± 0.69 (107)
7 to 14	14.25 ± 1.09 (107)	13.74 ± 0.73 (107)
14 to 21	−12.85 ± 1.10 (107)	−15.74 ± 0.74* (107)
1 to 21	22.61 ± 1.10 (107)	16.99 ± 0.91** (107)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Mean ± standard error (number of dams). The exposed group was compared to the control group using a t-test.

Feed consumption (g/animal/day) was marginally lower (≤ 3%) in the 300 ppm group compared to the control group at two intervals during gestation, but overall GD 6 to 21 feed consumption was unaffected (Table 31). Feed consumption by the 300 ppm female group during lactation was marginally less (~5%) than that of the control group from LD 1 to 14 (Table 31). Chemical consumption was 21.8 mg/kg/day during gestation and 48.3 mg/kg/day during days 1 to 14 of the lactational period.

Table 31. Feed Consumption by F_0 Females during Gestation and Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 2)[a]

Days	0 ppm	300 ppm
Gestation (g/animal/day)		
6 to 9	17.03 ± 0.20 (129)	16.52 ± 0.20** (123)
9 to 12	17.34 ± 0.13 (129)	17.58 ± 0.11 (123)
12 to 15	18.77 ± 0.15 (129)	18.27 ± 0.18** (123)
15 to 18	21.02 ± 0.21 (129)	21.50 ± 0.29 123)
18 to 21	21.24 ± 0.22 (124)	21.15 ± 0.26 (118)
6 to 21	19.11 ± 0.14 (124)	18.99 ± 0.12 (118)
Lactation (g/animal/day)		
1 to 4	41.24 ± 1.47 (115)	38.18 ± 1.11** (112)
4 to 7	40.00 ± 0.37 (108)	38.66 ± 0.30* (107)
7 to 10	47.89 ± 0.43 (107)	45.14 ± 0.34** (107)

Days	0 ppm	300 ppm
10 to 14	54.50 ± 0.41 (106)	53.25 ± 0.39* (107)
14 to 17	57.58 ± 0.60 (107)	55.66 ± 0.37** (107)
17 to 21	67.70 ± 0.81 (107)	66.83 ± 0.59 (107)
1 to 14	46.81 ± 0.42 (102)	44.62 ± 0.34** (107)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Mean ± standard error in grams/cage per day (number of dams). The exposed group was compared to the control group using the Wilcoxon rank-sum test.

Live litter sizes on PNDs 1 and 21 were not affected by exposure, and survival of F_1 pups was not affected between PNDs 4 poststandardization and 21 (Table 32). The mean pup body weight of the 300 ppm group was 4% less than that of the controls on PND 1. F_1 300 ppm male weights were less than control group weights throughout lactation, ranging from 3% lower at PND 1 to 7% lower at PND 21.

Table 32. Mean Litter Size, Survival Ratio, and Mean Body Weights of F_1 Rats during Lactation in the Two-year Perinatal and Postweaning Study of Perfluorooctanoic Acid (Study 2)

Postnatal Day	0 ppm	300 ppm
Live Litter Size[a]		
1	11.72 ± 0.26 (118)	11.52 ± 0.24 (112)
4 (Poststandardization)	8.00 ± 0.00 (118)	7.99 ± 0.01 (112)
21	7.81 ± 0.07 (118)	7.89 ± 0.03 (112)
Survival Ratio[a,b]		
4 to 21	0.98 ± 0.01 (118)	0.99 ± 0.00 (112)
Body Weight		
All Pups[a,c]		
1[c]	7.37 ± 0.06 (118)	7.07 ± 0.05** (112)
Male Pups		
1[c]	7.50 ± 0.06 (116)	7.26 ± 0.06** (111)
4[d]	10.57 ± 0.12 (436/108)	10.14 ± 0.10** (435/107)
7[d]	15.90 ± 0.18 (435/108)	15.03 ± 0.15** (435/107)
14[d]	31.64 ± 0.30 (427/107)	30.19 ± 0.24** (431/107)
21[d]	52.35 ± 0.48 (421/106)	48.84 ± 0.43** (428/107)

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.
**Statistically significant at $p \leq 0.01$.
[a]Mean ± standard error (number of dams). The exposed group was compared to the control group using the Wilcoxon rank-sum test.
[b]Number of live pups on PND 21/number of live pups on PND 4 poststandardization.
[c]Mean ± standard error (number of dams). The exposed group was compared to the control group using a t-test.
[d]Mean ± standard error (number of pups/number of dams). Values were adjusted for live litter size at PND 4 poststandardization; a mixed-litter effects model was used to compare the exposed group to the control group.

Sixteen-week Interim Evaluation in Males (Study 2)

All rats designated for interim evaluation survived until 16 weeks (CEBS, Study 2, E01). There were no clinical observations in any of the exposed groups (CEBS, Study 2, E05).

Group mean feed and compound consumption data are presented in CEBS (Study 2, E08). Feed consumption (g/animal/day) in all groups remained within 10% of the 0/0 ppm control group throughout the study. PFOA consumption for the first 13 weeks postweaning averaged 1.9 mg/kg/day for the 0/20 and 300/20 ppm groups, 4.0 mg/kg/day for the 0/40 and 300/40 ppm groups, and 7.9 and 8.0 mg/k/day for the 0/80 and 300/80 ppm groups, respectively. In general, chemical consumption increased in proportion with exposure concentration (ppm).

Plasma concentrations of PFOA in males were consistent between groups that were exposed postweaning (300 ppm) or not exposed to PFOA (Table 33). The differences between groups (e.g., 0/20 and 300/20 ppm) were 12% or less. Liver concentrations followed a similar pattern as the plasma concentrations.

Table 33. Summary of Plasma and Liver Concentration Data for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)[a,b]

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
n		10	10	10	10
Plasma Concentration (ng/mL)	0 ppm	BD	81,400 ± 2,715	130,780 ± 7,560	159,600 ± 8,303
	300 ppm	36 ± 12**	78,030 ± 2,976**	117,060 ± 4,189**	144,100 ± 5,480**
Plasma Concentration (μM)	0 ppm	BD	196.6 ± 6.6	315.8 ± 18.3	385.5 ± 20.1
	300 ppm	0.1 ± 0.0**	188.5 ± 7.2**	282.7 ± 10.1**	348.0 ± 13.2**
Liver Concentration (ng/g)	0 ppm	BD	83,550 ± 4,658	108,280 ± 5,412	147,400 ± 10,629
	300 ppm	BD	85,960 ± 3,635	109,210 ± 3,039	133,310 ± 4,625
Liver Concentration (μM)	0 ppm	BD	201.8 ± 11.2	261.5 ± 13.1	356.0 ± 25.7
	300 ppm	BD	207.6 ± 8.8	263.8 ± 7.3	322.0 ± 11.2
Liver/Plasma Ratio	0 ppm	BD	1.02 ± 0.03	0.84 ± 0.04	0.92 ± 0.03
	300 ppm	BD	1.11 ± 0.04	0.94 ± 0.03	0.94 ± 0.05

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 ppm or 300/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/20 vs. 300/20, 0/40 vs. 300/40, and 0/80 vs. 300/80 ppm) did not show any statistically significant differences.
**Statistically significant at $p \leq 0.01$.
Values adjusted for molar concentration were calculated by dividing by the molecular weight of 414.06.
BD = below detection; group did not have over 20% of its values above the limit of quantification. In these cases, no statistical analyses were performed.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).

Group mean body weights are provided in CEBS (Study 2, E04). Group mean body weights for the 0/20, 300/0, and 300/20 ppm groups were within 10% of the 0/0 ppm control group until the interim evaluation. At 16 weeks, group mean body weights for the 0/40, 300/40, 0/80, and 300/80 ppm groups were 18%, 14%, 19%, and 21% less than that of the 0/0 ppm control group, respectively (Table 34).

With the exception of the 300/0 ppm group, the group mean absolute and relative liver weights of all exposed groups were significantly greater than those of the 0/0 ppm control group (Table 34). Histological correlates were present in all groups that had liver weight changes (CEBS, Study 2, P03; P10; P18). No differences were observed between groups with and without perinatal exposures.

Acyl-CoA oxidase enzyme activity within the liver was increased in all postweaning exposed groups compared to the 0/0 ppm control group (Table 34). The magnitude of the increase was similar between groups that were perinatally exposed or not (e.g., 0/20 vs. 300/20 ppm). Aromatase activity within the liver was increased marginally in all postweaning exposed groups with roughly a doubling in activity compared to the 0/0 ppm control group (Table 34). Note that the 0/0 ppm control group and 300/0 ppm groups had similar acyl-CoA oxidase and aromatase activity. No differences were observed between groups with and without perinatal exposures.

Table 34. Summary of Hepatic Findings for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)[a,b]

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
n		10[c]	10	10	10
Necropsy Body Wt. (g)	0 ppm	429.8 ± 12.1**	389.5 ± 7.9**	351.8 ± 8.7**	348.4 ± 10.2**
	300 ppm	414.6 ± 7.5**	390.5 ± 7.7†	367.5 ± 7.2**††	338.4 ± 13.7**††
Liver Weight					
Absolute (g)	0 ppm	14.62 ± 0.43**	17.99 ± 0.42**	18.80 ± 0.76**	19.74 ± 0.90**
	300 ppm	13.57 ± 0.30**	17.33 ± 0.66**††	18.49 ± 0.47**††	18.64 ± 1.04**††
Relative (mg/g)	0 ppm	34.10 ± 0.81**	46.24 ± 0.90**	53.38 ± 1.49**	56.56 ± 1.71**
	300 ppm	32.73 ± 0.43**	44.27 ± 1.02**††	50.30 ± 0.76**††	55.07 ± 2.18**††
n		10	10	10	10
Acyl-CoA Oxidase Activity (nmol/min/mg)	0 ppm	2.636 ± 0.107**	11.436 ± 0.660**	19.360 ± 1.170**	25.010 ± 1.973**
	300 ppm	2.754 ± 0.073**	13.779 ± 1.484**††	18.420 ± 1.061**††	23.320 ± 2.438**††
Aromatase Activity (pmol/mg/min)	0 ppm	7.658 ± 0.718**	15.910 ± 0.842**	14.539 ± 1.075**	16.630 ± 0.766**
	300 ppm	8.837 ± 0.782**	15.951 ± 1.059**††	17.560 ± 1.477**††	16.640 ± 1.083**††

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 ppm or 300/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/20 vs. 300/20, 0/40 vs. 300/40, and 0/80 vs. 300/80 ppm) did not show any statistically significant differences.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
†Statistically significant at $p \leq 0.05$ for pairwise comparisons of 0/0 to 300/20 ppm, 0/0 to 300/40 ppm, and 0/0 to 300/80 ppm using a Wilcoxon rank-sum test with a Hommel adjustment (enzyme activity) or a t-test with a Hommel adjustment (body and organ weights); ††$p \leq 0.01$.
[a]Data presented as mean ± SEM.
[b]Statistical analysis of body and organ weights performed using Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests. Statistical analysis of enzyme activity performed using Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests.
[c]Number of animals examined.

With the exception of the relative weight of the 0/80 ppm group, the group mean absolute and relative spleen weights of all exposed groups were significantly less than those of the 0/0 ppm control group (Table 35; CEBS, Study 2, PA06). Absolute weights were decreased up to 29% compared to the 0/0 ppm control group without a histological correlate.

With the exception of the 300/0 ppm group, the group mean relative right kidney weights of all exposed groups were significantly greater than that of the 0/0 ppm control group (Table 35; CEBS, Study 2, PA06). The absolute right kidney weight of the 0/20 ppm group was significantly greater than that of the 0/0 ppm controls. There were no correlated histological findings to explain the increases in kidney weights. Overall, organ weight changes in the adrenal, heart, pituitary gland, lung, testis, thymus, and thyroid gland were considered secondary to body weight changes (CEBS, Study 2, PA06).

Table 35. Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)[a,b]

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
n		10[c]	10	10	10
Spleen Weight					
Absolute (g)	0 ppm	0.793 ± 0.029**	0.634 ± 0.025**	0.564 ± 0.014**	0.577 ± 0.031**
	300 ppm	0.648 ± 0.016**##	0.643 ± 0.025††	0.589 ± 0.015††	0.540 ± 0.035**††
Relative (mg/g)	0 ppm	1.85 ± 0.08*	1.63 ± 0.05*	1.61 ± 0.05*	1.66 ± 0.07
	300 ppm	1.57 ± 0.04#	1.64 ± 0.04†	1.60 ± 0.02†	1.59 ± 0.06†
Right Kidney Weight					
Absolute (g)	0 ppm	1.14 ± 0.04	1.31 ± 0.03**	1.26 ± 0.04	1.19 ± 0.04
	300 ppm	1.17 ± 0.02	1.27 ± 0.02†	1.23 ± 0.03	1.18 ± 0.05
Relative (mg/g)	0 ppm	2.66 ± 0.06**	3.36 ± 0.07**	3.59 ± 0.04**	3.42 ± 0.06**
	300 ppm	2.83 ± 0.04**	3.26 ± 0.05**††	3.35 ± 0.05**††	3.47 ± 0.05**††

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 ppm or 300/0 ppm control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
†Statistically significant at $p \leq 0.05$ for pairwise comparisons of 0/0 to 300/20 ppm, 0/0 to 300/40 ppm, and 0/0 to 300/80 ppm using a t-test with a Hommel p value adjustment (body and organ weights); ††$p \leq 0.01$.
#Statistically significant at $p \leq 0.05$ for pairwise comparisons of 0/0 to 300/0 ppm, 0/20 to 300/20 ppm, 0/40 to 300/40 ppm, and 0/80 to 300/80 pm using a t-test with a Hommel p value adjustment; ##$p \leq 0.01$.
[a]Data presented as mean ± SEM.
[b]Statistical analysis of body and organ weights performed using Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests.
[c]Number of animals examined.

Clinical chemistry data are presented in Table 36. Compared to the 0/0 ppm control group, urea nitrogen concentrations were significantly increased in the 0/40, 0/80, 300/40, and 300/80 ppm groups. Mild increases in urea nitrogen without concomitant increases in creatinine concentration usually indicate decreased water consumption (i.e., mild dehydration). Triglyceride concentrations were significantly decreased in the 0/20, 0/40, 300/20, 300/40, and 300/80 ppm groups. Cholesterol concentrations were significantly decreased in the 300/20 and 300/80 ppm groups. No differences were observed between groups with and without perinatal exposures.

In all exposed groups except the 300/0 ppm group, the globulin concentrations were significantly decreased compared to the 0/0 ppm control group; the degree of decrease in the globulin concentration resulted in significant decreases in total protein concentration in these groups. Albumin concentrations were significantly increased in all male groups, except the 0/20 and 300/0 ppm groups. The combination of these protein changes resulted in significant increases in the albumin:globulin (A/G) ratios in all groups except the 300/0 ppm group.

ALT, ALP, and SDH activities were significantly increased in all exposed groups except the 300/0 ppm group, and bile acid concentrations were significantly increased in the 300/20 and 300/40 ppm groups, compared to the 0/0 ppm control group.

Table 36. Select Clinical Chemistry Findings for Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)[a,b]

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
n		10[c]	10	10	10
Urea Nitrogen (mg/dL)	0 ppm	17.0 ± 0.3**	18.6 ± 0.7	20.4 ± 0.7**	19.8 ± 0.8**
	300 ppm	16.4 ± 0.5**	18.0 ± 0.4*	20.3 ± 0.7**††	22.4 ± 1.4**††
Total Protein (g/dL)	0 ppm	6.58 ± 0.06**	6.13 ± 0.10**	5.92 ± 0.07**	5.98 ± 0.07**
	300 ppm	6.45 ± 0.07	6.15 ± 0.07*††	5.96 ± 0.06**††	6.32 ± 0.09#†
Albumin (g/dL)	0 ppm	4.24 ± 0.06**	4.53 ± 0.10	4.61 ± 0.06**	4.65 ± 0.06**
	300 ppm	4.31 ± 0.04**	4.60 ± 0.07**††	4.51 ± 0.07*†	4.91 ± 0.06**#††
Globulin (g/dL)	0 ppm	2.34 ± 0.05**	1.60 ± 0.06**	1.31 ± 0.07**	1.33 ± 0.06**
	300 ppm	2.14 ± 0.06**	1.55 ± 0.07**††	1.45 ± 0.05**††	1.41 ± 0.10**††
A/G Ratio	0 ppm	1.82 ± 0.06**	2.87 ± 0.13**	3.60 ± 0.18**	3.55 ± 0.16**
	300 ppm	2.03 ± 0.05**	3.02 ± 0.13**††	3.14 ± 0.12**††	3.64 ± 0.27**††
Triglycerides (mg/dL)	0 ppm	98.8 ± 6.1	58.4 ± 4.3**	64.2 ± 4.5**	73.1 ± 6.9
	300 ppm	93.3 ± 7.9*	59.9 ± 4.6*††	67.5 ± 7.0††	62.2 ± 5.0*††
Cholesterol (mg/dL)	0 ppm	132.4 ± 5.4	114.3 ± 5.1	117.9 ± 4.9	120.7 ± 3.6
	300 ppm	132.6 ± 4.6	105.8 ± 4.1**††	118.9 ± 4.1	115.1 ± 3.9†
Alanine Aminotransferase (IU/L)	0 ppm	44.70 ± 2.62**	76.40 ± 5.25**	65.90 ± 2.85**	74.00 ± 3.64**
	300 ppm	51.20 ± 2.25**	67.00 ± 2.17**††	68.50 ± 7.95**††	70.00 ± 4.49**††

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
Alkaline Phosphatase (IU/L)	0 ppm	170.1 ± 4.9**	238.7 ± 10.8**	246.8 ± 14.8**	304.8 ± 15.7**
	300 ppm	173.4 ± 6.6**	241.4 ± 6.8**††	263.5 ± 13.0**††	270.8 ± 11.3**††
Sorbitol Dehydrogenase (IU/L)	0 ppm	9.4 ± 0.8**	15.3 ± 1.3**	12.7 ± 1.2*	16.8 ± 0.9**
	300 ppm	9.8 ± 0.6**	13.6 ± 1.3*†	18.3 ± 3.5*†	17.1 ± 1.9**††
Bile Salt/Acids (µmol/L)	0 ppm	28.4 ± 4.9	29.6 ± 4.2	48.2 ± 10.5	32.6 ± 4.3
	300 ppm	28.0 ± 2.0*	44.4 ± 6.3*†	41.8 ± 3.6*†	40.6 ± 4.5*

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 or 300/0 ppm control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
†Statistically significant at $p \leq 0.05$ for pairwise comparisons of 0/0 to 300/20, 0/0 to 300/40, and 0/0 to 300/80 using a Wilcoxon rank-sum test with a Hommel p value adjustment; ††$p \leq 0.01$.
[#]Statistically significant at $p \leq 0.05$ for pairwise comparisons of 0/0 to 300/0, 0/20 to 300/20, 0/40 to 300/40, and 0/80 to 300/80 using a Wilcoxon rank-sum test with a Hommel p value adjustment.
[a]Data presented as mean ± SEM.
[b]Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests (unless otherwise noted).
[c]Number of animals examined.

Liver: A spectrum of nonneoplastic hepatocellular lesions morphologically similar to those observed in the male and female 16-week interim evaluation of the first study also occurred in the male 16-week interim of the second study. The incidences of hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, and hepatocyte single cell death were significantly increased in all exposed groups except 300/0 ppm, compared to the 0/0 ppm control group (Table 37). The incidences of hepatocyte necrosis in the 0/40 ppm group and pigment in the 0/40, 0/80, 300/40, and 300/80 ppm groups were significantly increased.

Table 37. Incidences of Nonneoplastic Liver Lesions in Male Rats at the 16-week Interim of the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
n		10[a]	10	10	10
Hepatocyte, Cytoplasmic Alteration[b]	0 ppm	0**	10** (1.0)[c]	10** (1.8)	10** (2.0)
	300 ppm	0**	9** (1.2)	10** (1.7)	10** (1.9)
Hepatocyte, Hypertrophy	0 ppm	0**	10** (1.0)	10** (1.0)	10** (1.2)
	300 ppm	0**	9** (1.1)	10** (1.1)	10** (1.2)
Hepatocyte, Single Cell Death	0 ppm	0**	7** (1.0)	9** (1.0)	10** (1.0)
	300 ppm	0**	5** (1.0)	8** (1.0)	10** (1.0)
Necrosis	0 ppm	1* (1.0)	1 (1.0)	6* (1.0)	4 (1.5)
	300 ppm	0	2 (1.0)	3 (1.0)	1 (1.0)
Pigment	0 ppm	0**	2 (1.0)	8** (1.0)	9** (1.0)
	300 ppm	0**	3 (1.0)	7** (1.0)	10** (1.0)

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 or 300/0 ppm control group indicates a significant trend test.
Pairwise comparisons across perinatal exposures (0/20 vs. 300/20, 0/40 vs. 300/40, and 0/80 vs. 300/80 ppm) did not show any statistically significant differences.
*Significantly different ($p \leq 0.05$) from the 0/0 or 300/0 ppm control by the Poly-3 test; **$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Two-year Study in Males (Study 2)

Survival in treated groups was similar to that of the control groups (Table 38, Table 39; Figure 7). There were no treatment-related clinical observations in any of the exposed groups (CEBS, Study 2, E05).

Table 38. Survival of Male Rats Following Postweaning-only Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

	0/0 ppm	0/20 ppm	0/40 ppm	0/80 ppm
Animals Initially in Study	50	50	50	50
Moribund	7	6	8	3
Natural Deaths	7	2	8	11
Animals Surviving to Study Termination	36	42	34	36
Percent Probability of Survival at End of Study[a]	72.0	84.0	70.0	74.0
Mean Survival (days)[b]	683.5	721.5	675.4	668.8
Survival Analysis[c]	p = 0.733	p = 0.160N	p = 0.856	p = 0.882N

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and study termination).
[c]The result of the life table trend test (Tarone) is in the 0/0 ppm column, and the results of the life table pairwise comparisons (Cox) with the 0/0 ppm control group are in the exposed group columns. A lower mortality in an exposure group is indicated by N.

Table 39. Survival of Male Rats Following Perinatal and Postweaning Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

	300/0 ppm	300/20 ppm	300/40 ppm	300/80 ppm
Animals Initially in Study	50	50	50	50
Moribund	9	8	6	4
Natural Deaths	7	4	6	7
Animals Surviving to Study Termination	34	38	38	39
Percent Probability of Survival at End of Study[a]	70.0	76.0	76.0	78.0
Mean Survival (days)[b]	692.2	700.8	707.1	699.9
Survival Analysis[c]	p = 0.429N	p = 0.507N	p = 0.486N	p = 0.387N

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and study termination).
[c]The result of the life table trend test (Tarone) is in the 0/0 ppm column, and the results of the life table pairwise comparisons (Cox) with the 0/0 ppm control group are in the exposed group columns. A lower mortality in an exposure group is indicated by N.

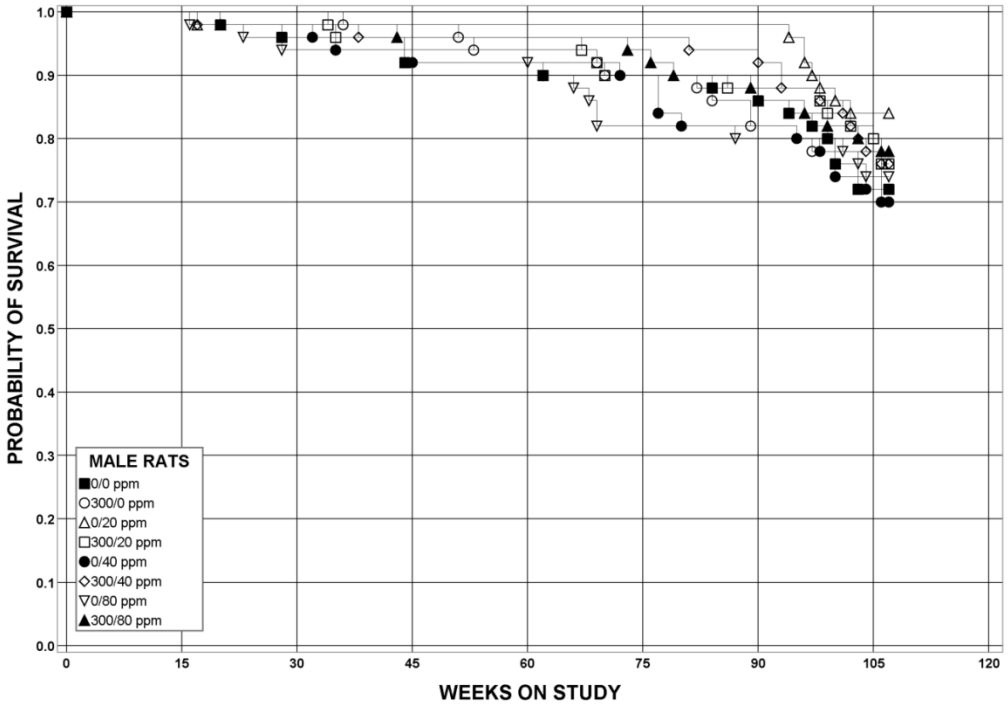


Figure 7. Kaplan-Meier Survival Curves for Male Rats in the Two-year Feed Study of Perfluorooctanoic Acid in Feed (Study 2)

At study termination, group mean body weights for the 0/20, 0/40, 0/80, 300/0, 300/20, and 300/40 ppm groups were within 10% of the 0/0 ppm or 300/0 ppm control group, respectively (Table 40, Table 41; Figure 8, Figure 9). The terminal mean body weight of the 300/80 ppm group was 13% less than that of the 0/0 ppm control group.

Table 40. Mean Body Weights and Survival of Male Rats Following Postweaning-only Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

	0/0 ppm		0/20 ppm			0/40 ppm			0/80 ppm		
Day	Av. Wt. (g)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	51.9	25	53.6	103.3	25	54.4	104.7	25	53.7	103.5	25
8	81.6	25	82.6	101.2	25	84.4	103.4	25	82.7	101.3	25
15	124.5	25	123.6	99.2	25	126.7	101.7	25	124.6	100.1	25
22	170.3	25	169.9	99.8	25	173.1	101.7	25	170.4	100.1	25
29	217.1	25	215.0	99.0	25	214.7	98.9	25	210.2	96.8	25
36	262.1	25	255.4	97.4	25	249.6	95.2	25	242.9	92.7	25
43	296.4	25	284.2	95.9	25	273.7	92.3	25	266.5	89.9	25
50	321.2	25	302.1	94.1	25	289.1	90.0	25	283.6	88.3	25
57	341.3	25	316.8	92.8	25	307.7	90.2	25	293.3	85.9	25
64	356.8	25	329.7	92.4	25	321.3	90.0	25	307.9	86.3	25
71	374.3	25	342.7	91.5	25	332.0	88.7	25	318.8	85.2	25
78	385.8	25	354.7	91.9	25	342.1	88.7	25	329.3	85.4	25
85	395.6	25	360.0	91.0	25	343.5	86.8	25	337.8	85.4	25
120	431.7	25	395.5	91.6	25	377.8	87.5	25	365.1	84.6	25
141	447.3	25	408.2	91.3	25	391.0	87.4	25	376.9	84.3	25
169	463.7	25	424.5	91.6	25	402.6	86.8	25	384.0	82.8	25
197	484.5	25	436.2	90.0	25	414.7	85.6	25	400.7	82.7	25
225	498.7	25	455.8	91.4	25	430.8	86.4	25	408.7	81.9	25
253	508.7	25	464.6	91.3	25	440.0	86.5	25	419.3	82.4	25
281	519.7	25	479.3	92.2	25	448.4	86.3	25	430.4	82.8	25
309	528.1	25	490.1	92.8	25	463.0	87.7	25	437.9	82.9	25
337	536.8	25	499.5	93.1	25	472.0	87.9	25	443.2	82.6	25
365	549.0	25	510.4	93.0	25	481.8	87.8	25	454.8	82.8	25
393	553.4	25	515.3	93.1	25	487.0	88.0	25	452.9	81.8	25
421	555.0	25	517.0	93.2	25	490.2	88.3	25	459.9	82.9	25
449	562.5	25	527.7	93.8	25	507.4	90.2	25	475.0	84.4	25
477	572.0	25	542.9	94.9	25	517.7	90.5	25	486.1	85.0	25
505	581.4	25	550.4	94.7	25	525.6	90.4	25	493.1	84.8	25
533	586.5	25	555.6	94.7	25	528.6	90.1	25	501.4	85.5	25
561	591.3	25	564.3	95.4	25	535.7	90.6	25	507.9	85.9	25
589	591.7	25	570.1	96.3	25	544.8	92.1	25	515.0	87.0	25
617	597.6	25	577.3	96.6	25	550.0	92.0	25	519.4	86.9	25
645	598.5	24	577.5	96.5	25	545.6	91.2	25	527.6	88.2	25
673	594.9	23	583.0	98.0	25	561.8	94.4	25	532.7	89.5	25
701	605.6	23	582.7	96.2	24	565.7	93.4	24	535.5	88.4	24
729	604.4	22	587.0	97.1	24	571.1	94.5	23	541.6	89.6	23
EOS	**598.3**	**22**	**588.1**	**98.3**	**24**	**578.7**	**96.7**	**21**	**541.3**	**90.5**	**23**

EOS = end of study.

Table 41. Mean Body Weights and Survival of Male Rats Following Perinatal and Postweaning Exposure in the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

	300/0 ppm		300/20 ppm			300/40 ppm			300/80 ppm		
Day	Av. Wt. (g)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	50.7	25	51.5	101.5	25	50.0	98.7	25	49.8	98.1	26
8	80.0	25	80.7	100.8	25	78.6	98.2	25	76.9	96.1	26
15	121.9	25	124.2	101.9	25	121.5	99.6	25	118.2	97.0	26
22	168.8	25	172.2	102.0	25	169.4	100.4	25	165.4	98.0	26
29	214.2	25	218.2	101.9	25	211.3	98.7	25	206.7	96.5	26
36	258.1	25	259.3	100.5	25	249.2	96.5	25	242.5	94.0	26
43	293.1	25	287.3	98.0	25	277.4	94.6	25	264.9	90.4	26
50	318.1	25	304.6	95.7	25	295.5	92.9	25	284.5	89.4	26
57	340.2	25	320.6	94.2	25	308.4	90.7	25	297.9	87.6	26
64	356.3	25	335.1	94.1	25	320.4	89.9	25	309.8	87.0	26
71	370.8	25	348.7	94.0	25	330.0	89.0	25	320.5	86.4	26
78	382.0	25	357.4	93.6	25	342.1	89.6	25	331.3	86.7	26
85	389.6	25	366.3	94.0	25	351.7	90.3	25	337.5	86.6	26
120	428.9	25	399.3	93.1	25	383.4	89.4	25	364.7	85.0	26
141	438.5	25	405.9	92.6	25	390.6	89.1	25	370.1	84.4	26
169	456.8	25	429.1	93.9	25	410.4	89.9	25	385.9	84.5	26
197	478.1	25	442.4	92.5	25	424.9	88.9	25	397.1	83.1	26
225	492.1	25	462.5	94.0	25	442.5	89.9	25	409.8	83.3	26
253	504.5	25	474.5	94.1	25	450.6	89.3	25	420.9	83.4	26
281	518.9	25	487.9	94.0	25	463.8	89.4	25	428.1	82.5	26
309	527.9	25	501.4	95.0	25	477.8	90.5	25	435.8	82.6	26
337	536.5	25	507.2	94.5	25	484.1	90.2	25	445.4	83.0	26
365	540.6	25	523.4	96.8	25	498.7	92.3	25	454.7	84.1	26
393	545.1	25	524.7	96.3	25	502.0	92.1	25	454.0	83.3	26
421	548.2	25	528.1	96.3	25	506.6	92.4	25	458.6	83.7	26
449	558.7	25	539.7	96.6	25	525.6	94.1	25	470.1	84.1	26
477	567.7	25	555.2	97.8	25	531.4	93.6	25	480.0	84.6	26
505	575.4	25	563.6	97.9	25	541.7	94.1	25	484.1	84.1	26
533	576.2	25	565.1	98.1	25	550.5	95.5	25	493.2	85.6	26
561	577.2	25	576.9	99.9	25	560.0	97.0	25	501.2	86.8	26
589	584.0	25	582.2	99.7	25	558.6	95.7	25	510.6	87.4	26
617	588.5	25	578.0	98.2	25	570.3	96.9	25	517.2	87.9	25
645	586.5	25	589.6	100.5	25	572.7	97.7	25	519.2	88.5	24
673	580.6	25	599.7	103.3	25	572.7	98.6	25	524.8	90.4	24
701	583.6	25	596.4	102.2	24	575.3	98.6	24	523.2	89.7	24
729	575.6	25	593.4	103.1	24	575.3	99.9	24	527.6	91.7	23
EOS	**581.7**	**23**	**586.1**	**100.7**	**24**	**573.2**	**98.5**	**24**	**529.5**	**91.0**	**22**

EOS = end of study.

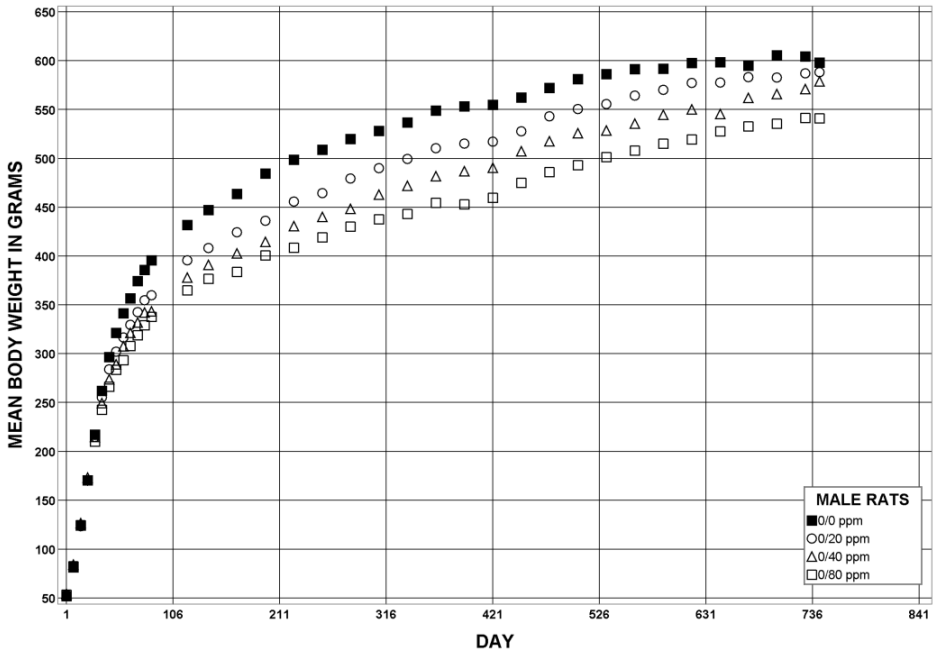


Figure 8. Growth Curves for Male Rats with Postweaning-only Exposure to 0/0, 0/20, 0/40, or 0/80 ppm Perfluorooctanoic Acid in Feed for Two Years (Study 2)

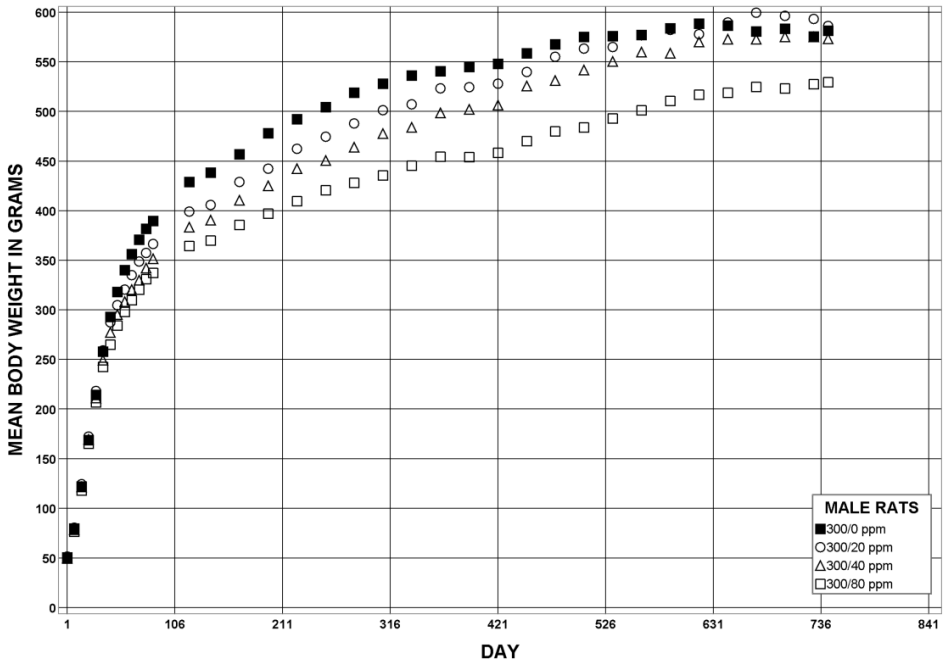


Figure 9. Growth Curves for Male Rats with Perinatal and Postweaning Exposure to 300/0, 300/20, 300/40, or 300/80 ppm Perfluorooctanoic Acid in Feed for Two Years (Study 2)

Group mean feed consumption by exposed groups was within 10% of the 0/0 ppm control group throughout the study (CEBS, Study 2, E08). After weaning, PFOA consumption for rats in the 0/20, 0/40, and 0/80 ppm groups and the 300/20, 300/40, and 300/80 ppm groups averaged 1.1, 2.2, and 4.6 mg/kg/day and 1.0, 2.1, and 4.6 mg/kg/day, respectively.

Pathology and Statistical Analyses

The morphologic features of the lesions discussed in this section are presented in the Histopathological Descriptions section following the Study 2 results.

Liver: Chronic exposure with and without perinatal exposure resulted in increases in the incidences of hepatocellular neoplasms (Table 42). Significant increases were observed in the incidences of hepatocellular adenoma (including multiple) in the 0/40 and 0/80 ppm groups compared to the 0/0 ppm control group, and in the 300/80 ppm group compared to the 300/0 ppm group (Table 42). The incidence of hepatocellular carcinoma was increased in the 300/80 ppm group; however, the increase was not statistically significant. Significant increases were observed in the incidences of hepatocellular adenoma or carcinoma (combined) in the 0/40, 0/80, and 300/80 ppm groups compared to their respective control.

Chronic exposure with and without perinatal exposure resulted in increases in the incidences of a spectrum of nonneoplastic hepatocellular lesions that in general increased with increasing exposure concentration (Table 42; CEBS, Study 2, P03; P10; P18). In general, the spectrum and the histological morphology of these lesions were similar to those that occurred in the female 2-year study.

The incidences of hepatocyte cytoplasmic alteration and hepatocyte hypertrophy were significantly increased in all groups of the chronic study, and in the 300/40 and 300/80 ppm groups of the perinatal and postweaning study. In general, the severity of these lesions tended to be minimum to moderate and were more severe in groups receiving the highest exposure concentrations. Hepatocyte hypertrophy and cytoplasmic alteration generally occurred in the same hepatocytes.

The incidences of hepatocyte single cell death were significantly increased in the 0/40 and 0/80 ppm groups compared to the 0/0 ppm control group and in the 300/80 ppm group compared to the 300/0 ppm group (Table 42). The incidences of hepatocyte necrosis were significantly increased in all postweaning exposed groups compared to their respective controls (Table 42). The incidences of pigment were significantly increased in all exposure groups of the chronic study and in the 300/40 and 300/80 ppm groups of the perinatal and postweaning exposure study (Table 42).

Compared to the 0/0 ppm control group, the incidences of mixed cell foci of cellular alteration were increased in the 0/40 and 0/80 ppm groups of the postweaning exposure study and in the 300/80 ppm group of the perinatal and postweaning exposure study (Table 42).

The incidences of focal inflammation were significantly increased in the 0/80 and 300/80 ppm groups compared to their respective controls (Table 42).

The incidences of cystic degeneration were significantly increased in the 300/80 ppm group of the perinatal and postweaning exposure study compared to the 300/0 ppm control (Table 42).

The incidences of bile duct hyperplasia were significantly less than those of the 0/0 or 300/0 ppm control group in all postweaning exposed groups (20, 40, and 80 ppm) (Table 42).

Table 42. Incidences of Select Neoplastic and Nonneoplastic Liver Lesions in Male Rats in the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
n		50[a]	50	50	50
Hepatocyte, Cytoplasmic Alteration[b]	0 ppm	0**	12** (1.5)	34** (1.6)	46** (1.8)
	300 ppm	0**	4 (1.0)	29** (1.4)	41** (1.7)
Hepatocyte, Hypertrophy	0 ppm	0**	13** (1.2)	34** (1.2)	43** (1.6)
	300 ppm	1** (4.0)[c]	4 (1.0)	29** (1.4)	42** (1.5)
Hepatocyte, Single Cell Death	0 ppm	1** (1.0)	1 (1.0)	11* (1.7)	24** (1.3)
	300 ppm	1** (4.0)	3 (2.7)	5 (1.6)	29** (1.3)
Necrosis	0 ppm	2** (1.5)	17** (1.2)	23** (1.4)	20** (1.3)
	300 ppm	1** (1.0)	11** (1.2)	14** (1.1)	21** (1.3)
Pigment	0 ppm	0**	7* (1.4)	15** (1.1)	30** (2.0)
	300 ppm	0**	4 (1.3)	11** (1.4)	26** (1.4)
Mixed Cell Focus	0 ppm	0*	4	9**	6*
	300 ppm	0**	4	4	9*
Eosinophilic Focus	0 ppm	3	6	5	9
	300 ppm	2	5	1	7
Inflammation, Focal	0 ppm	15* (1.0)	19 (1.0)	18 (1.1)	24* (1.0)
	300 ppm	13** (1.0)	11 (1.0)	19 (1.1)	24* (1.0)
Degeneration, Cystic	0 ppm	2* (1.0)	5 (1.2)	7 (1.0)	8 (1.1)
	300 ppm	0**	3 (1.0)	3 (1.0)	11** (1.3)
Bile Duct, Hyperplasia	0 ppm	24** (1.1)	3** (1.0)	3** (1.0)	1** (1.0)
	300 ppm	25** (1.2)	8** (1.0)	2** (1.0)	5** (1.0)
Hepatocellular Adenoma, Multiple	0 ppm	0	0	3	3
	300 ppm	0	0	0	5
Hepatocellular Adenoma (includes Multiple)[d]					
Overall Rate[e]	0 ppm	0/50 (0%)	0/50 (0%)	7/50 (14%)	11/50 (22%)
Litters Rate[f]		0/25 (0%)	0/25 (0%)	6/25 (24%)	8/25 (32%)
Rao-Scott Adjusted Poly-3 Test[g]		p < 0.001	(e)	p = 0.050	p = 0.010
Overall Rate	300 ppm	0/50 (0%)	1/50 (2%)	5/50 (10%)	10/50 (20%)
Litters Rate		0/25 (0%)	1/25 (4%)	5/25 (20%)	9/26 (35%)
Rao-Scott Adjusted Poly-3 Test		p < 0.001	p = 0.564	p = 0.070	p = 0.006

	Perinatal Exposure	Postweaning Exposure 0 ppm	20 ppm	40 ppm	80 ppm
Hepatocellular Carcinoma[h]					
Overall Rate	0 ppm	0/50	0/50	0/50	0/50
Litters Rate		0/25	0/25	0/25	0/25
Rao-Scott Adjusted Poly-3 Test		–[i]	–	–	–
Overall Rate	300 ppm	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Litters Rate		0/25 (0%)	0/25 (0%)	0/25 (0%)	3/26 (12%)
Rao-Scott Adjusted Poly-3 Test)		p = 0.049	(e)	(e)	p = 0.236
Hepatocellular Adenoma or Carcinoma (Combined)[j]					
Overall Rate	0 ppm	0/50 (0%)	0/50 (0%)	7/50 (14%)	11/50 (22%)
Litters Rate		0/25 (0%)	0/25 (0%)	6/25 (24%)	8/25 (32%)
Rao-Scott Adjusted Poly-3 Test		p < 0.001	(e)	p = 0.050	p = 0.010
Overall Rate	300 ppm	0/50 (0%)	1/50 (2%)	5/50 (10%)	12/50 (24%)
Litters Rate		0/25 (0%)	1/25 (4%)	5/25 (20%)	10/26 (38%)
Rao-Scott Adjusted Poly-3 Test		p < 0.001	p = 0.579	p = 0.077	p = 0.003

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 or 300/0 ppm control group indicates a significant trend test.
*Significantly different ($p \leq 0.05$) from the 0/0 or 300/0 ppm control by the Rao-Scott test; **$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 2/340 (0.67% ± 1.03%); range: 0% to 2%.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Number of litters with animals with neoplasm per number of litters necropsied.
[g]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the respective control and that exposed group. The Rao-Scott test adjusts the Poly-3 test for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[h]Historical control incidence: 0/340.
[i]Not applicable; no neoplasms in group.
[j]Historical control incidence: 2/340 (0.67% ± 1.03%); range: 0% to 2%.
(e) = value of statistic could not be computed.

Pancreas: Chronic exposure with and without perinatal exposure resulted in increased incidences of pancreatic acinar cell neoplasms (Table 43). The incidences of acinar cell adenoma including multiple adenomas were significantly increased in all exposed groups. The incidences of acinar cell adenocarcinomas were increased in all groups; however, the increases were not statistically significant. The incidences of acinar cell adenoma or adenocarcinoma (combined) in all exposed groups of the postweaning studies with and without perinatal exposure were significantly greater than those of the respective control groups. No differences between groups with perinatal and without perinatal exposures were observed.

The incidences of acinus hyperplasia were also significantly increased in all postweaning-only exposure groups of the chronic study (Table 43), and in the 300/40 and 300/80 ppm groups of the perinatal and postweaning study, and this lesion is considered a potentially preneoplastic lesion. No differences between groups with perinatal and without perinatal exposures were observed.

Table 43. Incidences of Select Neoplastic and Nonneoplastic Pancreas Lesions in Male Rats in the Two-year Feed Study of Perfluorooctanoic Acid (Study 2)

		Postweaning Exposure			
	Perinatal Exposure	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**
N		50[a]	50	50	50
Acinus, Hyperplasia[b]	0 ppm	18** (2.7)	32* (3.7)	37** (3.2)	31** (3.2)
	300 ppm	23* (2.7)	27 (3.2)	38** (3.3)	33 (3.4)
Acinar Cell Adenoma, Multiple	0 ppm	2	20	22	26
	300 ppm	4	18	22	27
Acinar Cell Adenoma (includes multiple)[d]					
Overall Rate[e]	0 ppm	3/50 (6%)	28/50 (56%)	26/50 (52%)	32/50 (64%)
Litters Rate[f]		3/25 (12%)	21/25 (84%)	20/25 (80%)	22/25 (88%)
Rao-Scott Adjusted Poly-3 Test[g]		p < 0.001	p < 0.001	p < 0.001	p < 0.001
Overall Rate	300 ppm	7/50 (14%)	18/50 (36%)	30/50 (60%)	30/50 (60%)
Litters Rate		6/25 (24%)	12/25 (48%)	21/25 (84%)	21/26 (81%)
Rao-Scott Adjusted Poly-3 Test		p < 0.001	p = 0.016	p < 0.001	p < 0.001
Acinar Cell Adenocarcinoma (includes multiple)[h]					
Overall Rate	0 ppm	0/50 (0%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Litters Rate		0/25 (0%)	3/25 (12%)	1/25 (4%)	3/25 (12%)
Rao-Scott Adjusted Poly-3 Test		p = 0.179	p = 0.188	p = 0.527	p = 0.154
Overall Rate	300 ppm	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Litters Rate		0/25 (0%)	2/25 (8%)	1/25 (4%)	3/26 (12%)
Rao-Scott Adjusted Poly-3 Test		p = 0.144	p = 0.299	p = 0.559	p = 0.172
Acinar Cell Adenoma or Adenocarcinoma (Combined)[i]					
Overall Rate	0 ppm	3/50 (6%)	29/50 (58%)	26/50 (52%)	32/50 (64%)
Litters Rate		3/25 (12%)	21/25 (84%)	20/25 (80%)	22/25 (88%)
Rao-Scott Adjusted Poly-3 Test		p < 0.001	p < 0.001	p < 0.001	p < 0.001
Overall Rate	300 ppm	7/50 (14%)	20/50 (40%)	30/50 (60%)	30/50 (60%)
Litters Rate		6/25 (24%)	13/25 (52%)	21/25 (84%)	21/26 (81%)
Rao-Scott Adjusted Poly-3 Test		p < 0.001	p = 0.006	p < 0.001	p < 0.001

Statistical significance for a treatment group indicates a significant pairwise test compared to the respective control group (0/0 or 300/0 ppm). Statistical significance for the 0/0 or 300/0 ppm control group indicates a significant trend test.
*Significantly different ($p \leq 0.05$) from the 0/0 or 300/0 ppm control by the Rao-Scott test; **$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 45/340 (12.33% ± 10.07%); range: 0% to 28%.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Number of litters with animals with neoplasm per number of litters necropsied.
[g]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the respective control and that exposed group. The Rao-Scott test adjusts the Poly-3 test (which accounts for differential mortality in animals that do not reach terminal euthanasia) for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[h]Historical control incidence: 2/340 (0.52% ± 0.85%); range: 0% to 2%.
[i]Historical control incidence: 45/340 (12.33% ± 10.07%); range: 0% to 28%.

Histopathological Descriptions

In general, the histopathological changes—when present in the liver, pancreas, uterus, kidney, stomach, forestomach, and thyroid gland—were morphologically similar across males and females in the first and second studies.

Liver

Hepatocellular adenomas were generally discrete, irregularly nodular masses that, at their margins, completely or partially compressed the surrounding normal hepatic parenchyma (Figure 10A). Adenomas were characterized by irregular cords of large, amphophilic to basophilic hepatocytes positioned tangentially to the surrounding normal hepatic parenchyma (Figure 10B). Portal areas were generally not present within adenomas.

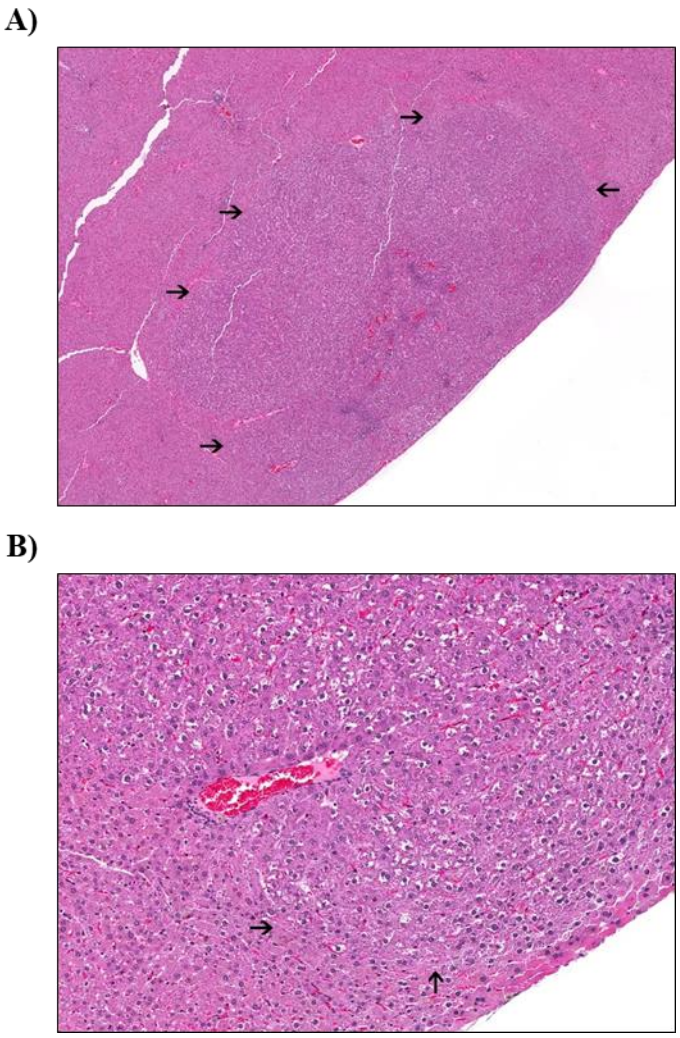
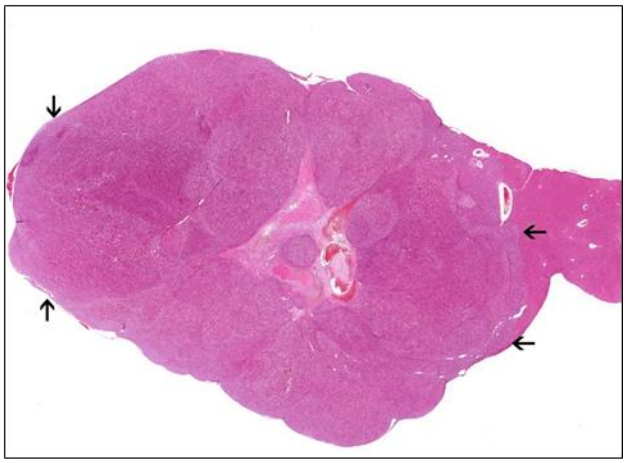


Figure 10. Hepatocellular Adenoma in a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 2)

A) The adenoma occurs as a discrete, irregularly nodular mass within the hepatic parenchyma (arrows). B) Higher magnification of panel A. The adenoma is characterized by irregular cords of amphophilic to basophilic hepatocytes positioned tangentially to the surrounding normal hepatic parenchyma. Note the partial compression of the adjacent hepatocytes at the margins (arrows).

Hepatocellular carcinomas were large, irregularly nodular, invasive masses that in some cases lacked distinct borders (Figure 11A). The neoplastic hepatocytes were pleomorphic and generally arranged in irregular clusters and islands. However, in focal areas they formed irregular trabeculae or cords that varied from two to six cells thick; in these areas, the hepatic sinusoids were dilated (Figure 11B**)**. Such patterns are hallmarks of hepatocellular carcinomas.

A)



B)

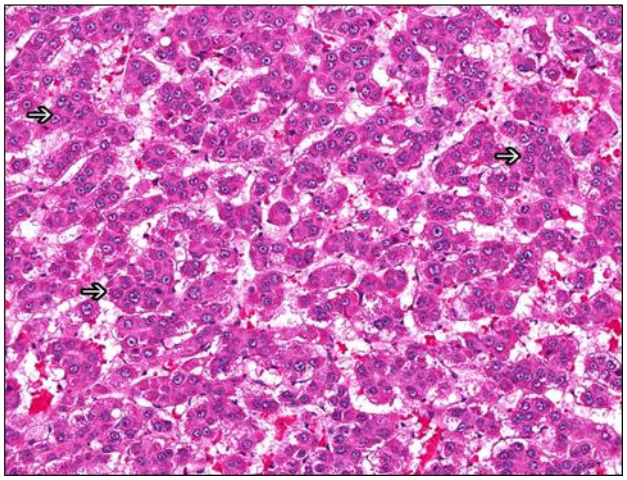


Figure 11. Hepatocellular Carcinoma in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

A) The carcinoma occurs as a large, irregularly discrete, pleomorphic, invasive mass that has effaced the hepatic parenchyma (arrows). B) Higher magnification of panel A. The neoplastic hepatocytes are pleomorphic and arranged in irregular clusters and islands with focal areas they formed irregular trabeculae or cords that varied from two to six cells thick (arrows).

In the male 2-year study, some adenomas (Figure 12) and carcinomas (Figure 13) were largely composed of neoplastic hepatocytes that contained prominent cytoplasmic microvesicular and macrovesicular cytoplasmic vacuoles.

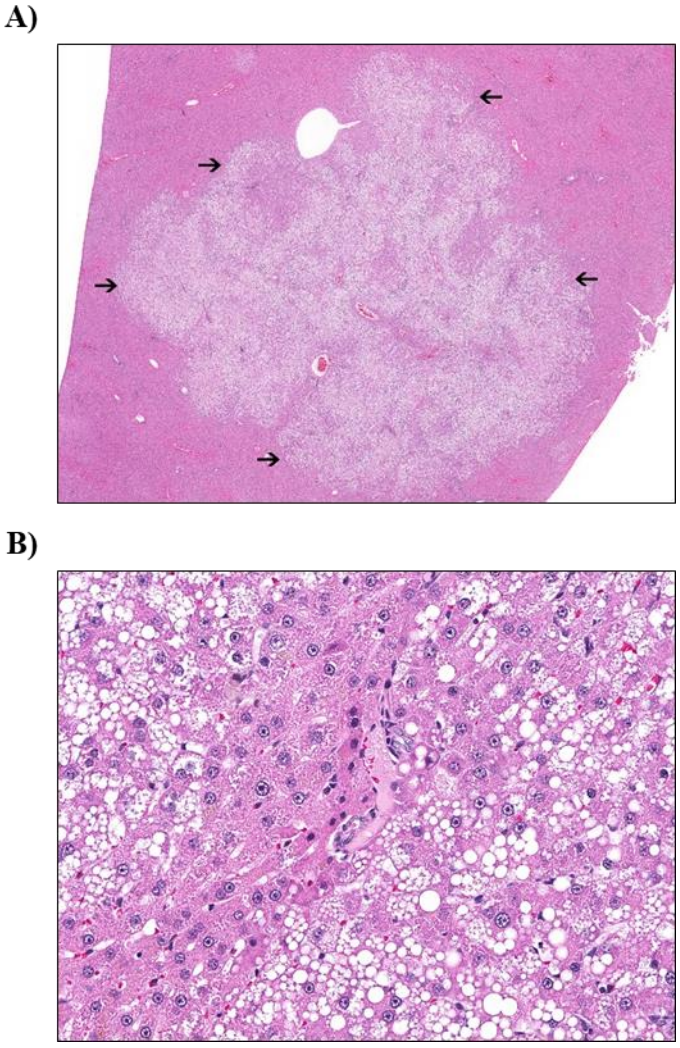


Figure 12. Hepatocellular Adenoma in a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 2)

A) The adenoma occurs as a discrete, irregularly nodular, vacuolated mass within the hepatic parenchyma. B) Higher magnification of panel A. The adenoma is composed of neoplastic hepatocytes that contain prominent cytoplasmic microvesicular and macrovesicular cytoplasmic vacuoles.

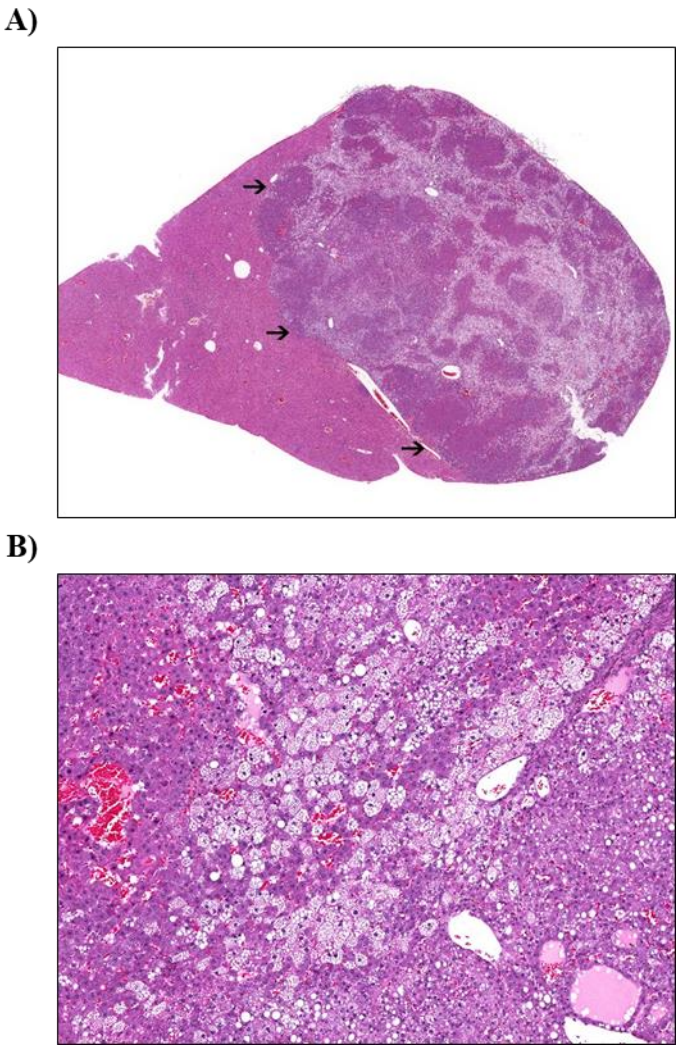


Figure 13. Hepatocellular Carcinoma in a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 2)

A) The carcinoma occurs as a large, irregularly nodular, pleomorphic, invasive mass that has effaced the hepatic parenchyma (arrows). B) Higher magnification of panel A. Similar to the adenoma in Figure 12, the carcinoma is composed largely of neoplastic hepatocytes that contain prominent cytoplasmic microvesicular and macrovesicular cytoplasmic vacuoles.

Mixed cell foci were focal proliferations of hepatocytes that tended to blend with the surrounding normal hepatic parenchyma at their margins (Figure 14A). They were composed of a mixture of eosinophilic to amphophilic hepatocytes mixed with hepatocytes that contained prominent cytoplasmic microvesicular and macrovesicular cytoplasmic vacuoles similar to those that occurred in hepatocellular adenomas (Figure 14B**)**. At times, the distinction between hepatocellular adenoma and mixed cell focus was not clear-cut and could only be based on lack of compression of the surrounding hepatic parenchyma along the margins of mixed cell foci. Mixed cell foci are considered potentially preneoplastic.

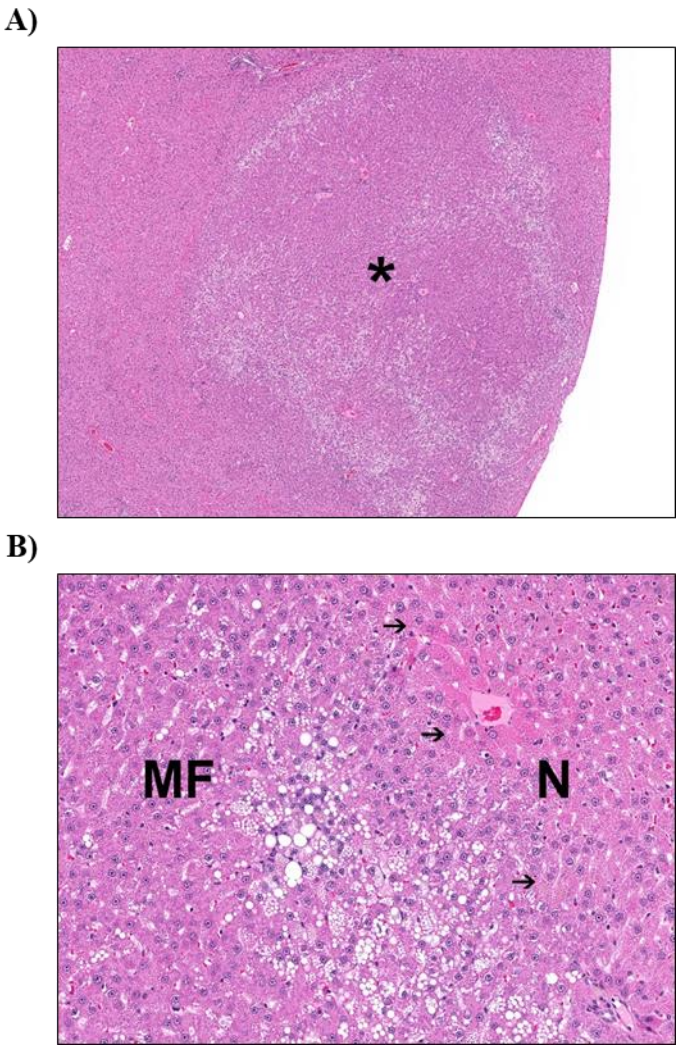


Figure 14. Mixed Cell Focus of Hepatocytes from the Liver of a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 2)

A) Mixed cell focus (MF), characterized by a focal, proliferation of hepatocytes that blends with the surrounding normal hepatic parenchyma at the margins (asterisk). B) Higher magnification of panel A. The MF is composed of a mixture of eosinophilic to amphophilic hepatocytes and hepatocytes that contain prominent microvesicular and macrovesicular cytoplasmic vacuoles similar to the cells that were present in hepatocellular adenomas. Also note how the hepatocytes in the foci blend with the normal hepatocytes (arrows).

Hepatocyte cytoplasmic alteration and hepatocyte hypertrophy were generally colocalized within hepatocytes and were generally of mild to moderate severity. These hepatocyte changes were observed throughout the liver sections but were often most prominent in centrilobular hepatocytes (Figure 15A). Hepatocyte with cytoplasmic alteration appeared granular and hypereosinophilic due to accumulations of eosinophilic granules in the cytoplasm (Figure 15B). Hepatocellular hypertrophy was characterized by hepatocyte enlargement by either accumulation of eosinophilic granules (when associated with cytoplasmic alteration) or homogenous eosinophilic cytoplasm, in the absence of cytoplasmic alteration.

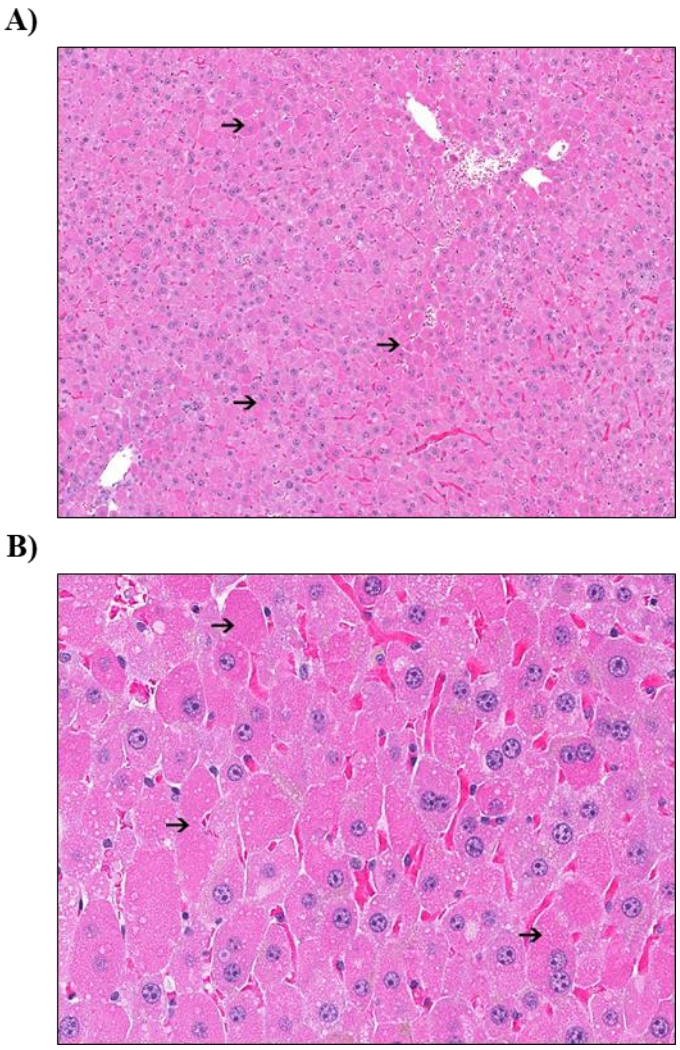


Figure 15. Hepatocyte Cytoplasmic Alteration and Hypertrophy in a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 2)

A) Affected hepatocytes are enlarged brightly eosinophilic (arrows). B) Higher magnification of panel A. Note enlarged hepatocytes that have brightly eosinophilic granules (arrows).

Hepatocyte single cell death consisted of randomly distributed, individual, irregularly round hepatocytes that were shrunken and had hypereosinophilic cytoplasm, and pyknotic nuclei with condensed, tortuous chromatin (Figure 16**)**. Some cells were surrounded by a clear space or halo.

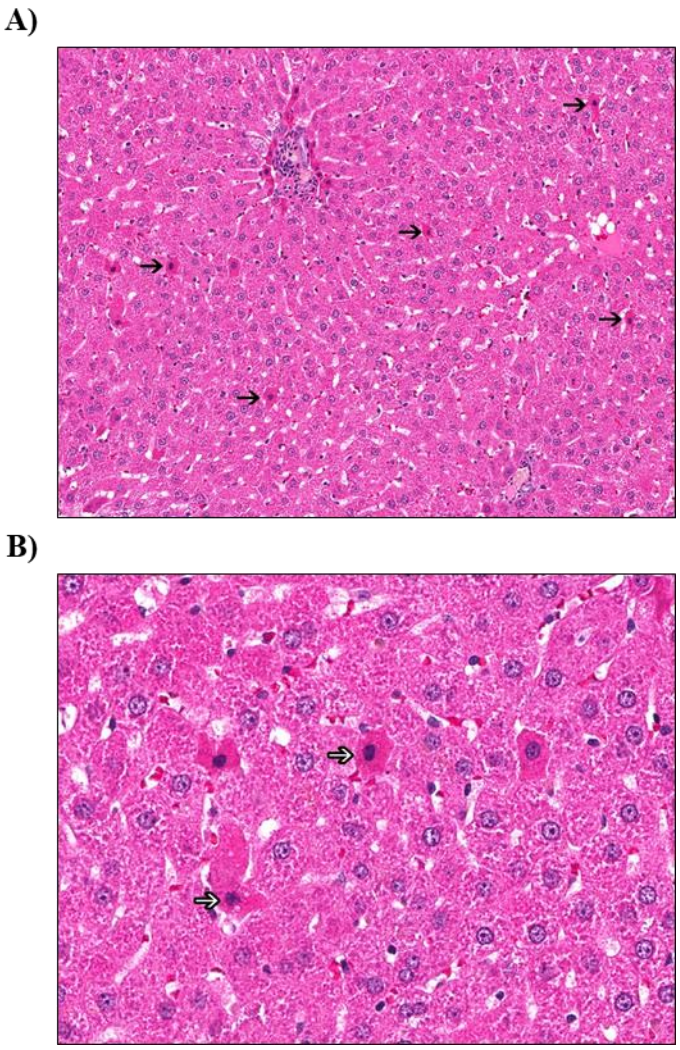


Figure 16. Hepatocyte Single Cell Death in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

A) Affected hepatocytes are randomly distributed, individual, hypereosinophilic hepatocytes (arrows). B) Higher magnification of panel A. Affected hepatocytes are shrunken and have hypereosinophilic cytoplasm and pyknotic nuclei with condensed chromatin (arrows).

Necrosis occurred as variably distributed, (focal, multifocal, diffuse), variably sized, irregular zones of coagulative necrosis in the parenchyma that were often prominent in the centribobular zones **(**Figure 17**).** In areas of necrosis, necrotic hepatocytes were swollen, pale, and had lost all cellular detail. Bridging between adjacent centrilobular zones of necrosis was sometimes evident.

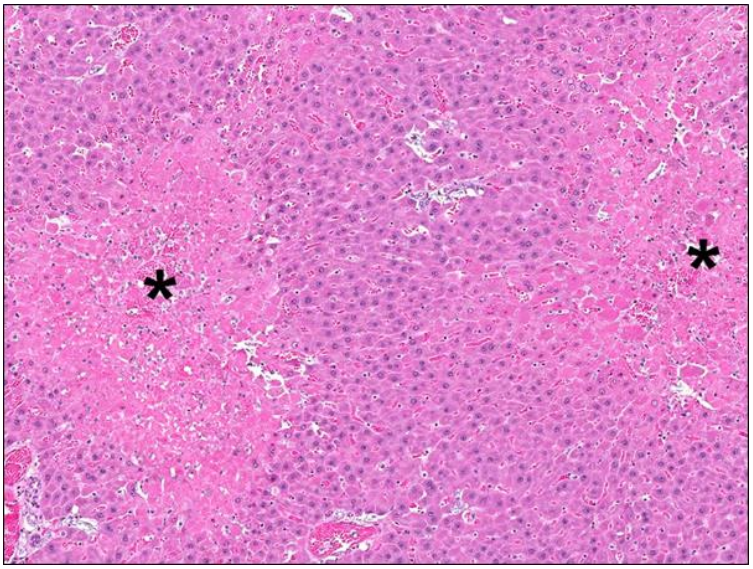


Figure 17. Hepatocyte Necrosis in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

Necrosis consisted of prominent, variably sized, irregular zones of coagulative necrosis (asterisks) that are sharply demarcated from the surrounding unaffected hepatic parenchyma. Compared to the surrounding hepatic parenchyma, in affected areas, the necrotic hepatocytes are swollen, lightly eosinophilic and lack cellular detail.

Hepatocyte pigment was characterized by the presence of fine golden-brown material in both hepatocytes and Kupffer cells (Figure 18). The pigment was considered likely to be lipofuscin due to the lack of staining with the histochemical stains for iron (Perl's Prussian blue stain) or for bilirubin (Hall's bilirubin stain).

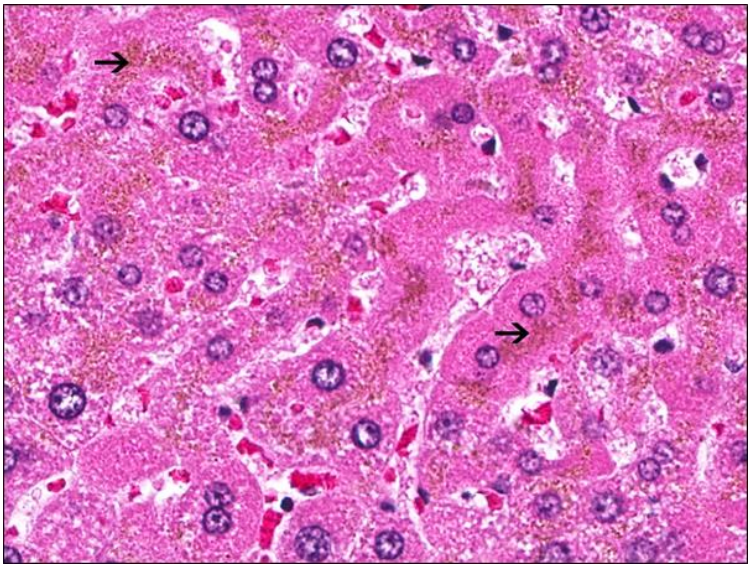


Figure 18. Hepatocyte Pigment in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

Hepatocyte pigment is characterized by the presence of fine golden-brown material in both hepatocytes and Kupffer cells (arrows).

Bile duct hyperplasia was multifocal in distribution and consisted of increased numbers of bile duct profiles within the portal areas (Figure 19). The hyperplastic ducts were sometimes surrounded by dense collagen.

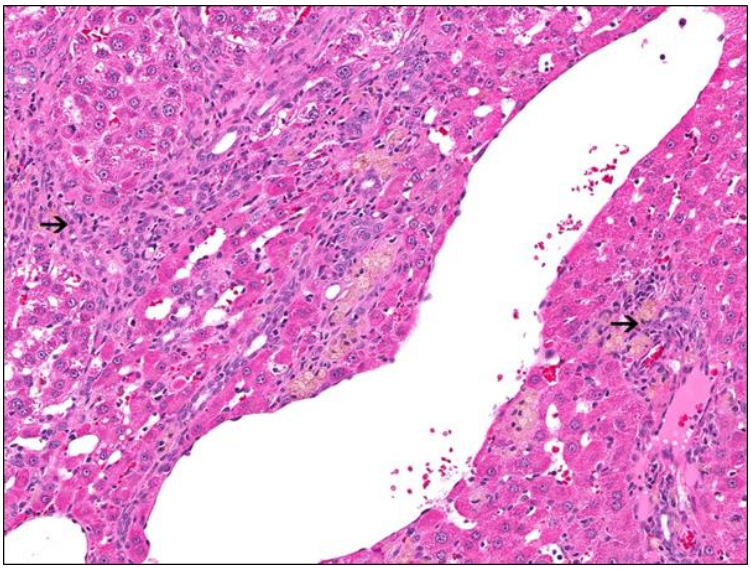


Figure 19. Bile Duct Hyperplasia in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

Bile duct hyperplasia is characterized by increased numbers of bile duct profiles within a portal area (arrows).

Hepatocyte mitoses consisted of increased numbers of hepatocytes in which the nuclei were undergoing mitoses (Figure 20).

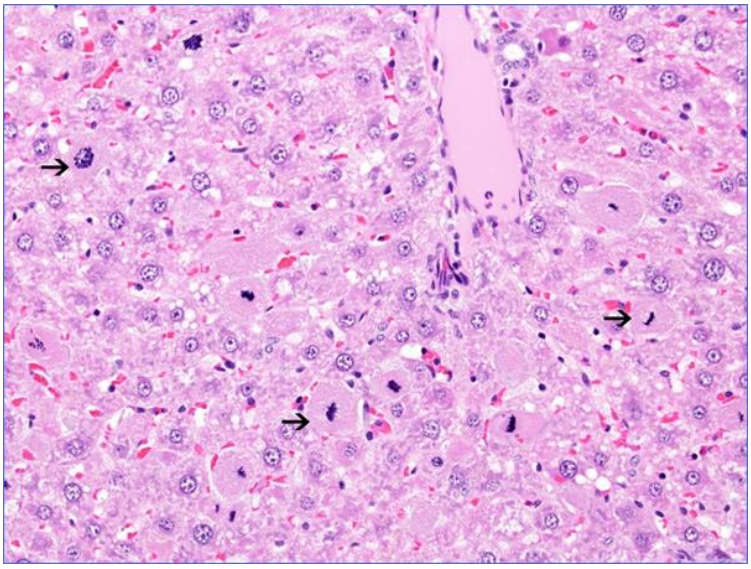


Figure 20. Hepatocyte Mitoses in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

Hepatocyte mitoses are characterized by increased numbers of hepatocytes in which the nuclei were undergoing mitosis (arrows).

Pancreas

Acinar cell hyperplasias often coexisted in tissue sections with adenomas but were also present in tissue sections that did not have adenomas (Figure 21A). Hyperplasia was morphologically similar to adenomas, that suggested a continuum from hyperplasia to adenoma. The distinction between acinar cell hyperplasia and adenoma was based largely upon size, with acinar cell hyperplasia diagnosed when they were less than 3 mm in the widest diameter[116]. Acinar cell hyperplasia was characterized by nodular proliferations of rounded clusters of normal-appearing pancreatic acinar cells, and relative normal-appearing acinar architecture (Figure 21B).

Acinar cell adenomas occurred as single or multiple discrete, irregularly nodular masses that were more than 3 mm in diameter, and sometimes compressed the adjacent pancreatic parenchyma (Figure 21A). Neoplasm cells were well differentiated and formed small, irregular acini (Figure 21).

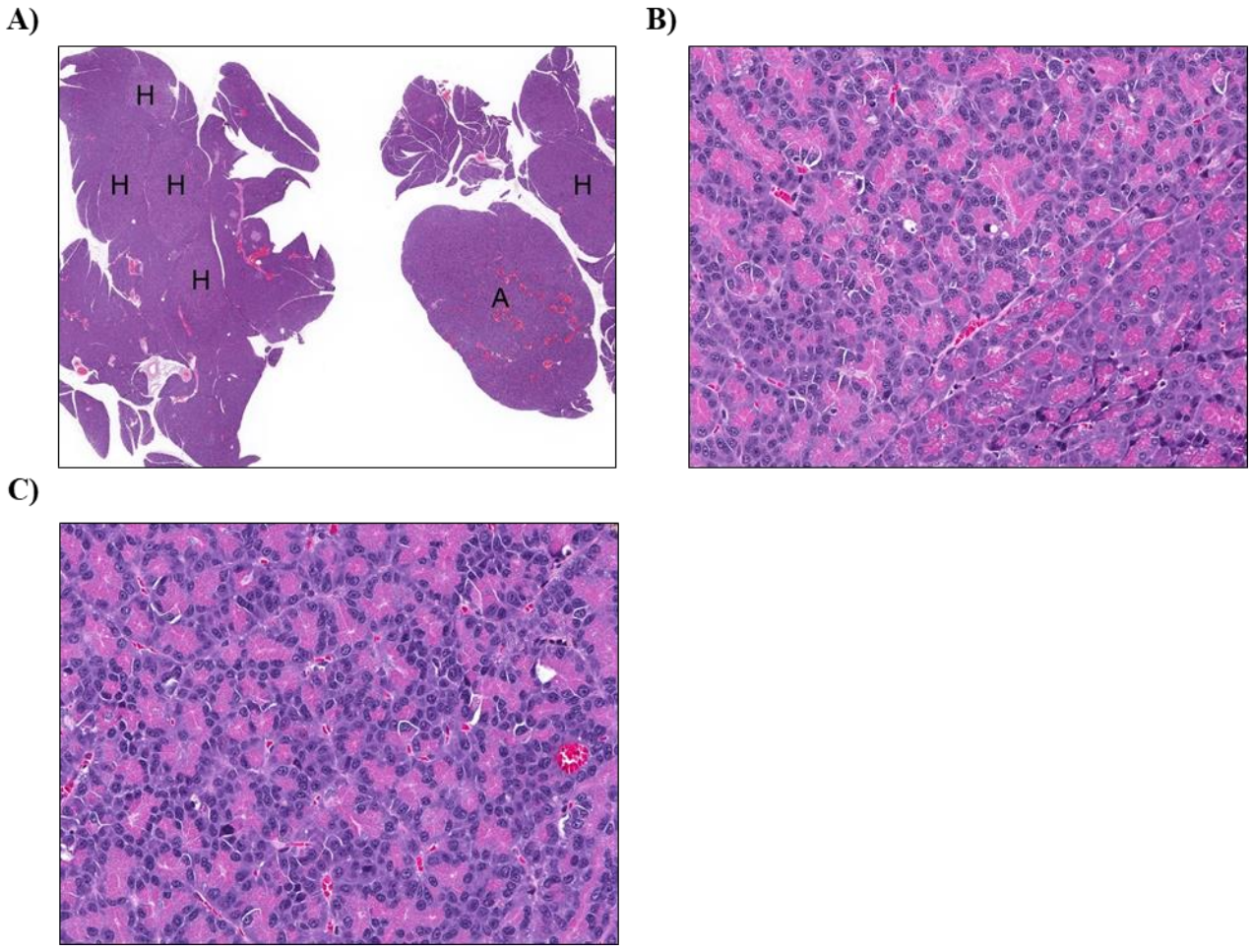


Figure 21. Acinar Cell Hyperplasia and Adenoma of the Pancreas in a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid for Two Years (Study 2)

A) Acinus hyperplasia (H) and acinus adenoma (A) were nodular lesions that often occurred in the same histological section and were distinguishable only by size. B) Higher magnification of the hyperplasia in panel A. Acinar hyperplasia was characterized by proliferation of normal-appearing pancreatic acinar cells with retention of the normal-appearing acinar architecture. C) Higher magnification of the adenoma in panel A. Acinar cell adenomas were morphologically similar to that of acinar hyperplasia but sometimes compressed the adjacent pancreatic parenchyma.

Acinar cell adenocarcinomas occurred as invasive, irregular multinodular masses separated by bands of fibrous or connective tissue (scirrhous response) (Figure 22A**)**. The growth patterns within adenocarcinomas were pleomorphic with well to poorly differentiated neoplastic acinar cells that formed sheets, small acini, gland-like structures, and trabeculae (Figure 22B**)**. Islets of Langerhans were absent within the acinar cell neoplasms.

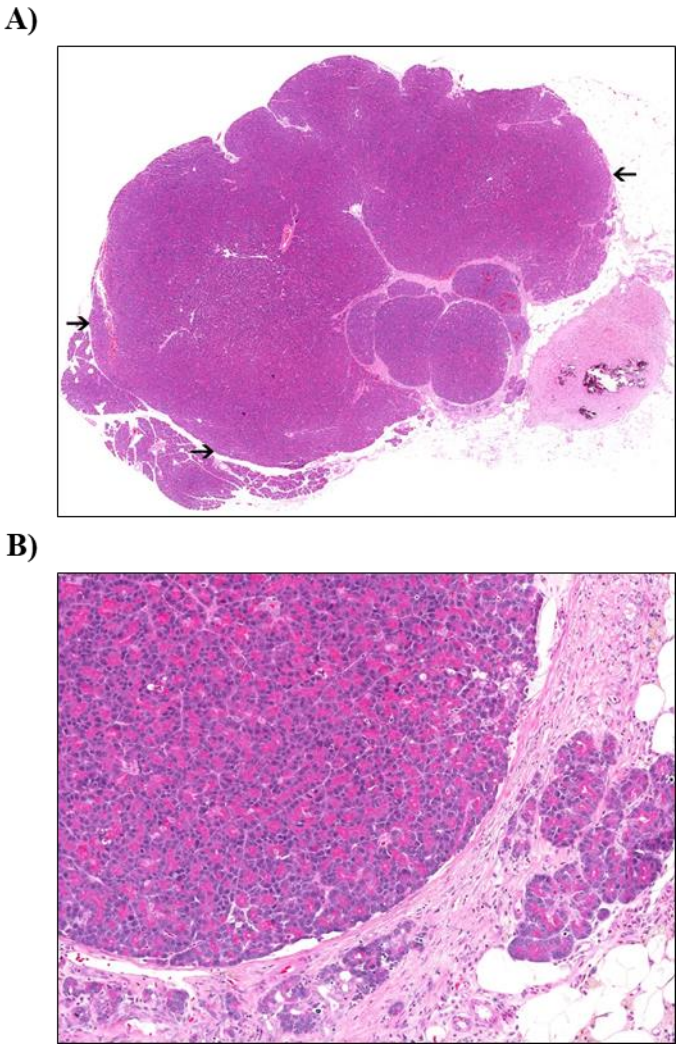


Figure 22. Adenocarcinoma of the Pancreas in a Male Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 2)

A) The pancreas adenocarcinoma occurs as a multinodular masse that has replaced most of the pancreatic parenchyma (arrows). B) Higher magnification of panel A. The pancreas adenocarcinoma is composed of relatively well differentiated acinar cells with retention of the normal-appearing acinar architecture.

Uterus

Uterine adenocarcinomas were poorly circumscribed, invasive masses that varied in size from small focal lesions to some larger lesions that effaced the entire endometrium and extended into or sometimes through the myometrium (Figure 23A**)**. The neoplastic epithelial cells had multiple morphologies that included solid nests, cords, and papillary or acinar structures (Figure 23B**)**.

Typically, the neoplastic epithelial cells had at least some degree of pleomorphism or atypia and mitotic figures were common.

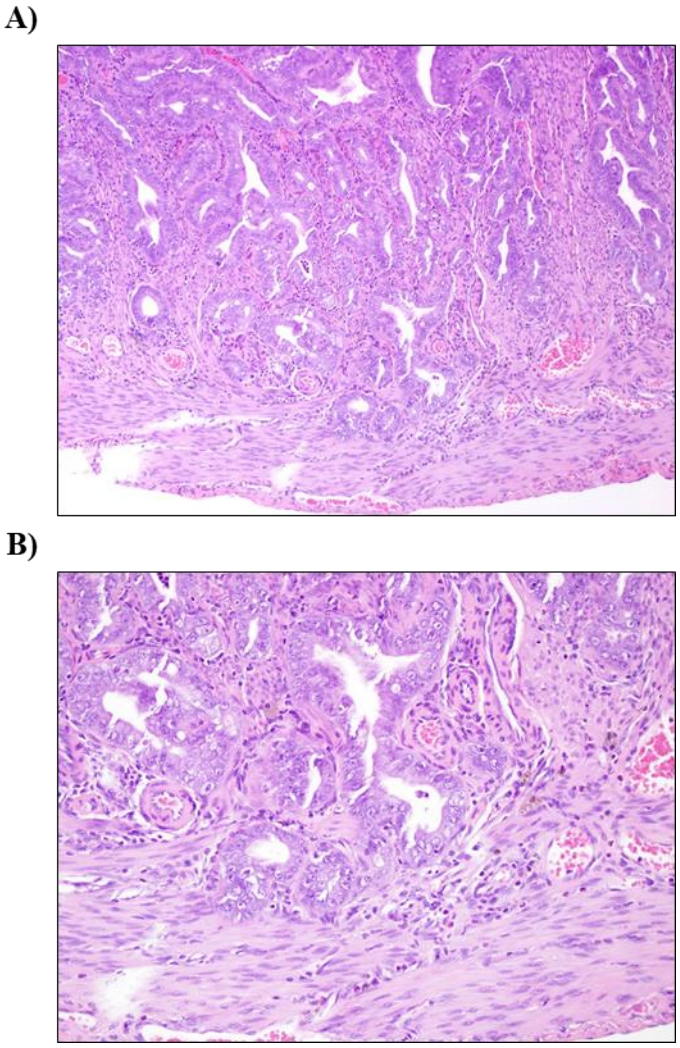


Figure 23. Adenocarcinoma of the Uterus in a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

A) The uterus adenocarcinoma occurs as a highly invasive mass that has completely effaced the architecture of the uterus. B) Higher magnification of panel A.

Kidney

Kidney papillary urothelial hyperplasia was characterized by minimal to moderate proliferative thickening of the urothelium lining the renal papilla (Figure 24A), which formed variably sized, irregular, papillary protuberances that extended into the lumen of the renal pelvis (Figure 24B). In some cases, the proliferative urothelium extended into the renal papilla forming variably sized, irregular gland-like structures (Figure 24C).

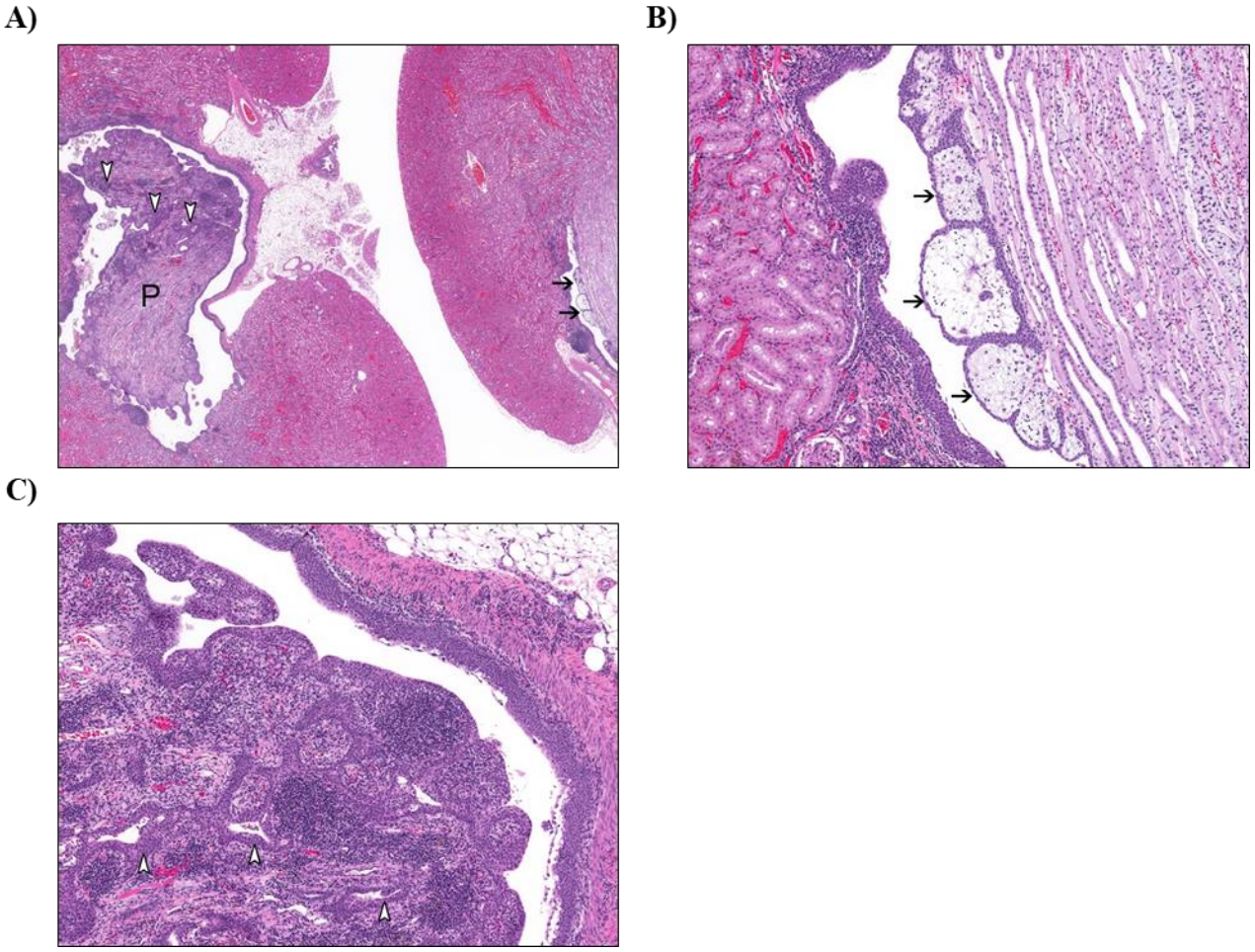


Figure 24. Papilla Urothelial Hyperplasia in the Kidney of a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

A) Papilla urothelial hyperplasia of the kidney is characterized by hyperplasia of the epithelium of the renal papilla in the form of papillary projections into the lumen of the renal pelvis (arrows), or within the interstitium (arrows) of the renal papilla (P). B) Higher magnification of panel A. The hyperplastic epithelium forms papillary projections that protrude into the lumen of the renal pelvis (arrows). C) Higher magnification of panel A. The hyperplastic epithelium forms irregular anastomosing tubule-like structures within the interstitium of the renal papilla (arrows).

Kidney papillary necrosis was characterized by coagulative necrosis of the distal one-third to one-half of the papilla with complete loss of architecture (Figure 25).

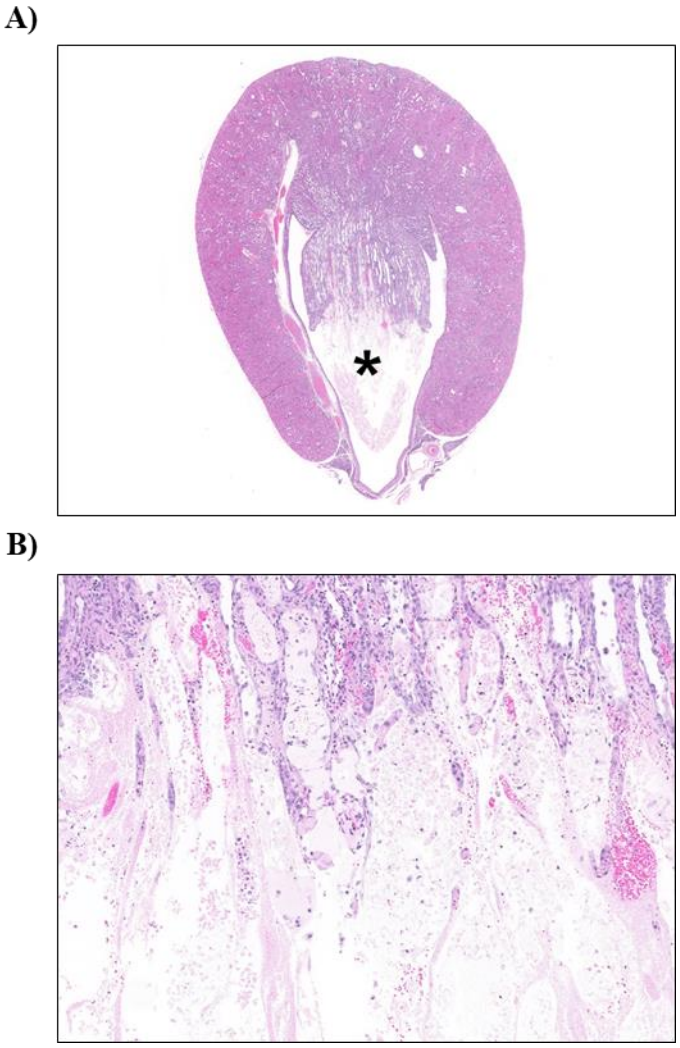


Figure 25. Papillary Necrosis in the Kidney of a Female Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

A) Papillary necrosis of the kidney is characterized by necrosis of the distal one-half of the renal papilla (asterisk). B) Higher magnification of the necrotic area in panel A (asterisk) demonstrating coagulative necrosis with complete loss of the architecture of renal papilla.

Tubular mineral was of minimal severity and characterized by the presence of randomly distributed basophilic mineral deposits in the epithelial cells lining the cortical and medullary tubules.

Forestomach

Forestomach ulceration, epithelial hyperplasia, and inflammation were associated lesions (Figure 26). Ulcers were focal lesions characterized by full-thickness loss of the lining mucosal squamous epithelium and accompanied by variable thickening (up to 6 to 10 cell layers) of the

adjacent squamous epithelium (hyperplasia) that extended from the margins, and the presence of a mixed inflammatory cell infiltrates (inflammation, chronic active) within the underlying submucosa, that sometimes extended into the muscularis mucosa below (Figure 26).

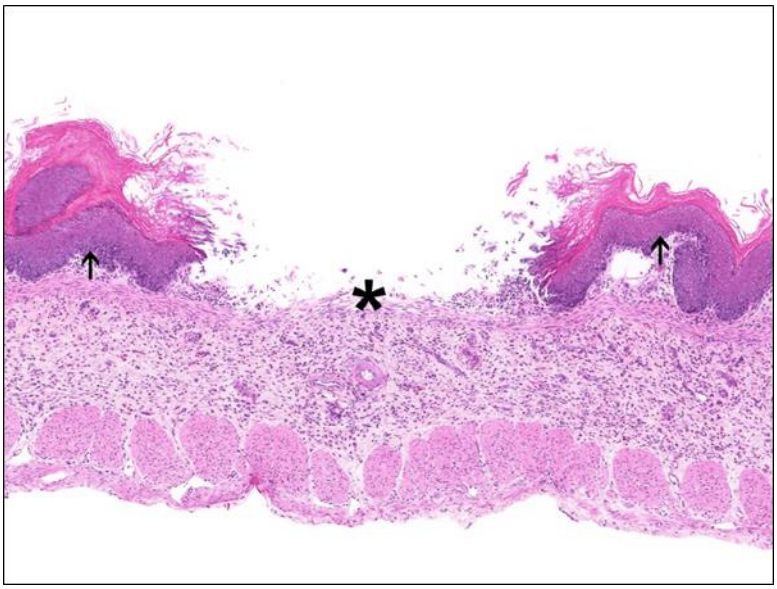


Figure 26. Forestomach Ulcer in the Stomach of a Female Sprague Dawley Rat Exposed to Perfluorooctanoic Acid in Feed for Two Years (Study 1)

Forestomach ulcer (asterisk) accompanied by epithelial hyperplasia (arrows) and inflammation.

Thyroid Gland

Follicular cell hypertrophy was of minimal to mild severity and diagnosed when the thyroid gland was composed predominantly of small follicles that had minimal amounts of lightly basophilic colloid. Affected follicles were lined by cuboidal rather than flattened epithelial cells. Such small follicles are typically present in the centers of normal thyroid glands and constituted less than 30% of the area of thyroid gland sections among control animals.

Discussion

Perfluorooctanoic acid (PFOA) is a widespread contaminant and the second most prevalent per/polyfluoroalkyl chemical (PFAS) measured in human plasma to date; second only to perfluorooctane sulfonic acid (PFOS). Its stability in the environment and long half-life in humans have led to decades of exposure, which continue despite its having been removed from use. Exposure likely occurs throughout development, starting in utero. Previous studies in rats have identified the carcinogenic activity of PFOA, but in these studies exposures were started in young adult animals after critical periods of development[67; 69]. Due to the concern of widespread exposure occurring through multiple life stages, the National Toxicology Program (NTP) tested the hypothesis that additional exposure during the perinatal period (in utero and during lactation up to weaning) would increase the incidence of neoplasms or lead to the appearance of different neoplasm types. To test the hypothesis, groups of rats exposed during the perinatal period and after weaning were compared to groups with postweaning exposure only. Because female rats are known to have higher PFOA elimination rates than male rats, higher postweaning feed exposures of up to 1,000 ppm were provided to females. Exposure groups were analyzed by pairwise comparison to assess potential differences

In the current studies, exposure during the perinatal period was up to 300 ppm, which led to plasma concentrations of 74–75 μM in the dam on gestation day (GD) 18 and postnatal day (PND) 4. The similar concentrations at different time periods suggest concentrations were at steady state. At 19 weeks of age, plasma concentrations were ~50 μM in the F_1 females at a similar exposure concentration (i.e., 300 ppm). The lower concentrations are likely due to the differences in F_0 feed consumption during gestation and lactation versus F_1 nonpregnant females.

F_1 exposure evaluations occurred on GD 18, PND 4, and at 19 weeks of age, and showed maternal transfer early in development and continued exposure at the later time periods. Fetuses pooled by litter had concentrations about a third of maternal plasma concentrations on GD 18, indicating some maternal transfer. Pooled whole pups had consistent concentrations between male and female pups indicating lactational transfer to the offspring early during postnatal development. No apparent sex differences were observed in the pups, which is consistent with previous reports that sex differences arise during puberty with increases in testosterone[26]. At 19 weeks of age, male and female adult plasma and liver concentrations were consistent between groups that were and were not perinatally exposed. As expected, plasma concentrations were about 12-fold lower in females compared to males with a similar exposure (0/300 ppm). Exposure-adjusted (mg/kg/day) plasma concentrations decreased in males as exposure increased from 20 ppm up to 300 ppm. This is consistent with the hypothesized saturation of a kidney reabsorption pathway[26; 117]. Exposure-adjusted plasma concentrations in females at 19 weeks of age did not appear to change with exposure concentration.

In general, the rat plasma PFOA concentrations were considerably higher than those attained in the general human population, for which 2015–2016 NHANES data yield a geometric mean of 4 nM (95th percentile = 10 nM) in human sera[5]. Human sera:plasma and sera:whole blood ratios are estimated to be 1:1 and 2:1, respectively[118]. The rat plasma concentrations were marginally higher than concentrations measured in the NTP 28-day toxicity studies (57 μM) with administered doses of 100 mg/kg/day via gavage route of exposure[83].

In the current studies, exposure during gestation and lactation up to 300 ppm (~22 and ~46 mg/kg/day, respectively) resulted in minimal to no changes in body weight of the F_0 dams and no effect on litter size or survival. Pup weights were at most 8% lower compared to the 0/0 ppm control group. These data are consistent with published feed studies in SD rats, which observed effects at 30 mg/kg/day in rats[48]. However, with continued exposure, body weights of exposed groups were significantly lower compared to the 0/0 ppm control group.

At the interim evaluation, liver toxicity was observed in the PFOA-exposed male rats with no difference between groups with and without perinatal exposure; similar liver effects were observed in the PFOA-exposed female rats, but to a much lesser degree. In males, liver weights were increased and hepatocyte pigment (most likely lipofuscin), hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, and hepatocyte necrosis were observed in the PFOA-exposed animals. The male portion of Study 1 was stopped because of reduced body weight and liver toxicity in males exposed to 150 or 300 ppm PFOA either perinatally and postweaning or postweaning alone. In female rats, liver weights were also increased, but the incidences of hepatocyte necrosis and hepatocyte hypertrophy were much lower compared to the males. These sex differences are likely in part due to the lower plasma concentrations in females compared to males.

In the NTP 28-day toxicity studies, PFOA activated PPARα and constitutive androstane receptor (CAR) in this animal model (Hsd:Sprague Dawley® SD® rats) as evident by increased expression of associated genes[83]. At the 16-week interim necropsy in this 2-year study, liver acyl-CoA oxidase enzyme activities were elevated in male and female rats. The hepatocellular hypertrophy and cytoplasmic alteration are likely due to peroxisome proliferation, but may also be mediated through CAR activation or possibly other mechanisms[45; 119-122]. Mild increases (less than twofold) in the biomarkers of hepatocellular injury, ALT and SDH activity, correlated with the liver histopathology (necrosis and single cell death). In male rats, bile acid concentrations were increased in the higher dosed groups with a greater than twofold increase compared to the 0/0 ppm control group. Greater than twofold increases in bile acid concentrations are consistent with cholestasis, the causes of which include physical disruption of bile flow through the biliary system or perturbation of bile acid formation and excretion at the cellular level[123; 124]. ALP was mildly increased (less than twofold) in male and female rats. These increases may be due to cholestasis; however, mild increases in ALP activity (and ALT activity) are also associated with the administration of hepatic microsomal enzyme inducer compounds, including PPARα agonist, when the only histological finding is centrilobular hypertrophy[123; 125; 126]. Additionally, these effects corresponded with large increases in the acyl-CoA oxidase activity in males compared to females. Similar to the observed liver toxicity, there were no differences in acyl-CoA oxidase activity between animals exposed perinatally and postweaning versus those only exposed after weaning.

Liver aromatase activity was not affected by PFOA exposure in females and males of Study 1, but there was a consistent twofold increase across postweaning exposure groups (20, 40, and 80 ppm) in males exposed to the lower concentrations in Study 2. This occurred in animals with and without perinatal exposure, with no obvious explanation for why this occurred in males of Study 2 and not in Study 1. Aromatase activity was measured to potentially understand the mechanism of Leydig cell neoplasms of the testis and other potentially endocrine-related effects; however, there was no increase in the incidence of Leydig cell neoplasms observed in this study. This may be due to differences in exposure concentrations among studies, as Leydig cell

neoplasms were observed at a higher exposure of 300 ppm[67; 69], or due to differences among rat stocks (Crl:COBS CD(SD)BR/Crl:CD BR (CD) versus Hsd:Sprague Dawley® SD®).

Other clinical chemistry changes included increases in total protein (female only) and albumin concentrations, and decreases in total protein (male only), globulin, cholesterol (male only), and triglyceride concentrations. In general, these clinical chemistry changes were similar with and without perinatal exposure, but in females were of lesser magnitude or absent compared to the male rats. Albumin concentrations may have been elevated due to decreased water intake (supported by the increases in blood urea nitrogen) or as an acute phase protein in response to (liver) inflammation. Globulins are produced by the liver or lymphocytes (immunoglobulins), and it is not clear if PFOA caused a perturbation of hepatic or lymphocytic production or metabolism of these proteins. The decreases in lipid concentrations (cholesterol, triglycerides) are consistent with the known effects of PPARα activation on lipid metabolism, which includes increases in peroxisomal fatty acid β-oxidation and effects on lipid transport[127-129]. CAR is also an important regulator of cholesterol homeostasis, and its activation may also be related to the observed lipid alterations[130]. The various clinical chemistry changes at the 16-week interim are similar to those observed in the NTP 28-day PFOA study[83].

In addition to the liver, increased incidences of thyroid gland hypertrophy were observed at the 16-week interim (male and female) and terminal evaluation (female) in the higher exposure groups compared to the controls. This may be a compensatory effect related to decreased circulating total thyroxine and triiodothyronine hormone concentrations, which were observed in the NTP 28-day toxicity study[83]. Increased submucosa inflammation of the glandular stomach was observed in the male postweaning exposure groups of 150 and 300 ppm at the 16-week interim, but not at lower exposure groups in the second study. This lesion was increased in the female 1,000 ppm postweaning exposure groups at the terminal evaluation, but an exposure-related increase was not observed at the 16-week interim. Kidney lesions occurred in female rats at the terminal evaluation, primarily in the 1,000 ppm postweaning exposure groups, with increased incidences of papilla urothelium hyperplasia, papilla necrosis, and renal tubule mineralization in the 1,000 ppm postweaning groups.

The following discussion on carcinogenic activity is based on a weight of evidence of several factors described in the preface of this report and on the consistent findings in animals exposed either perinatally and postweaning or postweaning alone. A few differences between exposure paradigms are noted, but in general, the additional effect of including perinatal exposure on the chronic toxicity or carcinogenic response of PFOA appeared to be minimal.

At the end of the 2-year study, several neoplastic and nonneoplastic liver lesions were observed. In males, the incidences of hepatocellular adenomas were increased in the 40 and 80 ppm groups with and without perinatal exposure and exceeded the historical control range. In addition, hepatocellular carcinomas, a rare neoplasm (0/340 historical control), occurred in the 300/80 group. Increased incidences of liver necrosis were observed in all exposure groups with and without perinatal exposure, as were incidences of hepatocyte pigment, cytoplasmic alteration, and hypertrophy. Hepatocyte single cell death occurred in the mid- and high-postweaning exposure groups of 0/40, 300/40, and 0/80, 300/80 ppm. The increase in the incidences of hepatocellular adenomas or carcinomas (combined) was related to exposure. The incidence of hepatocellular carcinoma in animals exposed perinatally (300/80 ppm) was higher compared to those that were not (0/80 ppm). Although this increase was not statistically significant, it was

considered an effect of the additional perinatal exposure based on the rarity of occurrence of this hepatocellular neoplasm. The increase in incidence of hepatocellular neoplasms is similar to that seen in a previous PFOA rat study with 300 ppm exposure[69] and could be related at least in part due to the PPARα activity[69].

In female rats, there were higher incidences of hepatocellular carcinomas, which are rare (1/340), in the 0/1,000 and 300/1,000 ppm groups, compared to the 0/0 ppm controls. Similar to males, this was accompanied by higher incidences of necrosis, pigment, cytoplasmic alteration, hypertrophy, increased mitosis, and hepatocyte single cell death. The magnitude of these findings was less than those in the males, which again might in part be due to the lower plasma concentrations of PFOA in females than in males. The marginally higher incidence of carcinomas compared to the controls may be related to exposure given the liver response in males, and that females had consistently lower incidences. No differences were observed between groups exposed perinatally and those that were not.

Increased incidences of pancreatic acinar cell adenomas and adenocarcinomas were observed in exposed males, as was the combined incidence of these neoplasms. Significantly increased incidences of adenomas in all postweaning exposed groups (36–64%) were higher than the historical control range for adenomas in males (45/340; 0–28%) and the occurrence of rare adenocarcinomas (historical control: 2/340; 0–2%) were observed in all postweaning exposure groups (20, 40, and 80 ppm). The combined incidence of these neoplasms was statistically significant at all postweaning exposure concentrations with and without perinatal exposure. In addition, there was an increase in the incidence of pancreatic acinus hyperplasia with and without perinatal exposure. Taken together, the increase in the incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms (predominately adenomas) was related to PFOA exposure. There was no difference in response between groups with combined perinatal and postweaning exposure compared to groups with postweaning exposure only. These findings are consistent with a previous PFOA chronic exposure study[69], and although not considered to be related to exposure in the earlier study[67], a reevaluation of tissue from the first PFOA chronic study indicated increased incidences of pancreatic acinar cell hyperplasia[70].

There were higher incidences of pancreatic acinar cell neoplasms in the current study that also occurred at lower exposure concentrations compared to previous studies[67; 69], for which several explanations are possible. In this study, the criterion used for diagnosis of pancreatic acinar cell neoplasms was larger than 3 mm at their widest diameter, whereas previous studies used a diameter of 5 mm for their criterion[70]. Additionally, the Hsd:Sprague Dawley® SD® rat appears to have a higher background incidence of pancreatic acinar neoplasms, up to 28%, historically, in controls, thus they may be more sensitive to these neoplasms compared to the other rat stocks used previously. For example, NTP studies with the Wistar Han and F344/N rats appear to have a lower background incidence for pancreatic acinar cell neoplasms[131; 132] and Crl:CD (SD) Sprague Dawley rats are reported to have a background incidence of <1%[133].

In females, low incidences of pancreatic acinar cell adenomas and adenocarcinomas were observed in the highest exposure groups of 0/1,000 and 300/1,000. These neoplasms are rare in females (adenomas = 0/340; carcinomas = 0/340). Unlike males, incidences of acinus hyperplasia were not increased. Although the occurrences of these neoplasms were low and not statistically significant, observations of increased incidences of pancreatic acinar cell neoplasms in males increased confidence that the occurrence of these rare neoplasms in PFOA-treated

female animals also was due to exposure and hence was considered to be some evidence of carcinogenic activity. Perinatal and postweaning exposure groups did not differ from the postweaning-only exposure groups.

Within the uterus, there was a higher incidence of adenocarcinomas in PFOA-exposed females. This occurred in groups that had perinatal and postweaning or postweaning-only exposure, although the increase in most of the groups was not statistically significant. Historical control data for adenocarcinomas for similarly sectioned uterine tissue is very limited, with the concurrent control having a 2% incidence and two other studies having a 10% background incidence, suggesting that this is not a rare neoplasm. The incidences of nonneoplastic lesions of the uterus (e.g., atypical hyperplasia of the endometrium) were not increased in response to exposure. The increase in the incidence of adenocarcinomas of the uterus may have been related to exposure, as incidences in exposed females were higher than those in the 0/0 ppm control group, but the strength of the response was marginal and there was a low concurrent control incidence that lowered confidence in the response.

Treatment-related increased incidences of hyperplasia of the urothelium that lines the renal papilla, renal papilla necrosis, and renal tubule mineralization occurred in the female rats. Organic anion transporters play a major role in predominant excretion of PFOA in urine, and the excretion is significantly faster in females than males[26]. As a result, female rats have higher PFOA concentrations in the urine compared to males[26]. Although the mechanisms of injury were not explored, direct cytotoxicity from the high concentration of PFOA in the urine is a possibility[134]. A positive association between higher serum concentrations of perfluoroalkyl chemical and chronic kidney disease has been reported in humans[135].

The lack of differences between exposure paradigms is consistent with the similar plasma concentrations measured at the interim evaluation between PFOA-exposed animals with and without perinatal exposure. A review of perinatal carcinogenic risks suggests that non-genotoxic chemicals or chemicals that require activation generally are less potent perinatal carcinogens during this time of development compared to genotoxic chemicals or direct-acting chemicals, which can lead to new neoplasm types and/or a greater response[136]. Previous NTP studies examined the contribution of perinatal exposure to carcinogenic activity and found some differences between groups with and without perinatal exposure[84-86]. These differences were marginal in some cases and it was unclear if the length of exposure, and not developmental exposure per se, contributed to these differences. There was a general consistency in response between male and female rats in this study, but it varied in degree. Female rats generally had a lower response compared to males, which is consistent with the lower internal dose measured in females than in males at 16 weeks and supports the hypothesis that female response differences are due to pharmacokinetic differences.

Conclusions

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity*[a] of PFOA in male Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas. The additional effect of combined perinatal and postweaning exposure was limited to a higher incidence of hepatocellular carcinomas in male rats compared to postweaning exposure alone.

There was *some evidence of carcinogenic activity* of PFOA in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure. The combined perinatal and postweaning exposure was not observed to change the neoplastic or nonneoplastic response compared to the postweaning exposure alone in female rats.

Exposure to PFOA resulted in increased incidences of nonneoplastic lesions in the liver and pancreas of male rats and the liver, kidney, forestomach, and thyroid gland of female rats.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity.

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Appendix A. Chemical Characterization and Dose Formulation Studies

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A.1. Procurement and Characterization of Perfluorooctanoic Acid

PFOA was obtained from Sigma-Aldrich (St Louis, MO) in a single lot (lot 03427TH). Identity, purity, and stability analyses were conducted under the analytical chemistry laboratory and study laboratory at Battelle (Columbus, OH). Reports on analyses performed in support of the PFOA studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

PFOA is a white crystalline solid. The lot was identified using infrared (IR) spectroscopy. In addition, the lot was analyzed using ^{19}F (including fluorine-fluorine correlation spectroscopy, COSY) and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and gas chromatography (GC) with mass spectrometry (MS) detection. The IR spectrum (Figure A-1) was in good agreement with the structure of PFOA and with the reference spectrum of PFOA from the National Institute of Advanced Industrial Science and Technology Spectral Database (No. 6075). Due to extensive coupling, the peak shapes in the ^{19}F spectrum were broad and poorly resolved, but consistent with the structure of PFOA. The ^{13}C spectrum was consistent with the structure of PFOA and also with the prediction from the Advanced Chemistry Development spectral prediction program (Version 10.02, Toronto, Ontario, Canada) for PFOA. The ^{19}F and ^{13}C spectra are provided in Figure A-2 and Figure A-3, respectively. GC/MS analysis, following conversion to its methyl ester, identified the major peak as PFOA using fragmentation pattern and comparison with the National Institute of Standards and Technology standard for methyl perfluorooctanoate.

The moisture content of lot 03427TH was determined by Karl Fisher titration performed at Prevalere Life Sciences, Inc. (Whitesboro, NY). The purity of lot 03427TH was evaluated using GC with flame ionization detection (FID), following conversion of PFOA to its methyl ester, and using ion chromatography (IC) with conductivity detection and by differential scanning calorimetry (DSC).

Karl Fisher titration yielded a water content of 0.24%. Purity by DSC was 98.96% with a melting point of ~55ºC. GC-FID analysis detected one major peak with an area of 98.3% of the total peak area, with four reportable impurities that had areas greater than or equal to 0.1% of the total peak area. IC detected one major peak with 98.8% of the total peak area, with three impurities that had areas greater than 0.1% of the total peak area. The overall purity of lot 03427TH was determined to be greater than 98%. GC with electron capture detection (ECD) showed that the impurities are likely fluorinated compounds. GC-MS analysis showed that two of the impurities representing ~1% were isomers of PFOA; the remaining impurities were perfluorooctenoic acid (0.51%) and a structurally dissimilar fluorinated compound (0.11%).

Accelerated stability studies of PFOA were conducted by the analytical chemistry laboratory using lot 027022E from Sigma-Aldrich (St Louis, MO) using GC-FID. Stability of PFOA was confirmed for at least 2 weeks when stored in sealed glass vials at temperatures up to 60°C. To ensure stability, PFOA lot 03427TH was stored in amber glass bottles at room temperature (25ºC). Periodic analyses of lot 03427TH of the test chemical were performed prior to and during the animal studies by the study laboratory using GC-ECD; no degradation of the test chemical was detected.

A.2. Preparation and Analysis of Dose Formulations

The base diet was meal feed purchased from Zeigler Brothers, Inc. (Gardners, PA). The 21-week study utilized NIH-07 (single lot, milled July 2008) and NTP-2000 (four lots, milled September through December 2008). The 2-year study utilized NIH-07 (two lots milled May and April 2009) and NTP-2000 (24 lots milled June 2009 through June 2011).

Dose formulations were prepared monthly by mixing PFOA with feed (Table A-1). For the 21-week study, formulations of 0, 150, and 300 ppm were prepared in NIH-07 meal feed in September 2008, and in NTP-2000 meal feed twice (October 2008 and January 2009). For the 2-year study, formulations of 0 and 300 ppm PFOA in NIH-07 meal feed were prepared in June 2009. Formulations of 0, 20, 40, and 80 ppm PFOA in NTP-2000 meal feed were also prepared monthly for the 2-year study (28 formulations, July 2009–July 2011).

The analytical chemistry laboratory at Battelle (Columbus, OH) confirmed the homogeneity of NIH-07 and NTP-2000 dosed feed at formulations of 150 and 300 ppm PFOA with GC-FID prior to conducting studies. The homogeneity of dosed feed was also assessed by the study laboratory for NIH-07 (150 and 300 ppm PFOA) and NTP-2000 (20, 80, 150, 300, and 1,000 ppm PFOA) formulations with GC-ECD. After mixing, each formulation was sampled from three distinct locations and analysis of PFOA content was conducted using the protocols described in the above chemical procurement and characterization section and detailed in Table A-2. Formulations were determined to be homogenous and of appropriate concentration.

Remaining control and treated formulations were stored in plastic bag-lined buckets. Formulations of NIH-07 were refrigerated (~5ºC), whereas formulations of NTP-2000 were stored at room temperature (~25ºC). Stability was confirmed for these conditions up to 42 days.

Analysis of preadministration and postadministration (animal room) dose formulations of PFOA were each conducted every 1–3 months over the course of the study to determine purity (Table A-3, Table A-4). All preadministration formulations in both the 21-week and 2-year studies were within 10% of the target concentration. In the 21-week study, the 300 ppm formulation prepared in October 2008 was 12.5% below the target concentration when evaluated from feeders 6 weeks later, but only 6.5% below target when sampled from the remainder in the bucket at that same time. Postadministration formulation samples were analyzed five times over the course of the 2-year study. With the exception of the July 2009 formulation evaluated in September 2009 from residual feed in feeders, the postadministration formulations were within 10% of the target concentrations. These analyses provide confidence that the administered formulations were reflective of the target concentrations throughout the studies.

Table A-1. Preparation and Storage of Dose Formulations in the 21-week and Two-year Feed Study of Perfluorooctanoic Acid

Preparation
Stock solutions of PFOA were created by weighing an appropriate amount of lot 03427TH and adding it to a volumetric flask. Acetone was used to clean the weighing container until clear (at least three times) and bring the solution to volume. Flasks of stocks solution were sealed and shaken until lot 03427TH was dissolved (at least 10 inversions). An initial formulation premix was created by weighing an appropriate amount of feed (NIH-07 or NTP-2000) into a mixing bowl. The stock PFOA solution was slowly poured onto the feed while the mixture was stirred using a Hobart mixer. The formulations were mixed for approximately one hour and acetone was used twice to rinse the sides of the bowl and incorporate any residuals. The entire procedure was conducted under a nitrogen stream to encourage cyclonic flow and ensure acetone fully evaporated. In a twin shell blender, half of the remaining untreated feed was evenly covered with the premix. The sides were "rinsed" with the remaining untreated feed in two increments. The final formulation was mixed in the blender for 15 minutes. Dose formulations were prepared monthly throughout the experiments for a total of four formulations in the 21-week study and 30 formulations in the 2-year study.
Chemical Lot Number
03427TH
Maximum Storage Time
42 days
Storage Conditions
Stored in sealed plastic bag-lined buckets at ~5ºC (NIH-07) or ~25ºC (NTP-2000)
Study Laboratory
Battelle (Columbus, OH)

Table A-2. Chromatography Systems Used in the 21-week and Two-year Feed Study of Perfluorooctanoic Acid

Chromatography	Detection System	Column	Mobile Phase
System A			
Gas Chromatography	Flame ionization detection	Restek RTX-5, 1.0 μm film thickness, 30 m × 0.32 mm (ID)	Helium, flow rate ~1 mL/min
System B			
Gas Chromatography	Electron capture detection	Restek RTX-5, 1.0 μm film thickness, 30 m × 0.32 mm (ID)	Helium, flow rate ~1 mL/min
System C			
Gas Chromatography	Mass selective detector	Restek RTX-5, 1.0 μm film thickness, 30 m × 0.32 mm (ID)	Helium, flow rate ~1 mL/min
System C			
Ion Chromatography	Suppressed conductivity/ 40ºC	Dionex (Sunnyvale, CA) IonPac NG1-5 uM, 150 × 4 mm (ID)	A: 0.1 M NaOH in water B: 70% isopropanol in water C: water Flow rate: 0.6 mL/min

ID = internal diameter.

Table A-3. Results of Analyses of Dose Formulations Administered to Rats in the 21-week Feed Study of Perfluorooctanoic Acid

Date Prepared	Data Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
September 3, 2008	September 5, 2008	0	BLOQ	NA
		150	149.5	−0.3
		300	296.5	−1.2
October 14, 2008	October 17, 2008	0	BLOQ	NA
		150	148.7	−0.9
		300	296.5	−1.2
January 7, 2009	January 9, 2009	0	BLOQ	NA
		150	148.0	−1.3
		300	302.3	0.8
Animal Room Samples[b]				
September 3, 2008	October 17, 2008	0	BLOQ	NA
		150	148/149	−1.6/−0.9
		300	310/301	3.5/0.3
October 14, 2008	December 2, 2008	0	BLOQ	NA
		150	140/144	−6.9/−4.0
		300	262/281	−12.7/−6.5

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average of triplicate analysis.
[b]Value to left of slash mark is a composite sample from formulation left in feeders. Value to the right of the slash mark is for sample collected from bucket.

Table A-4. Results of Analyses of Dose Formulations Administered to Rats in the Two-year Feed Study of Perfluorooctanoic Acid

Date Prepared	Data Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
June 3, 2009	June 5, 2009	0	BLOQ	NA
		300	297.2	−0.9
July 16, 2009	July 21, 2009	0	BLOQ	NA
		20	19.1	−4.8
		40	39.0	−2.6
		80	75.1	−6.2
October 6, 2009	October 9, 2009	0	BLOQ	NA
		20	19.7	−1.5
		40	40.1	0.2

Date Prepared	Data Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
		80	79.4	−0.7
December 29, 2009	December 30, 2009	0	BLOQ	NA
		20	19.7	−1.8
		40	40.8	2.0
		80	80.8	1.0
March 23, 2010	March 24, 2010	0	BLOQ	NA
		20	19.9	−0.5
		40	40.1	0.1
		80	83.1	3.9
June 15, 2010	June 16, 2010	0	BLOQ	NA
		20	20.2	0.7
		40	41.8	4.4
		80	81.9	2.3
September 7, 2010	September 10, 2010	0	BLOQ	NA
		20	20.9	4.5
		40	41.3	3.2
		80	82.7	3.3
November 30, 2010	December 1, 2010	0	BLOQ	NA
		20	20.6	2.7
		40	42.3	5.7
		80	81.4	1.8
February 23, 2011	February 24, 2011	0	BLOQ	NA
		20	21.4	7.0
		40	41.7	4.3
		80	85.2	6.5
May 23, 2011	May 24, 2011	0	BLOQ	NA
		20	20.0	0.0
		40	39.2	−2.0
		80	77.3	−3.4

Date Prepared	Data Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
July 18, 2011	July 19, 2011	0	BLOQ	NA
		20	20.5	2.3
		40	40.1	0.1
		80	81.3	1.6
Animal Room Samples[b]				
June 3, 2009	July 10, 2009	0	BLOQ	NA
		300	280/294	−6.7/−1.9
July 16, 2009	September 9, 2009	0	BLOQ	NA
		20	17.7/18.3	−11.5/−8.3
		40	35.3/37.4	−11.9/−6.5
		80	69.3/73.5	−13.4/−8.1
March 23, 2010	May 5, 2010	0	BLOQ	NA
		20	19.3/19.8	−3.7/−1.2
		40	39.2/39.7	−2.1/−0.8
		80	78.2/77.9	−2.3/−2.7
November 10, 2010	January 10, 2011	0	BLOQ	NA
		20	19.3/19.8	−3.7/−0.8
		40	40.1/41.5	0.2/3.8
		80	78.8/82.1	−1.4/2.7
July 18, 2011	August 31, 2011	0	BLOQ	NA
		20	19.6/19.2	−2.2/−4.2
		40	39.7/40.3	−0.7/0.8
		80	79.0/79.9	−1.2/−0.2

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average of triplicate analysis.
[b]Value to left of slash mark is a composite sample from formulation left in feeders. Value to the right of the slash mark is for sample collected from bucket.

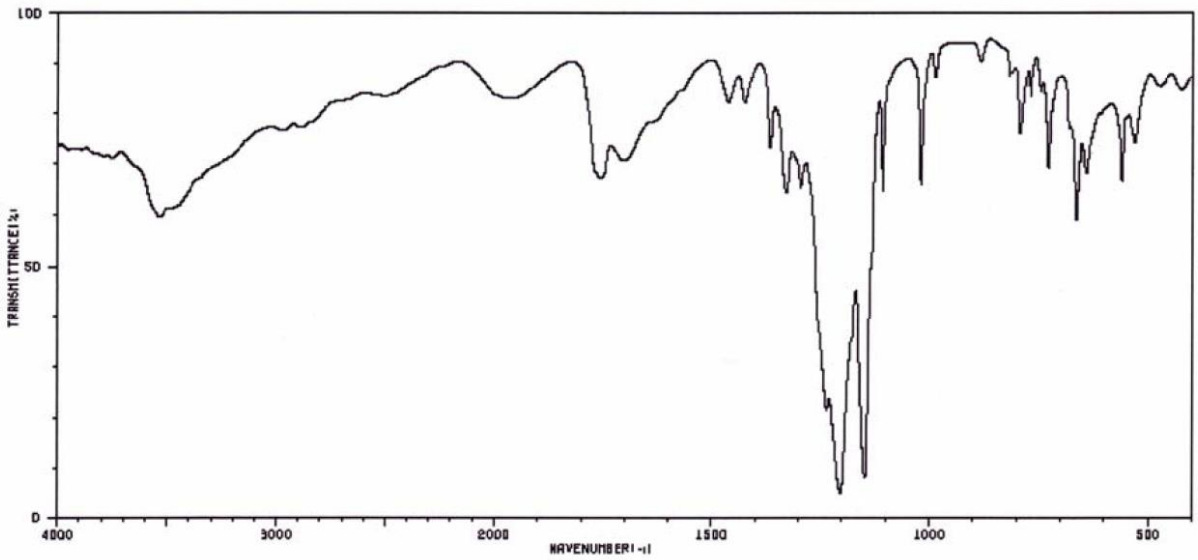

Figure A-1. Reference Infrared Absorption Spectrum of Perfluorooctanoic Acid

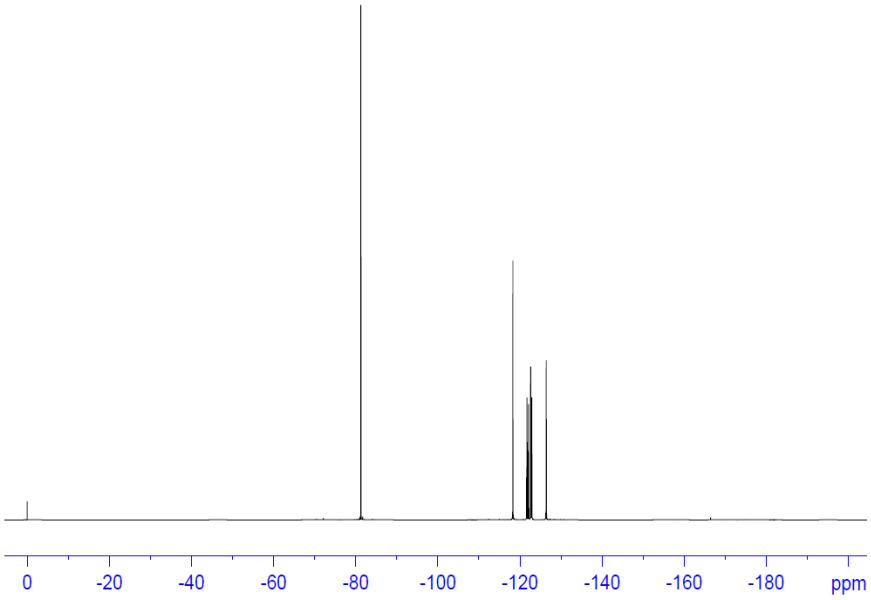

Figure A-2. ^{19}F-NMR Spectrum of Sample of Lot 03427TH of Perfluorooctanoic Acid

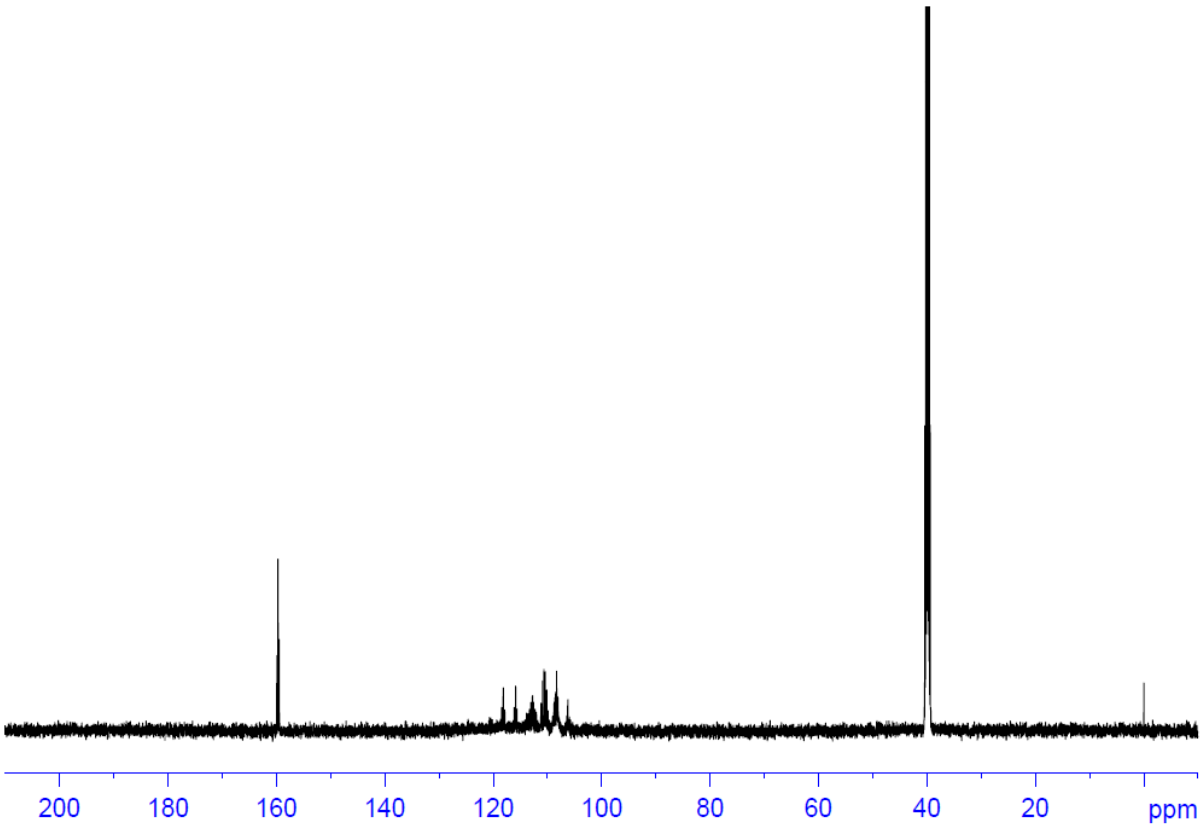

Figure A-3. ^{13}C NMR Spectrum of Sample of Lot 03427TH of Perfluorooctanoic Acid

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table B-1. Ingredients of NTP-2000 Rat Ration

Ingredient	Percent by Weight (Study 1)	Percent by Weight (Study 2)
Ground Hard Winter Wheat	22.26	22.26
Ground #2 Yellow Shelled Corn	22.18	22.18
Wheat Middlings	15.0	15.0
Oat Hulls	8.5	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5	7.5
Purified Cellulose	5.5	5.5
Soybean Meal (49% Protein)	5.0	5.0
Fish Meal (60% Protein)	4.0	4.0
Corn Oil (without Preservatives)	3.0	3.0
Soy Oil (without Preservatives)	3.0	3.0
Dried Brewer's Yeast	1.0	1.0
Calcium Carbonate (USP)	0.9	0.9
Vitamin Premix 1[a]	0.5	0.5
Mineral Premix 2[b]	0.5	0.5
Calcium Phosphate, Dibasic (USP)	0.4	0.4
Sodium Chloride	0.3	0.3
Choline Chloride (70% Choline)	0.26	0.26
Methionine	0.2	0.2

USP = United States Pharmacopeia.
[a]Wheat middlings as a carrier.
[b]Calcium carbonate as a carrier.

Table B-2. Vitamins and Minerals Added to NTP-2000 Rat Ration[a]

	Study 1		Study 2	
	Amount	**Source**	**Amount**	**Source**
Vitamins				
A	4,000 IU	Stabilized vitamin A palmitate or acetate	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione (MSBC)	1.0 mg	Menadione (MSBC)
α-Tocopheryl Acetate	100 IU	–	100 IU	–
Niacin	23 mg	–	23 mg	–
Folic Acid	1.1 mg	α-Calcium pantothenate	1.1 mg	α-Calcium pantothenate
α-Pantothenic Acid	10 mg	–	10 mg	–
Riboflavin	3.3 mg	Thiamine mononitrate	3.3 mg	Thiamine mononitrate
Thiamin	4 mg	–	4 mg	–
B_{12}	52 µg	–	52 µg	–

	Study 1		Study 2	
	Amount	**Source**	**Amount**	**Source**
Pyridoxine	6.3 mg	Pyridozine hydrochloride	6.3 mg	Pyridozine hydrochloride
Biotin	0.2 mg	α-Biotin	0.2 mg	α-Biotin
Minerals				
Magnesium	514 mg	Magnesium oxide	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate	0.2 mg	Chromium acetate

MSBC = menadione sodium bisulfite complex.
[a]Per kg of finished product.

Table B-3. Nutrient Composition of NTP-2000 Rat Ration

Nutrient	Study 1			Study 2		
	Mean ± SD	**Range**	**Number of Samples**	**Mean ± SD**	**Range**	**Number of Samples**
Protein (% by weight)	14.57 ± 0.389	13.9–15.8	23	14.6 ± 0.239	14.1–15.0	24
Crude Fat (% by weight)	8.46 ± 0.257	8.1–9.0	23	8.55 ± 0.289	8.1–9.1	24
Crude Fiber (% by weight)	9.3 ± 0.987	7.5–11.6	23	9.5 ± 0.952	7.5–11.6	24
Ash (% by weight)	5.11 ± 0.133	4.82–5.3	23	4.99 ± 0.184	4.61–5.22	24
Amino Acids (% of Total Diet)						
Arginine	0.802 ± 0.075	0.67–0.97	28	0.802 ± 0.075	0.67–0.97	28
Cystine	0.220 ± 0.022	0.15–0.25	28	0.220 ± 0.022	0.15–0.25	28
Glycine	0.703 ± 0.038	0.62–0.80	28	0.703 ± 0.038	0.62–0.80	28
Histidine	0.342 ± 0.071	0.27–0.68	28	0.342 ± 0.071	0.27–0.68	28
Isoleucine	0.549 ± 0.041	0.43–0.66	28	0.549 ± 0.041	0.43–0.66	28
Leucine	1.097 ± 0.064	0.96–1.24	28	1.097 ± 0.064	0.96–1.24	28
Lysine	0.700 ± 0.106	0.31–0.86	28	0.700 ± 0.106	0.31–0.86	28
Methionine	0.410 ± 0.042	0.26–0.49	28	0.410 ± 0.042	0.26–0.49	28
Phenylalanine	0.623 ± 0.047	0.47–0.72	28	0.623 ± 0.047	0.47–0.72	28
Threonine	0.512 ± 0.042	0.43–0.61	28	0.512 ± 0.042	0.43–0.61	28
Tryptophan	0.155 ± 0.027	0.11–0.20	28	0.155 ± 0.027	0.11–0.20	28
Tyrosine	0.420 ± 0.066	0.28–0.54	28	0.420 ± 0.066	0.28–0.54	28
Valine	0.666 ± 0.040	0.55–0.73	28	0.666 ± 0.040	0.55–0.73	28

Nutrient	Study 1			Study 2		
	Mean ± SD	Range	Number of Samples	Mean ± SD	Range	Number of Samples
Essential Fatty Acids (% of Total Diet)						
Linoleic	3.88 ± 0.455	1.89–4.55	28	3.88 ± 0.455	1.89–4.55	28
Linolenic	0.30 ± 0.065	0.007–0.368	28	0.30 ± 0.065	0.007–0.368	28
Vitamins						
Vitamin A (IU/kg)	3,731 ± 85	2,360–5,280	23	3,842 ± 77	2,360–5,290	24
Vitamin D (IU/kg)	1,000[a]	–		1,000[a]	–	
α-Tocopherol (ppm)	2,543 ± 13,044	27.0–69,100	28	2,543 ± 13,044	27.0–69,100	28
Thiamine (ppm)[b]	7.45 ± 1.24	6.1–12.4	23	8.14 ± 1.87	3.9–12.5	24
Riboflavin (ppm)	8.06 ± 2.83	4.20–17.50	28	8.06 ± 2.83	4.20–17.50	28
Niacin (ppm)	78.6 ± 8.26	66.4–98.2	28	78.6 ± 8.26	66.4–98.2	28
Pantothenic Acid (ppm)	26.6 ± 11.22	17.4–81.0	28	26.6 ± 11.22	17.4–81.0	28
Pyridoxine (ppm)[b]	9.78 ± 2.08	6.44–14.3	28	9.78 ± 2.08	6.44–14.3	28
Folic Acid (ppm)	1.58 ± 0.44	1.15–3.27	28	1.58 ± 0.44	1.15–3.27	28
Biotin (ppm)	0.32 ± 0.09	0.20–0.704	28	0.32 ± 0.09	0.20–0.704	28
Vitamin B12 (ppb)	50.6 ± 35.5	18.3–174.0	28	50.6 ± 35.5	18.3–174.0	28
Choline (as Chloride) (ppm)	2,615 ± 635	1,160–3,790	28	2,615 ± 635	1,160–3,790	28
Minerals						
Calcium (%)	0.915 ± 0.046	0.797–0.99	23	0.896 ± 0.046	0.797–0.99	24
Phosphorus (%)	0.553 ± 0.031	0.487–0.613	23	0.563 ± 0.025	0.487–0.613	24
Potassium (%)	0.667 ± 0.030	0.626–0.733	28	0.667 ± 0.030	0.626–0.733	28
Chloride (%)	0.393 ± 0.045	0.300–0.517	28	0.393 ± 0.045	0.300–0.517	28
Sodium (%)	0.197 ± 0.026	0.160–0.283	28	0.197 ± 0.026	0.160–0.283	28
Magnesium (%)	0.217 ± 0.055	0.185–0.490	28	0.217 ± 0.055	0.185–0.490	28
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	191.6 ± 36.8	135–311	28	191.6 ± 36.8	135–311	28
Manganese (ppm)	50.1 ± 9.59	21.0–73.1	28	50.1 ± 9.59	21.0–73.1	28
Zinc (ppm)	57.4 ± 26.0	43.3–184.0	28	57.4 ± 26.0	43.3–184.0	28
Copper (ppm)	7.53 ± 2.53	3.21–16.3	28	7.53 ± 2.53	3.21–16.3	28
Iodine (ppm)	0.531 ± 0.201	0.158–0.972	28	0.531 ± 0.201	0.158–0.972	28
Chromium (ppm)	0.684 ± 0.258	0.330–1.380	27	0.684 ± 0.258	0.330–1.380	27
Cobalt (ppm)	0.225 ± 0.154	0.086–0.864	26	0.225 ± 0.154	0.086–0.864	26

SD = standard deviation.
[a]From formulation.
[b]As hydrochloride.

Table B-4. Contaminant Levels in NTP-2000 Rat Ration[a]

	Study 1			Study 2		
	Mean ± SD[b]	**Range**	**Number of Samples**	**Mean ± SD[b]**	**Range**	**Number of Samples**
Contaminants						
Arsenic (ppm)	0.25 ± 0.046	0.159–0.341	23	0.257 ± 0.059	0.172–0.385	24
Cadmium (ppm)	0.059 ± 0.007	0.042–0.069	23	0.059 ± 0.011	0.042–0.094	24
Lead (ppm)	0.086 ± 0.016	0.06–0.119	23	0.091 ± 0.033	0.06–0.194	24
Mercury (ppm)	0.019 ± 0.004	0.019–0.004	23	0.017 ± 0.008	0.01–0.049	24
Selenium (ppm)	0.211 ± 0.051	0.16–0.333	23	0.191 ± 0.049	0.145–0.333	24
Aflatoxins (ppm)	<5.00	–	23	<5.00	–	24
Nitrate Nitrogen (ppm)[c]	16.64 ± 7.97	0.61–34.6	23	16.33 ± 6.09	10.0–29.6	24
Nitrite Nitrogen (ppm)[c]	0.61	–	23	0.61	–	24
BHA (ppm)[d]	<1.0	–	23	<1.0	–	24
BHT (ppm)[d]	1.09 ± 0.446	1.0–3.14	23	1.09 ± 0.437	1.0–3.14	24
Aerobic Plate Count (CFU/g)	<10.0	–	23	<10.0	–	24
Coliform (MPN/g)	3.0	–	23	3.0	–	24
Escherichia coli (MPN/g)	<10.0	–	23	<10.0	–	24
Salmonella (MPN/g)	Negative	–	23	Negative	–	24
Total Nitrosamines (ppb)[e]	10.0 ± 4.5	3.2–21.7	23	9.8 ± 4.5	3.2–18.9	24
N-Ndimethylamine (ppb)[e]	2.5 ± 2.6	0–11.8	23	2.8 ± 2.0	0–6.8	24
N-Npyrrolidine (ppb)[e]	7.5 ± 2.4	3.2–11.6	23	7.0 ± 3.1	2.1–15.15	24
Pesticides (ppm)						
α-BHC	<0.01	–	23	<0.01	–	24
β-BHC	<0.02	–	23	<0.02	–	24
γ-BHC	<0.01	–	23	<0.01	–	24
δ-BHC	<0.01	–	23	<0.01	–	24
Heptachlor	<0.01	–	23	<0.01	–	24
Aldrin	<0.01	–	23	<0.01	–	24
Heptachlor Epoxide	<0.01	–	23	<0.01	–	24
DDE	<0.01	–	23	<0.01	–	24
DDD	<0.01	–	23	<0.01	–	24
DDT	<0.01	–	23	<0.01	–	24
HCB	<0.01	–	23	<0.01	–	24
Mirex	<0.01	–	23	<0.01	–	24
Methoxychlor	<0.05	–	23	<0.05	–	24
Dieldrin	<0.01	–	23	<0.01	–	24

	Study 1			Study 2		
	Mean ± SD[b]	**Range**	**Number of Samples**	**Mean ± SD[b]**	**Range**	**Number of Samples**
Endrin	<0.01	–	23	<0.01	–	24
Telodrin	<0.01	–	23	<0.01	–	24
Chlordane	<0.05	–	23	<0.05	–	24
Toxaphene	<0.10	–	23	<0.10	–	24
Estimated PCBs	<0.20	–	23	<0.20	–	24
Ronnel	<0.01	–	23	<0.01	–	24
Ethion	<0.02	–	23	<0.02	–	24
Trithion	<0.05	–	23	<0.05	–	24
Diazinon	<0.10	–	23	<0.10	–	24
Methyl Chlorpyrifos	0.138 ± 0.126	0.02–0.567	23	0.13 ± 0.129	0.02–0.567	24
Methyl Parathion	<0.02	–	23	<0.02	–	24
Ethyl Parathion	<0.02	–	23	<0.02	–	24
Malathion	0.122 ± 0.102	0.02–0.385	23	0.142 ± 0.101	0.02–0.385	24
Endosulfan I	<0.01	–	23	<0.01	–	24
Endosulfan II	<0.01	–	23	<0.01	–	24
Endosulfan Sulfate	<0.03	–	23	<0.03	–	24

CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; PCB = polychlorinated biphenyl.
[a]All samples were irradiated.
[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.

Appendix C. Sentinel Animal Program

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Tables

C.1. Methods

Rodents used in 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 toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals are from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected from the rodents and allowed to clot, and the serum was separated. All samples were processed appropriately with serology testing and sent to IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed. Blood was collected per the following methods and as shown in Table C-1 and Table C-2.

Study 1: 10 females at arrival; 5 females, 4 weeks postarrival; 5 animals per sex at 6, 12, and 18 months; 10 females at study termination.

Study 2: 10 females at arrival; 5 females, 4 weeks postarrival; 5 males at 6, 12, and 18 months, and study termination.

Table C-1. Methods and Results for Sentinel Animal Testing (Study 1)

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
Kilham's Rat Virus	Arrival[a]; 4 weeks postarrival[b]; 6, 12, and 18 months; study termination
Mycoplasma pulmonis	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Parvo NS-1	Arrival; 4 weeks postarrival; 6 and 12 months
Pneumonia Virus of Mice	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Coronavirus/Sialodacryoadenitis Virus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Minute Virus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Parvovirus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Theilovirus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Sendai	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Theiler's Murine Encephalomyelitis Virus Strain GDVII	Arrival; 4 weeks postarrival; 6 and 12 months
Toolan's H-1	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination

[a]Age-matched nonpregnant females.
[b]Time-mated females that did not have a litter.

Table C-2. Methods and Results for Sentinel Animal Testing (Study 2)

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
Kilham's Rat Virus	Arrival[a]; 4 weeks postarrival[b]; 6, 12, and 18 months; study termination
Mycoplasma pulmonis	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Parvo NS-1	Arrival; 4 weeks postarrival; 6 months
Pneumonia Virus of Mice	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Coronavirus/Sialodacryoadenitis Virus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Minute Virus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Parvovirus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Rat Theilovirus	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Sendai	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Theiler's Murine Encephalomyelitis Virus Strain GDVII	Arrival; 4 weeks postarrival; 6 months
Toolan's H-1	Arrival; 4 weeks postarrival; 6, 12, and 18 months; study termination
Immunofluorescence Assay	
Mycoplasma pulmonis	Study termination
Pneumocystis carinii	Study termination

[a]Age-matched nonpregnant females.
[b]Time-mated females that did not have a litter.

C.2. Results

All test results were negative.

Appendix D. Summary of Peer Review Panel Comments

This section will appear in a future draft of the report