United States Environmental Protection Agency

Health Effects Document for Perfluorooctanoic Acid (PFOA)

ED_002330_00118754-00001

Health Effects Document

for

Perfluorooctanoic Acid (PFOA)

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

EPA Document Number: 822R14001
Date: February 2014

ED_002330_00118754-00002

ACKNOWLEDGMENT

This document was prepared under the U.S. EPA Contract No. DW-8992342701, Work Assignment No. 2011-001 with Oak Ridge National Laboratory. The Lead U.S. EPA Scientist is Joyce Morrissey Donohue, Ph.D., Health and Ecological Criteria Division, Office of Science and Technology, Office of Water.

The Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

ED_002330_00118754-00003

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ED_002330_00118754-00004

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ABBREVIATIONS AND ACRONYMS

ADAF	age-Dependent Agjustment Factor
ADHD	attention deficit hyperactivity disorder
ADX	adrenalectomized
AIC	Akaike's Information Criterion
ALP	alkaline phosphatase
ALT	alanine transaminase
ANOVA	analysis of variance
Areg	amphiregulin
AP	alkaline phosphatase
AST	aspartate aninotransferase
ATSDR	Agency of Toxic Substances and Disease Registry
AUC	area under the plasma concentration time curve
AUC_{inf}	area under the plasma concentration time curve, extrapolated to infinity
AWWA	American Water Works Association
AwwaRF	American Water Works Association Research Foundation
BMD	benchmark dose
BMDL	benchmark dose – lower 95th percentile confidence bound
BMDS	benchmark dose software
BMI	body mass index
BMR	benchmark response
BQL	below quantifiable limit
BSA	bovine serum albumin
BSEP	bile salt export pump
BUN	blood urea nitrogen
bw	body weight
C	Celsius
Cal-EPA	California Environmental Protection Agency
CaMKII	calcium/calmodulin-dependent protein kinase II
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
CAT	carnitine aceyltransferase
CCK	cholecystokinin
CCL	Contaminant Candidate List
CCL 3	Contaminant Candidate List 3
CDC	Centers for Disease Control and Prevention
CFSE	6-carboxyfluorescein succinimidyl ester
ChAT	choline acetyltransferase
CHO	Chinese hamster ovary
CI	confidence interval
Cl_p	plasma clearance
CL	clearance
CL_R	renal clearance
C_{max}	peak plasma concentration at the first intestinal absorption loci
CoA	coenzyme A

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ConA	concanavalinA
CORT	corticosterone
Cox II	cytochrome c oxidase subunit II
Cox IV	cytochrome c oxidase subunit IV
CPT	carnitine palmitoyltransferase
CRL	Charles River Laboratory
CSF	Cancer Slope Factor
CWS	community water system
d	day
DCDQ	Developmental Coordination Disorder Questionnaire
DCFH-DA	2',7'-dichlorofluorescein diacetate
DES	diethylstilbestrol
DHT	5α-dihydroxy-testosterone
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTH	delayed-type hypersensitivity
DWI	drinking water intake
E2	17-β estradiol
E3S	estrone-3-sulfate
EC_{50}	half maximal effective concentration
ECF	Electro-Chemical Fluorination
EFSA	European Food Safety Authority
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
ER	endpplasmic reticulum
ERα	estrogen receptor α
Errα	estrogen-related receptor α
FID	flame ionization detector
FSH	follicle-stimulating hormone
FXR	farnesoid receptor
g	gram
GAP-43	growth-associated protein-43
GD	gestation day
GGT	gamma-glutamyl transpeptidase
GJIC	gap junction intercellular communication
GlyT	glycogen tropholblast cell
GnRH	gonadotropin releasing hormone
GSD	geometric standard deviation
hCG	human chorionic gonadotropin
HDL	high density lipoprotein
HED	human equivalent dose
HEK	human embryonic kidney
HET	heterozygous
HGFα	hepatocyte growth factor
HL-60	human promyelocytic leukemia cell line
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HRBC	horse red blood cells

HRL	health reference level
HSA	human Serum Albumin
HSD17β1	hydroxysteroid 17β dehydrogenase 1
HSD3β1	hydroxysteroid 3β dehydrogenase 1
HSDB	Hazardous Substances Database
IC_{50}	half-maximal Inhibiting Concentration
ICR	imprinting control region
IGF-I	insulin like growth factor I
IHD	ischemic heart disease
IL-6	interleukin 6
IRR	incidence rate ratio
IRIS	Integrated Risk Information System
IU	international unit
IV	intravenous
K_a	adsorption rate constant
K_d	dissociation constant
K_e	elimination rate constant
kg	kilogram
K_m	substrate concentration at which the initial reaction rate is half maximal
K_{oc}	organic carbon water partitioning coefficient
K_t	affinity constant
L	liter
LC_{50}	lethal concentration for 50% of animals
LC-MS	liquid chromatography – mass spectrometry
LCT	Leydig cell tumors
LD	lactation day
LD_{50}	lethal dose for 50% of animals
LDH	lactic dehydrogenase
LDL	low density lipoprotein
L-FABP	liver fatty acid binding protein
LH	luteinizing hormone
LHWA	Little Hocking Water Association
LLOQ	lower limit of quantification
LOAEL	lowest observed adverse effect level
LOQ	Limit of Quantitation
LPS	lipopolysaccharide
m	meter
MCAD	medium chain acyl-CoA dehydrogenase
MCLG	Maximum Contaminant Level Goal
Mdr2	mulidrug resistance protein 2
µg	microgram
mg	milligram
min	minute
mL	milliliter
µm	micrometer
MMAD	mass median aerodynamic diameter
MOA	mechanism of action

mol	mole
mPL	mouse placental lactogen
mPLP	mouse prolactin like protein
MRL	minimum reporting level
MRP	multidrug restistance-associated protein
MTBE	methyl tertiary-butyl ether
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAWQA	National Water Quality Assessment
NDWAC	National Drinking Water Advisory Council
ng	nanogram
NAS	National Academy of Sciences
ND	not detected, no differences, or no data
Nd2	NADH dehydrogenase 2
Ndufs8	NADH dehydrogenase iron-sulfur protein 8
NHANES	The National Health and Nutrition Examination Survey
NIRS	National Inorganics and Radionuclides Survey
NK	natural killer
NM	not monitored
NMR	Nuclear Magnetic Resonance
NMRI	Naval Medical Research Institute
NOAEL	no observed adverse effect level
NPDWR	National Primary Drinking Water Regulation
NRC	National Research Council
Nrf1	nuclear respiratory factor 1
Nrf2	nuclear respiratory factor 2
NTCP	sodium-taurocholate contransporting polypeptide
OAT	organic anion transporter
OATp	organic anion transporting peptide
OGWDW	Office of Ground Water and Drinking Water
OPP-RED	Office of Pesticide Programs – Reregistration Eligibility Decision
OR	odds ratio
OVA	ovalbumin
OVX	ovariectomized
OW	Office of Water
P	progesterone
PACT	pancreatic acinar cell tumors
PAH	polycyclic aromatic hydrocarbon
PB	phenobarbital
PBMC	peripheral blood mononuclear cells
PBPK	pharmacologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PenH	enhanced pause airway respiration
PFAA	perfluoroalkyl acid
PFC	perfluorinated carboxylate acids
PFDA	perfluorodecanoic acid
PFHxS	perfluorohexanesulfonic acid
PFOA	perfluorooctanoic acid

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PFOS	perfluoroocatane sulfonate
Pgc-1α	peroxisome proliferator-activated receptor gamma coactivator 1α
PH	peroxisomal bifunctional protein
PHA	phytohemagglutinin
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
pKa	acid dissociation constant
PPAR	peroxisome proliferator activated receptor
Ppb	parts per billion
ppm	parts per million
PT	peroxisomal thiolase
PWG	Pathology Working Group
PWS	public water system
PXR	pregnane X receptor
Q	flow in and out of tissues
Q_{filc}	median fraction of blood flow to the filtrate
Reg Det 2	Regulatory Determinations on the Second CCL
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
RSA	rodent serum albumin
RSC	relative source contribution
RTECS	The Registry of Toxic Effects of Chemical Substances
RT-PCR	reverse transcription polymerase chain reaction
RXRα	retinoid X receptor alpha
SD	standard deviation
SDH	sorbitol dehydrogenase
SDQ	Strengths and Difficulties Questionnaire
SDWA	Safe Drinking Water Act
SHBG	sex hormone binding globulin
SIAR	SIDS Initial Assessment Report
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SPI	Society of the Plastics Industry
SRBC	sheep red blood cells
S-TGC	sinusoidal trophoblast giant cells
T_{max}	time of maximum plasma concentration
T3	triiodothyronine
T4	thyroxine
$T_{1/2}$	elimination half-time
T_m	transporter maximum
T_{max}	time of maximum plasma concentration
TC	total cholesterol
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)] benzene

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Tfam	transcription factor A
TG	triglycerides
TH	tyrosine hydroxylase
TNFα	tumor necrosis factor α
TRR	total reactive residues
TSH	thyroid stimulating hormone
TTP	time to pregnancy
TTR	thyroid hormone transport protein, transthyretin
UA	uric acid
UCMR	Unregulated Contaminant Monitoring Rule
UCMR 1	Unregulated Contaminant Monitoring Rule 1
UCMR 2	Unregulated Contaminant Monitoring Rule 2
UF	uncertainty factor
URAT	urate trasnporter
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Service
V_d	volume of distribution
V_{max}	maximum initial rate of an enzyme catalysed reaction
VLCAD	very long chain acyl-CoA dehydrogenase
VLDL	very low density lipoprotein
VOC	volatile organic compound
WHO	World Health Organization
WRF	Water Research Foundation
WT	wild type

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1.0 EXECUTIVE SUMMARY PFOA

Perfluorooctanoic acid (PFOA) is a completely fluorinated organic synthetic acid used to produce fluoropolymers. Because of strong carbon-fluorine bonds, PFOA is stable to metabolic and environmental degradation and is resistant to biotransformation. Extensive data in humans and animals demonstrate ready absorption of PFOA and distribution of the chemical throughout the body by noncovalent binding to plasma proteins. The liver is an important binding site with increased liver weight in laboratory animals one of the early, low-dose manifestations of exposure. At least three transport families seem to play a role in PFOA absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting peptides (OATps) and multidrug resistance-associated proteins (MRPs). PFOA is not readily eliminated from humans as evidenced by the half-life of 2.3 years. In contrast, half-life values for the monkey, rat, and mouse are 20.8 days, 11.5 days, and 15.6 days, respectively. Differences in transporters may explain species differences in elimination.

PFOA is known to activate the peroxisome proliferator activated receptor (PPAR) pathway by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. However, based on transcriptional activation of many genes in PPARα null mice, the effects of PFOA involve more than activation of PPAR. Also activated are the constitutive androstane receptor (CAR), farnesoid receptor (FXR), and pregnane X receptor (PXR).

Epidemiology studies have examined occupational and residential populations at or near large-scale PFOA production plants in the United States in an attempt to determine the relationship between serum PFOA concentration and various health outcomes suggested by the standard animal toxicological studies. Exposures were mainly through contaminated drinking water and to multiple PFCs. These studies have found a positive association between serum PFOA concentration and higher cholesterol in the general population and in worker populations but no consistent trends for the low-and high-density protein lipids. A positive association has been shown between serum PFOA concentrations and increased liver enzymes and/or decreased bilirubin in both worker and general populations, chronic kidney disease in the general population, and the odds of experiencing early menopause. Maternal or child plasma levels of PFOA were positively associated with decreased antibody titers in children after vaccination, obesogenic effects in female children at 20 years of age, and parent reported Attention Deficit Hyperactivity Disorders.

No consistent associations were identified between serum PFOA and hyperglycemia, type II diabetes, thyroid homeostasis, fertility, fecundity, and birth outcome. Maternal or child plasma PFOA levels were not associated with changes in children reaching motor or mental developmental milestones, child risk of hospitalization for infectious diseases, or attainment of puberty.

In most animal studies, short-term and chronic exposure to PFOA resulted in an increase in liver weight as at least one of the critical effects. Co-occurring effects in these studies included changes in spleen, thymus, liver and/or developmental endpoints. In repeated dosing studies, rats given 0.64 mg/kg/day for 13 weeks and monkeys given 3 mg/kg/day for 26 weeks had increased liver weight accompanied by hepatocellular hypertrophy. As part of a two-

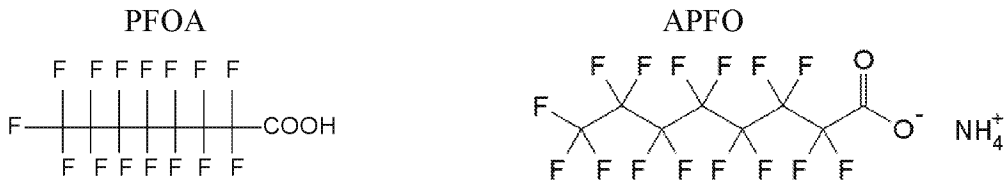
generation study, male rats had increased liver and kidney weights at 1 mg/kg/day. In shorter-term studies, slightly higher doses to rats also resulted in increased liver weight and liver lesions. In mice, developmental toxicity and increased spleen weight at a dose of 1 mg/kg/day accompanied increased liver weight. Slightly higher doses resulted in decreased immunoglobulin levels.

U.S. EPA has selected 0.00002 mg/kg/day as the RfD for PFOA based on the consistency of the response and with recognition of the use of liver weight as a common denominator for loss of homeostasis and protection against co-occurring adverse effects. This value is the outcome for modeled serum values from three rat studies and one mouse study. In two of the rat studies, liver effects were accompanied by developmental effects and kidney weight increases. A pharmacokinetic model was used to predict a serum area under the curve (AUC) concentration for each LOAEL and NOAEL for liver weight effects. From each AUC, the average serum concentration was calculated which was then used to calculate a human equivalent dose (HED). The total uncertainty factor (UF) applied to the HED_{NOAEL} from rat study was 30 which included a UF of 10 for intrahuman variability and a UF of 3 to account for toxicodynamic differences between animals and humans. For the critical studies that lacked an HED_{NOAEL}, and additional 10-fold UF was added to adjust for the use of a HED based on a LOAEL. Four of six candidate studies resulted in the same RfD. All of the studies supporting the RfD, except one, are studies with exposures to PFOA for $\geq$ 84 days meeting the duration requirements for determination of a lifetime exposure value; the exception is a 17-day developmental toxicity study in the mouse.

Under the U.S. EPA 2005 cancer guidelines, the evidence for the carcinogenicity of PFOA is considered *suggestive* because only one species has been evaluated and studies for gene mutations were negative. Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. The dose-response data for Leydig cell tumors in rats in was analyzed using the multistage cancer model for a dichotomous dataset to predict the dose at which a 4% increase in tumor incidence would occur. The 4% was chosen as the low-end of the observed response range within the study results. The resulting $BMDL_{04}$ was 1.99 mg/kg/day which yields a HED of 0.58 mg/kg/day and a slope factor of 0.07 $(mg/kg/day)^{-1}$. The HED of 0.58 mg/kg/day is nearly 30,000-fold greater than the RfD. Thus, the proposed RfD, based on increased liver weight as a common denominator for loss of homeostasis and protection of co-occurring effects, will also be protective of Leydig cell tumors.

2.0 IDENTITY: CHEMICAL AND PHYSICAL PROPERTIES

Perfluorooctanoic acid (PFOA) is a completely fluorinated organic synthetic acid used to produce fluoropolymers. It is manufactured by the Simons Electro-Chemical Fluorination (ECF) process or by telomerization. In the ECF process, the carbon-hydrogen bonds on molecules of organic feedstock are replaced with carbon-fluorine bonds when an electric current is passed through a solution of hydrogen fluoride and the organic feedstock. In the telomerization process, fluorine-bearing chemicals and tetrafluoroethylene react to produce fluorinated intermediates that are converted into PFOA (HSDB, 2006). Ammonium perfluorooctanoate (APFO) is the salt of PFOA (Figure 2-1) and is a processing aid in the manufacture of certain fluoropolymers, especially as an emulsifier in aqueous solution during the emulsion polymerisation of tetrafluoroethylene. APFO is not consumed during the polymerization process (SPI, 2005). Some sources of PFOA in the atmosphere result from the atmospheric degradation or transformation or surface deposition of precursors, including related fluorinated chemicals (fluorotelomer alcohols, olefins, and perfluoroalkyl sulfonamido substances) (Wallington et al., 2006).



Sources: SIAR (2006); http://commons.wikimedia.org/wiki/File:Ammonium_perfluorooctanoate.png

FIGURE 2-1 Chemical Structures of PFOA and APFO

Although PFOA is not a polar molecule, each of the carbon fluoride bonds is a dipole as a result of the electronegativity difference between fluoride (4.1) and carbon (2.5) placing a partial negative charge on each of the covalently bound fluorines and a partial positive charge on each of the fluorinated carbons. Charge repulsion of the partially negative fluorines and steric factors favor a PFOA conformation in which carbons 2 through 7 adopt an *anti* arrangement of substituents (Figure 2-2) resulting in a fairly linear molecular shape (Figure 2-3) as the lowest energy conformer.

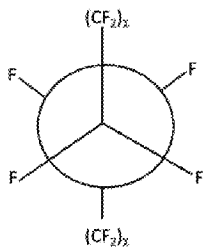


FIGURE 2-2 PFOA Anti-Conformer

ED_002330_00118754-00019

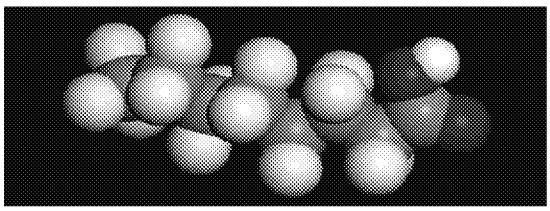


FIGURE 2-3 PFOA Lowest Energy Conformer

The favored PFOA conformer is very similar to the preferred conformation of the eight-carbon fatty acid, octanoic acid (also known as caprylic acid) except for the sphere of partial negative charge on the fluorines of the exterior surface. The ionized carboxylate grouping and the fluorine's partial negative charges favor electrostatic interactions between PFOA and positively charged surfaces on proteins and other macromolecules.

Some commercial PFOA is a combination of linear and branched chain isomers (80% linear, 20% branched; Loveless et al., 2006). The samples studied by Loveless et al. (2006) had the following mole percents of branched chain isomers: 12.6% internal monomethyl (non-alpha), 9% isopropyl, 0.2%, *tert*-butyl, 0.1% gem-dimethyl, and 0.1% alpha monomethyl. A study by Yoshikane et al. (2010) reported finding perfluoro-6-methylheptanoic acid (the isopropyl isomer) using mass spectroscopy analysis of environmental fluorosurfactants in Japan.

The physical and chemical properties and other reference information for PFOA and its salt APFO are provided in Table 2-1. These properties help to define the behavior of PFOA in living systems and the environment. PFOA and its salt are highly stable compounds. They are solids at room temperature with low vapor pressures. The melting point for PFOA is identified as 40-50°C and vapor pressures increase at temperatures near the melting point.

PFOA is moderately soluble in water and APFO is even more soluble. Both compounds are considered insoluble in nonpolar solvents which results in their being described as olephobic. Water solubility is increased by the presence of other ions and is an important factor governing solubility in body fluids. As the concentration of PFOA in aqueous solution increases, it forms colloidal micelles with the carboxyl functional groups on the exterior and the fluorocarbon chain on the interior. The critical micelle concentration has been identified as 3.6-3.7 g/L.

The pKa (acid dissociation constant) for PFOA has been reported as 1.5-2.8. As a result, it will be present in most biological fluids (gastric secretions excluded) primarily as the perfluorooctanoate anion. This is an important feature in governing absorption and membrane transport.

TABLE 2- 1 Chemical and Physical Properties of PFOA and APFO		
Property	**Perfluorooctanoic Acid**	**Ammonium Perfluorooctanoate**
Chemical Abstracts Registry (CAS) No.	335-67-1	3825-26-1
Synonyms	PFOA; Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-; Pentadecafluoro-1-octanoic acid; Pentadecafluoro-n-octanoic acid; Octanoic acid, pentadecafluoro-; Perfluorocaprylic acid; Pentadecafluoroocanoic acid; Perfluoroheptanecarboxylic acid;	APFO; C-8; Ammonium pentadecafluoroctanoate; Ammonium perfluorocarpylate; DS 101; FC 1015; FC 143; FX 1006; Fluorad® FC 143; Perfluorooctanoic acid ammonium salt; Unidyne® DS 101-20
Chemical Formula	$C_8HF_{15}O_2$	$C_8HF_{15}O_2NH_3$
Molecular Weight	414.09	431.10
Color/Physical State	White powder	White powder
Boiling Point	189°C	Decomposition
Melting Point	45-50 °C	Decomposition starts above 105°C
Density (at 20°C)	1.7921 g/cm^3	0.6-0.7 g/cm^3
Vapor Pressure:	4.2 (25°C) 2.3 (20°C) 128 (59.3°C)	3.7 (90.1°C) 0.0081 (20°C)
pKa	2.5 2.8 1.5-2.8	-
pH value	2.6, 1 g/L (20°C)	~5
K_{oc}	27,000 estimated	
Solubility in water ($g \cdot L^{-1}$)	9.5 (25°C) 4.1 (22°C)	> 500
Solubility in organic solvents	-	Heptane, Toluene: 0 Methanol, Acetone: > 500
Conversion Factors for vapor phase	1 ppm = 17.21 mg/m^3	1 ppm = 17.21 mg/m^3

Sources: HSDB (2006); SIAR (2006), EFSA (2008); RTECS (2008)

3.0 TOXICOKINETICS

Because of strong carbon-fluorine bonds, PFOA is stable to metabolic and environmental degradation. PFOA, is resistant to biotransformation, making the toxicity of the parent compound the concern, not that of a metabolite. However, because of their impact on cellular receptors and proteins, they possess the ability to impact the biotransformation of dietary molecules, intermediate metabolites, and other xenobiotic chemicals and may alter enzyme activities and transport kinetics. PFOA is known to activate the peroxisome proliferator activated receptor (PPAR) pathway by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. However, based on transcriptional activation of many genes in PPARα null mice, the effects of PFOA involve more than activation of PPAR. They may also activate the constitutive androstane receptor (CAR), farnesoid receptor (FXR), and pregnane X receptor (PXR).

PFOA is not readily eliminated from humans and other primates. Toxicokinetic profiles and the underlying mechanism for half-life differences are not completely understood although many of the differences appear to be related to elimination kinetics and factors that control membrane transport. Thus far, three transport families seem to play a role in PFOA absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting peptides (OATps) and multidrug resistance-associated proteins (MRPs) (Launay-Vacher et al., 2006). The transporters play a critical role in gastrointestinal absorption, uptake by the tissues, and excretion via the kidney. These transport systems are located at the blood brain barrier, blood placental barrier, blood testes barrier, and mammary gland where they function to protect the brain, fetus, and male reproduction organs (Ito and Alcorn, 2003; Zaïr et al., 2008). There are differences in transporters across species, genders, and individuals. There is more PFOA-specific information regarding the OAT and OATp families than the MRPs. Nomenclature abbreviations for the various transporters are not totally standardized, and there are inconsistencies across individual publications.

3.1 Absorption

Absorption data are available for oral, inhalation, and dermal exposure in laboratory animals. There are extensive data from humans demonstrating the presence of PFOA in serum. These data demonstrate absorption by one or more routes but do not quantify the amounts absorbed relative to dose. The human serum data are included in Section 3.5 of this report.

The absorption processes requires transport across the interface of the gut, lung, and skin with the external environment. Since PFOA is moderately soluble in aqueous solutions and oleophobic (minimally soluble in body lipids), movement across the apical and basal membrane surfaces of the lung, gastrointestinal tract, and skin involves transporters or mechanisms other than simple diffusion across the lipid bilayer. As discussed above, there are data that identify involvement of OATs, OATps, and MRPs in entrocytes (Zaïr et al., 2008). OAT2, OAT3, OATp2b1, and MRP2 are located in the apical membrane of the microvilli, and MRP1, 3, and 4 are located along the basolateral membrane. Together they function in the uptake of organic anions from gastrointestinal contents and transport of those anions into the portal blood supply (Zaïr et al., 2008). No primary data sources relative to the specificity of these transporters for PFOA in humans or laboratory animals were identified.

Oral Exposure

PFOA is well absorbed following oral exposure. Gibson and Johnson (1979) administered a single dose of ^{14}C-PFOA averaging 11.4 mg/kg by gavage to groups of 3 male 10-week old CD rats. Twenty-four hours after administration, at least 93% of total carbon-14 was absorbed. In another study, Cui et al. (2010) exposed male Sprague-Dawley rats (10/group) to PFOA (96% a.i.) at 0, 5, and 20 mg/kg/day once daily by gavage for 28 days. The percent of the dose absorbed was 92.8% and 92.3% for the low and high dose, respectively under the assumption that fecal excretion over the first 24-hours after dosing was estimated to be unabsorbed material.

Steady state serum PFOA levels were reached within 4-6 weeks in cynomolgus monkeys dosed with oral capsules containing 3, 10, or 20 mg/kg PFOA for 6 months (Butenhoff et al., 2004b). Urine steady state levels were reached after 4 weeks. Serum PFOA concentration in male rats fed diets containing 0.06, 0.64, 1.94, or 6.5 mg PFOA/kg for 90 days was 7.1, 41.2, 70.3, and 137.6 µg/mL, respectively (Palazzolo, 1993). Peak blood levels of PFOA were attained 1-2 hours following a 25 mg/kg dose to male and female rats (Kennedy et al., 2004). Blood levels of PFOA over time were similar in female rats given a single dose of 25 mg PFOA/kg when compared to a female rat given 10 daily doses of 25 mg PFOA/kg (Kennedy et al., 2004). Plasma PFOA concentrations in male Sprague-Dawley rats fed a diet containing 300 ppm PFOA for 1, 7, or 28 days were 259, 234, and 252 µg/mL, respectively (Elcombe et al., 2010). In rats, a marked gender difference in serum and tissue levels exists following PFOA administration. Males consistently have much higher levels than females with the difference maintained and becoming more pronounced over time. Correspondingly, female rats show much greater urinary excretion of PFOA than do male rats with serum half-life values in hours for females compared with days for males. These differences are discussed in more detail in the following sections.

Inhalation Exposure

Hinderliter (2003) measured the serum concentrations of PFOA following single and repeated inhalation exposures in Sprague-Dawley rats. For the single exposure study, male and female rats (3/sex/group) were exposed to a single nose-only exposure of an aerosol of 0, 1, 10, or 25 mg/m^3 PFOA. Preliminary range-finding studies demonstrated that aerosol sizes were 1.8 - 2.0 µm mass median aerodynamic diameter (MMAD) with geometric standard deviations ranging from 1.9 - 2.1 µm. Blood samples were collected before exposure, at 0.5, 1, 3 and 6 hours during exposure, and at 1, 3, 6, 12, 18 and 24 hours after exposure. Plasma was analyzed by liquid chromatography-mass spectrometry (LC-MS). PFOA plasma concentrations were proportional to the inhalation exposure concentrations.

The male Cmax values were approximately 2-3 times higher than the female Cmax. The female Cmax occurred approximately one hour after the exposure period with plasma concentrations then declining. In males, Cmax was observed immediately after the exposure period ended and persisted for up to six hours. The data are illustrative of absorption of PFOA via inhalation.

Dermal Exposure

There is evidence that PFOA is absorbed following dermal exposure. Kennedy (1985) treated rabbits and rats dermally with a total of 10 applications of PFOA at doses of 0, 20, 200 or 2,000 mg/kg. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner. O'Malley and Ebbens (1981) treated groups of 2 male and 2 female New Zealand White rabbits dermally with doses of 100, 1,000 and 2,000 mg/kg PFOA for 14 days. Mortality among the exposed animals demonstrated dermal uptake. All of the animals died at the highest dose, 3 of 4 died in the mid-dose group and none from the low-dose group. Although these data demonstrate dermal absorption they do not provide quantitative data.

The results of *in vitro* percutaneous absorption studies of PFOA through rat and human skin have been reported (Fasano et al, 2005). The permeability coefficient for PFOA was calculated to be $3.25 \pm 1.51 \times 10^{-5}$ cm/h and $9.49 \pm 2.86 \times 10^{-7}$ cm/h in rat and human skin, respectively.

3.2 Distribution

Distribution of absorbed material requires vascular transport from the portal of entry to receiving tissues. It has been suggested that PFOA circulates in the body by noncovalently binding to plasma proteins. Several studies have investigated the binding of PFOA to plasma proteins in rats, humans or monkeys to gain an understanding of its absorption, distribution and elimination, plus information on species and gender differences.

Protein Binding. Protein binding in plasma from cynomolgus monkeys, rats, and humans was tested with PFOA via in vitro methods (Kerstner-Wood et al., 2003). The results are summarized in Table 3-1. Rat, human, and monkey plasma proteins were able to bind 97 to 100% of the PFOA added at concentrations ranging from 1-500 ppm. Human serum albumin carried the largest portion of the PFOA among the protein components of human plasma. Serum albumin is a common carrier of hydrophobic materials in the blood including short and medium chain fatty acids, inorganic ions, and some pharmaceuticals. Approximately 60% of the serum protein in humans and rats is albumin (Saladin, 2003; Harkness and Wagner, 1983). At 68%, the percentage bound to albumin in mice is slightly higher than in humans and rats (Harkness and Wagner, 1983).

TABLE 3-1. Protein Binding in Rat, Human, and Monkey Plasma

PFOA Concentration (ppm)	Rat (%)	Monkey (%)	Human (%)
1	~100	~100	~100
10	99.5	99.8	99.9
100	98.6	99.8	99.9
250	97.6	99.8	99.6
500	97.3	99.5	99.4

From Kerstner-Wood et al., 2003
%Binding values reported as "~100" reflect a nonquantifiable amount of test article in the plasma water below the quantifiable limit (BQL)<6.25 ng/mL

Han et al. (2003) investigated the binding of PFOA to rat and human plasma proteins in vitro. The authors concluded that there was no correlation between the PFOA persistence and

binding of the PFOA to rat serum. The primary PFOA binding protein in plasma was serum albumin. However, the method used (ligand blotting) would not theoretically allow the identification of low abundance proteins with high affinity for PFOA. Further investigation of purified rodent and human serum albumin binding using labeled ^{19}F nuclear magnetic resonance (NMR) allowed the calculation of disassociation constants for PFOA binding to rodent and human serum albumin. No significant difference in binding to the serum albumin of rat versus human was detected (Table 3-2).

Male and female rats treated in vivo showed no gender difference in the binding of PFOA to serum proteins though the persistence of PFOA in vivo is much greater in male than female rats.

TABLE 3-2. Dissociation Constants (Kd) of Binding Between PFOA and Albumin

Parameter	Method	RSA	HSA
K_d (mM)	NMR[a]	0.29 ± 0.10[c]	
K_d (mM)	micro-SEC[b]	0.36 ± 0.08[c]	0.38 ± 0.04
Number of Binding Sites	micro-SEC[b]	7.8 ± 1.5	7.2 ± 1.3

From Han et al., 2003
RSA= Rodent Serum Albumin; HSA= Human Serum Albumin
a Average of the two *Kd* values (0.31 ± 0.15 and 0.27 ± 0.05 mM) obtained by NMR.
b Values were obtained from three independent experiments and their standard deviations are shown.
c On the basis of the result of unpaired t-test at 95% confidence interval, the difference of *Kd* values determined by NMR and micro-SEC is statistically insignificant.

Wu et al. (2009) examined the interaction of PFOA and human serum albumin (HSA). The authors tested their hypothesis that PFOA, after absorption, was transported bound to albumin by dialyzing PFOA solutions in the presence and absence of HSA. In the absence of HSA, 98% of the dissolved PFOA crossed the dialysis membrane into the dialysate within 4 hours. In the presence of HSA, the amount of PFOA found in the dialysate after 4 hours decreased in direct proportion to the albumin concentration, demonstrating binding to the protein. No albumin was identified in the dialysate.

Using the dialysis data and thermodynamic considerations, the authors concluded that albumin could bind up to 12 PFOA molecules on its surface via chemical monolayer absorption with a 13th molecule bound noncovalently in the more hydrophobic interior of the protein. The surface nature of the binding may well indicate potential binding to other serum proteins as well. Circular dichroism measurements of the albumin/PFOA complex suggested a conformational change in the protein as a result of the PFOA binding. The beta-pleated sheet content of the albumin decreased, and the alpha-helix content increased by 15%. These conformational changes could interfere with the functional properties of serum albumin or other serum proteins impacted by surface monolayers of PFOA. For example, albumin's ability to transport its natural ligands could be decreased by the presence of PFOA on the protein surface. The interaction of albumin with target cellular receptors could also be altered.

MacManus-Spencer et al. (2010) used a variety of approaches to quantify the binding of PFOA to serum albumin (surface tension measurements, ^{19}FNMR spectroscopy, and fluorescence spectroscopy). When taken together, the results from these analyses suggest the presence of primary and secondary binding sites on albumin. The PFOA-albumin association constants for the primary site (K_1^a) was about $1.5 \pm 0.2 \times 10^5$/mol bovine serum albumin (BSA)

while the association constant for the secondary site (K_2^a) was $0.8 \pm 0.1 \times 10^2$/mol BSA at a concentration of 1μM. When the concentration of BSA increased to 10 μM, the binding per mole of BSA decreased: $K_1^a = 0.33 \pm 0.004$; $K_2^a = 0.53 \pm 0.1$. Qin et al. (2010) also used fluorescence spectroscopy quenching analysis to study PFOA binding to BSA and concluded that van der Waals forces and hydrogen bonds were the dominant intermolecular binding forces.

The results of the fluorescence spectroscopy suggested a conformational change in BSA following binding of PFOA that moved a tryptophan residue (#214) from a slightly polar region of the protein to a less polar region. The shift in a tryptophan position is consistent with the observations of Wu et al. (2009) and Qin et al. (2010) who reported that BSA underwent a conformational change following the binding of PFOA. The authors considered the results from the fluorescence spectroscopy to be the most relevant to the potential physiological impact of environmental levels of PFOA.

A modeling study by Salvalaglio et al. (2010) was conducted to determine the binding sites of PFOA on HSA and classify them by their interaction energy using molecular modeling; this study builds on the binding studies of Wu et al. (2009) and MacManus-Spencer et al. (2010). It was estimated that maximum number of PFOA binding sites on HSA was 9. The site locations were common to the natural binding sites for fatty acids, thyroxine, warfarin, indol, and benzodiazepine (Figure 3-1; Salvalaglio et al., 2010). The binding site closest to tryptophan residue #214 had the highest binding affinity (-8.0 kcal/mol) compared to other sites (~-6--7 kcal/mol).

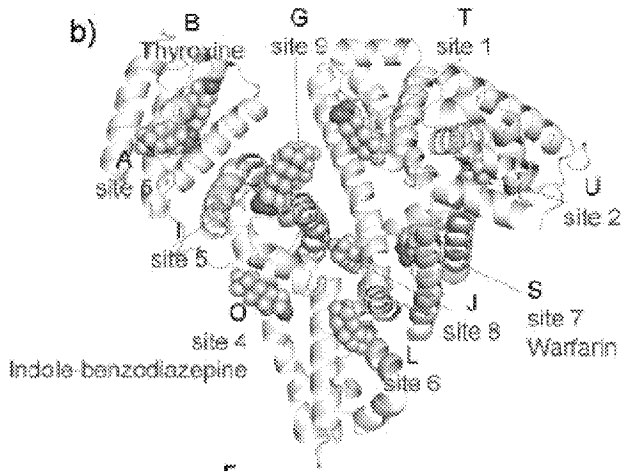


FIGURE 3-1. PFOA binding sites on human serum albumin

Weiss et al. (2009) screened several perfluorinated compounds (n=30), differing by carbon chain length C4-18, fluorination degree, and functional groups for potential binding to the serum thyroid hormone transport protein, transthyretin (TTR) using a radioligand-binding assay. The natural ligand of TTR is thyroxine (T4). PFOA was one of the chemicals evaluated.

Human TTR was incubated overnight with ^{125}I-labeled T4, unlabled T4, and 10-10,000 nM PFOA as a competitor for the T4 binding sites. The unlabeled T4 was used as a reference compound. The levels of T4 in the assay were close to the lower range for total T4 measured in healthy adults. The authors concluded that binding affinity for TTR was highest for the fully fluorinated compounds tested and those having at least an eight carbon length chain,

characteristics that apply to PFOA. PFOA demonstrated a high binding affinity for TTR with 949 nM causing a 50% inhibition of T4 binding to the TTR.

It is also possible that PFOA will display nonspecific binding to proteins within the cellular matrix as well as in the serum but little work has been done to investigate this probability. Luebker et al. (2002) conducted *in vitro* studies of the ability of a variety of perfluorinated chemicals to displace a fluorescent substrate (11-(5-dimethylamino-napthalenesulphonyl)-undecanoic acid) from liver fatty acid binding protein (L-FABP). L-FABP is an intracellular lipid carrier protein that reversibly binds long chain fatty acids, phospholipids and an assortment of peroxisome proliferators (Erol et al., 2003). It constitutes 2 to 5% of the cytosolic protein in hepatocytes. Luebker et al. (2002) reported that PFOA (IC_{50}>10μM) exhibited some binding to L-FABP, but the binding potential was only about 50% of that for perfluorooctane sulfonate (PFOS) (IC_{50} = 4.9 μM) and far less than that of oleic acid (IC_{50} = 0.01 μM).

Oral Exposure

Tissue distribution

Human. No clinical studies are available in humans on administration of a controlled dose of PFOA or its distribution. However, samples collected in biomonitoring and epidemiological studies provide data showing distribution of PFOA. Olsen et al. (2001c) analyzed human sera and postmortem liver samples and found that more than 90% of the liver samples (n=30) were <Limit of Quantitation (LOQ). Serum levels ranged from <LOQ-7.0 ng/mL. PFOA concentrations above the LOQ were detected in 5/6 postmortem liver samples from men in Catalonia, Spain. In females, only 1/6 liver samples was above LOQ (Kärrman et al., 2009). Pirali et al. (2009) measured intrathyroidal PFOA levels (0.4-6.0 ng/g) in thyroid surgical patients and found no correlation between serum and thyroid PFOA concentration. PFOA has been detected in breast milk samples (Tao et al., 2008; Völkel et al., 2008) and cord blood samples (Apelberg et al., 2007; Monroy et al., 2008) at concentrations above the LOQ. These studies indicate that PFOA is distributed within the body and that maternal transfer to offspring can occur.

Animal. Studies of tissue distributions are available for several species including monkeys, rats, and mice. The data are categorized by species in the sections that follow. The distribution data derived from studies during pregnancy and lactation follow the data on nonpregnant animals.

Monkey. Butenhoff et al. (2002; 2004b) studied the fate of PFOA in cynomolgus monkeys in a six-month oral exposure study. Groups of four to six male monkeys each were administered PFOA daily via oral capsule at dose rates of 0, 3, 10, or 20 mg/kg. The highest concentration was initially 30 mg/kg, but due to its toxicity, it was suspended after 12 days. Dosing was resumed on test day 22 using the 20 mg/kg/day dose for the remainder of the six month period, resulting in a normalized dose of 20 mg/kg/day for the study. Serum, urine and fecal samples were collected at two-week intervals and were analyzed for PFOA concentrations. Liver samples were collected at time of sacrifice.

Serum concentrations reached steady-state levels within four to six weeks in all dose groups. Steady-state concentrations of PFOA in serum were 77 ± 39, 86 ± 33, and 158 ± 100 µg/ml after six weeks (Butenhoff et al., 2002) and 81 ± 40, 99 ± 50, and 156 ± 103 µg/ml (Butenhoff et al., 2004b) after six months for the 3, 10, and 20 mg/kg dose groups, respectively. The mean serum concentration of PFOA in control monkeys was 0.134 - 0.203 µg/ml. Urine PFOA concentrations reached steady-state after 4 weeks and were 53 ± 25, 166 ± 83, and 181 ± 100 µg/ml in the 3, 10, and 20 mg/kg dose groups, respectively, for the duration of the study. Liver PFOA concentrations at terminal sacrifice in the 3 mg/kg and 10 mg/kg dose groups were similar and ranged from 6.29 to 21.9 µg/g. Liver PFOA concentrations in two monkeys exposed to 20 mg/kg were 16.0 and 83.3 µg/g. Liver PFOA concentrations in two monkeys dosed with 10 mg/kg-day at the end of a 13-week recovery period were 0.08 and 0.15 µg/g (Butenhoff et al., 2004b).

Rat. Ylinen et al. (1990) administered newly weaned Wistar rats (18/sex/group) doses of 3, 10, and 30 mg/kg-day PFOA by gavage for 28 days. At necropsy, serum was collected as well as the brain, liver, kidney, lung, spleen, ovary, testis, and adipose tissue. The concentration of PFOA in the serum and tissues was determined with capillary gas chromatography equipped with a flame ionization detector (FID). A mass spectrometer was used in the selected ion monitoring mode when the PFOA concentration was below the quantitation limit of the FID (1 µg/ml).

The concentration of PFOA in the serum and tissues following 28 days of administration is presented in Table 3-3. PFOA was not detected in the adipose tissue. The concentrations of PFOA in the serum and tissues were much higher in males than in females. In the males, the levels of PFOA in the serum were generally lower in the 30 mg/kg-day dose group than in the 10 mg/kg-day dose group due to increased urinary elimination in the 30 mg/kg-day group. The tissue levels were similar for the 10 and 30 mg/kg/day doses. In females there was a dose-related increase in tissue levels while the serum levels were comparable for the 10 and 30 mg/kg/day dose-groups. Among solid tissues, the liver had the highest tissue concentration followed by the testes, spleen, lung, kidney and brain, respectively. In females the concentration in the kidneys exceeded that in the liver for the 10 and 30 mg/kg-day doses but not at the lowest dose. Ovary and spleen had similar concentrations followed by lower levels in the lung and brain.

TABLE 3-3.Tissue Distribution of PFOA in Wistar Rats After 28 Days of Treatment

	Dose (Males[a]) mg/kg-day			Dose (Females[a]) mg/kg-day		
Tissue	**3**	**10**	**30**	**3**	**10**	**30**
Serum	48.6±10.3	87.27±20.09	51.65±1.47	2.4[b]	12.47±4.07	13.92±6.06
Liver	39.9±7.25	51.71±11.18	49.77±10.76	1.81±0.49	3.45±1.36	6.64±2.64
Kidney	1.55±0.71	40.56±14.94	39.81±17.67	0.06±0.02	7.36±3.19	12.54±8.24
Spleen	4.75±1.66	7.59±3.5	4.1±1.57	0.15±0.04	0.38±0.17	1.59±0.49
Lung	2.95±0.54	22.58±4.59	23.71±5.42	0.24[b]	0.22±0.15	0.75±0.26
Brain	0.398±0.144	1.464±0.211	0.71±0.32	< LOQ[c]	0.029±0.019	0.044±0.018
Ovary				< LOQ	0.41±0.27	1.16±0.58
Testis	6.24±2.04	9.35±4.02	7.22±3.17			

FromYlinen et al., 1990
[a] N = 6, Mean ± SD, µg/ml tissue
[b] N=3, no SD
[c] below the limit of quantitation of 1µg/mL

Kemper (2003) examined the distribution of PFOA in tissues of male and female Sprague-Dawley rats following administration by gavage. Rats were administered 1, 5, and 25 mg/kg ^{14}C-PFOA by oral gavage. Tissue concentrations were determined at the time of maximum plasma concentration (T_{max}) and at the time that plasma concentration had fallen to one half the maximum ($T_{max/2}$). Values for T_{max} and $T_{max/2}$ for male and female rats were determined from pharmacokinetic experiments. In those experiments, plasma was collected over the course of several days and PFOA concentration was analyzed. Non-compartmental pharmacokinetic models were applied to identify T_{max} and elimination half-time ($T_{1/2}$) from the data. The $T_{max/2}$ was calculated as the time (hr) for the maximum plasma concentration plus the elimination half-time (hr) ($T_{max} + T_{1/2}$). In some cases, elimination may occur in a rapid phase followed by a slower elimination phase. For cases in which biphasic elimination was evident, the rapid phase $T_{1/2}$ was used for calculation of $T_{max/2}$.

Tissues from male rats were collected at 10.5 hours (T_{max}) and 171 hours ($T_{max/2}$) after dosing. Tissues from female rats were collected at 1.25 hours (T_{max}) and 4 hours ($T_{max/2}$) after dosing. The results are summarized in Table 3-4 and Table 3-5 for males and females, respectively. Liver, kidney and blood were the primary tissues for distribution of ^{14}C-PFOA. In males the fraction of the dose found in liver increased between T_{max} and $T_{max/2}$, but remained constant or decreased in other tissues. In females, the fraction of the dose present in all tissues remained constant or decreased between T_{max} and $T_{max/2}$. Liver-to-blood concentration ratios for ^{14}C at T_{max} in males were greater than 1, and increased between T_{max} and $T_{max/2}$. Blood to kidney concentration ratios in females were between 1 and 2 at all dose levels and remained relatively constant between T_{max} and $T_{max/2}$. In males the blood to kidney ratios were 10 or higher at T_{max} and declined slightly at $T_{max/2}$.

TABLE 3-4. Distribution of PFOA in Male Sprague-Dawley Rats After Oral Exposure						
	Dose					
Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$
Prostate	0.083±0.039	0.030±0.002	0.071±0.045	0.057±0.020	0.067±0.018	0.028±0.012
skin[a]	14.77±2.135	6.061±0.274	15.565±0.899	7.233±0.430	13.836±0.969	5.419±0.237
blood[a]	22.148±0.692	8.232±1.218	24.919±1.942	11.140±0.624	22.905±1.177	7.904±1.032
Brain	0.071±0.018	0.022±0.002	0.051±0.021	0.023±0.008	0.063±0.007	0.019±0.002
fat[a]	2.281±0.467	0.593±0.136	2.815±0.225	0.916±0.205	2.153±0.430	0.628±0.110
Heart	0.451±0.119	0.195±0.024	0.443±0.037	0.252±0.030	0.461±0.053	0.164±0.032
Lungs	0.74±0.147	0.341±0.043	0.593±0.376	0.344±0.194	0.863±0.103	0.303±0.057
Spleen	0.086±0.011	0.045±0.006	0.096±0.017	0.060±0.007	0.106±0.015	0.042±0.005
Liver	21.708±5.627	32.627±3.601	18.750±2.434	25.231±1.289	17.528±0.900	20.145±3.098
Kidney	1.949±0.402	1.14±0.215	2.170±0.354	1.212±0.115	2.293±0.286	1.003±0.122
G.I. tract	2.930±0.929	0.980±0.300	2.508±0.713	1.052±0.202	2.784±0.608	0.808±0.189
G.I. contents	2.083±0.625	0.239±0.025	2.632±0.934	0.270±0.028	4.186±1.349	0.210±0.084
Thyroid	0.008±0.005	0.004±0.003	0.011±0.006	0.004±0.002	0.009±0.002	0.005±0.001
Thymus	0.085±0.008	0.051±0.018	0.085±0.012	0.053±0.003	0.120±0.025	0.045±0.010
Testes	0.755±0.079	0.356±0.037	0.693±0.180	0.372±0.062	0.623±0.098	0.224±0.031
adrenals	0.019±0.004	0.010±0.001	0.022±0.004	0.009±0.001	0.026±0.004	0.009±0.003
muscle[a]	12.025±0.648	4.984±0.745	13.565±0.576	6.429±0.648	12.855±0.841	4.253±0.358
bone[a]	3.273±0.538	1.120±0.094	3.047±0.544	1.375±0.169	3.062±0.438	0.906±0.100
Total[b]	85.465±6.426	57.026±3.379	88.033±1.420	56.031±1.025	83.937±3.680	42.112±4.740

From Kemper, 2003
Percent of dose recovered at T_{max} and $T_{max/2}$ in tissues
[a] Percent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.
[b] Totals are calculated from individual animal data.

Examination of the residuals from the administered PFOA in the male tissues at $T_{max/2}$ (171 hours) indicate that the 40 to 60% percent of the dosed PFOA retained was present in the liver, blood, skin, and muscle tissues in decreasing amounts (Table 3-4). In males, about 1% of the label was present in the gastrointestinal tissues and contents at $T_{max/2,}$ while the value for females was about 10%. However, the samples were collected at 1.25 and 4 hours in females and 10.5 and 171 hours in males providing more time for absorption in the males.

In the female tissues at $T_{max/2}$ (4 hours), approximately 30% percent of the dosed PFOA retained was present in the liver, blood, kidney, muscle, and skin tissues in decreasing amounts (Table 3-5). About 14% of the administered dose remained in the gastrointestinal tissues and contents. Based on the timing of the measurements and the results, females appear to absorb and excrete PFOA more rapidly than males.

TABLE 3-5. Distribution of PFOA in Female Sprague-Dawley Rats After Oral Exposure						
	Dose					
	1 mg/kg		5 mg/kg		25 mg/kg	
Tissue	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$
skin[a]	0.434±0.162	0.403±0.096	0.624±0.142	0.307±0.121	0.380±0.166	0.415±0.175
blood[a]	5.740±1.507	4.438±1.625	8.089±2.080	5.411±1.466	7.158±2.232	6.407±1.406
Brain	0.037±0.009	0.047±0.008	0.066±0.019	0.045±0.010	0.058±0.008	0.058±0.018
fat[a]	0.134±0.032	0.164±0.079	0.220±0.111	0.110±0.069	0.147±0.053	0.148±0.065
Heart	0.198±0.079	0.253±0.055	0.388±0.057	0.236±0.051	0.317±0.035	0.287±0.069
Lungs	0.454±0.148	0.546±0.082	0.827±0.102	0.570±0.179	0.678±0.067	0.775±0.204
Spleen	0.063±0.027	0.058±0.006	0.101±0.021	0.060±0.012	0.091±0.007	0.070±0.002
Liver	7.060±1.266	6.817±1.537	11.190±2.192	7.176±0.982	10.538±1.723	9.080±0.895
Kidney	3.288±0.948	2.769±0.784	4.293±0.771	2.685±0.736	5.867±0.946	4.749±0.393
G.I. tract	10.699±9.066	8.462±6.519	7.142±2.594	8.255±8.967	6.923±1.846	3.547±1.306
G.I. contents	21.956±13.48	3.891±2.395	2.896±2.305	5.601±6.165	2.491±1.548	1.121±1.010
Thyroid	0.010±0.003	0.016±0.021	0.008±0.002	0.006±0.002	0.009±0.003	0.007±0.002
Thymus	0.052±0.017	0.058±0.024	0.105±0.030	0.068±0.021	0.091±0.032	0.077±0.020
Ovaries	0.047±0.019	0.048±0.006	0.071±0.012	0.041±0.012	0.071±0.012	0.070±0.012
adrenals	0.014±0.005	0.018±0.004	0.026±0.005	0.015±0.004	0.031±0.005	0.021±0.001
muscle[a]	0.170±0.051	0.258±0.089	0.325±0.010	0.229±0.031	0.441±0.116	0.304±0.099
Uterus	0.243±0.091	0.374±0.247	0.354±0.046	0.247±0.068	0.358±0.124	0.365±0.029
bone[a]	0.101±0.017	0.153±0.052	0.174±0.057	0.142±0.078	0.157±0.072	0.181±0.090
Total[b]	50.698±16.48	28.772±10.98	36.897±3.187	31.201±12.63	35.803±2.554	27.680±2.569

From Kemper, 2003
Percent of dose recovered at T_{max} and $T_{max/2}$ in tissues
[a] Percent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.
[b] Totals are calculated from individual animal data.

Lau et al. (2006) studied PFOA's toxicokinetic properties in rats as part of a larger study. The authors gavage dosed adult male and female Sprague-Dawley rats (n=8) with 10 mg/kg for 20 days, and sacrificed them 24 hours after the last treatment. After 20 days of treatment, male rats had serum PFOA levels of 111 µg/mL compared to 0.69 µg/mL in female rats.

Martin et al. (2007) administered 20 mg PFOA/kg to adult male Sprague-Dawley rats (n= 4 or 5) for 1, 3, or 5 days by oral gavage and determined the liver and serum levels of PFOA. Blood was collected via cardiac puncture and PFOA concentration was determined by high-performance liquid chromatography-electrospray tandem mass spectrometry. The mean liver PFOA concentration was 92 ± 6, 250 ± 32, and 243 ± 23 µg/g after 1, 3, or 5 daily doses of 20 mg PFOA/kg/day, respectively. The mean serum concentration was 245 ± 41 µg/mL after 3 daily doses of 20 mg PFOA/kg/day. Serum PFOA concentration was not determined after 1 day and 5 days of dosing due to sample availability.

Mouse. Lau et al. (2006) gavage dosed adult male and female CD-1 mice (5-7/group) with 20 mg/kg for 7 or 17 days. The animals were sacrificed 24 hours after the last treatment. After seven days of treatment male mice had serum PFOA levels of 181 µg/mL and females had 178 µg/mL. After 17 days of treatment, male mice had serum PFOA levels of 199 µg/mL and females had levels of 171 µg/mL. These data suggest that the gender difference observed by Lau et al (2006) in rats was not seen in the mice under the conditions of this study.

As part of a physiologically based pharmacokinetic (PBPK) modelling exercise, Lou et al. (2009) administered single doses of 1 or 10 mg/kg to groups of 3 male and 3 female CD-1 mice. The mice were sacrificed for analysis of plasma, liver, and kidney tissues after 4, 8, or 12 hours and at 1, 3, 6, 9, 13, 20, 27, 34, or 48 days after dosing. This study was repeated for a second analysis which extended the sacrifice times to 55, 62, 70, and 80 days.

Measures of PFOA in serum were presented graphically and indicate that the order of magnitude difference between the doses was generally reflected in an order of magnitude difference between serum concentration for males and females across the 80 day observation period. [The study procedures indicated that serum was collected and analyzed, but the graphic presentation described the values as plasma values. Contact with one of the authors confirmed that the values should have been listed as serum rather than plasma.] The peak concentrations were 10 and 100 mg/L for the 1 and 10 mg/kg/day, respectively. Declines in serum concentrations for females were roughly parallel reaching concentrations of about 2 mg/L and <0.2 mg/L for the high and low doses, respectively, at the end of 80 days. Peak serum concentrations were slightly lower in the males (~8 and 80 mg/L) than in the females, and final serum concentrations were higher in the males (~0.5 and 8 mg/L). Liver and kidney concentrations were also higher in males than females for each of the two doses.

Lou et al. (2009) also collected serum data for up to 28 days after administration of a 60 mg/kg dose to groups of 3 female mice. Based on the graphic presentation of the data, the 60 mg/kg dose was cleared from the serum much more rapidly than the 1 and 10 mg/kg doses. For example, a serum concentration of about 0.4 mg/L was reached in about 28 days for the 60 mg/kg dose, 61 days for 10 mg/kg dose, and 70 days for the 1 mg/kg dose (values estimated from the Figure 3 of the published paper). No measurements were made for liver or kidney in the high dose animals.

In the final experimental portion of the study, Lou et al. (2009) exposed groups of 5 female CD-1 mice to 20 mg/kg/day for 17 days. Serum samples were collected 24 hours after the final dose and analyzed for PFOA. The mean serum concentration was 130 ± 23 mg/L which is comparable to that of 171 μg/mL reported by Lau et al. (2006).

Minata et al. (2010) orally administered 0, 12.5, 25, or 50 μmol/kg PFOA (~0, 5.4, 10.8, or 21.6 mg/kg PFOA) to groups of male wild-type 129S4/SvlmJ mice (n=39) and PPARα- null 129S4/SvJae-Pparα^{tm1Gonz}/J mice (n=40) for 4 weeks. Blood, liver, and bile were collected for determination of PFOA concentration at the end of 4 weeks as shown in Table 3-6. The PFOA concentration in whole blood and the liver were similar between wild-type and PPARα-null mice at the same dose level and appeared to increase in proportion to dose. In bile, PFOA concentration in wild-type mice increased by a factor of 13.8 from 12.5 to 25 μmol/kg and by a factor of 38 from 25 to 50 μmol/kg. In the bile of PPARα-null mice, PFOA concentration increased by a factor of 3.2 from 12.5 to 25 μmol/kg and by a factor of 19.5 from 25 to 50 μmol/kg. The data suggested the existence of a capacity-limited and PPARα independent PFOA transport from the liver to the bile duct.

TABLE 3-6. PFOA Concentration in Wild-type and PPARα-null Mice						
PFOA Concentration (μg/mL)						
Dose	Whole Blood		Bile		Liver	
μmol/kg	Wild-type	PPARα-null	Wild-type	PPARα-null	Wild-type	PPARα-null
0	ND	ND	ND	ND	ND	ND
12.5	20.6 ± 2.4	12.9 ± 2.2	56.8 ± 26.9	19.6 ± 2.2	181.2 ± 6.3	172.3 ±8.9
25	46.9 ± 3.2	36.4 ± 2.7*	784 ± 137.6	62.9 ± 16.7*	198.8 ± 15.4	218.3 ± 14.5
50	64.2 ± 6.5	71.2 ± 8.0	2174 ± 322.4	383 ± 109.9*	211.6 ± 13.3	239.7 ± 25.0

From Minata et al., 2010
Mean ± SD; ND= not detected (<0.001 μg/mL)

Tissue transporters. The liver is an important binding site for PFOA. Increased liver weight is one of the early, low-dose manifestations of exposure. OATps and MRPs, at least one OAT and the sodium-taurocholate cotransporting polypeptide (NTCP) have been identified at the interphace of the liver with the portal blood and the canalicular membranes within the liver (Kim, 2003; Kusuhara and Sugiyama, 2009; Zaïr et al., 2008). OATs and MRPs have been identified along the hepatocyte interface with systemic circulation.

The impact of PFOA on several membrane transporter systems linked to biliary transport was studied by Maher et al. (2008) as part of a more detailed study of perfluorodecanoic acid (PFDA). A dose of 80 mg/kg by i.p. injection (propylene glycol: water vehicle) was found to significantly increase ($p<0.05$) the expression of MRP3 and MRP4 in the livers of C57BL/6 mice two days after treatment as reflected in quantification of their DNA transcripts. MRP3 and MRP4 are believed to protect the liver from accumulation of bile acids, bilirubin, and potentially toxic exogenous substances by promoting their excretion in bile. Conversely, there were significant decreases ($p<0.05$) in the protein levels for OATp1a1, OATp1a4, and OATp1b2 (Cheng and Klaassen, 2008) as determined by Western Blot analysis and mRNA measurements. There was no significant impact on NTCP protein or the serum levels of bile acids. The OATps are transporters responsible for the uptake of bile acids and other hydrophobic substances such as steroid conjugates, ecosinoids, and thyroid hormones into the liver.

These studies, by the same laboratory, were carried out at high, single dose exposures which limit their value in extrapolating to low and repeat dose scenarios. The results suggest a decrease in the uptake of favored substrates into the liver and an increase in removal of favored substrates from the liver via bile. Based on the results with the more extensive evaluation of PFDA including mouse strains null for several receptors (PPARα, CAR, PXR, and FXR), the authors concluded that the changes in receptor proteins were primarily linked to activation of PPARα.

Impact of developmental age. Han (2003) administered groups of four to eight week old Sprague-Dawley rats (10/sex/age) a single dose of 10 mg/kg-day PFOA by oral gavage. Blood samples were collected 24 hours after dosing and the plasma concentration of PFOA was measured by HPLC-MS. In the 4 week old rats the concentration of plasma PFOA was approximately 2.7 times higher in males than in the females (Table 3-7). In the 5 and 6 week old female rats, the plasma PFOA concentrations were about 2 fold lower than in the 4-week old rats. However, in the 5 week old males, the concentration of plasma PFOA was about 5-fold higher than in the 4 week group suggesting a developmental change in excretion rate. Plasma concentrations did not differ appreciably among 5-, 6-, 7- and 8-week old rats within each sex

but did differ between sexes. In fact, PFOA plasma concentrations were 35-65-fold higher in males than in females at every age except at 4 weeks. Thus, it appears that maturation of the factor(s) responsible for the gender difference in elimination occurs between the ages of 4 and 5 weeks in the rat.

TABLE 3-7. Plasma PFOA Concentrations (µg/ml) in Postweaning Sprague-Dawley Rats

Age (weeks)	Males	Females
4	7.32 ± 1.01[a]	2.68 ± 0.64
5	39.24 ± 3.89	1.13 ± 0.46
6	43.19 ± 3.79	1.18 ± 0.52
7	37.12 ± 4.07	0.57 ± 0.29
8	38.55 ± 5.44	0.81 ± 0.27

From Han, 2003

[a] mean ± SD; samples from 10 animals/sex/group

Hinderliter (2004, 2006a) continued the investigation of the relationship between age and plasma PFOA in male and female Sprague-Dawley rats. Immature rats 3, 4, or 5 weeks of age were administered PFOA via oral gavage as a single dose of 10 or 30 mg/kg. Rats were not fasted prior to dosing. Two hours after dosing, 5 rats/sex/ per age group and dose group were sacrificed and blood samples were collected. The remaining 5 rats/sex/per age and dose group were placed in metabolism cages for 24-hour urine collection. These rats were sacrificed at 24 hours and blood samples were collected.

In the male rats, plasma PFOA concentrations for either the 10 or 30 mg/kg dosage groups did not differ significantly by sample time (at 2 and 24 hours) or by animal age (3, 4 or 5 weeks), except at 2 hours for the five week age group ($p<0.01$) which showed the lowest PFOA level (Table 3-8). PFOA plasma concentrations following a 30 mg/kg dose were 2-3 times higher than those following a 10 mg/kg dose. These data do not demonstrate a difference between the 5-week old rats and the younger 3- and 4-week age groups at 24 hours after dosing and thus do not support the observations from the Han (2003) study.

TABLE 3-8. Plasma PFOA Concentrations in Male Rats

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/ml)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
3	10	41.87	4.01	34.22	7.89
4	10	39.92	4.45	42.94	5.33
5	10	26.32*	6.89	40.60	3.69
3	30	120.65	12.78	74.16	18.23
4	30	117.40	18.10	100.81	13.18
5	30	65.66*	15.53	113.86	23.36

From Hinderliter 2004

*Statistically significantly different by sample time and animal age ($p<0.01$).

In the female rats, plasma PFOA concentrations were significantly lower in the 5-week age group than in the 3- or 4-week age groups at the 24-hour time period for both doses and at the 30 mg/kg dose group at 2-hours (Table 3-9). Plasma PFOA concentrations following a 30 mg/kg dose were approximately 1.5 to 4 times higher than those observed following a 10 mg/kg dose. At 24 hours post dose, plasma PFOA levels in the female rats was significantly lower than the plasma PFOA levels in male rats, especially at 5 weeks of age. The data for the 5-week old

female rats compared to the 3- and 4-week age groups at 24 hours are consistent with the Han (2003) data in that they demonstrate a decline in plasma levels compared to their earlier measurements. Thus, the developmental change is one that appears to be unique to the female rat.

TABLE 3-9. Plasma PFOA Concentrations in Female Rats

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/ml)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
3	10	37.87	5.77	13.55 [b]	3.83
4	10	29.88	12.15	18.98 [b]	7.01
5	10	33.23	7.41	1.36 [a, b]	0.87
3	30	84.86	10.51	51.43 [b]	13.61
4	30	80.67	14.10	28.01 [b]	9.90
5	30	56.90 [a]	29.66	3.42 [a, b]	1.95

From Hinderliter, 2004

a Statistically significantly different from the 3 and 4 week values ($p<0.01$).

b Statistically significantly different from 2 hour values ($p<0.01$).

In a supplemental study to determine the effect of fasting (Hinderliter, 2004, 2006a), 4 week old rats, 4 rats/sex, were administered 10 mg/kg PFOA via oral gavage. Animals (2/sex) were fasted overnight for 12 hours before dosing with PFOA. All the rats were sacrificed at 24 hours post dosing and blood was collected for analysis of PFOA in plasma. Plasma PFOA concentrations in male rats were 64.95 and 30.00 µg/ml for the fasted and non-fasted animals, respectively. Plasma PFOA concentrations in the female rats were 68.16 and 26.54 µg/ml for the fasted and non-fasted animals, respectively. Given the consistency in the 4-week old rat plasma PFOA concentrations, the authors concluded that age dependent changes in female PFOA elimination are observable between 3 and 5 weeks of age.

Distribution during pregnancy and lactation

Rat. An oral two-generation reproductive toxicity study of PFOA in rats was conducted (York, 2002; Butenhoff et al., 2004a). Five groups of rats (30 sex/group) were administered PFOA by gavage at doses of 0, 1, 3, 10, and 30 mg/kg-day. At scheduled sacrifice, after completion of the cohabitation period in F0 male rats and on lactation day (LD) 22 in F0 female rats, blood samples (3/sex/group-control; 10/sex/group-treated) were collected from animals dosed with 0, 10, or 30 mg/kg for analysis of PFOA. Serum analysis for the F0 generation males in the control, 10 and 30 mg/kg-day groups sampled at the end of cohabitation showed that PFOA was present in all samples tested, including controls. Control males had an average concentration of 0.0344±0.0148 µg/ml PFOA. Levels of PFOA were similar in the two male dose groups; treated males had 51.1±9.30 and 45.3±12.6 µg/ml, respectively for the 10 and 30 mg/kg-day dose groups. In the F0 female controls, serum PFOA was below the limits of quantitation (0.00528 µg/ml). Levels of PFOA found in female sera increased between the two dose groups; treated females had an average concentration of 0.37±0.0805 and 1.02±0.425 µg/ml, respectively for the 10 and 30 mg/kg-day dose groups.

PFOA levels during gestation and lactation were studied by Hinderliter et al. (2005) and Mylchreest (2003). Groups of 20 pregnant Sprague-Dawley rats were dosed with 0, 3, 10 or 30 mg/kg-day PFOA during days 4-10, 4-15, or 4-21 of gestation, or from gestation day 4 to

lactation day 21. Maternal blood samples were collected at 2 hours ± 30 minutes post-dose on a daily basis. Clinical observations and body weights were recorded daily. Five animals per dose group were sacrificed at specific time periods to harvest the conceptus and/or placenta and amniotic fluid. On gestation day (GD) 10 only embryos were recovered and on GD 15 and 21 the placentas, amniotic fluid, and embryos/fetuses were collected.

The remaining 5 rats per group were allowed to deliver their pups. On lactation days 0, 3, 7, 14, and 21, the pups were counted, weighed (sexes separate), and examined for abnormal appearance and behavior. Randomly selected pups were sacrificed and blood samples were collected. On lactation days 3, 7, 14, and 21, the dams were anesthetized and milk and blood samples were collected; dams were removed from their litters 1-2 hours prior to collection.

Plasma, milk, amniotic fluid extract, and tissue homogenate (placenta, embryo, and fetus) supernatants were analyzed for PFOA concentrations by HPLC-MS. Maternal PFOA plasma levels during gestation and lactation are presented in Table 3-10. Maternal plasma levels at 2 hrs post-dosing (approximately the time of peak blood levels following a gavage dose) were fairly similar during the course of the study with a mean level of 11.2, 26.8, and 66.6 µg/ml in the 3, 10, and 30 mg/kg-day groups, respectively; PFOA levels in the control group were below the limit of quantitation (0.05 µg/ml).

TABLE 3-10. Maternal Plasma PFOA Levels (µg/ml) During Gestation and Lactation

Exposure Period	Sample Time	Dose		
		3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
GD 4 - GD 10	GD 10 plasma	8.53 ± 1.06	23.32 ± 2.15	70.49 ± 8.94
GD 4 - GD 15	GD 15 plasma	15.92 ± 12.96	29.40 ± 14.19	79.55 ± 3.11
GD 4 - GD 21	GD 21 plasma	14.04 ± 2.27	34.20 ± 6.68	76.36 ± 14.76
GD 4 - LD 3	LD 3 plasma	11.01 ± 2.11	22.47 ± 2.74	54.39 ± 17.86
GD 4 - LD 7	LD 7 plasma	10.09 ± 2.90	25.83 ± 2.07	66.91 ± 11.82
GD 4 - LD 14	LD 14 plasma	9.69 ± 0.92	23.79 ± 2,81	54.65 ± 11.63
GD 4 - LD 21	LD 21 plasma	9.04 ± 1.01	28.84 ± 5.15	64.13 ± 1.45
NA	Average plasma	11.19 ± 2.76	26.84 ± 4.21	66.64 ± 9.80

From Hinderliter et al., 2005 and Mylchreest, 2003
Mean ± SD; samples were from 5 dams/group/time point and were collected 2 hrs post-dosing

PFOA levels in the placenta, amniotic fluid, and embryo/fetus are presented in Table 3-11. The levels of PFOA in the placenta on gestation day 21 were approximately twice the levels observed on gestation day 15, and the levels of PFOA in the amniotic fluid were approximately four times higher on day 21 than on day 15. The concentration of PFOA in the embryo/fetus was highest in the day 10 embryo and lowest in the day 15 embryo; PFOA levels in the day 21 fetus were intermediate.

TABLE 3-11. Placenta, Amniotic Fluid, and Embryo/Fetus PFOA Concentrations (µg/ml)				
Exposure Period	**Tissue**	**Dose**		
		3 mg/kg-day	**10 mg/kg-day**	**30 mg/kg-day**
GD 4 - GD 10	GD 10 - embryo	1.40 ± 0.30	3.33 ± 0.81	12.49 ± 3.50
GD 4 - GD 15	GD 15 - placenta	2.22 ± 1.79	5.10 ± 1.70	13.22 ± 1.03
	- amniotic fluid	0.60 ± 0.69	0.70 ± 0.15	1.70 ± 0.91
	- embryo	0.24 ± 0.19	0.53 ± 0.18	1.24 ± 0.22
GD 4 - GD 21	GD 21 - placenta	3.55 ± 0.57	9.37 ± 1.76	24.37 ± 4.13
	- amniotic fluid	1.50 ± 0.32	3.76 ± 0.81	8.13 ± 0.86
	- fetus	1.27 ± 0.26	2.61 ± 0.37	8.77 ± 2.36

From Hinderliter et al., 2005 and Mylchreest, 2003
Mean ± SD; samples were pooled by litter and were collected 2 hrs post-dosing

The concentrations of PFOA in the plasma of the day 21 fetus were approximately half the levels observed in the maternal plasma (Table 3-10). The values were about twice as high in the dams as in the pups with mean values of 14.04, 34.20 and 76.36 µg/ml, respectively in the 3, 10, and 30 mg/kg-day groups for the dams and 5.88, 14.48, and 33.11 µg/ml in the, respectively for the pups. Pup plasma levels decreased between birth and lactation day 7 (Table 3-12), and were thereafter similar to the levels observed in the milk (see Table 3-13 below). The pups were not separated by gender.

TABLE 3-12. Fetus/Pup PFOA Concentration (µg/ml) During Gestation and Lactation				
Exposure Period	**Tissue**	**Dose**		
		3 mg/kg-day	**10 mg/kg-day**	**30 mg/kg-day**
GD 4 - GD 21	GD21 fetal plasma	5.88 ± 0.69	14.48 ± 1.51	33.11 ± 4.64
GD 4 - LD 3	LD 3 - pup plasma	2.89 ± 0.70	5.94 ± 1.44	11.96 ± 1.66
GD 4 - LD 7	LD 7 - pup plasma	0.65 ± 0.20	2.77 ± 0.58	4.92 ± 1.28
GD 4 - LD 14	LD 14 - pup plasma	0.77 ± 0.10	2.22 ± 0.38	4.91 ± 1.12
GD 4 - LD 21	LD 21 - pup plasma	1.28 ± 0.72	3.25 ± 0.52	7.36 ± 2.17

From Hinderliter et al., 2005 and Mylchreest, 2003
Mean ± SD; samples were pooled by litter and were collected 2 hrs post-dosing

The concentration of PFOA in the milk was also fairly similar throughout lactation and was approximately 1/10th of the PFOA levels in the maternal plasma (see Table 3-11 above); the mean values for maternal milk were 1.1, 2.8, and 6.2 µg/ml in the 3, 10, and 30 mg/kg-day groups, respectively (Table 3-13).

TABLE 3-13. PFOA Levels (µg/ml) in Sprague-Dawley Maternal Milk During Lactation				
Exposure Period	**Sample Time**	**Dose**		
		3 mg/kg-day	**10 mg/kg-day**	**30 mg/kg-day**
GD 4 - LD 3	LD 3 - milk	1.07 ± 0.26	2.03 ± 0.33	4.97 ± 1.20
GD 4 - LD 7	LD 7 - milk	0.94 ± 0.22	2.74 ±0.91	5.76 ±1.26
GD 4 - LD 14	LD 14 - milk	1.15 ± 0.06	3.45 ± 1.18	6.45 ± 1.38
GD 4 - LD 21	LD 21 - milk	1.13 ± 0.08	3.07 ± 0.51	7.48 ± 1.63
NA	Average milk	1.07 ± 0.09	2.82 ± 0.60	6.16 ± 1.06

From Hinderliter et al., 2005 and Mylchreest, 2003
Mean ± SD; samples were from 5 dams/group/time point and were collected 2 hrs post-dosing

Mouse. Fenton et al. (2009) orally dosed pregnant CD-1 mice (n=25/group) with 0, 0.1, 1, or 5 mg PFOA/kg on GD17. On GD18, 5 dams/group were sacrificed and trunk blood, urine, amniotic fluid, and the 4th and 5th mammary gland were collected. One fetus/dam was

euthanized and retained for whole pup analysis. The remaining dams were allowed to litter. Biological samples as described above excluding amniotic fluid were also collected on postnatal days 1, 4, 8, and 18. As before, at each time-point, a single pup was euthanized and retained for whole pup analysis. Blood from the remaining pups was collected and pooled. Milk was collected from dams on postnatal days 2, 8, 11, and 18 following a 2 hour separation of the pups from the dam.

The concentration of PFOA in dam serum was approximately twice that detected in amniotic fluid (Table 3-14). Compared to the amniotic fluid, the concentration of PFOA in the fetuses was increased by 2.3-, 3.1-, and 2.7-fold at 0.1, 1, and 5 mg/kg, respectively. The highest concentration of PFOA was detected in the serum of nursing dams. In the dams, the concentration of PFOA in the serum exhibited a U-shaped response curve; the lowest serum concentration was observed at the time of peak lactation. Dam mammary tissue and milk PFOA concentrations showed a U-shaped response that mirrored to that found in the dam's serum. The concentration of PFOA in pup's serum was significantly higher than PFOA concentration in dam's serum, and appeared to decrease as the time for weaning approached. When pup PFOA concentration was calculated with consideration for pup body weight gain, PFOA body burden increased through the peak of lactation, and began to decrease by PND18 showing an inverse U-shaped response curve.

TABLE 3-14. PFOA Levels (ng/ml) During Gestation and Lactation in Selected Fluids and Tissues				
Tissue	**Day**	**Dose**		
		0.1 mg/kg	**1 mg/kg**	**5 mg/kg**
Dam Serum	GD18	143 ± 19	1697 ± 203	7897± 663
	PND1	217.5 ± 35	1957.0 ± 84	9845.6 ± 1478
	PND4	110.0 ± 12	1269.4 ± 235	6776.6 ± 561
	PND8	46.7 ± 21	360.8 ± 98	1961.8 ± 414
	PND18	123.3 ± 41	1035.2 ± 305	5156.5 ± 1201
Amniotic Fluid	GD18	99.0 ± 28	865.3 ± 191	3203.8 ± 492
Dam Urine	GD18	21.9 ± 8.6	104.9 ± 69.7	666.7 ± 169
	PND1	7.7 ± 1.7	116.8 ± 64	492.3 ± 119
	PND4	8.4 ± 6.4	53.5 ± 15	401.5 ± 117
	PND8	0.8 ± 0.22	11.6 ± 6.2	40.1 ± 17
	PND18	1.8 ± 1.1	18.7 ± 8.6	91.7 ± 49
Mammary Gland	GD18	18.9 ± 1.9	307.2 ± 30.4	1429± 186
	PND1	27.4 ± 6.8	343.8 ± 53	1933.5 ± 194
	PND4	9.6± 8.4	239.2 ± 53	1461.8 ± 267
	PND8	2.4 ± 3.8	71.7 ± 22	411.8 ± 78
	PND18	17.1 ± 10	239.9 ± 76	1372.8 ± 240
Milk	PND2	32.5 ± 12	716.7 ± 145	1236.6 ± 1370
	PND8	11.6 ± 8.1	77.4 ± 19	245.1 ± 26
	PND11	5.4 ± 1.0	42.3 ± 9.1	282.5 ± 162
	PND18	43.5 ± 19	251.8 ± 147	909.8 ± 308
Whole Pup	GD18	136.3 ± 15	1665.8 ± 213	6256.5 ± 751
	PND1	150.9 ± 21	1606.9 ± 288	7134.5 ± 1097
	PND4	91.8 ± 8.9	1183.2 ±187	5071.4 ± 267
	PND8	60.9 ± 16	729.0 ± 92	3118.5 ± 424
	PND18	17.5 ± 11	251.9 ± 112	1391.5 ± 118
Pup Serum	PND1	324.7 ± 36	3926.8 ± 480	16,286.4 ± 1372
	PND4	267.6 ± 47	3020.8 ± 223	11,925.2 ± 1077
	PND8	260.2 ± 56	2548.2 ± 245	9215.8 ± 594
	PND18	111.8 ± 30	1124.8 ± 236	5894.3 ± 743

From Fenton et al., 2009

Pregnant C57BL/6/Bkl mice were fed diets containing 0.3 mg PFOA/kg/day from GD1 through the end of pregnancy. At birth, the PFOA concentrations in the offspring were 0.7 ± 0.1 μg/g in the brain and 16.3 ± 4.1 μg/g in the liver (Onishchenko et al., 2011).

Macon et al. (2011) gavage dosed CD-1 mice with 0, 0.3, 1.0, or 3.0 mg PFOA/kg from GD1 to GD17 or with 0, 0.01, 0.1, or 1.0 mg PFOA/kg from GD10 to GD17. In the full gestation experiment (GD1-17), offspring were were sacrificed on postnatal day (PND) 7, 14, 21, 28, 42, 63, and 84, and in the half gestation experiment (GD10-17), female offspring were sacrificed on PND1, 4, 7, 14 and 21. Serum, liver, and brain from the offspring were analyzed for PFOA by HPLC-MS/MS.

At the lowest dose, PFOA concentration in the serum peaked around PND7, but the two higher doses peaked around PND 14 (Table 3-15). Calculated blood burdens which take into account the increasing blood volumes and body weights for females showed an inverted U-shaped curve peaking at PND14 for all doses. In the liver, PFOA concentration decreased over time with the highest concentration observed at PND7. Lower concentrations of PFOA were detected in the brain of the offspring on PND 7 and 14.

TABLE 3-15. Female Offspring PFOA Levels (ng/ml) After GD1-17 Exposure

Tissue	Day	Dose		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Female Serum	PND7	4980 ± 218	11026 ± 915	20700 ± 3900
	PND14	4535 ± 920	16950 ± 3606	26525 ± 2446
	PND21	1194 ± 394	377 ± 607	8343 ± 1078
	PND28	630 ± 162	1247 ± 208	4883 ± 1378
	PND42	377± 81	663 ± 185	2058 ± 348
	PND63	55 ± 17	176 ± 85	–
	PND84	16 ± 5	71 ± 8	125
Female Liver	PND7	2078 ± 90	8134 ± 740	16700 ± 749
	PND14	972 ± 124	4152 ± 483	10290 ± 1028
	PND21	1188 ± 182	1939 ± 637	2339 ± 1241
	PND28	678 ± 130	2007 ± 560	7124 ± 1081
	PND42	342 ± 87	617 ± 145	1145 ± 274
	PND63	118 ± 22	320 ± 113	417 ± 160
	PND84	43 ± 12	55± 12	235 ± 79
Female Brain	PND7	150 ± 26	479 ± 41	1594 ± 162
	PND14	65 ± 12	241 ± 20	650 ± 44
	PND21	<LOQ	31 ± 5	133 ± 23
	PND28	<LOQ	<LOQ	62 ± 93
	PND42	<LOQ	<LOQ	<LOQ
	PND63	<LOQ	<LOQ	<LOQ
	PND84	<LOQ	<LOQ	<LOQ

From Macon et al., 2011
– = not measured
LOQ: serum full gestation=10-20 ng/g; liver=35 ng/g; brain=35 ng/g; late gestation serum=5 ng/mL

After an exposure to low doses of PFOA from GD10 to GD17, serum PFOA concentration in the female offspring declined from PND1 through the end of the experiment (Table 3-16). Calculated blood burden showed a gradual increase from PND1 through PND14, followed by a decline through PND21.

TABLE 3-16. Female Offspring Serum PFOA Levels (ng/ml) After GD10-17 Exposure				
Tissue	**Day**	**Dose**		
		0.01 mg/kg	**0.1 mg/kg**	**1.0 mg/kg**
Female Serum	PND1	284.5 ± 21.0	2303.5 ± 114.4	16305.5 ± 873.5
	PND4	184.1 ± 12.1	–	–
	PND7	150.7 ± 20.9	1277.8 ± 122.6	11880.3 ± 1447.6
	PND14	80.2 ± 13.9	645.4 ± 114.2	6083.7 ± 662.6
	PND21	16.5 ± 2.1	131.7 ± 24.5	2025.1 ± 281.9
Female Blood Burden (calculated)	PND1	15.2 ± 1.7	114.3 ± 5.4	926.0 ± 47.6
	PND4	20.6 ± 0.1	–	–
	PND7	27.3 ± 3.8	221.7 ± 24.9	1965.9 ± 256.7
	PND14	27.0 ± 4.6	218.5 ± 39.8	2033.6 ± 293.5
	PND21	7.9 ± 1.0	66.4 ± 12.8	984.7 ± 142.8

From Macon et al., 2011
– = not measured, blood burden determined by (body weight x (58.5/1000) x serum x 0.55)

White et al. (2011) measured serum PFOA concentrations in three generation of CD-1 mice (Table 3-17). Pregnant mice (F0, n=10-12 dams/group) were gavage dosed with 0, 1, or 5 mg PFOA/kg from GD1-17. A separate group of pregnant mice (n=7-10 dams/group) were gavage dosed with either 0 or 1 mg PFOA/kg from GD1-17 and received drinking water containing 5 ppb PFOA beginning on GD7 and continuing until the end of the study for their offspring, except during breeding and early gestation, to simulate a chronic low-dose exposure. An increase in serum PFOA concentration was observed in the control + 5 ppb PFOA groups in the F1 and F2 generations and in the 1 mg/kg + 5ppb PFOA group of the F2 generation. A decrease was observed for the remaining groups.

TABLE 3-17. Serum PFOA Levels (ng/ml) Over Three Generations					
	Generation/ Day	**Dose**			
		0 mg/kg + 5 ppb	**1 mg/kg**	**1 mg/kg + 5 ppb**	**5 mg/kg**
Dams at weaning	F0/ PND22	74.8 ± 11.3	6658.0 ± 650.5	4772.0 ± 282.4	26980.0 ± 1288.2
	F1/~PND91	86.9 ± 14.5	9.3 ± 2.6	173.3 ± 36.4	18.7 ± 5.2
Offspring	F1/PND22	21.3 ± 2.1	2443.8 ± 256.4	2743.8 ± 129.7	10045 ± 1125.6
	F1/PND42	48.9 ± 4.7	609.5 ± 72.2	558.0 ± 55.8	1581.0 ± 245.1
	F1/PND63	66.2 ± 4.1	210.7 ± 21.9	187.0 ± 24.1	760.3 ± 188.3
	F2/PND22	26.6 ± 2.4	4.6 ± 1.2	28.5 ± 3.7	7.8 ± 1.9
	F2/PND42	57.4 ± 2.9	0.4 ± 0.0	72.8 ± 5.8	0.4 ± 0.0
	F2/PND63	68.5 ± 9.4	1.1 ± 0.5	69.2 ± 4.3	1.2 ± 0.5

From White et al., 2011

Subcellular Distribution. Han et al. (2005) examined the subcellular distribution of PFOA in the liver and kidney of male and female rats. Male and female Crl:CD (SD)IGS BR rats were gavage dosed with 25 mg/kg [^{14}C]PFOA and sacrificed 2 hours after dosing. Blood was collected and the liver and kidneys were removed. Five subcellular fractions (nuclei and cell debris, lysosome and mitochondria, microsome, light microsome and ribosome, and membrane-free cytosol) were obtained by differential centrifugation. The radioactivity per gram of each fraction and the total radioactivity were measured.

In the male liver, the highest proportion of total reactive residues (TRR) of PFOA was located in the nuclei and cell debris (40%). The TRR for the other subcellular fractions were as follows: membrane-free cytosol 26% TRR, lysosome and mitochondria ~14% TRR, microsome ~16% TRR. The level of PFOA in the light microsome and ribosome was ~1% TRR. In the female liver, the highest proportion of PFOA was found in the membrane-free cytosol, 48% TRR. The TRR were nuclei and cell debris ~31% TRR, lysosome and mitochondria ~12% TRR, and microsome ~8% TRR. As observed in the males, the level of PFOA in the light microsome and ribosome was ~1% TRR.

In the male kidney, the level of PFOA was 79% TRR in the membrane-free cytosol, 15% TRR in the nuclei and cell debris, and 4% TRR in the lysosome and mitochondria/microsome/ light microsome and ribosome (combined). In the female kidney, the level of PFOA was 71% TRR in the cytosol, 21% TRR in the nuclei and cell debris, and 8% TRR in the lysosome and mitochondria/ microsome/light microsome and ribosome (combined). Further examination showed that in both sexes 98% of PFOA in the plasma was protein bound and the protein bound fraction of PFOA in the liver cytosol was 56% TRR. In the kidney, the protein bound fraction of PFOA in males was 42% TRR and 17% TRR in the females.

Based on the results, the authors concluded that subcellular distribution of PFOA in the rat liver was sex-dependent because the proportion of PFOA in the liver cytosol of female rats was almost twice that in the male. They hypothesized that the female may have a greater amount than males of an unknown liver cytosolic binding protein with an affinity for perfluorinated acids. They also hypothesized that the unknown protein or protein complex might normally aid in transport of fatty acids from the liver. In the kidney, the subcellular distribution did not show the gender difference seen with the liver, however the protein bound fraction for the males (42%) was about twice that for thefemales (17%).

Inhalation Exposure

In a repeated exposure study, Hinderliter (2003, 2006b) exposed 6-8 week old male and female rats (5/sex/group) to 0, 1, 10, or 25 mg/m^3 aerosol concentrations of PFOA for 6 hours/day, 5 days/week for 3 weeks. Blood was collected immediately before and after the daily exposure period three days per week. The aerosols had mass median aerodynamic diameters (MMAD) of 1.3 - 1.9 µm with geometric standard deviations of 1.5 - 2.1. PFOA plasma concentrations were proportional to the inhalation exposure concentrations, and repeated exposures produced little plasma carryover in females, but significant day-to-day carryover in males. Male rats reached steady state plasma levels of 8, 21, and 36 µg/ml for the 1, 10 and 25 mg/m^3 groups, respectively by three weeks. In females, the post-exposure plasma levels were 1, 2, and 4 µg/ml for the 1, 10, and 25 mg/m^3 groups, respectively. When measured immediately before the daily exposure, plasma levels had returned to baseline in females demonstrating clearance within 24 hours of each daily dose.

Dermal Exposure

No data were identified on tissue distribution following dermal exposures.

3.3 Metabolism

Several studies have examined metabolism of PFOA. However, no studies show clear evidence of metabolism. Ophaug and Singer (1980) found no change in fluoride ion level in the serum or urine following oral administration of PFOA to female Holtzman rats. Ylinen et al. (1989) found no evidence of phase II metabolism of PFOA following a single intraperitoneal PFOA dose (50 mg/kg) in male and female Wistar rats. The free anionic and possible conjugated forms of PFOA in the urine were separated using BondElut tubes. The tubes contain NH_2 which is a weaker anion exchange sorbent and a good choice for retaining strong anions. The samples were aspirated through the tube, washed with water, and eluted with sodium bicarbonate/ carbonate-buffer. The aspirate and eluate from the separation method were analyzed by gas chromatography. PFOA was not detected in the aspirate, but was retained with the cationic animo phase found in the eluate. This also occurred in control blanks spiked with PFOA. The authors concluded that because the PFOA anion was completely bound to the weak cationic amino phase in both the spiked controls and urine samples, PFOA is secreted in the urine as such and has a neglible rate of phase II metabolism (Ylinen et al., 1989). The potential for reduction of the carboxyl group does not appear to have been evaluated but is not likely to occur.

3.4 Excretion

Excretion data are available for oral exposure in humans and laboratory animals. Several studies have investigated the elimination of PFOA in humans, cynomolgus monkeys, and rats. In human females, elimination pathways include pregnancy (cord blood) and lactation (breastmilk) (Apelberg et al., 2007; Tao et al., 2008; Thomsen et al., 2010; Völkel et al., 2008; von Ehrenstein et al., 2009).

Urinary elimination of PFOA was reported in a case history of of a single human male with high serum levels of perfluorinated chemicals (Genuis et al., 2010). Treatment with cholestyramine, a bile acid sequestrant for 20 weeks (4g/day, three times a day) lowered his serum PFOA concentration from 5.9 ng/g serum to 4.1 ng/g serum. More dramatic decreases were observed with serum PFOS (23-14.4 ng/g serum) and perfluorohexanesulfonic acid (PFHxS) (58-46.8 ng/g serum) which were present at higher levels in the serum. This observation that suggests excretion with bile and possible enterohepatic resorption via intestinal transporters limiting the loss of absorbed PFOA via feces in the absence of a binding agent such as cholestyramine.

Elimination half-lives differ among species. There are also significant gender differences in the elimination of PFOA in some laboratory animal species. Information from humans does not at present provide sufficient data to determin the magnitude of interindividual and gender differences in excretory half lives. The organic acid transport system appears to play an important role in renal excretion of PFOA and possibly biliary elimination.

Oral Exposure

Monkey. Butenhoff et al. (2004b) studied the fate of PFOA in cynomolgus monkeys in a six-month oral exposure study. Groups of four to six male monkeys each were administered PFOA daily via oral capsule at dose rates of 0, 3, 10, or 30/20 mg/kg for six months. Two monkeys exposed to 10 mg/kg and three monkeys exposed to 20 mg/kg were monitored for 21 weeks (recovery period) following dosing. Urine and fecal samples were collected at two-week intervals and were analyzed for PFOA concentrations.

Urine PFOA concentrations over the duration of the study were 53 ± 25, 166 ± 83, and 181 ± 100 μg/ml in the 3, 10, and 30/20 mg/kg dose groups, respectively, and reached steady-state after 4 weeks. Within two weeks of recovery, urine PFOA concentrations were <1% of the value measured during treatment and decreased slowly thereafter. Fecal PFOA concentrations were 6.8 ± 5.3, 28 ± 20, and 50 ± 33 μg/g in the 3, 10, and 20 mg/kg dose groups, respectively. Within two weeks of recovery, fecal PFOA concentrations dropped to less than 10% of the last value during treatment, and then declined slowly. These results are consistent with both renal and biliary excretion in male monkeys

Rat. There have been a number of studies of excretion in rats because of the gender differences noted in serum levels. Hinderliter (2004, 2006a) investigated the relationship between age and urine PFOA concentrations in male and female Sprague-Dawley rats. Immature rats 3, 4, or 5 weeks of age were administered PFOA via oral gavage as a single dose of 10 or 30 mg/kg. Two hours after dosing, 5 rats/sex/age group and dose group were sacrificed and blood samples were collected (See section 3.2). The remaining 5 rats/sex/ age and dose group were placed in metabolism cages for 24-hour urine collection. Urinary output (volume) was not quantified or standardized for creatinine levels.

Urine PFOA concentrations differed significantly with age, dose and sex ($p<0.01$, Table 3-18). Female rats had higher urine PFOA concentrations than males, and the female urine PFOA concentrations increased with age. In male rats, 24-hour urine PFOA concentrations decreased with age up to five weeks. In both sexes, urine PFOA was higher (2.5 to 6.5 times) at the 30 mg/kg dose as compared to the 10 mg/kg dose.

There was a difference in urinary excretion between the 3 week and 4/5 week male rats with the older rats excreting ~50% less PFOA in the urine compared to the younger rats at 10 mg/kg and 30 mg/kg. If the data from urine are integrated with the plasma data in the same study (Table 3-8), the male plasma levels increased from the 3-week value and were relatively stable for weeks 4 and 5. In the females, urine excretion increased gradually with age (Table 3-18) and plasma concentrations decreased (Table 3-9).

TABLE 3-18. Urine PFOA Concentrations in Male and Female Rats					
Age (weeks)	Dose (mg/kg)	Urine PFOA (μg/ml at 24 hours post-dose)			
		Male		Female	
		Mean	SD	Mean	SD
3	10	9.57	4.86	21.17	8.95
4	10	4.53	2.45	23.26	15.27
5	10	4.03	2.36	49.77	24.64
3	30	51.76	28.86	94.89	26.26
4	30	28.70	18.84	104.12	28.97
5	30	15.65	6.24	123.16	51.56

From Hinderliter, 2004

Hundley et al. (2006) examined excretion of PFOA in one male and one female CD rat (sexually mature). Each was given a single dose of 10 mg/kg ^{14}C-PFOA and housed in a metabolism cage. Urine and feces were collected at 12, 24, 48, 72, 96, and 120 hours post-dose. The female rat excreted more PFOA over the 120 hour collection period than the male rat. In the male rat, 25.6% and 9.2% ^{14}C-PFOA were excreted in the urine and feces, respectively. In the female rat, 73.9% and 27.8% ^{14}C-PFOA were excreted in the urine and feces, respectively. The female rat excreted almost all of the PFOA by 48 hours compared with only 19% of the dose excreted by the male rat over the same amount of time. The cumulative percent of the dose excreted is shown in Table 3-19.

TABLE 3-19. Cumulative Percent ^{14}C-PFOA Excreted in Urine and Feces by Rats						
	Hours After Dosing					
Rat	12	24	48	72	96	120
Male	0.6	8.7	19.2	23.4	30.2	34.3
Female	52.5	96.4	99.8	100.0	100.0	100.0

From Hundley et al., 2006

Adult male Sprague-Dawley rats (n=7) were given a single gavage dose of 0.5 mg PFOA/kg and monitored for 38 days (Benskin et al, 2009). Over the course of the study, the rats were held in metabolic cages and urine and feces were collected. The mean blood PFOA concentration was 1.1 μg/mL 24 hours post dose. During the first 24 hours post dose, 65% of PFOA was excreted in the urine; most of the PFOA that was not absorbed was excreted in the feces. After that time period, 91-95% of the daily excreted PFOA was eliminated in the urine. On day 3, the mean PFOA concentration in urine and feces were 265 ng/g and 28 ng/g. The half-life for elimination from plasma in male rats was 13.4 days.

Cui et al., (2010) exposed 2-month old male Sprague-Dawley rats (10/group) to PFOA (96% a.i.) at 0, 5, and 20 mg/kg/day once daily by gavage for 28 days. Urine and fecal samples were collected through use of metabolism cages at 24 hour intervals immediately following dosing on days 1, 2, 5, 7, 10, 14, 18, 21, 24, and 28 of the study. Daily urine volume and fecal weight were comparable across all groups throughout the study. As measured by excretion 24-hours after the first dose, 17.9% of the applied dose was excreted in the urine of the low-dose group and 22% for the high dose group. The percent of the absorbed dose was 92.8% and 92.3% for the low and high doses, respectively when the fecal excretion over the 24-hours following dosing was estimated to be unabsorbed material. During week 1, a sharp increase in urinary and

fecal excretion expressed as percent of dose/day was observed in rats of both groups. The excretion rate leveled off at about 50% for the low-dose animals for the remainder of the 28 days. In the case of the high-dose animals, the urinary excretion remained level at about 80% for the second and third weeks and then increased sharply to about 140% at 28 days. The fecal excretion rates were 7.2% and 7.7% for rats in the 5 and 20 mg/kg groups, respectively during the first 24 hours post dosing and continued an upward trend throughout the 28 days with the terminal percent/day about 25% for the low dose group and 40% for the high dose group.

Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination of ^{14}C after oral administration of a single dose of ^{14}C-PFOA (Gibson and Johnson, 1983). At 8 or 9 days after conception, four pregnant CD rats and two nonpregnant female CD rats were given a mean dose of 15 mg/kg ^{14}C-PFOA. Individual urine samples were collected at 12, 24, 36, and 48 hours post dose and analyzed for ^{14}C content. Essentially all of the ^{14}C was eliminated via the urine within 24 hours for both groups of rats

Other Species. Hundley et al. (2006) examined excretion of PFOA in CD mice, BIO-15.16 hamsters, and New Zealand White rabbits. One male and female of each species was given a single dose of 10 mg/kg ^{14}C-PFOA and housed in metabolism cages. Urine and feces were collected at 12, 24, 48, 72, 96, and 120 hours post-dose. Additional samples were collected from rabbits at 144 and 168 hours post-dose.

Over 120 hours, the male mouse excreted 3.4% and 8.3% ^{14}C-PFOA, and the female mouse excreted 6.7% and 5.7% ^{14}C-PFOA in urine and feces, respectively. The mice were similar in amount excreted. The male hamster excreted 90.3% and 8.2% ^{14}C-PFOA, and the female hamster excreted 45.3% and 9.3% ^{14}C-PFOA in urine and feces, respectively. The male hamster excreted a greater amount of ^{14}C-PFOA than the female hamster. Over 84% of ^{14}C-PFOA was excreted 24 hours after dosing by the male hamster compared to less than 25% of ^{14}C-PFOA excreted by the female hamster at 24 hours after dosing. Over 168 hours, the male rabbit excreted 76.8% and 4.2% ^{14}C-PFOA, and the female rabbit excreted 87.9% and 4.6% ^{14}C-PFOA in the urine and feces, respectively. Both rabbits excreted most of the dose by 24 hours. The cumulative percentage of ^{14}C-PFOA excreted is shown in Table 3-20.

TABLE 3-20. Cumulative Percent ^{14}C-PFOA Excreted in Urine and Feces

		Hours After Dosing						
Species	**Sex**	**12**	**24**	**48**	**72**	**96**	**120**	**168**
Mouse	Male	0.4	4.1	6.7	8.6	9.1	10.8	-
	Female	0.2	4.1	6.5	8.4	9.0	11.0	-
Hamster	Male	67.3	84.5	96.1	97.4	98.2	98.4	-
	Female	11.3	24.6	36.4	43.9	50.1	54.0	-
Rabbit	Male	77.8	80.2	80.4	80.4	80.4	80.4	80.4
	Female	86.7	90.5	92.0	92.2	92.7	92.9	93.0

From Hundley et al., 2006

Inhalation Exposure

Although no data were identified on urine or fecal excretion of PFOA following inhalation exposures, the Hinderliter (2003) study provides evidence of clearance following single and repeated inhalation exposures in Sprague-Dawley rats. Plasma PFOA concentrations

following a single exposure to 1, 10, or 25 mg/m^3 PFOA declined 1 hour after exposure in females and 6 hours after exposure in males. In females, the elimination of PFOA was rapid at all exposure levels, and by 12 hours after exposure the plasma levels had dropped below the analytical limit of quantitation (0.1 μg/ml). In males, the plasma elimination was much slower and at 24 hours after exposure, the plasma concentrations were approximately 90% of the peak concentrations at all exposure levels. In the repeated exposure study, male and female rats were exposed to the same concentrations for 6 hr/day, 5 days/week for 3 weeks. Steady state plasma levels were reached in males by 3 weeks, but plasma PFOA levels in females returned to baseline with 24 hours of each dose. The data are illustrative of distinct toxicokinetic differences between male and female rats in their response to PFOA exposure (Hinderliter, 2003).

Dermal Exposure

No data were identified on excretion following dermal exposures. Little to no fecal excretion is anticipated for the dermal route of exposure.

3.4.1 Mechanistic Studies of Renal Excretion

Several studies have been conducted to elucidate the cause of the gender difference in the elimination of PFOA for rats. Many of the studies have focused on the role of transporters in the kidney tubules. Most studies have examined the OAT transporters located in the proximal portion of the descending tubule. They are located in other tissues as well and were discussed earlier for their role in absorption and distribution. In the kidney they are responsible for delivery of organic anions including a large number of medications from the serum into the kidney tubule for excretion as well as reabsorption of anions from the glomerular filtrate. The transporters are particularly important in excretion of PFOA because its binding to surfaces of serum proteins (particularly albumin) make much of it unavailable for removal during glomerular filtration. Other transporter families believed to be involved in renal excretion are the OATps and the MRPs. However, they have not been evaluated as extensively as the OATs for their role in the kidney.

OATs are located on both the basolateral (serum interphase) and apical surfaces of the brush boarder of the proximal tubule inner surface. At the basolateral surface the OATs transport the perfluorooctanoate anion from the serum to the tubular cells (Anzai et al, 2006; Cheng and Klaassen 2008; Nakagawa et al. 2007, 2009; Klaassen and Lu 2008). OATs 1, 2, and 3 are located on the basolateral membrane surface. The OAT 4 and 5 transporters are located on the apical surface of the tubular cells where they function to reabsorb the PFOA anions from the glomerular filtrate. Figure 3.2 diagrams the flow of organic anions such as PFOA anion from serum to the glomerular filtrate for excretion and resorption of organic acids from the glomerular filtrate with transport back to serum. OATs can function for uptake into the cell across both the basolateral and apical surfaces.

There are several MRP transporters that also appear to function in the kidney and move organic anions in and out of cells at both the basolateral (e.g. MRP2/4) and apical (e.g. MRP1) surfaces (Launay-Vacher et al., 2006; Klaassen and Lu, 2008; Cheng and Klaassen, 2009; Kusuhara and Sugiyama, 2009) and one or more OATps on each surface (Cheng and Klaassen,

2009; Kusuhara and Sugiyama, 2009; Yang et al., 2009). Bidirectional movement of PFOA across both the basolateral and apical surfaces can occur as driven by concentration gradients and/or active transport. There are far more data on the PFOA and OATs in the kidneys than on OATps and MRPs. Abreviations for individual transporters on the basolateral and apical surfaces differ across publications.

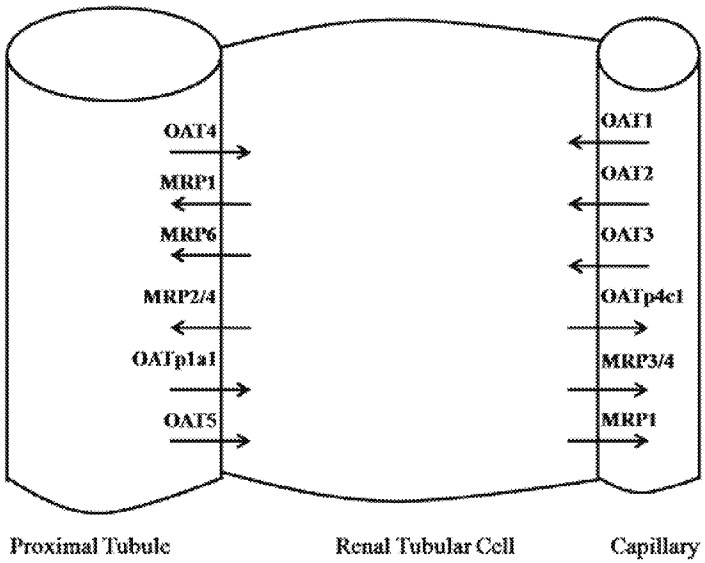


FIGURE 3-2. Renal Organic Acid Transporters

From Anzai et al., 2006; Luanay-Vacher et al., 2006; Cheng and Klaassen, 2006; Klaassen and Lu, 2007; Cheng et al., 2008; Zaïr et al., 2008; Kusuhara and Sugiyama, 2009

Knowledge about specific OAT, OATp and MRP transporters in the kidneys is rapidly evolving. A low membrane density or blockage of basolateral OATs will decrease PFOA excretion while low membrane densities or blockage of apical OATs will increase excretion because they decrease resorption of anions from the glomerular filtrate.

The earliest studies of the impact of gender on urinary excretion were conducted by Hanhijarvi et al (1982) using probenecid, an inhibitor of renal excretion of organic acids on PFOA excretion in male and female Holtzman rats. The female rats that had not received the probenecid excreted 76% of the administered dose of PFOA, while males excreted only 7.8% of the administered dose over a 7-hr period. Probenecid administration modified the cumulative excretion curve for males only slightly. However, in females probenecid markedly reduced PFOA elimination to 11.8%. The authors concluded that the female rat possesses an active secretory mechanism which rapidly eliminates PFOA from the body while male rats do not.

Kudo et al. (2002) examined the role of sex hormones and organic anion transporters on the renal clearance (CL_R) of PFOA. Renal mRNA levels of specific organic anion transporters in castrated male and ovariectomized female Wistar rats were also determined. Castration of male rats caused a 14-fold increase in CL_R of PFOA. The elevated PFOA CL_R in castrated males was reduced by treating them with testosterone. Treatment of male rats with estradiol increased the CL_R of PFOA. In female rats, ovariectomy caused a significant increase in CL_R of PFOA (2-fold

increase), but the administration of estradiol to ovariectomized female rats returned CL_R of PFOA to normal values. Treatments of female rats with testosterone reduced the CL_R of PFOA.

Treatment with probenecid, a known inhibitor of OATs 1-6 and 8, markedly reduced the CL_R of PFOA in male rats, castrated male rats, and female rats (Kudo et al., 2002). Accordingly, the male sex hormones appear to decrease the presence of OATs in the renal basolateral membranes while the female sex hormones appear to increase the receptors.

To identify the transporter molecules that are responsible for PFOA transport in the rat kidney, renal mRNA levels of specific organic anion transporters were determined in male and female rats under various hormonal states and compared with the CL_R of PFOA (Kudo et al. 2002). The level of OAT2 mRNA in male rats was only 13% that in female rats. Castration or estradiol treatment increased the level of OAT2 mRNA whereas treatment of castrated male rats with testosterone reduced it. Ovariectomy of female rats significantly increased the level of OAT3 mRNA. Multiple regression analysis of the data suggested that OAT2 and OAT3 are responsible for urinary elimination of PFOA in the rat; however, the possibility of a resorption process mediated by the organic anion-transporting polypeptide (OATp1) was mentioned as a possible factor in male rat retention of PFOA. OAT2 and OAT3 are located on the basolateral cell surface. OATp1 is located on the apical surface of the renal tubule cells.

Cheng et al. (2006) examined whether sex hormones influenced gender specific OATp expression in the kidneys of adult male and female C57BL/6 mice. Gonadectomized mice were used for these studies in conjunction with hormone replacement measures (5α-dihydroxy-testosterone (DHT) or 17-β estradiol (E2)). OATp1a1 and OATp3a1 were evaluated. Treatment with DHT resulted in significant increase in both OATps in the kidneys of male and female gonadectomized mice. In both cases, the change in males was greater than that in the females. Treatment with E2 almost abolished the expression of OATp1a1 in the kidneys and caused no significant change in OATp3a1. In the intact control animals there was almost no expression of OATp1a1 in the kidneys of females and a significantly lower ($p<0.05$) expression of OATp3a1. In the gonadectomized control animals, there was litle or no expression of OATp1a1 in either sex, and expression of OATp3a1 was equivalent in both sexes.

Nakagawa et al. (2007) investigated the role of OATs in the renal excretion of PFOA using in vitro methods. HEK293 transformed cells, derived from human embryonic kidney, were transfected with human or rat OAT1, OAT2, or OAT3 constructs. Cells from the S2 segment of the proximal tubule were transfected with human or rat OAT2 constructs. HEK293 and S2 cells transfected with the vector only served as control cells. The transfected HEK293 cells were incubated for one minute with or without 0, 10, or 100 μM [^{14}C]PFOA and/or varying concentrations of favored OAT substrates as follows: 5 μM [^{14}C]para-aminohippuric acid (OAT1), 20 nM [^{14}C]estrone sulfate (OAT3), or 10 nM [^{14}C]prostaglandin $F_{2\alpha}$ (OAT2) to determine inhibitory effects of PFOA.

PFOA significantly inhibited para-aminohippuric acid and estrone sulfate uptake mediated by OAT1 and OAT3, respectively. At 10 μM PFOA, uptake of 5 μM [^{14}C]para-aminohippuric acid was inhibited by 75-85% compared to control, and 100 μM PFOA inhibited it to 35-45% of control. Estrone sulfate uptake by human OAT3 was inhibited by 65% compared to control at 10 μM PFOA and 40% of control at 100 μM PFOA. Estrone sulfate uptake by rat

OAT3 was inhibited to 15% of control in the presence of 10 μM PFOA and by 100% at 100 μM PFOA. Prostaglandin $F_{2\alpha}$ uptake by OAT2 was inhibited moderately by PFOA, 75-85% of control at 10 μM PFOA and 65% of control at 100 μM PFOA.

In the second part of their study Nakagawa et al. (2007) incubated HEK293 and S2 transfected cells with 10 μM [^{14}C]PFOA for 1 minute to determine uptake. Time dependent uptake of 5 μM [^{14}C]PFOA from 0-30 minutes was conducted in the HEK293 cells transfected with human or rat OAT1 OAT2 or OAT3. Experiments were conducted in triplicate. Uptake of PFOA was stimulated ($p<0.001$) in cells transfected with human or rat OAT1 or OAT3, while no uptake was stimulated in cells transfected with OAT2 in either cell line. In the time dependent experiments, uptake by human or rat OAT1 or OAT3 increased linearly up to 2 minutes and reached a plateau in about 15 minutes. Kinetic evaluations resulted in K_m (substrate concentration at which the initial reaction rate is half maximal) values of 48.0, 51.0, 49.1, and 80.2 μM for human OAT1, rat OAT1, human OAT3, and rat OAT3, respectively. The authors showed that both human and rat OAT1 and OAT3 transport PFOA in the kidney while human and rat OAT2 does not.

Yang et al. (2009) investigated the role of organic anion transporter polypeptide 1a1 (OATp1a1) in the renal elimination of PFOA. The polypeptide is located on the apical side of proximal tubule cells and may be the mechanism for renal reabsorption of PFOA in rats. The level of mRNA of OATp1a1 in male rat kidney is 5-20-fold higher than in female rat kidney, OATp1a1 protein expression is higher in male rat kidneys, and it is regulated by sex hormones. One of its known substrates is estrone-3-sulfate (E3S). A substantial presence of OATp1a1 in male rats would favor resorption of PFOA in the glomerular filtrate and reduce excretion. Chinese hamster ovary (CHO) cells were transfected with rat OATp1a1 cDNA. The transfected CHO cells were incubated with 4 μM [^{14}C]PFOA for up to 10 minutes or with 0-1000 μM [^{14}C]PFOA for 2 minutes to determine uptake. The difference between the uptake velocities of CHO OATp1a1-transfected cells and CHO vector-transfected cells was defined as active PFOA uptake by the tubular epithelium. The transfected CHO cells were incubated with 5 μM [^{14}C]PFOA for 2 minutes in the absence or presence of inhibitors (sulfobromophthalein, taurocholate, probenecid, *p*-aminohippurate, and naringin) for inhibition studies. The transfected CHO cells were incubated with 2 μM E3S and 0, 0.1, or 1 mM perfluorocarboxylates with carbon chain lengths ranging from 4 to 12 including PFOA (C8) for 30 seconds for E3S inhibition studies.

In time-dependent uptake experiments, uptake of PFOA by OATp1a1-transfected cells increased proportionally to time during the first 2 minutes of incubation. Vector-transfected cells had a significant level of uptake of PFOA attributed to non-specific passive diffusion. In the concentration-dependent uptake experiments, uptake velocity of PFOA in OATp1a1-transfected cells increased with increasing concentration and saturation levels were not reached. In vector-transfected cells, uptake velocities increased linearly with increasing concentration of PFOA demonstrating a passive diffusion mechanism. Active PFOA uptake, the difference between the uptake of the OATp1a1 cells and the vector-transfected cells, was described by the Michaelis-Menton equation and exhibited saturable kinetics.

Inhibition experiments with substrates of OATs and OATps showed that sulfobromophthalein, taurocholate, and naringin inhibited PFOA uptake to 10-30% of control

and *p*-aminohippurate inhibited PFOA uptake to 62% of control. Probenecid, an OAT inhibitor, did not inhibit PFOA uptake at all. In OATp1a1-transfected cells, uptake of E3S was inhibited to less than 10% of control uptake following incubation with 1 mM [^{14}C]PFOA. Inhibition of E3S was less than 50% of control uptake after incubation with 0.1 mM [^{14}C]PFOA. Based on the results of the uptake and inhibition experiments, the authors suggested that passive diffusion may be an important route of PFOA distribution, and that renal reabsorption in the male rat may be mediated by OATp1a1.

Nakagawa et al. (2009) investigated the role that the human organic acid transporter (OAT4) plays in transporting PFOA. Human OAT4 is located on the apical side of proximal tubule cells and mediates re-absorption of organic anions. Transformed cells derived from human embryonic kidney cells, HEK293, were transfected with human OAT1, OAT3, or OAT4 constructs. HEK293 cells transfected with only the vector served as control cells. The transfected HEK293 cells were incubated with 10 μM [^{14}C]PFOA for 15 minutes to determine uptake. Transfected cells were also incubated with 10 μM [^{14}C]PFOA for 15 minutes and then washed with incubation medium containing 1%, 3%, and 5% BSA to investigate the contribution of non-specific binding of PFOA on the cell membrane. Experiments were conducted in triplicate.

Uptake of PFOA was significantly stimulated ($p<0.01$) in cells transfected with human OAT1, OAT3, and OAT4. Uptake of PFOA in human OAT1 transfected cells was 1.6 fold higher than in control cells. In human OAT3 cells, PFOA uptake was ~2.4 fold higher than in control cells. In human OAT4 transfected cells, PFOA uptake was 2.7 fold higher than in control cells. Accumulation of PFOA in transfected human OAT4 cells was also significantly greater than in human OAT1 cells ($p<0.01$). Washing the cells with BSA reduced PFOA uptake by 30% at most, suggesting mediation by the transporters into the transfected cells. The experiments showed that human OAT4 transports PFOA and that human OAT4 activity may play a role in reabsorption of PFOA from the tubule resulting in poor urinary excretion.

Yang et al. (2010) examined cellular uptake of PFOA by OATp1A2, OAT4, and URAT1 to determine their roles in mediating human renal reabsorption. Chinese hamster ovary (CHO) and HEK293 cells were transfected with OATp1A2, OAT4, and URAT1 plasmid DNA or vector DNA (control). In uptake studies, PFOA incubation times were 10 seconds (OAT4) or 30 seconds (URAT1). Cells transfected with OAT4 were incubated with 5 μM PFOA for up to 1 minute in time-dependent uptake experiments. In inhibition studies, cells transfected with OAT4 were incubated with 5 μM C^{14}-PFOA for 10 seconds in the presence and absence of 100 μM BSP, probenecid, glutarate, or polycyclic aromatic hydrocarbon (PAH). Perfluorochemicals with differing chain lengths (C4-C12) were used in chain-length dependent inhibition experiments. Incubations with ^{3}H-E3S (OAT4, OATp1A2) or 6 μM C^{14}-uric acid(URAT1) in the presence and absence of 100 μM perfluorinated carboxylate acids (PFCs) lasted 10 seconds (OAT4), 30 seconds (OATp1A2), or 1 minute (URAT1).

PFOA uptake in OATp1A2 transfected HEK293 cells was not different than uptake in control cells. At 100 μM PFCs, E3S uptake was inhibited ~30% by PFOA (C8), ~62% by C9, ~70% by C10, ~42% by C11, and ~18% by C12. E3S uptake was not inhibited by C4-C7. In CHO cells transfected with OAT4, time-dependent uptake experiments showed a saturation phase after an incubation time of approximately 10 seconds. A pH-dependent increase in PFOA

uptake was observed with approximately 90% uptake at pH 8 and 250% at pH 5.5. In concentration-dependent uptake experiments, uptake increased with increasing PFOA concentration (0-1000 μM) in OAT4-transfected CHO cells at pH 7.4 and 6. PFOA uptake was cis-inhibited by BSP and probenecid and trans-stimulated by PAH and glutarate at pH 7.4. A chain length-dependent effect was observed in E3S inhibition on OAT4–expressing cells in the presence of C7 (30%) through C10 (~80%). Inhibition in the presence of C11 and C12 were ~52% and ~30%, respectively. Inhibition of E3S in the presence of C4, C5 and C6 was less than 20% for each. PFOA uptake in HEK293 cells transfected with URAT1 was not statistically different from control cells in the presence and absence of Cl^-. Under both conditions, PFOA intake was enhanced especially in the absence of Cl^- in which PFOA uptake was greater than 4-fold compared to uptake in control cells. Time-dependent PFOA (5 μM) uptake by URAT1 increased with time during the 5 minute incubation period, and a concentration-dependent increase in PFOA uptake was observed (0-700 μM). Urate uptake was inhibited in a chain length-dependent manner. Inhibition in the presence of C7-C10 was ~70% each, ~60% in the presence of C6 and C11, ~50% in the presence of C5, ~30% in the presence of C12, and ~25% in the presence of C4. Based on the results, the authors concluded that PFOA was not a substrate for OATp1A2, but that OAT4 and URAT1 were probably involved in the renal reabsorption of PFOA.

Weaver et al. (2010) published in vitro studies on the transport activities of the rat renal transporters OAT1, OAT2, OAT3, OATp1a1, and URAT1. The transporters were transfected into one of several cell lines and exposed to a series of PFCs having chain lengths ranging from 2 to 18 carbons (C). The activity of the PFC on the transporters was quantified on the basis of its ability to inhibit the transport of a favored radiolabeled substrate. The PFC inhibition of the individual transporters varied with chain length. The PFCs with 6, 7, and 8 carbon chains caused a significant decrease in OAT1 transport of tritiated p-aminohippurate with the C7 acid having the strongest effect. The PFCs with 5- through 10- carbon chains caused a significant decrease in transport of tritiated estrone-3-sulfate by OAT3 with C8 and C9 acids having the strongest effect. The transport of tritiated estadiol-17β-glucuronide by OATp1a1 was significantly inhibited by PFCs with 6 through 11 carbon chains with C10 acid having the strongest effect. The PFCs did not inhibit OAT2 or URAT1 transport of favored substrates.

The kinetic response of the OAT1, OAT3, and OATp1a1 transporters to increasing concentrations of selected PFCs was also evaluated by Weaver et al. (2010). The change in transport velocity (ng/mg protein/min) with increasing concentrations of the PFCs exhibited a Michaelis-Menton type response. The kinetic data were analyzed to determine the K_m and V_{max}, and those data are summarized in Table 3-21 below.

TABLE 3-21. Kinetic Parameters of PFC Transport by OAT1, OAT3 and OATp1a1

Transporter	PFC	Km (μM)	Vmax (nmol/mg protein/min)
OAT 1	C7	50.5 ± 13.9	2.2 ± 0.2
	C8	43.2 ± 15.5	2.6 ± 0.3
OAT 3	C8	65.7 ± 12.1	3.8 ± 0.5
	C9	174.5 ± 32.4	8.7 ± 0.7
OATp1a1	C8	126.4 ± 23.9	9.3 ± 1.4
	C9	20.5 ± 6.8	3.6 ± 0.5
	C10	28.5 ± 5.6	3.8 ± 0.3

From Weaver et al., 2010

The Michaelis-Menton kinetic data [K_m and V_{max} (maximum initial rate of an enzyme catalyzed reaction)] indicate that there are substantial differences in the affinity of the PFCs with 8 and 9 carbon chains for OAT3, with the C8 acid favored over the C9 acid. OAT3 is an export transporter located on the basolateral side of the tubular cells; thus when present in a mixture consisting of comparable concentrations of both, renal tubular excretion of the C8 acid would tend to decrease excretion of the C9 acid. For OATp1a1, a resorption transporter located on the apical side of the renal tubular cells, the C9 and C10 acid have a greater affinity for the transport protein than the C8 acid. The kinetic data suggest that the net impact of these relationships would be to favor excretion of the C8 acid over the C9 acid and possibly the C10 acid when all three fluorocarbons are present in the exposure matrix at approximately equal concentrations. There were minimal kinetic differences between transport of the C7 and C8 acids by OAT1, an export transporter on the basolateral surface of the renal tubular cells.

Based on the Hinderliter (2004) study, a developmental change in renal transport occurs in female rats between 3 and 5 weeks of age that allows for expedited excretion of PFOA. When the transporters become active, there is a decrease in plasma PFOA levels and an increase in urinary excretion (Table 3-22). The developmental change in male rats appears to have the opposite effect. Sexual maturity appears to influence these events because castrated male rats become more like females and ovariectomized females become more like males in their PFOA excretion capabilities. The change in female rats seems to involve the OATs (Kudo et al., 2002) while that in males seems to involve the OATps (Cheng et al., 2006).

TABLE 3-22. Plasma and Urine PFOA Concentration 24 hr After Treatment with 30 mg/kg PFOA

Age (weeks)	Female		Male	
	Plasma (µg/ml)	Urine (µg/ml)	Plasma (µg/ml)	Urine (µg/ml)
3	51.43±13.61	94.89±26.26	74.16±18.23	51.76±28.86
4	28.01±9.90	104.12±28.97	100.81±13.18	28.70±18.84
5	3.42±1.95	123.16±51.56	113.86±23.36	15.65±6.24

From Hinderliter (2004)

When considered together, the studies of the transporters suggest that female rats are efficient in transporting PFOA across the basolateral and apical membranes of the proximal kidney tubules into the glomerular filtrate but male rats are not. Males, on the other hand, have a higher rate of resorption for the smaller amount they can transport into the glomerular filtrate via OATp1a1 in the apical membrane than females. This scenario may explain the inverse relationship between the levels of PFOA in female urine and plasma and the plateau of plasma PFOA in male rats when compared to their losses via urine.

Unfortunately much work remains to be done in order to explain the gender differences between male and female rats and to determine whether it is relevant to humans. Similarities are possible because of the long half-life in humans suggests that they may be more like the male rat rather than the female rat. There is a broad range of half-lives in human epidemiology studies suggesting a variability in human transport capabilities resulting from genetic variations in structures and consequently in function. Genetic variations in human OATs and OATps have been identified as described in a review by Zaïr et al. (2008).

3.5 Toxicokinetic Considerations

3.5.1 PK Models

One of the earliest PK models (Andersen et al., 2006) was done using the post-dosing plasma data from the Butenhoff et al. (2004b) study in cynomolgus monkeys. In this study, groups of 6 monkeys (3/sex/group) were dosed for 26 weeks with 0, 3, 10, or 20 mg/kg PFOA (high-dose =30 mg/kg PFOA for the first 12 days) and followed for >160 days after dosing. Metabolism cages were used for overnight urine collection. Since urine specimens could account for only overnight PFOA excretion, the total volume and total PFOA were extrapolated to 24-hour values based on the excretion rate (volume/hour) for the volume collected and the hours of collection.

The Andersen et al. (2006) model was based on the hypothesis that saturable resorption capacity in the kidney would possibly account for the unique half-life properties of PFOA across species and genders. The model structure (Figure 3-3; Andersen et al.2006) was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells.

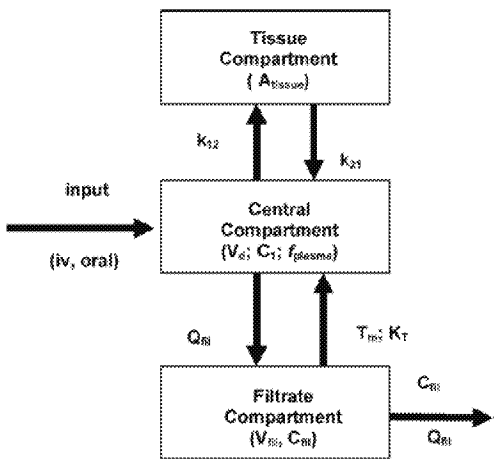


FIGURE 3-3. Schematic for a physiologically-motivated renal resorptions pharmacokinetic model.

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption and transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was less resorption and greater excretion at high serum PFOA concentrations than at low concentrations.

The model was parameterized using the body weight and urine output of cynomolgus monkeys (Butenhoff et al., 2004) and a cardiac output of 15 L/h-kg from the literature (Corley et al., 1990). A 20% blood flow rate to the kidney was assumed based on data from humans and dogs. Other parameters were optimized to fit the data for plasma and urine at lower concentrations and then applied for the 20 mg/kg/day dose which was assumed to represent a concentration where renal resorption was saturated. Based on the data for the dose of 20

mg/kg/day, the model was able to predict the decline in plasma levels after the cessation of dosing. The predictions were fairly adequate for one of the three modeled monkeys; for the other two monkeys, the model predicted higher levels than were observed. This could be because the model did not allow for efflux of PFOA into the glomerular filtrate through transporters on the basolateral surface of the tubular cells. The authors observed that three monkeys had faster renal clearance of PFOA than the other three monkeys.

Tan et al. (2008) divided the second compartment in the Anderson et al. (2006) model into a liver compartment and a tissue compartment. A storage compartment was added between the filtrate compartment and urinary excretion (Figure 3-4; Tan et al., 2008).

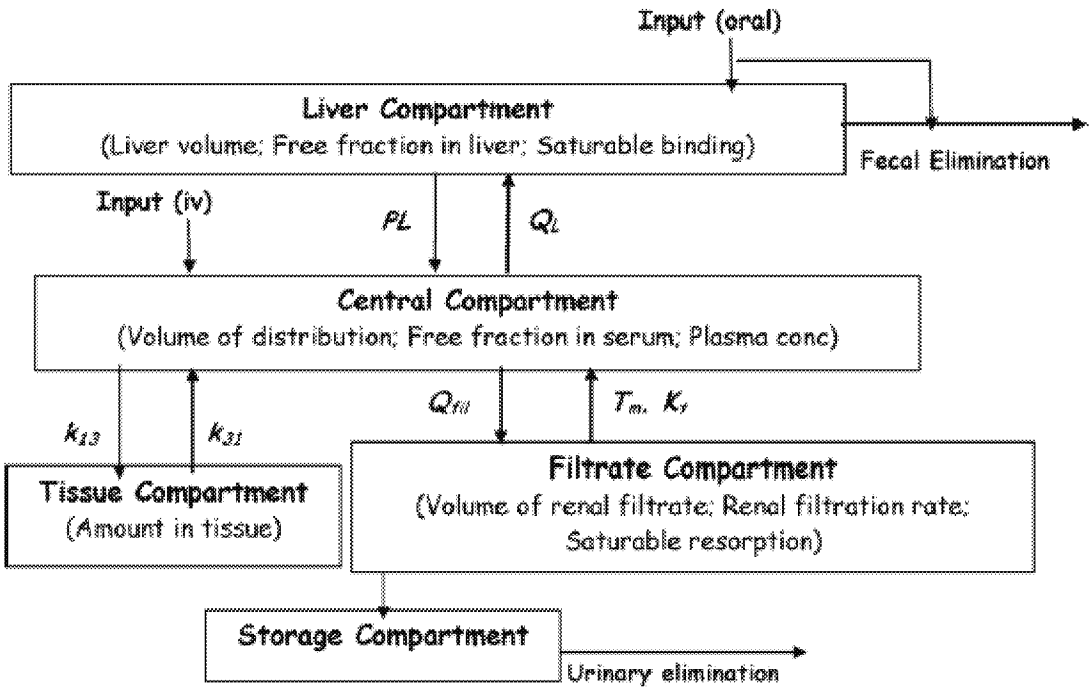


FIGURE 3-4. Physiologically-motivated pharmacokinetic model schematic for PFOA-exposed rats

The models were parameterized and applied to the Kemper (2003) data for male and female CD rats given doses of 1, 5, or 25 mg/kg/day. The model did not provide a satisfactory fit between the predictions of plasma concentration or urine + fecal excretion and experimental data for either sex.

Lou et al. (2009) utilized the data they collected on the serum, liver, and kidney PFOA concentration (see section 3.2) in CD-1 mice to examine if one (Figure 3-5) or two compartment pharmacokinetic (PK) models would fit the experimental data for 1, 10, and 60 mg/kg/day single gavage doses. Both models assumed first order absorption and elimination. The two compartment model included a central compartment that received PFOA after absorption and transferred it to a second compartment for excretion. The excretion compartment was coupled with bidirectional flow between the two compartments. The net loss from the central compartment differed during and after distribution. The models were fit using a general nonlinear squares approach. A likelihood ratio squared approach was applied to determine which model achieved the best fit to the data.

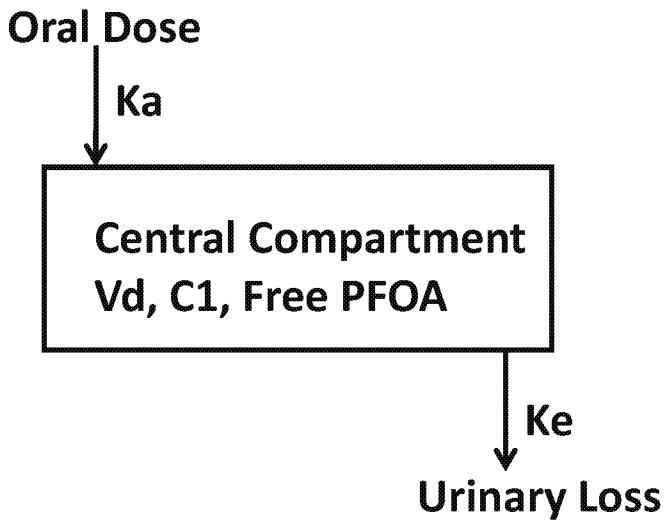


FIGURE 3-5. Schematic for one compartment model.

The one compartment model performed well for serum, liver, and kidney in this analysis and output was not significantly improved with use of a two compartment model. The input parameters for the one compartment model included volume of distribution (Vd), serum half-life, and absorption (Ka) and elimination (Ke) rate constants for serum, liver, and kidney. There were slight differences in the fitted values between males and females for some parameters. The Ke values were consistently higher in the female mice (Table 3-23). The quantitative measures for liver and kidney were only available for the 1 and 10 mg/kg/day doses.

TABLE 3-23. Model Parameters for 1 and 10 mg/kg Single Doses of PFOA to CD1 Mice

Tissue	Parameter (abbreviation)	Females	Males
Serum	Volume of distribution (Vd)	0.135 L/kg	0.266 L/kg
	Adsorption rate constant (Ka)	0.537 L/hr	
	Elimination rate constant (Ke)	0.00185 L/hr	0.00133 L/hr
	Half life (T ½)	15.6 days	21.7 days
Liver	Volume of distribution (Vd)	0.161 L/kg	0.120 L/kg
	Adsorption rate constant (Ka)	0.5170 L/hr	
	Elimination rate constant (Ke)	0.00161 L/hr	0.00129 L/hr
Kidney	Volume of distribution (Vd) – 1 mg/kg	0.822 L/kg	1.280 L/kg
	Volume of distribution (Vd) - 10 mg/kg	1.092 L/kg	1.170 L/kg
	Adsorption rate constant (Ka)	0.527 L/hr	
	Elimination rate constant (Ke)	0.00151 L/hr	0.00113 L/hr

From Lou et al., 2009

The one compartment model described above was not able to predict serum concentration in female mice given a single 60 mg/kg dose. This suggests a change in kinetics with the 60 mg/kg dose compared to the 1 and 10 mg/kg doses. This conclusion is supported by comparison of the serum measurements made during the 30 day post dosing period for all three doses. The serum PFOA concentration at the 60 mg/kg dose declined more rapidly with time than serum

PFOA concentrations at the 1 and 10 mg/kg doses. For example, a serum concentration of about 0.4 mg/L was reached in about 28 days at 60 mg/kg dose, 61 days at 10 mg/kg, and 70 days at 1 mg/kg (values estimated from Figure 3, Lou et al., 2009). The one compartment model also produced a poor fit for the serum level measurements taken 24 hours after the cessation of a 17-day exposure to 20 mg/kg/day. The two compartment model provided a better fit with experimental serum concentration data for the single 60 mg/kg dose and the repeat 20 mg/kg/day dose, but the fit was still unsatisfactory.

The authors also tried the Andersen et al. (2006) renal-resorption model to determine if it provided an improved fit for the data. The Andersen et al. (2006) model fit to the data was superior to that of the one compartment and two compartment models of Lou et al. (2009) for the 60 mg/kg single dose and the 20 mg/kg/day repeat dose scenarios.

The Andersen et al. (2006) model includes a second tissue compartment that articulates with the central compartment but not the filtrate compartment. In addition to values for Vd, Ka, and Ke, the model includes values for cardiac output, volume for the renal filtrate, renal blood filtration rate, intercompartmental clearance, transport maximum, transport affinity constant, and the proportion of free PFOA in serum. With the exception of body weight and cardiac output, the input parameters for the model were either assumed (volume of renal filtrate and proportion of free serum PFOA) or optimized for the model. The wide confidence bounds around the optimized values are indicative of considerable parameter uncertainty.

The Lou et al. (2009) parameter estimates indicate that there may be several biological limitations to the Anderson et al. (2006) PK model for adult mice including the fact that it requires an unreasonably high portion of the cardiac output to pass through the kidneys in order to optimize fit to the experimental data. It also does not include excretion via export transporters in the renal tubular cells and does not consider that the bound fraction in the serum could vary with the magnitude of the dose and duration of dosing. Much of the emerging data is consistent with a variety of tubular transporters functioning in both efflux and resorption from the glomerular filtrate. In addition, there may also be opportunities for protein binding within organs, including the liver, which could function to retard distribution especially at low doses with changes occurring when binding sites become saturated.

A model has also been developed that applied to female CD-1 mice during gestation and lactation (Rodriguez et al., 2009). The gestational model includes two compartments, one for the dam and the other for the litter. They are linked by placental blood flow. The biological data used to set the parameters for the two compartments were based on the data from the Lau et al. (2006) and Abbott et al. (2007) studies in CD-1 and 129S1/SvlmJ mice, respectively. Exposure was assumed to be limited by blood flow, and only the experimental doses that did not impact litter size (0.1-1.0 mg/kg/day for CD-1 mice and 1-10 mg/kg/day for 129S1/SvlmJ mice) were used in model development.

Lactational exposure was modeled as a dynamic relationship between the dam (n=10) and the litter, and were connected by a milk compartment. Milk yield information was obtained from the literature. Milk was assumed to be consumed as it was produced without any circadian impact on consumption patterns. PFOA excreted in pup urine was routed back to the dam.

Both adsorption and excretion were assumed to be first order processes as was lactation transfer from the dam to the litter (Figure 3-6; Rodriguez et al., 2009). Resorption of a portion of the PFOA urinary efflux was included in the model. The renal excretion/resorption was parameterized for cardiac output, kidney blood flow, glomerular filtration rate, urine flow rate, volume of renal plasma (fraction of body weight), and volume of renal filtrate (fraction of body weight). The fraction of free PFOA in serum reaching the glomerulus was assumed to be 0.01 based on protein binding information. As was the case with the Lou et al. (2009) model, Rodriguez et al. (2009) did not include parameters to adjust for transporter-mediated efflux from the renal tubular cells into the glomerular filtrate.

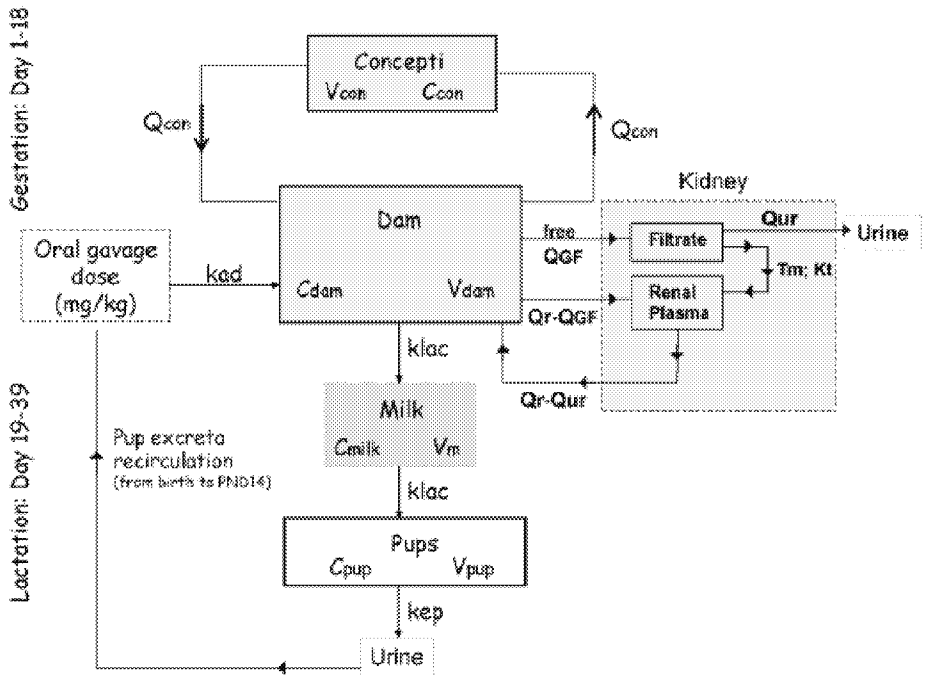


FIGURE 3-6. Pharmacokinetic model of gestation and lactation in mice.

One of the limitations of the Rodriguez et al. (2009) modeling effort was the limited amount of laboratory data against which to evaluate projections. Serum measures from the Lau et al. (2006) and Abbott et al. (2007) studies were available for only a few time points. Nevertheless, the authors reached several conclusions based on the model projections as follows:

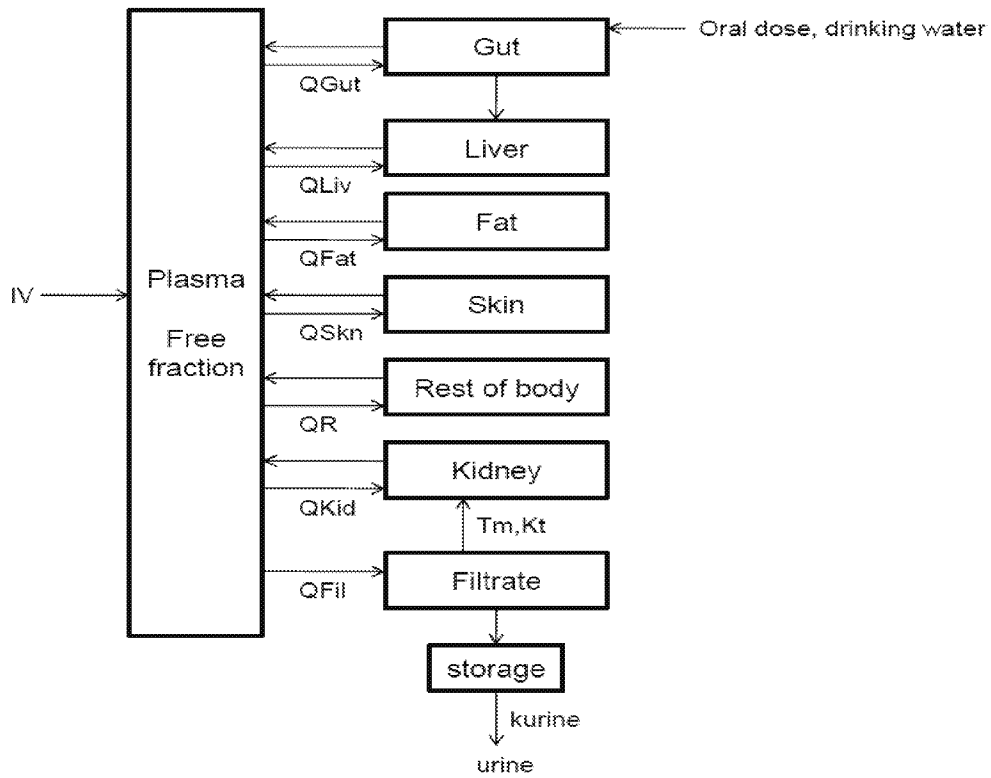
- The model had a tendency to overestimate serum levels suggesting nonlinearity as doses increased.
- Gestation and lactation as a source of exposure contributed about equally to the pups of 129S1/SvlmJ dams exposed only during gestation.
- The contributions to the pups from gestation exceeded those from lactation in the CD-1 mice.
- Exposure to the pups via lactation increased over time.
- Lactation is a clearance pathway for the dam.

The authors caution that the model is accompanied by a number of uncertainties because of the assumptions regarding the flow limitation on transport to the fetus and to maternal milk, the first order elimination rate constant for the pups, the milk: maternal serum partition

coefficient, and the limited knowledge regarding the renal tubular transporters. They caution that the model should not be applied for cross-species or high to low dose extrapolation.

Loccisano et al. (2011) developed a PFOA physiologically-based pharmacokinetic (PBPK) model for monkeys based on the Anderson et al (2006) and Tan et al. (2008) models, and then extrapolated it for use in humans (Figure 3-7). The model reflects saturable renal absorption of urinary PFOA by the proximal tubule of the kidney. This is represented in Figure 3-7 by the interactions between the plasma and kidney plus the interaction of the filtrate compartment with both plasma and kidney.

The fraction of PFOA free in plasma and available for glomerular filtration was based on data fit and estimated to be less than 10% because of binding to serum proteins, especially albumin. Lacking the kinetic data on tubular resorption, the rate was based on the best fit to the plasma/urine data. A storage compartment was added to the model between filtrate and urine. Tissue plasma partition coefficients were derived from the data by Kudo et al. (2007) following the disposition pattern of a single IV dose to male Wistar rats.



Tm = transporter maximum, Kt= affinity constant and Q= flow in and out of tissues

FIGURE 3-7. Structure of the PFOA PBPK model in monkeys and humans

Existing i.v. and oral data sets from Butanhoff et al. (2004b) for the cynomolgus monkey were used to develop the monkey model. In the oral study (Section 3.2), animals were dosed for six months and followed for 90 days after dosing. Plasma and urine samples were analyzed periodically during dosing and recovery. The model projections for the oral study were in good agreement with the Butanhoff et al. (2004b) data for the 10 mg/kg dose showing a rapid rise to

plasma steady state and a slow terminal half-life. The model performance for the high dose (30/20 mg/kg/day) did not fit as well, partially as consequence of the observed toxicity with the initial 30 mg/kg/day dose that necessitated cessation of dosing on study day 12, followed by resumption of dosing at 20 mg/kg/day on study day 22.

The structure of the human model was similar to that used for the monkeys. Human serum data (means with standard deviations or medians) for PFOA are available for occupational and general populations (Emmett et al., 2006; Calafat et al., 2007 a,b; Olsen et al., 2005; Hölzer et al., 2008; Steenland et al., 2009; Bartell et al., 2010). The fact that the serum data were the results from measurements made following uncertain routes and uncertain exposure durations presented a challenge in the assessment of model fit. The human half-lives used for the model (3.8 and 2.3 years) came from an occupational study (Olsen et al., 2005) and a study of the Little Hocking population after reduction of the PFOA in drinking water as a result of treatment (Bartell et al., 2010); see section 3.5.2. In each case the major source of exposure was assumed to be the drinking water. Both half-life values were used in evaluating the model's ability to predict serum concentration at the time the serum samples were collected.

The model produced results that can be characterized as fair to good when compared to the reported average serum measurements. For the Little Hocking Population studied by Emmett et al. (2006), the model indicated the need for a 30 year exposure to reach steady state concentrations. The model indicated that both half-life values provided reasonable results compared to the measured serum values. The authors concluded that more data are needed on the kinetics of renal transporters, intra-human variability, and more definitive information on exposures in order to further refine the human model.

Loccisano et al. (2012a) also utilized the saturable resorption hypothesis when developing a model for adult Sprague Dawley Rats (Figure 3-8). The structure of the model is similar to that for the monkey/human model depicted in Figure 3-7 but lacks the fat and skin compartments and includes a storage compartment to accommodate fecal loss of unabsorbed dietary PFOA as well as that from biliary secretions. Oral and IV data used in model development came from studies by Kemper (2003), Kudo et al. (2007) and Perkins et al. (2004). Partition coefficients for liver:plasma, kidney:plasma, and rest of the body:plasma were derived from unpublished data on mice by DePierre (2009) through personal communication with the authors. Most of the other kinetic parameters were based on values providing the best fit to the experimental data. Because a number of the renal transporters involved with PFOA resorption are known; available kinetic information was utilized where appropriate. Model performance was evaluated primarily based on its ability to predict plasma and liver concentrations from the studies identified above. As was the case for the monkey model, performance was generally good given the limitations in the primary data sources.

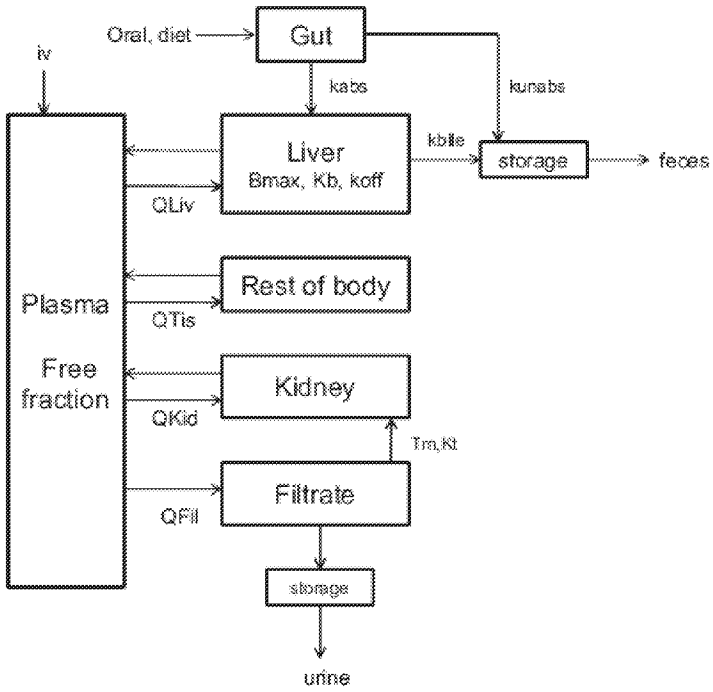


FIGURE 3-8. Structure of the PBPK Model for PFOA in the Adult Sprague Dawley Rat

Loccisano et al. (2012b) expanded the adult Sprague Dawley rat model described above to cover gestational and lactational exposure to the fetus and pups through their dams. The data from Hinderletter et al. (2005) were used in model development for both the gestation and lactation periods. The gestational model structure for the dams is similar to Figure 3-8. The model was expanded to include the fetuses linked to the dams by way of the placenta. Uptake from the placenta was described by simple diffusion; the fetal plasma compartment was separate from the dams as was distribution to fetal tissues and amniotic fluid. Based on the transporter data for PFOA, elimination differed for male and female rats and was considered to be developmentally regulated resulting in faster elimination for female fats than male rats after sexual maturation. The lactation model linked the pups to their dams through the mammary gland secretions. Pup compartments included the gut, liver, kidney, renal filtrate, plasma and rest of the body.

Model performance was judged by its ability to predict concentrations in maternal and fetal plasma, amniotic fluid and milk. The predictive capability of the model ranged from fair to good depending on the medium. The fit of the projections to the data was weakest for the whole embryo during gestation where measured levels were greater than projection for two of three data points and for neonate plasma during lactation where all data points fell below the predictions.

3.5.2 Half-Life Data

Human. There have been several studies of half-lives in humans and all support a long residence time for serum PFOA with estimates measured in years rather than months or weeks. Bartell et al. (2010) determined an average half-life of 2.3 years based on a study of the decreases in human serum levels after treatment of drinking water for PFOA removal was instituted by the Lubeck Public Services District and the Little Hocking Water Association in West Virginia (WV). Source waters for these systems had become contaminated with PFCs from the Dupont Works Plant in Washington, WV between 1951 and 2000.

The Bartell et al. (2010) study was based on a series of serum measurements (8 over 4 years) from 200 individuals who agreed to participate in the study. Inclusion criteria for the participants included: serum PFOA concentrations ≥ 50 ng/mL, residential water service provided by one of the two treatment plants, never employed at the Dupont plant, not growing their own vegetables, and signed acceptance of the study consent form. The participants were almost equally divided between males and females with an average age of about 50 years (range 18-89 years). Most of the participants consumed public tap water (172) as their primary cource, but a small number (28) consumed bottled water as their source.

The participants were required to report primary use of home tap water for cooking, bathing, and showering between 2005-2007. The tap water users had to report public water as their primary source of residential water consumption, and bottled water users had to report the use of bottled water as their primary source of residential water consumption. The initial blood draw for serum occurred in June 2007, with subsequent samples at 1, 2, 3, 6, and 12 months after the initial sample. Samples were analyzed by the Centers for Disease Control and Prevention (CDC), and 19 samples from the 2-month blood draw were not analyzed due to mislabeling.

A linear mixed model was used to determine the decline in serum PFOA concentration over time. With these models, the decline from baseline by the participants was essentially first order. The serum PFOA concentration was the only time-varying measurement entered into the model. Serum concentrations were log normally distributed, as described by the following equation:

$\ln C + \ln C0 - kt$
where C= serum concentration at time t
C0 = baseline serum concentration
k = elimination rate constant
t = time point for the measurement.

The results of this assessment showed a 26% decrease in PFOA concentration/year after adjustment for covariates and a half-life of 2.3 years [confidence interval (CI) = 2.1-2.4]. The covariates considered included the water treatment system, the time exposed before and after filtration, public vs. bottled water, sex, age, consumption of local or home grown vegetables, and exposure to the public water supply at work. The only potential confounders determined to be significant were the treatment plant ($p=0.03$) and home grown vegetable consumption ($p<0.001$).

Identification of consumption of home grown vegetables as a significant confounder revealed a weakness in the study design because it had been an exclusion factor, yet was identified as an exposure source at the 12-month interview of the study participants. The researchers concluded that this problem was a result of the way the exclusion question was phrased for the original interview, "Do you grow your own vegetables?". When the question was asked later in the study, it was phrased, "Do you eat any fruits and vegetables grown at your own home?" Some people who answered no to the original questions andwered yes to the second question.

Changes in the source of drinking water during the study could have also impacted the results. When baseline interview data were compared with the results from the 12 month interview, 39% of the bottled water group reported using public water at home. Some of the public water drinkers (10%) reported using primarily bottled water at the 6 month interview.

In another study, the drinking water supply was contaminated with a mixture of perfluorinated chemicals when a soil-improver mixed with industrial waste was applied upriver to agricultural lands in Arnsberg, Germany (Brede et al., 2011). The PFOA levels in the finished drinking water were measured as 500-640 ng/L in 2006. PFOS and PFHxS were also present. The plasma PFOA levels in the Arnesberg population were 4.5 to 8.3 times higher than those in a reference community at the time the problem was discovered. Charcoal filtration was added to the potable treatment train and succeeded in reducing the PFCs in the drinking water.

The authors used the differences in plasma 2008 PFOA measurements from a subset of the participants (children and adults) initially exposed in 2006 to determine the PFOA half-life. The 2008 subjects included 66 men women and children from Arnsberg and 73 from the reference community in their evaluation. The drinking water concentration monitoring results (not detects estimated as ½ the LOD, 10 ng/L) and drinking water intake estimates obtained by questionnaire and interview, were used to estimate PFOA exposures. Plasma PFOA samples were collected during a two month period in late 2008. Plasma PFOA had declined in the serum for both the Arnsberg residents (39.2%) and those from the reference community (13.4%). In Arnsberg the decrease was greater for the exposed women and children than the men when compared to the reference community, an observation that appeared to reflect the reported lower drinking water intakes of the Arnsberg women and children (0.3±0.2 and 0.8±0.6 L/day compared to 0.7±0.5 and 1.6±0.8 L/day, respectively). The estimate for the human half-life was 3.26 years (geometric mean; range 1.03-14.67 years). Regression analysis of the data also suggested that the elimination rate may have been greater in the younger subjects and older subjects.

Seals et al. (2011) determined half-life estimates for 602 residents of Little Hocking, OH and 971 residents of Lubeck, WV who were part of the C8 study but had relocated to a different area of the country. The half-life estimate was based on the decline in serum PFOA levels after the time of the initial measurement and the years since the change in residential location occurred. A background estimate (5 ng/ml) was subtracted from the serum measurements before analysis. On average, the initial serum PFOA concentrations were higher in Little Hocking (60.6 ng/mL) than in Lubeck (31.0 ng/mL). Due to the nonlinearity in scatter plots of the natural log for adjusted serum PFOA concentrations versus the years elapsed since relocation, the authors used a two segment (Little Hocking- 4 years, Lubeck-9 years) linear spline regression approach

in their analysis of the data. The slope of the line decreased for the second time segment as compared to the first. In former residents of Little Hocking, a -21.4% change in serum PFOA was observed in the first 4 years after leaving Little Hocking, and a -7.6% change was observed after 4 years. In former Lubeck residents, the serum PFOA change was -7.8% for the first 9 years and 0.2% (a slight increase) afterwards. The half-life estimates for Little Hocking ranged from 2.5-3.0 years (average 2.9 years) and those for Lubeck ranged from 5.9-10.3 years (average 8.5 years).

Based on their analysis, the authors suggested that, if their assumptions were correct, a simple first order elimination model may not be appropriate for PFOA given that the rate of elimination appeared to be influenced by both concentration and time. There was a difference in the clearance for the two locations even though the range of years elapsed since relocation was the same for both communities. The authors identified three potential limitations of their analysis: the cross-sectional design, the assumption that exposure was uniform within a water district, and a potential bias introduced by exclusion of individuals with serum values <15 ng/mL.

3M (Burris et al., 2000; Burris et al., 2002) conducted a half-life study on 26 retired fluorochemical production workers from their Decatur, Alabama (n = 24) and Cottage Grove, Minnesota (n = 3) plants. Blood was collected from the subjects between 1998 and 2004, a period during which serum samples were drawn every 6 months over a 5-year period, depending on the facility at which the subject had worked. Responses on questionnaires determined whether any of the retirees had occupational exposures after retirement. The average number of years that participants worked was 31 (range 20-36 years) and they had been retired an average of 2.6 years at study initiation (range 0.4 – 11.5 years). The mean age of the retirees was 61 years (range 55-75) at the beginning of the study.

The initial mean serum PFOA concentration of all of the subjects was 0.691 μg/ml (range, 0.072 – 5.1 μg/ml). At the completion of the study, the mean PFOA concentration was 0.262 μg/ml (range, 0.017 – 2.435 μg/ml). Two of the retirees died during the study period; therefore, they were only followed for 4.2 years. The mean serum elimination half-life of PFOA in these workers was 3.8 years (1378 days, 95% CI, 1131-1624 days) and the median was 3.5 years (Olsen et al., 2005). The range was 1.5 - 9.1 years (561-3334 days). No association was reported between the serum elimination half-life and with initial PFOA concentrations, age or sex of retiree, the number of years retired or worked at the production facility, or medication use or health conditions.

Harada et al. (2005a) studied the relationship between age, sex and serum PFOA concentration in residents of Kyoto, Japan. They found that females in the 20-50-year-old age group (all with regular menstrual cycles) had serum PFOA concentrations that were significantly lower than those in females over age 50 (all postmenopausal). Mean serum PFOA concentration in the younger females was 7.89 ± 3.61 ng/ml versus 12.63 ± 2.42 ng/ml in the older females. This age difference in serum PFOA concentrations was not seen in males, and serum PFOA concentrations in males were comparable to those of the older females.

Harada et al. (2005b) also estimated the renal clearance rate of PFOA in humans and found it to be only about 0.001% of the glomerular filtration rate, indicating the absence of active

excretion in human kidneys. There was no significant difference in renal clearance of PFOA with respect to sex or age group, and the mean value was 0.03 ± 0.013 ml/day/kg.

Animal. Kemper (2003) examined the plasma concentration profile of PFOA following gavage administration in sexually mature Sprague-Dawley rats. Male and female rats (4/sex/group) were administered single doses of PFOA by gavage at dose rates of 0.1, 1, 5, and 25 mg/kg PFOA. After dosing, plasma was collected for 22 days in males and 5 days in females. Plasma concentration vs. time data were then analyzed by non-compartmental pharmacokinetic methods (Table 3-24 and Table 3-25). To further characterize plasma elimination kinetics, animals were given oral PFOA at a rate of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (extended time).

Plasma elimination curves were linear with respect to time in male rats at all dose levels. In males, plasma elimination half-lives were independent of dose level and ranged from approximately 138 hours to 202 hours. To further characterize plasma elimination kinetics, particularly in male rats, animals were given oral PFOA at a dose of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (2016 hours in males). The estimated plasma elimination half-life in this experiment was approximately 277 hours (11.5 days) in male rats.

TABLE 3-24. Pharmacokinetic Parameters in Male Rats Following Administration of PFOA

Parameter	Dose					
	0.1 mg/kg	**1 mg/kg**	**5 mg/kg**	**25 mg/kg**	**1 mg/kg (i.v.)**	**0.1 mg/kg extended time**
T_{max} (hr)	10.25 (6.45)	9.00 (3.83)	15.0 (10.5)	7.5 (6.2)	NA	5.5 (7.0)
C_{max} (µg/ml)	0.598 (0.127)	8.431 (1.161)	44.75 (6.14)	160.0 (12.0)	NA	1.08 (0.42)
Lambda z (1/hr)	0.004 (0.001)	0.005 (0.001)	0.0041 (0.0007)	0.0046 (0.0012)	0.004 (0.000)	0.0026 (0.0007)
$T_{1/2}$ (hr)	201.774 (37.489)	138.343 (31.972)	174.19 (28.92)	157.47 (38.39)	185.584 (19.558)	277.10 (56.62)
AUC_{INF} (hr·µg/ml)	123.224 (35.476)	1194.463 (215.578)	6733.70 (1392.83)	25,155.61 (7276.96)	1249.817 (113.167)	206.38 (59.03)
AUC_{INF}/D (hr·µg/ml/mg/kg)	1096.811 (310.491)	1176.009 (206.316)	1221.89 (250.28)	942.65 (284.67)	1123.384 (100.488)	2111.28 (586.77)
Cl_p (ml/kg·hr)	0.962 (0.240)	0.871 (0.158)	0.85 (0.21)	1.13 (0.31)	0.896 (0.082)	0.51 (0.17)

From Kemper, 2003
(Mean (SD))
AUC_{INF}: area under the plasma concentration time curve, extrapolated to infininty; AUC_{INF}/D: AUC_{INF} normalized to dose; Cl_p: plasma clearance; C_{max}: maximum plasma concentration; Lambda z: terminal elimination constant; $T_{1/2}$: terminal elimination half-life; T_{max}: time to C_{max}.

Plasma elimination curves were biphasic in females at the 5 mg/kg and 25 mg/kg dose levels. In females, terminal elimination half-lives ranged from approximately 2.8 hours at the lowest dose to approximately 16 hours at the high dose. The estimated plasma elimination half-life in the extended time experiment was approximately 3.4 hours in females.

TABLE 3-25. Pharmacokinetic Parameters in Female Rats Following Administration of PFOA						
Parameter	**Dose**					
	0.1 mg/kg	**1 mg/kg**	**5 mg/kg**	**25 mg/kg**	**1 mg/kg (i.v.)**	**0.1 mg/kg extended time**
T_{max} (hr)	0.56 (0.31)	1.13 (0.63)	1.50 (0.58)	1.25 (0.87)	NA	1.25 (0.50)
C_{max} (µg/ml)	0.67 (0.07)	4.782 (1.149)	20.36 (1.58)	132.6 (46.0)	NA	0.52 (0.08)
Lambda z (1/hr)	0.231 (0.066)	0.213 (0.053)	0.15 (0.02)	0.059 (0.037)	0.250 (0.047)	0.22 (0.07)
$T_{1/2}$ (hr)	3.206 (0.905)	3.457 (1.111)	4.60 (0.64)	16.22 (9.90)	2.844 (0.514)	3.44 (1.26)
AUC_{INF} (hr µg/ml)	3.584 (0.666)	39.072 (10.172)	114.90 (11.23)	795.76 (187.51)	33.998 (7.601)	3.34 (0.32)
AUC_{INF}/D (hr µg/ml/mg/kg)	31.721 (5.880)	38.635 (10.093)	20.78 (2.01)	29.54 (6.92)	30.747 (6.759)	34.39 (3.29)
Cl_p (ml/kg·hr)	32.359 (6.025)	27.286 (7.159)	48.48 (4.86)	35.06 (.88)	34.040 (9.230)	29.30 (3.06)

From Kemper, 2003
(Mean (SD))

Gibson and Johnson (1979) administered a single dose of ^{14}C-PFOA averaging 11.4 mg/kg by gavage to groups of 3 male 10-week old CD rats. The elimination half-life of carbon-14 from the plasma was 4.8 days. NRC (2005) reported half-lives of 4-6 days for male rats and 2-4 hours for female rats; there was no mention of the strains studied. These values are consistent with the half-lives reported by Kemper (2003) for male and female Sprague-Dawley rats.

Lou et al. (2009) determined values of 21.7 days (95% confidence interval: 19.5-24.1) for male CD1 mice and 15.6 days (95% confidence interval: 14.7-16.5) for females for use in their pharmacokinetic model (see Section 3.5.1). NRC (2005) provided values of 12 days for males and 20 days for females without any information on strains.

Butenhoff et al. (2004b) looked at the elimination half-life in monkeys treated for 6 months with 0, 3, 10 or 20 mg/kg/day via capsules. Elimination of PFOA from serum after cessation of dosing was monitored in recovery monkeys from the 10 and 20 mg/kg dose groups. For the two monkeys exposed to 10 mg/kg, serum PFOA elimination half-life was 19.5 (R^2=0.98) days and indicated first-order elimination kinetics. For three monkeys exposed to 20 mg/kg, serum PFOA elimination half-life was 20.8 days (R^2=0.82) and also indicated first-order elimination kinetics although dosing was suspended at different time points because of weight loss. The data from NRC (2005) which were provided by Butenhoff were about 21 days for females and 30 days for males.

3.5.3 Volume of Distribution Data

Several researchers have attempted to characterize PFOA exposure and intake in humans (Thompson et al., 2010; Lorber and Egeghy, 2011) through pharmacokinetic modeling. As an integral part of model validation, the parameter for volume of distribution of PFOA within the body was calibrated from the available data. In the models discussed below, volume of

distribution was defined as the total amount of PFOA in the body divided by the blood or serum concentration.

Two groups of researchers defined a volume of distribution of 170 ml/kg body weight for humans for use in a simple, single compartment, first-order pharmacokinetic model (Thompson et al., 2010; Lorber and Egeghy, 2011). The models developed by these groups were designed to estimate intakes of PFOA by young children and adults (Lorber and Egeghy, 2011) and the general population of urban areas on the east coast of Australia (Thompson et al., 2010). In both models, the volume of distribution was calibrated using human serum concentration and exposure data from the National Health and Nutrition Examination Survey (NHANES) and assumes that most PFOA intake is from contaminated drinking water. Thus, in using the models to derive an intake from contaminated water, the value of volume of distribution was calibrated so that model prediction of elevated blood levels of PFOA matched those seen in residents.

Butenhoff et al. (2004b) calculated a volume of distribution from non-compartmental pharmacokinetic analysis of data from cynomolgus monkeys. Three males and three females were administered a single intravenous dose of 10 mg/kg and serum PFOA concentrations were measured in samples collected up to 123 days post-dosing. The volumes of distribution of PFOA at steady state (Vdss) were similar for both sexes at 181 ± 12 ml/kg for males and 198 ± 69 ml/kg for females.

4.0 HAZARD IDENTIFICATION

4.1 Human Effects

4.1.1 Long-Term and Epidemiological Studies

Large-scale production of PFOA occurred in the United States for several decades. The employees of the main production companies and the residents near the plants were exposed to the compound over time. There are several studies that have examined the data available on these occupational and residential populations. Both 3M and DuPont have been the primary U.S. producers and users of perfluorinated compounds, and both companies have been offering voluntary fluorochemical medical surveillance programs for many years to workers at plants that produce or use perfluorinated compounds. The monitoring data collected by 3M and DuPont were used in conjunction with mortality and health effect information in a number of epidemiological studies on their worker populations. 3M discontinued manufacturing PFOA in 2000, but a subsidiary in Europe continued to manufacture and sell it through 2008.

The data from NHANES have been the subject of several studies investigating the association between PFOA and health effects in the general population of the United States. The NHANES studies have examined representative members of the U.S. population (~5000 adults and children/year) through their surveys focusing on different health topics. The studies consist of an interview (demographic, socioeconomic, dietary, and medical questions) and examination (medical including blood and urine collection, dental, and physiological). Serum samples from the studies were analyzed for PFOA concentration by solid-phase extraction coupled to isotope dilution/high-performance liquid chromatography/ tandem mass spectrometry.

Members of the general population living in the vicinity of the West Virginia DuPont Washington Works PFOA production plant in Parkersburg, WV, are the focus of an ongoing study titled the C8 Health Project. Releases from the Washington Works plant, where PFOA (C8) was used as a processing aid in the manufacture of fluoropolymers, contaminated the ground water from six water districts near the plant resulting in exposures to the general population. The purpose of the C8 Health Project is to assess if there are any "probable links" between PFOA exposure and disease. During August 2005, through July 2006, about 69,000 study participants were identified. Eligible participants included those who had consumed drinking water for at least one year up to and including December 4, 2004 from the Lubeck and Mason County water districts in West Virginia, Belpre, Little Hocking, Tuppers Plains-Chester, and Pomeroy water districts in Ohio, or private water source within the geographical boundaries of the public water sources. The participants (n=69,030; 33,242 males, 35,788 females; <10 to 70+ years) donated a blood sample, filled out an extensive questionnaire, and received $400 in compensation. Serum was analyzed for perfluorochemicals, including PFOA by liquid chromatography separation with tandem mass spectrometry. Clinical chemistry tests were carried out if serum quantities were sufficient. Medical records were used to validate diseases reported by participants.

The results of these studies along with other population studies are described in the following sections.

4.1.1.1 Non Cancer Systemic Toxicity Studies

Several studies have been undertaken that examine the relationship between serum PFOA concentration and various health outcomes suggested by the standard animal toxicological database. Many of these monitored standard clinical chemistry parameters used within the occupational and public health communities as indicators of health status. In the studies of worker cohorts, the data collected were focused towards measures of cardiovascular risk, signs of organ damage, and standard haematological endpoints. Within the general population, data were focused on cardiovascular risk factors and diabetic or prediabetic conditions as well as reproductive and developmental endpoints. In reviewing the epidemiological monitoring data for PFOA it is important to remember that many individuals were exposed to other fluorocarbons and, although only serum PFOA measurements are presented in the study summaries that follow, many individuals had other PFCs in their serum as well.

Serum Lipids and Cardiovascular-Related Outcomes

Occupational Population Studies

Serum lipids have been a focus in many of the occupational studies because data from animal studies shows alterations in standard profiles for total cholesterol and triglycerides following exposure to PFOA. A number of the studies discussed in the following paragraphs also examined endpoints in addition to those associated with cardiovascular disease. Descriptions of the outcomes related to standard clinical biochemistry endpoints and hematological effects are found in the sections that follow the lipid and cardiovascular-related observations.

Olsen et al. (2000) analyzed data from voluntary medical surveillance examinations conducted in 1993, 1995, and 1997, in PFOA production plant workers to determine if serum PFOA concentration in workers was associated with serum lipid alterations. Cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides were measured in male workers; n=111 in 1993; n= 80 in 1995, n= 74 in 1997. Multivariable regression analyses were adjusted for age, body mass index (BMI), alcohol use, and cigarette use. Employees' serum PFOA levels were stratified into 3 categories (<1, 1- <10, and ≥10 μg/mL). Categories were chosen in order to maximize the number of employees in the highest exposure category. Serum PFOA levels ranged from 0.0-80.0, 0.0-114.1, and 0.1-81.3 μg/mL for the years 1993, 1995, and 1997, respectively. No association was observed between serum PFOA concentration and total cholesterol, LDL, HDL, or triglycerides in the workers in 1993, 1995, or 1997.

A cross-sectional analysis of the data from the 2000 medical surveillance program at the Decatur and Antwerp manufacturing plants was undertaken to determine if there were any associations between PFOA exposure and serum lipids of monitored employees (Olsen et al., 2001a, 2003). Multivariable regression analyses were conducted to adjust for possible confounders including the following variables: production job (yes or no), plant, age, BMI, cigarettes/day, drinks/day and years worked at the plant. Mean PFOA serum levels were 1.03 μg/mL for all male employees at the Antwerp plant and 1.90 μg/mL for all male employees at the Decatur plant. Male production employees at the Decatur plant had significantly higher ($p < 0.05$) mean serum levels (2.34 μg/mL) than those at the Antwerp plant (1.28 μg/mL). Mean

PFOA serum concentrations were 0.07 μg/mL for female employees at the Antwerp plant and 1.23 μg/mL for female employees at the Decatur plant. A positive significant association was reported between serum PFOA and cholesterol ($p = 0.05$) and triglycerides ($p = 0.002$). Age was also significant in both analyses. Alcohol consumed per day was significant in the cholesterol model, while BMI and cigarettes smoked per day was significant for triglycerides.

A longitudinal analysis of the above data and previous medical surveillance results was carried out to determine whether occupational exposure to fluorochemicals over time was related to changes in serum lipids in employees of the Antwerp and Decatur facilities (Olsen et al., 2001b, 2003). Medical surveillance data from 1995, 1997, and 2000 were analyzed using multivariable regression analysis. The plants were analyzed using 3 subcohorts that included those who participated in 2 or more medical exams between 1995 and 2000. A total of 175 male employees voluntarily participated in the 2000 surveillance and at least one other. Only 41 employees were participants in all 3 surveillance periods.

When mean serum PFOA levels were compared by surveillance year, PFOA levels in the employees participating in medical surveillance at the Antwerp plant increased between 1994/95 and 1997 and then decreased slightly between 1997 and 2000. At the Decatur plant, PFOA serum levels decreased between 1994/95 and 1997 and then increased between 1997 and 2000. When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was a statistically significant positive association between PFOA and serum cholesterol ($p = 0.0008$) and triglycerides ($p = 0.0002$) over time. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees ($p = 0.005$) and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years ($p = 0.001$). However, the association between PFOA and triglycerides was also statistically significant ($p = 0.02$) for the subgroup in which employees participated in biomonitoring in 1994/95 and 2000. There was not a significant association between PFOA and triglycerides among Decatur workers. There were no significant associations between PFOA and changes over time in HDL. Limitations to the 2000 cross-sectional and longitudinal studies included but were not limited to the consistent differences in serum PFOA concentration and demographics between employees of the Decatur and Antwerp plants, possible confounding by other perfluorinated chemicals, and differences in analytical techniques.

A cross-sectional study was undertaken to determine the relationship between serum PFOA and lipids in a large population of active employees (Washington Works fluoropolymer production plant) with potential exposure to PFOA (Sakr et al., 2007a). The employees who volunteered (n=1025, 782 males, 243 females) to participate in the study had a physical examination, provided fasting blood samples, and answered a medical and occupation history questionnaire in 2004. The mean age and years of work were 46.5 and 19.6 years, respectively, for males and 44.4 and 15.9 years, respectively, for females. Serum lipids including cholesterol, HDL, LDL, very low density lipoprotein (VLDL), triglyceride, uric acid (risk factor for hypertension) and serum PFOA concentrations were determined from blood samples. Serum PFOA concentration was analyzed by liquid chromatography tandem mass spectrometry. The relationship between PFOA exposure and lipid levels was evaluated by analysis of variance (ANOVA), χ^2 test, *t* test, and linear regression models. Confounders including age, BMI, gender, alcohol consumption, and parental heart attack were considered in the models.

Serum PFOA concentration in the workers ranged from 0.0046 to 9.55 μg/mL. For those with current occupational exposure to PFOA, the range was 0.0174 to 9.55 μg/mL, and 0.0081 to 2.07 μg/mL for workers with intermittent occupational exposure. The range was 0.0086 to 2.59 μg/mL for workers with past occupational exposure and 0.0046 to 0.963 μg/mL for workers with no occupational exposure. Serum PFOA was positively associated with cholesterol, VLDL, and LDL ($p<0.03$) in the participating workers whether or not they were taking lipid-lowering medication. An association between uric acid and serum PFOA was also observed. No association was observed between serum PFOA and HDL or triglycerides. Limitations of the study included the study design (cross-sectional surveys which cannot be used to determine causality), and the limitations of the questionnaire which was not designed or validated for the study.

Sakr et al. (2007b) conducted a longitudinal study of serum lipids in workers of the Washington Works plant from 1979 to 2004. Employee medical records from the medical surveillance program were used to obtain blood lipid (total cholesterol, LDL, HDL, triglycerides, and uric acid), height, and weight data. As part of the medical surveillance program, employees gave a detailed medical history and had a physical examination at least every 3 years. Serum PFOA concentration was measured every 1 to 2 years in PFOA-exposed workers and every 3 to 5 years in non PFOA-exposed workers on a volunteer basis. This study included 454 workers who had 2 or more serum PFOA measurements. The study population included 334 males and 120 females ranging in age from 24 to 66 years who had worked at the plant for at least one year since 1979. A linear mixed effects regression model was used to analyze the data. Age, gender, BMI, and decade of hire were potential confounders.

Serum PFOA concentration ranged from 0 to 22.66 μg/mL with a mean of 1.13 μg/mL over the 23 year monitoring period in the study population. Serum PFOA concentration was positively associated with total cholesterol after age, BMI, gender, and decade of hire adjustment in the model. Limitations of the study included inclusion of workers who had at least 2 PFOA measurements (only 16% of all workers in the PFOA area), lack of information on lipid-lowering medications and alcohol intake, and the exposure and outcome were not measured on the same date.

Olsen and Zobel (2007) examined the association between serum PFOA concentration and serum lipids in fluorochemical workers based on data collected from the 3M medical surveillance program in 2000. The fluorochemical workers consisted of males (age 21-67) from the Antwerp, Belgium (n=196), Cottage Grove, MN (n=122), and Decatur, AL (n=188) production facilities who volunteered to participate in the medical surveillance program and did not take cholesterol lowering medication. Blood was collected for fluorochemical concentration determination and serum lipid parameters including cholesterol, LDL, HDL, and triglycerides. PFOA concentration was determined by liquid chromatography/tandem mass spectrometry. Analysis of variance, analysis of covariance, logistic regression, and multiple regression models were used to analyze the data with age, BMI, and alcohol consumption as covariates.

Serum PFOA concentration ranged from 0.04 to 92.03 μg/mL for the male workers (all sites combined). The range was 0.01-7.04, 0.01-92.03, and 0.04-12.7 μg/mL for the Antwerp, Cottage Grove, and Decatur sites, respectively. The mean serum PFOA concentration was 2.21, 1.02, 4.63, and 1.89 for all sites combined, and the Antwerp, Cottage Grove, and Decatur sites,

respectively. Serum PFOA (all sites combined) was not associated with total cholesterol or LDL. A negative association was observed between serum PFOA concentration (all sites combined) and HDL. However, no association was observed at the individual sites for HDL. It was hypothesized that the results may be due to uncontrolled residual confounding. Serum triglyceride was positively associated with serum PFOA at all sites combined and the Antwerp site. Non-adherence to fast requirement for blood collection and non-causal positive correlation were provided as possible hypotheses for the results.

Costa et al. (2009) provided additional serum lipid data using 30 years of medical surveillance data from workers of a PFOA production plant in Italy. The workers (n=53 males, 20-63 years of age) participated in the medical surveillance program yearly from 1978 to 2007. The length of work exposure was 0.5 to 32.5 years. In 2007, 37 men were active workers, and 16 men were retired or had transferred to other departments and were no longer being exposed. Non-exposed male workers, (n=107, 12 executives and 95 blue collar workers) from different departments also participated in the medical surveillance program and served as controls. The medical surveillance program consisted of a yearly physical examination and blood chemistry tests including Apo-A and Apo-B lipoproteins, total cholesterol, HDL, triglycerides, and uric acid analysis. Beginning in 2000, serum PFOA was monitored yearly except in 2005. Determination of serum PFOA concentration was made by HPLC-electrospray-tandem mass spectrometry. The relationship between PFOA exposure and serum lipids was evaluated by ANOVA, *t* test, and multiple linear regression models. Confounders including age, BMI, smoking, alcohol consumption, and shift work were considered in the models.

Serum PFOA concentration of the exposed workers ranged from 1.54-86.3, 0.73-91.9, 0.34-91.9, 0.38-74.7, 0.54-46.3, 0.54-41.9, and 0.2-47.0 μg/mL for the years 2000, 2001, 2002, 2003, 2004, 2006, and 2007, respectively. Serum PFOA concentrations in the workers decreased after plant renovations partially automated the PFOA production process and adopted strict working procedures with the use of protective devices in 2002. Total cholesterol and uric acid were significantly increased ($p<0.05$) in 34 workers currently exposed to PFOA when compared to 34 non-exposed workers (age and work matched). Regression analysis comparing those same 34 exposed workers to all non-exposed workers (n=107) revealed a significant effect ($p<0.05$) of PFOA exposure on total cholesterol and uric acid levels. Serum PFOA concentration was correlated ($p<0.05$) with total cholesterol and uric acid levels in 56 workers (formally, currently, and never exposed) assessed concurrently from 2000-2007. No correlations were observed between serum PFOA concentration and Apo-A or B lipoproteins, HDL, or triglycerides in any of the analyses. Due to the small number of subjects, the authors were hesitant to draw firm conclusions, but suggested further exploration into these correlations.

Leonard et al. (2008) examined mortality in employees at the DuPont Washington Works plant in West Virginia after a cross sectional survey of 1,025 active employees revealed a positive association between serum PFOA and cholesterol and LDL. The association led the authors to target ischemic heart disease (IHD) as well as many other causes of death as outcomes. The cohort consisted of 6,027 employees (4,872 males and 1,155 females) who had ever worked at the plant from 1948 through 2002. The DuPont Epidemiology Registry and US National Death Index were used to obtain causes of death. Standardized mortality ratios were estimated using three reference populations; the populations of the US and West Virginia and the DuPont regional worker reference population excluding workers at the Washington Works plant.

A non-signifcant elevation for IHD mortality (SMR= 109, 95% CI: 96, 124) and a significant increase in diabetes mortality [standardized mortality ratio (SMR) = 197, 95% CI: 123, 298] were observed for Washington Works plant workers compared to the DuPont regional worker reference population. No increases were observed in these two outcomes when compared to the national and state populations. No significant associations were observed for the other causes of death. Using populations spanning from 1948 to 2002 increased the possibilities for loss to follow-up and inadequate records. Also caution must be exercised in interpreting the results of mortality analyses that examine diseases that do not uniformly cause death.

Sakr et al. (2009) further examined IHD mortality in workers at the Washington Works plant because of the association between PFOA exposure and increased serum lipid levels, a risk factor for heart disease. The cohort consisted of 4,747 employees (4,460 males and 102 females) who had worked in the plant from 1948 through 2002. The average duration of employment was 22.5 years. Serum PFOA levels were linked with job titles and used to determine PFOA exposure for each job. Jobs were divided into categories for low, medium, or high exposures, and intensity factors were assigned that corresponded to worker mean serum PFOA concentration for all the jobs in each category. The cumulative exposure was calculated by multiplying the time spent in each job by the mean intensity estimate from the job exposure matrix. The exposure-response analysis of cumulative exposure to PFOA was used to estimate relative mortality risks. There were 773 total deaths in employees and 239 of the deaths were from IHD. Males accounted for 235 of the 239 deaths from IHD. Mortality from IHD was not found to be associated with PFOA exposure. These results were similar to those reported by Leonard et al. (2008) in which a non-significant elevation for IHD mortality was observed relative to expected deaths for a regional DuPont employee population.

Lundin et al. (2009) also investigated occupational exposure to PFOA and death from IHD and other vascular diseases in workers at the Cottage Grove manufacturing plant as reported by Gilliland and Mandel (1993). Cerebrovascular disease, diabetes, and ischemic heart disease cases were included as the causes of deaths of 3,993 workers. A total of 807 workers died in the follow-up period. The cohort differed from Gilliland and Mandel (1993) in that the period of enrolment was extended to 1997 versus 1983, later follow-up (2002 versus 1989), and the inclusion criteria were modified. Standardized mortality ratios (SMRs) and 95% confidence intervals (CIs) were calculated. Mortality from cerebrovascular disease and ischemic heart disease in PFOA workers was not elevated compared to the general population of Minnesota. Within the cohort, the risk for cerebrovascular disease, SMR=1.6, 95% CI 0.5-3.7, was elevated in workers who had ever worked a job in which they were exposed to PFOA. The authors found diabetes mortality to be associated with moderate PFOA exposure (Hazard Ratio 3.7, 95% CI: 1.4-10.1) among subjects whose cause of death had been identified as diabetes. No deaths from diabetes had occurred among workers with high exposure, based on job classification.

The studies discussed above did not uniformly monitor the same endpoints. Table 4-1 provides a summary of the results to assist the reader in integrating the findings of the studies.

TABLE 4-1. Association of Serum PFOA with Serum Lipids and Uric Acid in Studies of Occupational Populations						
Study	**TC**	**VLDL**	**LDL**	**HDL**	**TG**	**UA**
Olsen et al., 2000	↔	NM	↔	↔	↔	NM
Olsen et al., 2001a, 2003	↑	NM	NM	↔	↑	NM
Olsen et al., 2001b, 2003	↑	NM	NM	↔	↑	NM
Sakr et al., 2007a	↑	↑	↑	↔	↔	↑
Sakr et al., 2007b	↑	NM	↔	↔	↔	NM
Olsen and Zobel, 2007	↔	NM	↔	↓	↑	NM
Costa et al., 2009	↑	NM	NM	↔	↔	↑

↑= positive association; ↓= negative association; ↔= no association; TC= total cholesterol; VLDL=very low density lipoprotein; LDL= low density lipoprotein; HDL=high density lipoprotein; TG= triglycerides; UA=uric acid; NM= not monitored

General Population Studies

Several epidemiology studies examined serum lipids, lipoproteins, and/or uric acid among cohorts from the general population. Three studies focused on populations serviced by water districts contaminated by the Washington Works PFOA production plant in Ohio and West Virginia, and two utilized data from the NHANES program.

Emmett et al. (2006) examined the association of serum PFOA concentration with serum total cholesterol in residents of the Little Hocking water district in Ohio. The study population (n=371, 2->60 years of age) was a random sample of the population served by the Little Hocking water service. The subjects completed questionnaires (demographic, occupational, health conditions, etc) and provided blood samples. PFOA concentration was determined by high performance liquid chromatography tandem mass spectrometry. Regression models were used to analyze the data. The median serum PFOA concentration was 354 ng/mL. No association was observed between serum PFOA and total cholesterol.

Steenland et al. (2009) examined the association of PFOA with serum lipids in adult participants of the C8 Health Project (n=46,294; 18 to ≥80 years). Serum samples were separated into deciles or quartiles for analysis. Total cholesterol, HDL, triglycerides, LDL, and non-HDL (total cholesterol minus HDL cholesterol) were measured or calculated from blood samples. The data were analyzed by linear regression using the log-transformed values for all variables. Covariates of the model included gender, quartile body mass index, education, smoking, regular exercise, and alcohol consumption. A logistic model was used to analyze high cholesterol and serum PFOA concentration (quartiles). The mean serum PFOA concentration was 80 ng/mL.

All lipid outcomes, except for HDL showed significant increasing trends with increasing serum PFOA decile. There was a positive association between mean levels of serum PFOA and total cholesterol, LDL cholesterol, triglycerides, total cholesterol/HDL ratio, and non-HDL. The predicted increase in total cholesterol from lowest to highest serum PFOA concentration decile was 11-12 mg/dL. No association between mean level of serum PFOA and HDL cholesterol was observed.

The odds ratio for high cholesterol (≥240 mg/dL) increased from the lowest to the highest quartile of serum PFOA concentrations: 1.00, 1.21 (95% CI: 1.12-1.31), 1.33 (95% CI: 1.23-1.43), and 1.40 (95% CI: 1.29-1.51). The results of the study were consistent with occupational studies that found a positive association between PFOA exposure and serum lipids. Data interpretation was limited by the lack of cumulative PFOA exposure data for the individual subjects and by the cross-sectional study design which prevented determination of whether an increase in PFOA serum levels corresponded with an increase in cholesterol levels.

Frisbee et al. (2010) examined the association of PFOA with serum lipids in children (n=6536; 1-11.9 years) and adolescent (n=5934; 12.0-17.9 years) participants of the C8 Health Project. Total cholesterol, HDL, LDL, and triglycerides were measured from serum samples. The data were analyzed by linear regression using the natural log-transformed values for all variables or by analysis of covariance. For some analyses, data were grouped into quintiles with age-group and sex-specific groupings. Covariates of the model included age, gender, body mass index *z* score, exercise, and length of fast. Triglyceride values were used only if fast had lasted 6 hours or more. The mean serum PFOA concentration for children was 77.7 ng/mL (82.1 ng/mL for males; 73.1 ng/mL for females) and for adolescents, the mean was 61.8 ng/mL (69.3 ng/mL for males; 53.7 ng/mL for females).

Total cholesterol, LDL, and triglycerides were positively associated ($p \leq 0.02$) with serum PFOA concentration after covariable adjustment. Assessment of the quintile trends showed significant differences ($p \leq 0.02$) between the first and fifth quintile for total cholesterol and LDL for children and adolescents of both sexes combined and separated. A significant difference ($p=0.04$) was observed for fasting triglycerides in female children only. An increased risk of abnormal total cholesterol and LDL were positively associated with serum PFOA. The odds ratios were 1.0 first (reference), 1.1 (95% CI: 1.0-1.3, second), 1.2 (95% CI: 1.0-1.4, third), and 1.2 (95% CI: 1.1-1.4, fourth and fifth) for total cholesterol, and 1.0 (reference, first), 1.2 (95% CI: 1.0-1.5, second), 1.2 (95% CI: 1.0-1.4, third and fourth), and 1.4 (95% CI: 1.2-1.7, fifth) for LDL. An increased risk of abnormal fasting triglyceride and HDL was not associated with serum PFOA.

Steenland et al. (2010) examined the association of serum PFOA concentrations with uric acid levels in adult subjects (n=53,458; 20 to ≥80 years) participating in the C8 Health Project from 2005-2006. The reference range for uric acid is 2.0 to 8.5 mg/dL. Serum samples were separated into deciles or quintiles for analysis. The data were analyzed by linear and logistic regression with uric acid as the outcome and PFOA as the exposure variable. Covariates of the model included age, gender, body mass index, education, smoking, alcohol consumption, and serum creatinine. The mean serum PFOA concentration was 86.4 ng/mL.

The mean uric acid level was 5.58 mg/dL with an interquartile range of 4.5-6.6 mg/dL. The increase in uric acid from lowest to highest serum PFOA concentration decile was 0.2-0.3 mg/dL. The odds ratio for high serum uric acid levels increased from the lowest to the highest quintile of PFOA serum concentrations: 1.00, 1.33 (95% CI: 1.24-1.43), 1.35 (95% CI: 1.26-1.45), 1.47 (95% CI: 1.37-1.58), and 1.47 (95% CI: 1.37-1.58). The study showed that higher serum PFOA concentrations were associated with higher incidence of high serum uric acid levels. As was the case for the Steenland et al. (2009a) report, data interpretation was limited by the lack of cumulative PFOA exposure data and by the cross-sectional study design.

Lin et al. (2009) investigated the association between serum PFOA, HDL cholesterol, and triglycerides in adolescents (12-20 years) and adults (>20 years) of the United States by analyzing data from the 1999-2000 and 2003-2004 National Health and Nutrition Examination Survey (NHANES) study. The study population included 1443 subjects (474 adolescents, 969 adults) at least 12 years of age who had a morning examination and triglyceride measurement. There were 266 male and 208 female adolescents and 475 male and 493 female adults. Multiple linear regression and logistic regression models were used to analyze the data. Covariates included age, sex, race, smoking status, alcohol intake, and household income. Log-transformed PFOA concentration was 1.51 and 1.48 ng/mL for adolescents and adults, respectively. Serum PFOA concentration was not associated with HDL or triglycerides. Data interpretation was limited by the cross-sectional study design which did not permit causal inference.

Nelson et al. (2010) examined the relationship between polyfluoroalkyl chemical serum concentration, including PFOA, and lipid and weight outcomes in the general population of the United States by analyzing data from the 2003-2004 NHANES study. The population (n=1445; 773 male, 672 female) included persons between the ages of 12 years and 80 years with no missing covariate information who were not pregnant, breast-feeding, taking insulin or cholesterol medicine, or undergoing dialysis. Cholesterol (total, HDL, LDL, VLDL) was measured from serum samples. Weight, height, and waist circumference were used in consideration of body size outcome. Data for covariates predicting cholesterol and body weight including age, sex, race/ethnicity, socioeconomic status, saturated fat intake, exercise, alcohol consumption at ≥ 20 years of age, smoking, and parity were obtained from the questionnaires. Regression analyses were performed for sex and the age groups 12-19 years, 20-59 years, and 60-80 years.

A positive association was found between total (TC) and non-HDL (TC-HDL, ~70-80% TC) cholesterol and serum PFOA (9.8; 95% CI, -0.2 to 19.7). No association was found between serum PFOA concentration and HDL, LDL, or VLDL. No association was found between serum PFOA concentration and body weight. Data interpretation was limited by the cross-sectional study design and lack of multiple cholesterol measurements.

Table 4-2 provides a summary of the results from the general population studies using the same format as Table 4-1.

TABLE 4-2. Association of Serum PFOA with Serum Lipids and Uric Acid in Studies of General Populations

Study	TC	VLDL	LDL	HDL	Non-HDL	TG	UA
Emmett et al., 2006	↔	NM	NM	NM	NM	NM	NM
Steenland et al., 2009a, b	↑	NM	↑	↔	↑	↑	↑
Frisbee et al., 2010	↑	NM	↑	↔	NM	↔	NM
Lin et al., 2009	NM	NM	NM	↔	NM	↔	NM
Nelson et al., 2010	↑	↔	↔	↔	↑	NM	NM

↑= positive association; ↓= negative association; ↔= no association; TC= total cholesterol; VLDL=very low density lipoprotein; LDL= low density lipoprotein; non-HDL= TC(VLDL,IDL, LDL)-HDL; HDL=high density lipoprotein; TG= triglycerides; UA=uric acid; NM= not monitored

Clinical Chemistries (Biochemical and Hematological)

Occupational Population Studies

Many of the studies that investigated alterations in serum lipids also looked at alterations in clinical chemistry and hematological parameters. The results that apply to occupational cohorts are described below.

Olsen et al. (2000; 2001a,b; 2003) examined alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine transaminase (ALT), total and direct bilirubin, renal enzymes, blood glucose, and hematology in the workers of PFOA production plants as described above. No association was observed between serum PFOA concentration and the parameters explored in the workers in 1993, 1995, 1997, or 2000 by cross-sectional or longitudinal analyses.

Sakr et al. (2007a) examined the relationship between serum PFOA and several clinical chemistry parameters. A complete blood count, metabolic panel (glucose, BUN, creatinine, iron, uric acid, electrolytes, creatinine kinase, lactic dehydrogenase (LDH), alkaline phosphatase (ALP), protein, albumin, C-reactive protein), liver enzyme panel (AST, ALT, GGT, bilirubin), and serum PFOA concentration were determined from the blood samples. Serum PFOA was positively associated ($p<0.05$) with GGT in all of the participating workers. An association between serum PFOA concentration and iron, LDH, calcium, and potassium was observed; however, the authors concluded that the associations were unlikely to have any clinical significance attributable to PFOA because of the small magnitude of the effect based on the parameter estimates, and the expectation of finding statistically significant associations by chance alone due to the number of comparisons performed.

Sakr et al. (2007b) also conducted a longitudinal study of liver enzymes in workers of the Washington Works plant described previouly. Hepatic clinical chemistry (GGT, AST, ALT, ALP, total bilirubin), height, and weight data were analyzed. Serum PFOA concentration was positively associated with AST ($p=0.009$) and negatively associated with total bilirubin ($p=0.006$) after adjustment for age, BMI, gender, and decade of hire in the model. No association was observed between serum PFOA concentration and GGT, ALT, and ALP. A limitation of the study was the lack of alcohol consumption data for the workers which could lead to confounding of the liver enzyme results.

In addition to serum lipids, Olsen and Zobel (2007) examined the association between serum PFOA concentration and liver enzymes in fluorochemical workers. Serum samples were analyzed for glucose, ALP, GGT, AST, ALT, and bilirubin concentrations. Serum PFOA was not associated with ALP or ALT. A negative association between total bilirubin and serum PFOA concentration ($p<0.05$) was observed at all sites combined. At the Decatur site, serum PFOA was positively associated with ALP, ALT, and GGT, and negatively associated with total bilirubin ($p<0.05$). The authors suggested the associations may be due to demographics and lifestyle rather than due to serum PFOA concentrations.

Costa et al. (2009) also examined correlations between serum PFOA concentration and hematological parameters, α2 globulins, immunoglobulins (IgA, IgG, IgM), glucose, liver

enzymes, and kidney enzymes in workers of a PFOA production plant in Italy. Serum PFOA concentration was positively correlated with ALT, GGT, ALP, and α2 globulins ($p<0.05$), and negatively correlated with total bilirubin ($p<0.01$) in 56 workers assessed concurrently over the last 7 years. Body mass index was also correlated with ALT and ALP suggesting a possible interaction. The authors hypothesized that PFOA may interfere with metabolism, but were hesitant to draw firm conclusions due to the small number of subjects. No associations were observed between serum PFOA concentration and hematology, immunoglobulins, glucose, and kidney enzymes.

The occupational epidemiology studies that examined standard biochemical and hematological endpoints looked for elevated levels of one or more enzyme indicative of effects on the liver, but did not always examine the same enzymes. Two of the studies did not report on renal enzymes, blood glucose levels, or hematology. Table 4-3 summarizes the observations from the studies described above.

TABLE 4-3. Associations of Serum PFOA with Serum Clinical Biochemistry and Hematology Measures

Study	Liver Enzymes	Bilirubin	Renal Enzymes	Glucose	Hematology
Olsen et al., 2000, 2001a,b; 2003	↔	↔	↔	↔	↔
Sakr et al., 2007a	↑GGT	↔	↔	↔	↔
Sakr et al., 2007b	↑AST	↓	NM	NM	NM
Olsen and Zobel, 2007	↑GGT, ALP, ALT (Decatur)	↓	NM	NM	NM
Costa et al., 2009	↑GGT, ALP, ALT	↓	↔	↔	↔

↑= positive association; ↓= negative association; ↔= no association The entries for liver enzymes identify only those that were observed to have a positive association with serum PFOA. NM = Not Monitored

General Population Studies

Emmett et al. (2006) found no association between serum PFOA and liver enzymes, renal enzymes, and hematologic parameters in 371 residents of the Little Hocking water district in Ohio.

Lin et al. (2010) investigated the association between low-dose serum PFOA and liver enzymes in the adult population of the United States by analyzing data from the 1999-2000 and 2003-2004 NHANES study. The study population included 2,216 adults (1076 men, 1140 women) older than 20 years who were not pregnant or nursing, had fasted more than 6 hours at the time of examination, were negative for hepatitis B or C virus, had body weight, height, educational attainment, and smoking status data available, and had serum tests for PFCs, liver function, or the five components of metabolic syndrome. Regression models were used to analyze the data and adjust for confounders.

Serum PFOA concentration was divided into quartiles (Q1: ≤ 2.90; Q2: ≤ 4.20; Q3: ≤ 5.95; Q4: > 5.95 ng/ml). Unadjusted liver enzymes, serum ALT and log-GGT increased across quartiles of PFOA ($p \leq 0.012$) but total bilirubin showed no trend. The linear regression models were adjusted for:

- age, gender, and race/ethnicity
- age, gender, race/ethnicity, lifestyle (smoking status, drinking status, education level),
- biomarker data (BMI, metabolic syndrome, iron saturation status, insulin resistance).

A positive association was found between serum PFOA concentration and serum ALT and log-GGT. One unit increase in serum log-PFOA was associated with an increase of 1.86 units of serum ALT and a 0.08 unit increase in log-GGT. An association between serum PFOA concentration and serum ALT was significant in the following categories: non-Hispanic Caucasians, lower education level, higher BMI, non-smoking, lower alcohol consumption, higher insulin resistance, and those with metabolic syndrome. An association between serum PFOA concentration and GGT was significant in the following categories: non-Hispanic Caucasians, higher BMI, lower alcohol consumption, and higher insulin resistance. The ALT level trend across serum PFOA quartiles was significant (p=0.003) in adjusted models with subjects having a BMI $\geq$ 30 kg/m^2. Data interpretation were limited by the cross-sectional study design, and the fact that other environmental chemicals (possible covariates or explanatory variables) were not included in the study, medication use was not included, and liver tissue status was unknown.

Similar to the above study, Gallo et al. (2012) investigated the correlation between serum PFOA levels and liver enzymes in a total of 47,092 samples collected from members enrolled in the C8 Health Project. The association of ALT, GGT, and direct bilirubin with PFOA was assessed using linear regression models adjusted for various confounders. The ln-transformed values of ALT were significantly associated with ln-PFOA and showed a steady increase in fitted levels of ALT per decile of PFOA, leveling off after approximately 30 ng PFOA/mL. Fitted values of GGT by deciles of PFOA showed a slight positive trend when adjusted for insulin resistence and BMI, but this was not confirmed in the logistic model analysis. No association was seen with direct bilirubin and PFOA levels. Limitations of the study include the cross-sectional design and self-reported lifestyle characteristics. Only a small number of ALT values were outside the normal range making the results difficult to interpret in terms of health.

Only the data from Lin et al. (2010) and Gallo et al. (2012) provide any suggestion of effects on the liver at levels of serum PFOA observed in the general population. A significant association with ALT and GGT in some population groups and in individuals with a higher BMI was observed in the US adult population.

Although there were no changes in kidney enzymes in the studies discussed of worker populations above, there is the potential for an impact of PFOA on the on the function of tubular resorption as a result its utilization of tubular transporters for excretions (See Section 3-4). Shankar et al. (2011) used data from the NHANES study to determine whether there was a relationship between serum PFOA levels and chronic kidney disease. A total of 4,587 adult participants (51.1% women) with PFOA measurements available from the 1999-2000 and 2003-2008 cycles of the survey were examined. Chronic kidney disease was defined as glomerular filtration rate of less than 60 mL/minute/1.73 m^2. Serum PFOA levels were categorized into quartiles: quartile 1 = <2.8 ng/mL; quartile 2 = 2.8-4.1 ng/mL; quartile 3 = 4.2-5.9 ng/mL; quartile 4 = >5.9 ng/mL. The multivariable odds ratio for chronic kidney disease for individuals in quartile 4 was 1.73 (95% CI: 1.04, 2.88; p for trend = 0.015) compared with individuals in quartile 1. This association was shown to be independent for confounders of age, sex, race/ethnicity, body mass index, diabetes, hypertension, and serum cholesterol level. However, the authors noted that because of the cross-sectional nature of the study, the possibility of reverse causality could not be excluded. A low glomerular filtration rate would diminish the removal of PFOA from serum for excretion by the kidney, thus increasing the serum PFOA levels. Had

glomerular filtration rate been the variable, those in the highest PFOA quartiles could have had been those in the quartile with the lowest glomerular filtrations rates.

Diabetes and Related Endpoints

General Population Studies

MacNeil et al. (2009) examined the association of PFOA with type II diabetes in adult participants of the C8 Health Project (n=54,468; 20 to ≥80 years). Serum PFOA concentration was divided into deciles using the population distribution. Serum PFOA (deciles), body mass index, gender, family history of diabetes, race, use of cholesterol-lowering medicine, and use of blood pressure-lowering medicine were used to analyze the data in categorical and logistic regression models for the outcome of type II diabetes. Serum fasting glucose levels were the focus for a linear regression analysis of the study population (n=21,643) excluding type II diabetics and those who had provided non-fasting blood samples. The mean serum PFOA concentration for the entire study population was 86.8 ng/mL and 91.3 ng/mL for subjects with type II diabetes validated by medical review (n=3539).

There was no association was between serum PFOA concentration and fasting serum glucose level in participants who were not characterized as diabetics. The mean serum PFOA concentration in subjects who self-reported type II diabetes (n=4278) was 92.9 ng/mL and 122.7 ng/mL in subjects diagnosed in the last 10 years (n=1055). No association was observed between type II diabetes and serum PFOA concentration. The odds ratio by decile was 1.00, 0.71, 0.60, 0.72, 0.65, 0.65, 0.87, 0.58, 0.62, and 0.72. The results of the analysis indicated that PFOA exposure is not associated with type II diabetes among population studied. Data interpretation was limited by the cross-sectional study design which made it difficult to determine if PFOA exposure preceded disease.

Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease and diabetes. Lin et al. (2009) investigated the association between serum PFOA and glucose homeostasis and metabolic syndrome in adolescents (12-20 years) and adults (>20 years) as described previously. The National Cholesterol Education Program Adult Treatment Panel III guidelines were used to define adult metabolic syndrome and the modified guidelines were used to define adolescent metabolic syndrome. In adults, serum PFOA concentration was associated with increased β-cell function (β coefficient 0.07, $p<0.05$). Serum PFOA concentration was not associated with metabolic syndrome, metabolic syndrome waist circumference, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents.

Nelson et al. (2010) examined the relationship between polyfluoroalkyl chemical serum concentration, including PFOA, and insulin resistance as previously described. Fasting insulin and fasting glucose were used to determine the homeostatic model assessment for insulin resistance. No association was found between serum PFOA concentration and insulin resistance.

4.1.1.2 Thyroid Effects

Occupational Population Studies

Thyroid hormones were monitored in the medical surveillance programs at the various PFOA manufacturing plants and an association with serum PFOA concentration was examined in several occupational studies.

Serum PFOA levels were obtained from volunteer workers of the Cottage Grove, Minnesota PFOA plant in 1993 (n = 111) and 1995 (n = 80) as part of the medical surveillance program (Olsen et al., 1998) and analyzed to determine a relationship between thyroid stimulating hormone (TSH) and PFOA concentration. Employees were placed into 4 exposure categories based on their serum PFOA levels: 0-1 ppm, 1- < 10 ppm, 10- < 30 ppm, and >30 ppm. Statistical methods used to compare PFOA levels and hormone values included: multivariable regression analysis, ANOVA, and Pearson correlation coefficients. TSH was significantly ($p = 0.002$) elevated in 10-<30 ppm exposure category for 1995 only (mean serum TSH level was 2.9 ppm). However, mean TSH levels for the other exposure categories, including the ≥30 ppm category, were all the same (1.7 ppm). In 1993, TSH was elevated in this same exposure category, but was not statistically significant ($p = 0.09$) when compared to the other exposure categories.

Olsen et al. (2001a, 2003) and Sakr et al. (2007a) found no association between serum PFOA concentration and TSH, T4, and T3 levels in the Decatur, Antwerp, and Washington Works plant employees. Costa et al. (2009) also found no association between serum PFOA concentration and thyroid hormones levels in workers at a PFOA production plant in Italy.

Olsen and Zobel (2007) looked at the relationship between serum PFOA concentration and TSH, serum and free T4, and T3 levels in workers at the Decatur, Antwerp, and Cottage Grove production plants as described previously. No association between TSH, serum T4, and PFOA concentration was observed. A negative association ($p<0.01$) between free T4 and serum PFOA concentration was observed in the unadjusted and adjusted (age, BMI, and alcohol) models for all locations combined; no association was observed for the individual locations. A positive association ($p<0.05$) was observed between T3 and serum PFOA concentration in the unadjusted and adjusted models for all locations combined, the Antwerp plant, and the Decatur plant. The authors noted that the results were not considered clinically relevant because the results were within normal reference range.

General Population Studies

Emmett et al. (2006) examined the association of serum PFOA with thyroid disease in residents of the Little Hocking, Ohio, water district as described previously. No association was observed between serum PFOA and thyroid disease. Serum PFOA was decreased (not significantly different) in subjects with thyroid disease (387 ng/mL) compared to subjects without thyroid disease (451 ng/mL).

Pirali et al. (2009) measured intrathyroidal levels of PFOA in thyroid surgical specimens to determine if a relationship existed between PFOA and the clinical, biochemical, and

histological phenotype of thyroid disease patients. Serum PFOA concentration was also measured to determine if a relationship existed between thyroid tissue and serum PFOA levels. Patients (n=28; 8 males, 20 females; 33-79 years) with benign multinodular goiters (n=15), Graves' disease (n=7), malignant papillary carcinoma (n=5), and malignant follicular carcinoma (n=1) were included in the study. Informed consent, clinical examination, work history, thyroid hormone and antibody measurements, thyroid ultrasound, fine-needle aspiration of nodules greater than 1 cm, and serum samples (n=21) were performed or collected prior to surgery. The control group consisted of thyroid tissues collected at autopsy from subjects with no history of thyroid disease (n=7; 5 males, 3 females; 12-83 years) and serum samples from 10 subjects with no evidence of thyroid disease. The Student's *t*-test, Mann-Whitney *U*-test, Pearson and Spearman's correlation tests, and Chi^2 test with Fisher's correction were used to compare group results. Regression analysis was used to test the effect of different variables independently of a covariate.

The patients were divided into three different groups: Group I (toxic and nontoxic multinodular goiter, n=12), Group II (differentiated thyroid cancer, n=6), and Group III (Hashimoto's thyroiditis or Graves' disease, n=10). Thyroid PFOA concentration for the control group, Group I, Group II, and Group III ranged from 1.0-6.0, 0.4-4.4, 1.4-4.0, and 1.0-4.6 ng/g, respectively. Serum PFOA concentration for the control group, Group I, Group II, and Group III ranged from 4-13.7, 1.2-16.6, 5.1-9.6, and 3.9-12.5 ng/ml, respectively. The concentration of PFOA in the thyroid and serum was similar between control and thyroid patients at the time of measurement. Age, sex, residence, working activity, malignant/nonmalignant conditions, antibodies, thyroid hormone concentrations, and ultrasound parameters were not associated with thyroid or serum PFOA concentration. There was also no correlation between serum and thyroid PFOA concentration.

Bloom et al. (2010) investigated the associations between serum PFCs, including PFOA, and thyroid stimulating hormone (TSH) and free thyroxine (T4). The serum samples came from 31 participants (27 males, 4 females; mean age 39 years) of the 1995-1997 New York State Angler Cohort Study Dioxin Exposure Substudy. The study subjects completed a questionnaire and provided a blood sample for serum analysis. The questionnaire contained questions about sport-fish and game consumption, lifestyle, demographic factors, and medical history. The serum samples were analyzed for TSH and free T4 in 2003 by immunometric chemiluminescent sandwich assay and for PFCs in 2006 by ion pair extraction high-performance chromatography with electrospray tandem mass spectrometry. Regression models were used to analyze the data and adjust for confounders.

No subjects reported use of thyroid medication or physician-diagnosed goiter or thyroid conditions. Mean TSH concentration (range 0.43-15.70 μIU/mL) was within normal range (0.40-5.00 μIU/mL) with the exception of one subject. Mean free T4 (0.90-1.55 ng/dL) was within normal range (0.80-1.80 ng/dL) for all subjects. The mean serum PFOA concentration was 1.33 ng/mL and ranged from 0.57 to 2.58 ng/mL. The males had a significantly higher serum PFOA concentration compared to females (1.47 ng/mL vs. 1.05 ng/mL; $p = 0.047$). There was no association between serum PFOA concentration and TSH or free T4.

Melzer et al. (2010) examined the association between serum PFOA concentration and thyroid disease in the general population of the United States by analyzing data from the 1999-

2000, 2003-2004, and 2005-2006 NHANES study. The population included 3,966 adults (2066 women, 1900 men) older than 18 years. The participants answered a questionnaire, had a physical examination, and provided blood and urine samples for analysis. Serum samples were analyzed for PFOA concentration by solid-phase extraction coupled to isotope dilution/high-performance liquid chromatography/ tandem mass spectrometry. Data on diseases diagnosed by a physician and confounding factors including year of NHANES study, age, gender, race/ethnicity, education, smoking status, BMI, and alcohol consumption were obtained from the questionnaire. Regression models were used to analyze the data and adjust for confounders.

Serum PFOA concentration was divided into quartiles for each sex. In women, serum PFOA concentration ranged from 0.1-123.0 ng/ml (Q1: 0.1-2.6; Q2: 2.7-4.0; Q3: 4.1-5.7; Q4: 5.7-123.0), and in men, serum PFOA concentration ranged from 0.1-45.9 ng/ml (Q1: 0.1-3.6; Q2: 3.7-5.2; Q3: 5.3-7.2; Q4: 7.3-45.9). Women in PFOA quartile 4 were more likely to report current thyroid disease [odds ratio (OR) = 2.24, 95% CI: 1.38-3.65, p=0.002] compared to women in quartiles 1 and 2. No association between serum PFOA concentration and thyroid disease was observed in men. Data interpretation was limited by the cross-sectional study design, lack of specific thyroid disease diagnosis, and single serum samples for PFOA measurements taken at the same time as disease status.

Chan et al. (2011) examined the association between hypothyroxinemia and serum PFOA concentration in pregnant Canadian women (n=≥271; 20.1-45.1 years of age, ≥22 weeks of gestation) in a matched case-control study. Maternal serum from the second trimester was collected between Dec 15, 2005 and June 22, 2006 as part of an elective prenatal screen for birth defects. Serum samples were anlayzed for TSH and free T4 concentrations and PFOA. The cases (n=96) had normal TSH concentrations and free T4 concentrations in the lowest 10th percentile (≤8.8 pmol/L). The controls (n=175) had normal TSH concentrations and free T4 concentrations between the 50th and 90th percentiles (12-14.1 pmol/L). Maternal age, weight, and gestational age at blood draw, dichotomized at 50th percentiles were included as confounders, and race was included at a covariate. Chi-square tests and regression models were used to analyze the data. The geometric mean serum PFOA concentration in the cases was 3.10 nmol/L and 3.32 nmol/L in the controls. There was no association between serum PFOA concentration and hypothyroxinemia in pregnant women. The authors noted that hormone levels change over pregnancy and time of blood could have influenced the results. They also noted that the population may have been skewed because only women who chose to be screened were included.

There are no strong data linking serum PFOA to changes in thyroid hormones or thyroid disorder and indicated by the Table 4-4 compilation of the studies in the occulational and general population.

TABLE 4-4. Association of serum PFOA with the prevalence of thyroid disease and thyroid hormone levels in studies of general and worker populations					
Study	**Population**	**Thyroid Disease**	**TSH**	**T3**	**T4**
Bloom et al. (2010)	General	↔	↔	NM	↔
Chan et al. (2011)	General	↔	↔	NM	↔
Costa et al. (2009)	Occupational	NM	↔	↔	↔
Emmett et al. (2006)	General	↔	NM	NM	NM
Melzer et al. (2010)	General	↔ men ↑ women	NM	NM	NM
Olsen et al. (1998)	Occupational	NM	↑	NM	NM
Olsen et al. (2001a, 2003)	Occupational	NM	↔	↔	↔
Olsen and Zobel (2007)	Occupational	NM	↔	↑	↔ serum ↓ free
Pirali et al. (2009)	General	↔	NM	NM	NM
Sakr et al. (2007a)	Occupational	NM	↔	↔	↔

↑= positive association; ↓= negative association; ↔= no association; NM = Not Monitored

4.1.1.3 Steroid Hormones

Occupational Population Studies

Olsen et al. (1998) examined several hormones including cortisol, estradiol, follicle stimulating hormone, dehydroepiandrosterone sulfate, 17 gamma-hydroxyprogesterone (a testosterone precursor), free testosterone, total testosterone, luteinizing hormone, prolactin, and sex hormone-binding globulin in male workers at the Cottage Grove, Minnesota, production plant for 1993 and 1995. No association between serum PFOA and any hormone was observed, but some interesting trends were noticed. When the mean measures of the various hormones were compared by exposure categories, there was a statistically significant ($p = 0.01$) elevation in prolactin in 1993 only for the 10 workers whose serum PFOA levels were between 10 and 30 ppm compared to the lower 2 exposure categories.

Estradiol levels in the >30 ppm PFOA group in both years were 10% higher than the other PFOA groups, but the difference was not statistically significant. These results were confounded by estradiol being correlated with BMI ($r = 0.41$, $p < 0.001$ in 1993, and $r = 0.30$, $p < 0.01$ in 1995). The authors postulate that the study may not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (55 ppm PFOA in the CD rat). Only 3 employees in this study had PFOA serum levels this high. They also suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol.

In the Sakr et al. (2007a) study, an association was observed between serum PFOA and serum estradiol ($p=0.017$) and testosterone ($p=0.034$) in male workers of the Washington Works plant; however, circadian variations of hormones were not taken into consideration during analysis. The biological signifificance of the results is unknown.

Costa et al. (2009) found no association between serum PFOA concentration and estradiol or testosterone in workers at a PFOA production plant from 2000 to 2007.

General Population Studies

Knox et al. (2011) examined the endocrine disrupting effects of perfluorocarbons in women from the C8 Health by analyzing the relationship between serum PFOA, serum estradiol concentration, and menopause onset. The population included women over age 18 years (n=25,957). Serum PFOA and estradiol concentrations were determined from blood samples. Women who were pregnant, had full hysterectomies, and taking any prescription hormones or selective estrogen receptor modulators and fertility agents were excluded from estradiol analysis. Serum PFOA concentration was grouped into quintiles (natural log transformation): 1=0.25-11.2; 2=11.3-19.8; 3=19.9-36.7; 4=36.8-84.9; and 5=85.0-22412.0 ng/mL. Estradiol analysis was calculated by age group, 18-42 years, >42 ≤ 51 years, and >51 ≤ 65 years. Menopause was determined by questionnaire. Menopause analysis was calculated by age group, 30-42 years, >42 ≤ 51 years, and >51 ≤ 65 years, and excluded those who reported hysterectomies. Logistic regression models were adjusted for smoking, age, BMI, alcohol consumption, and regular exercise. PFOA concentration in women who had a hysterectomy was significantly higher than women who had not had a hysterectomy. Serum PFOA and estradiol concentrations were not associated. Menopause analysis in the oldest group of women showed that all quintiles were significantly higher than the lowest, and in women between the ages of 42 and 51 years, quintiles 3-5 were significantly higher than the lowest. The authors suggested that PFOA may be associated with endocrine disruption in women. Data interpretation was limited by the cross-sectional study design and survey-reported menopause without age or independent confirmation.

7.1.1.4. Immunotoxicity

As described previously, no association between serum PFOA concentrations and immunoglobin levels was found in male workers (Costa et al., 2009).

Fei et al. (2010b) examined the association between prenatal PFOA exposure and hospitalizations for infectious diseases in early childhood. A total of 577 hospitalizations occurred with 363 being due to infectious disease. A lower risk of hospitalization due to infections was associated with higher maternal PFOA concentration. The incidence rate ratio (IRR) was significantly different only for the second quartile of exposure (IRR=0.71, 95% CI 0.53-0.94) compared to the first. A slightly higher risk for hospitalizations was observed in girls with higher maternal PFOA concentrations (IRR= 1.00, 1.20, 1.63, 1.74 for first, second, third, and fourth quartiles, respectively). The risk for boys was below 1.0 for each quartile. Overall, there was no association between hospitalizations due to infectious diseases and maternal PFOA exposure.

Okada et al. (2012) investigated the relationship between maternal PFOA concentration and infant allergies and infectious diseases during the first 18 months of life as well as cord blood IgE levels. The prospective birth cohort was based on infants delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan between July 2002 and October 2005. PFOA levels were measured in maternal serum taken after the second trimester (n=343) and total IgE concentration was measured in cord blood (n=231) at the time of delivery. Infant allergies and infectious diseases were assessed in a maternal self-administered questionnaire at 18 months post-delivery. Polynomial regression analyses, adjusted for potential confounders, were performed on log-transformed data. Mean maternal PFOA concentration was 1.4 ng/mL and

cord blood IgE level was 0.62 IU/mL. Cord blood IgE level decreased significantly with high maternal PFOA concentration among female infants, but not male infants. No significant associations were observed between maternal PFOA levels incidence of food allergy, eczema, wheezing, or otitis media in infants at 18 months of age. Limitations of the study include the small sample size, potential selection bias of the population, and accurate diagnosis of disease in the infants.

Antibody responses to diphtheria and tetanus toxoids following childhood vaccinations were assessed in context of exposure to perfluorinated compounds (Grandjean et al., 2012). The prospective study included a birth cohort of 587 singleton births during 1999-2001 from the National Hospital in the Faroe Islands. Serum antibody concentrations were measured in children at age 5 years prebooster, approximately 4 weeks after the booster, and at age 7 years. Prenatal exposures to perfluorinated compounds were assessed by analysis of serum collected from the mother during week 32 of pregnancy; postnatal exposure was assessed from serum collected from the child at 5 years of age. Multiple regression analyses with covariate adjustments were used to estimate the percent difference in specific antibody concentrations per 2-fold increase in PFOA concentration in both maternal and 5-year serum. Maternal PFOA serum concentration was negatively associated with antidiphtheria antibody concentration (-16.2%) at age 5 before booster. The biggest effect was found in comparison of antibody concentrations at age 7 with serum PFOA concentrations at age 5 where a 2-fold increase in PFOA was associated with differences of -36% (95% CI, -52% to -14%) and -25% (95% CI, -43% to -2%) for tetanus and diphtheria, respectively. Additionally at age 7, a small percentage of children had antibody concentrations below the clinically protective level of 0.1 IU/mL. The odds ratios of antibody concentrations falling below this level were 4.20 (95% CI, 1.54 to 11.44) for tetanus and 3.27 (95% CI, 1.43 to 7.51) for diphtheria when age 7 antibody levels were correlated with age 5 PFOA serum concentrations.

The association between serum levels of perfluorinated compounds and childhood asthma was investigated by Dong et al. (2013). The cross-sectional study included a total of 231 children aged 10-15 years with physician-diagnosed asthma and 225 age-matched non-asthmatic controls. Between 2009 and 2010, asthmatic children were recruited from two hospitals in Northern Tiawan, while the controls were part of a cohort population in seven public schools in Northern Tiawan. Serum was collected for measurement of ten perfluorinated compounds, absolute eosinophil counts, total IgE, and eosinophilic cationic protein. A questionnaire was administered to asthmatic children to assess asthma control and to calculate an asthema severity score (including frequency of attacks, use of medicine, and hospitilazation) during the previous four weeks. Associations of perflourinated compound quartiles with concentrations of immunological markers and asthma outcomes were estimated using multivariable regression models. Nine of ten perfluorinated compounds were detectable in ≥84.4% of all children with levels generally higher in asthmatic children compared with non-asthmatics. Serum concentrations of PFOA in asthmatic and non-asthmatic children were 1.5±1.3 and 1.0±1.1 ng/mL, respectively; four other compounds were measured at higher concentrations with the highest levels for PFOS and perfluorotetradecanoic acid. The adjusted odds ratios for asthma association with the highest versus lowest quartile levels were significantly elevated for seven of the compounds. For PFOA the odds ratio was 4.05 (95%CI: 2.21, 7.42). In asthmatic children, absolute eosinophil counts, total IgE, and eosinophilic cationic protein concentration were postitively associated with PFOA levels with a significant monotonic trend with increasing

serum concentration. None of these biomarkers was significantly associated with PFOA levels in non-asthematic children. Serum PFOA levels were not significantly associated with asthma serverity scores among the children with asthma.

4.1.1.4 Reproductive & Developmental Endpoints

Several studies have examined the relationship between PFOA exposures and reproductive, gestational, and developmental endpoints as well as postnatal growth and maturation. The reproductive endpoints included sperm count (Joensen et al., 2009) and fecundity (Fei et al., 2009). The gestational endpoints included gestational age (Nolan et al., 2009), and fetal growth (Fei et al., 2007, 2008a; Washino et al., 2009). The developmental endpoints included birth weight (Apelberg et al., 2007; Monroy et al., 2008; Nolan et al., 2009; Stein, et al., 2009), miscarriage or pre-term birth (Stein, et al., 2009), birth defects (Stein et al., 2009), and infant development during the first two years (Anderson et al., 2010; Fei et al., 2008b, 2010a,b). Postnatal growth and maturation included behavioural assessment (Fei and Olsen, 2011; Hoffman et al., 2010), onset of puberty (Lopez-Espinosa et al., 2011; Christensen et al., 2011), and risk of adult obesity (Halldorsson et al., 2012). As a group, the studies do not suggest an effect of serum PFOA at the levels examined on reproductive and developmental outcomes. A few studies indicate an impact on anthropometric measures (e.g. body weight, birth length) but the associations are not strong.

Andersen et al. (2010) and Fei et al. (2007, 2008a, b, 2009, 2010a,b) conducted a series of studies examining the association of maternal plasma PFOA concentration and various reproductive and developmental outcomes in Danish women and their offspring. The women (n=1400) and their infants were randomly selected from the Danish National Birth Cohort, and the study included those who provided their first blood samples between gestational weeks 4 and 14 and gave birth to a single live-born child without congenital malformation. The women participated in telephone interviews (at 12 and 30 weeks gestation and when children were 6 and 18 months of age) and filled out a food frequency questionnaire. As the children aged, more questionnaires were completed by the mothers with regard to behavioral health and motor coordination. Highly structured questionnaires were used to gather information on possible confounders including infant sex, maternal age, parity, socio-occupational status, prepregnancy BMI, and smoking during pregnancy. The National Hospital Discharge Register was used to obtain birth weight, gestational age, placental weight, birth length, head and abdominal circumference data, Apgar scores based on heart rate, respiratory effort, reflex, irritability, muscle tone, and skin color. Plasma PFOA concentration was determined from the first blood samples of 1399 women, from the second blood samples of 200 women, and from cord blood samples of 50 infants by solid phase extraction high performance liquid chromatography-tandem mass spectrometry. PFOA concentrations were divided into quartiles (Fei et al., 2009, 2010a), with the lowest quartile designated as the reference group, as follows: <lower limit of quantification (LLOQ)-3.90, 3.91-5.20, 5.21-6.96, and ≥ 6.97 ng/mL. Regression models were used to analyze the data. Results of these studies are included in the discussion of results for specific endpoints below.

Reproductive Outcome

Fei et al. (2009) examined the association between plasma PFOA concentration and longer time to pregnancy as a measure of fecundity in 1240 women. The time to pregnancy (TTP) was categorized as follows: immediate pregnancy (<1 month), 1-2, 3-5, 6-12, and >12 months. Having >12 months TTP or having used fertility treatment to get pregnant were used to define infertility. A total of 620 women had a TTP within the first 2 months of trying to get pregnant and 379 had a TTP of ≥6 months with 188 of those women having a TTP of >12 months. The mean plasma PFOA concentration was 5.6 ng/mL for women who planned their pregnancies, and for TTP <6 months, 6-12 months, and >12 months was 5.4, 6.0, and 6.3 ng/mL, respectively. Plasma PFOA concentration was significantly greater ($p<0.001$) in women who had TTP >6 months compared to those whose TTP was <6 months. The women with a TTP >6 months were more likely to be older, have middle socio-occupational status, and have a history of spontaneous miscarriage or irregular menstrual cycles. The adjusted odds for infertility increased 60-154% among women with >3.91 ng/mL plasma PFOA concentration compared to women with <3.91 ng/mL plasma concentration. The fecundity odds ratio was 0.72, 0.73, and 0.60 for the three highest PFOA concentration quartiles. In the likelihood ratio test, the trend was significant ($p<0.001$). Although the results of the study suggest that plasma PFOA concentration may reduce fecundity, the authors noted that selection bias, the unknown quality of the sperm, unknown frequency and timing of intercourse, and abnormal hormone levels may have an impact on the results and fecundity.

Nolan et al. (2009, 2010) published two studies that examined birth outcome in infants born to mothers whose partial or full public water source was from the Little Hocking Water Association (LHWA) in Washington County, OH, or from the same geographic area but with water not provided by the LHWA. The population serviced by LHWA had serum levels of PFOA (6.78 μg/L) that were approximately 50 times higher than serum levels of the general population. Archival data for births, including birth weight, gestation age, plurality, neonatal sex, race, mother's age, and mother's zip code, in Washington County, OH, from January 1, 2003 to September 1, 2005 were obtained for analysis. For mothers with public water service provided completely, partially, or not at all by LHWA, the incidence of preterm births was 10.7%, 11.3%, and 13.4%, respectively (Nolan et al., 2009). The national incidence of preterm birth is 12.7%. No differences were found between gestational age of infants born to mothers whose partial or full water source was serviced by LHWA and infants born to mothers whose water source was serviced by another provider. Exposure to high levels of PFOA through the drinking water was not associated with preterm births.

Congenital anomalies were diagnosed in 1.8%, 1.9%, and 2.0% of the mothers with water provided completely, partially, or not at all by LHWA, respectively (Nolan et al., 2010). When adjusted for confounders, no statistically significant differences were found. Complications with labor and delivery were observed in 32.5%, 35.9%, and 41.9% of the mothers with water provided completely, partially, or not at all by LHWA, respectively. Mothers with water provided by LHWA did have in increased likelihood of having dysfunctional labor, but the reported cases were low in number. Mothers with one or more maternal risk factors were 37.5%, 34.4%, and 39.3% of the populations with water provided completely, partially, or not at all by LHWA, respectively. Adjusted regression models showed no statistical differences across water service. An increased likelihood (crude OR 11, 95% CI: 1.8-64) of anemia and dysfunctional

labor (crude OR 5.3, 95% CI: 1.2-24) in mothers with water provided by LHWA was found, but the reported cases were low in number. No association was found between PFOA and increased incidence of congenital anomalies, most labor and delivery complications, and maternal risk factors.

Stein et al. (2009) examined the association between PFOA serum levels and pregnancy outcome in a population of women living in the Mid-Ohio Valley near Parksburg, WV from 2000 to 2006. The women were part of the C8 Health Project. Pregnancies were restricted to those occurring in the 5 years prior to C8 Health Project enrollment (August 2005 through July 2006) to ensure that the serum PFOA levels measured during enrollment were the same levels that occurred during pregnancy. Restrictions on residency including living in the same water district from pregnancy to enrollment were also applied. Preeclampsia and birth defects were included in the analysis as well. Maternal age, education, and smoking status were possible cofounders. A total of 1,845 pregnancies for 1,505 women met the requirements for the study. Regression models were used for statistical analysis. The median PFOA serum level was 21.2 ng/mL, and the mean serum PFOA level was 48.8 ng/mL.

No association was observed between serum PFOA levels and miscarriage, low weight birth, or pre-term birth. Serum PFOA levels above the median were weakly associated with preeclampsia. A weak association (odds ratio 1.7, CI: 0.8-3.6) was observed between PFOA levels above the 90th percentile and birth defects. Data interpretations were limited by the quality of the self-reported pregnancy outcome especially in preeclampsia, miscarriage, and preterm births.

Joenson et al. (2009) examined the association between PFCs, including PFOA, and testicular function in 105 Danish men who provided semen and blood samples as part of reporting for the military draft in 2003. The men chosen had the highest (ranging from 30.1 to 34.8 nmol/L; n=53; 18.2-24.6 years) and lowest (ranging 10.5-15.5 nmol/L; n=52; 18.2-25.2 years) testosterone concentrations. Regression models were used to analyze associations between PFCs and testicular function. Median serum PFOA concentration was 4.4, 5.0, and 4.9 ng/mL in the high testosterone, low testosterone, and combined groups, respectively. A nonsignificant negative association was observed between serum PFOA concentration and semen volume, sperm concentration, sperm count, sperm motility, or sperm morphology. No association was observed between serum PFOA concentration and testosterone, estradiol, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and inhibin B.

Anthropometric Endpoints

Apelberg et al. (2007) measured PFOA in the cord blood of 293 newborns (singleton births) born November 26, 2004 through March 16, 2005 in Baltimore, MD, at Johns Hopkins Hospital. Maternal and infant data including maternal birth cohort, social class, place of residence, past pregnancies, insurance type, BMI, age, race, education, marital status, parity, gestational age, smoking status, and infant sex were collected from the hospital database and forms filled out at time of delivery. All samples of cord blood contained PFOA (geometric mean 1.6, range 0.3-7.1 ng/mL). Higher concentrations ($p<0.03$) of PFOA were measured in infants born to obese mothers. Concentration was evenly distributed by race, but not by sex. Male

infants had significantly lower ($p<0.01$) PFOA concentrations than females. Lower concentrations ($p<0.02$) were also measured in instances of multiparous birth. No correlations were observed between PFOA and maternal age, gestational age, education, insurance type, marital status, smoking status, and place of residence. Birth weight, head circumference, and ponderal index were negatively associated with PFOA.

At birth, Fei et al. (2007, 2008a) examined the association between plasma PFOA concentration and infant birth weight, gestation age, placental weight, birth length (skeletal growth), head circumference (brain), and abdominal circumference (liver size). The mean plasma PFOA concentration from the first blood sample, the second blood sample, and the cord blood sample was 5.6, 4.5, and 3.7 ng/mL, respectively. Plasma PFOA concentration ranged from below the limit of quantitation (≤1.0 ng/ml) to 41.5 ng/ml. Mean infant birth weight was 3623 g; 24 infants were categorized as low birth weight infants (<2500 g). The mean gestational age was 280.4 days, and 53 were preterm births and 139 were post-term births. Plasma PFOA concentration was significantly inversely associated with birth weight among normal-weight women (adjusted β=-10.63 g; 95% CI, -20.79 to -0.47 g). A statistically significant decrease was observed in birth length in the second and fourth quartiles of PFOA exposures compared to the first quartile after adjustment for confounders. When maternal PFOA concentration was considered as a continuous variable in relation to fetal growth indicators, a statistically significant negative association between abdominal circumference and birth length were observed after adjustment for confounders. The authors noted that any associations may not be causal, but rather due to chance or confounding factors.

Monroy et al. (2008) examined the association between maternal serum and umbilical cord PFCs, including PFOA, and birth weight. The population (n=101 pregnant mothers) was selected from those recruited from January 2004 to June 2005, to participate in the Family Study in Canada. As part of the study, the women provided blood samples during the second trimester of pregnancy and filled out an obstetrical history questionnaire. Another blood sample was provided at delivery as well as blood from the umbilical cord. Age, occupation, pre-pregnancy height and weight, education, and infant gender and birth weight were obtained post delivery. Paired t-test and linear regression analysis were used to analyze the data. The mean serum PFOA concentration was 2.54, 2.24, and 1.94 ng/mL at 24-28 weeks, delivery, and in the umbilical cord, respectively. There was no association between serum or umbilical cord PFOA concentration and birth weight.

As mentioned above, Nolan et al. (2009, 2010) published two studies that examined birth outcome in infants born to mothers whose partial or full public water source was from the Little Hocking Water Association (LHWA) in Washington County, OH, or from the same geographic area but with water not provided by the LHWA. The population serviced by LHWA had serum levels of PFOA (6.78 μg/L) that were approximately 50 times higher than serum levels of the general population. Archival data for births, including birth weight, gestation age, plurality, neonatal sex, race, mother's age, and mother's zip code, in Washington County, OH, from January 1, 2003 to September 1, 2005 were obtained for analysis. Analysis of variance and regression models or logistic regression models were used for statistical analysis. The noted limitations included lack of individual exposure levels, possible misclassification of mothers by zip code, misclassification of socioeconomic status, possible reporting error in the database used to abstract the data, and using data from only live births for analysis.

Nolan et al. (2009) examined the association between birth weight with PFOA in a total of 1,555 singleton neonates (777 male, 778 female): 11% were born to mothers fully serviced by LHWA, 13% born to mothers partially serviced by LHWA, and 76% born to mothers not serviced by LHWA. For mothers with public water service provided completely, partially, or not at all by LHWA, the incidence of low birth weight was 3.6%, 3.8%, and 8.1%, respectively. The national incidence of low birth weight is 8.2%. No differences were found between birth weights of infants born to mothers whose partial or full water source was serviced by LHWA and infants born to mothers whose water source was serviced by another provider.

Washino et al. (2009) investigated the correlation between prenatal exposure to perfluorinated chemicals and reduced fetal growth in a population of women (n=428, average age 30.5 years) living in or near Sapporo, Japan. Blood samples were obtained from the women either after the second trimester or after delivery for PFOA and PFOS concentration determination. PFOA was detected in 92.8% of the blood samples, and PFOS was detected in all samples. Maternal serum PFOA concentrations ranged from <0.5 (limit of detection) to 5.2 ng/mL. A regression model adjusted only for gestational age showed a correlation between $\log_{10}$-transformed PFOA concentrations and decreased birth weight. The same model showed a correlation between $\log_{10}$-transformed PFOA concentrations and decreased birth weight in male infants. However, when the regression model was adjusted for multiple factors, including maternal age, education level, smoking status, BMI, parity, how infant was delivered, infant sex, gestational age, and when blood sample was taken, no correlation was found. No correlations were observed between PFOA and birth length, chest circumference, or head circumference. Data interpretation is limited by small sample size, selection bias, and possible errors in birth length, chest circumference, and head circumference measured due to molding during birth.

Hamm et al. (2009) investigated whether increasing maternal exposure to PFCs, including PFOA, was associated with fetal growth and gestation length. Pregnant women living in and around the city of Edmonton, Alberta, Canada, (n=252; age ≥18 years) submitted serum samples at 15-16 weeks gestation as part of a screening for birth defects between December 15, 2005 and June 22, 2006. The serum samples were included in the study if the mother had given birth to a live singleton with no evidence of malformations at greater than or equal to 22 weeks of gestation. Maternal serum PFOA concentrations ranged from below the limit of detection to 18 ng/mL and had a mean of 2.1 ng/mL. A total of 21 births (8.3%) were classified as preterm deliveries and 16 infants (6.3%) were considered small for gestational age. The mean infant birth weight was 3349 g and the mean birth weight *z*-score was 0.062. The natural log of maternal serum PFOA concentration was associated with changes in birth weight of -37.4 g (95% CI: -86.0-11.2) after adjustment for covariates. No association was observed between the untransformed serum PFOA concentration and birth weight. There was no association between maternal serum PFOA concentration and birth weight *z*-score, gestation length, or preterm delivery. Data interpretation may have been limited by the small sample size, selection bias, and possible errors in birth length, chest circumference, and head circumference measurements.

Andersen et al. (2010) examined the association between maternal plasma PFOA concentration and offspring weight, length, and BMI at 5 and 12 months of age. The mothers (n=1010) reported the information during an interview and weight and length measurements were used to calculate BMI. Maternal plasma PFOA concentration was inversely associated

with weight at 5 months (β -30.2, 95% CI -59.3– -1.1), BMI at 5 months (β -0.067, 95% CI -0.129– -0.004), weight at 12 months (β -43.1, 95% CI -82.9– -3.3), and BMI at 12 months in boys (β -0.078, 95% CI -0.144– -0.011) in models adjusted for maternal age, parity, prepregnancy BMI, smoking, gestational age at blood draw, socioeconomic status, and breastfeeding. No associations were observed between maternal plasma PFOA concentration and the endpoints for female children in the adjusted models.

Neurodevelopmental Endpoints

Fei et al. (2008b) examined the association between plasma PFOA concentration in pregnant women and motor and mental developmental milestones of their children. The mothers self-reported the infant's fine and gross motor skills and mental development at 6 and 18 months of age. There was no association between maternal plasma PFOA concentration and Apgar score or between maternal plasma PFOA concentration and fine motor skills, gross motor skills, or cognitive skills at 6 and 18 months of age. The children born to women having higher plasma PFOA concentrations reached developmental milestones at the same times as children born to women having lower plasma PFOA concentrations. The authors concluded that there was no association between maternal early pregnancy levels of PFOA and motor or mental developmental milestones in offspring.

Fei and Olsen (2011) examined the association between prenatal PFOA exposure and behavior or coordination problems in the children at age 7. Behavioral problems were assessed using the Strengths and Difficulties Questionnaire (SDQ), and coordination problems were assessed using the Developmental Coordination Disorder Questionnaire (DCDQ) completed by the mothers. A total of 787 mothers completed the SDQ and 537 completed the DCDQ for children aged 7.01-8.47 years (mean age 7.15 years). The mean maternal PFOA concentration was 5.7 ng/mL, and PFOA levels were divided into quartiles: <LLOQ-3.95, 3.96-5.32, 5.35-7.11, and 7.14-21.90 ng/mL. A child having higher scores in total difficulties, emotional symptoms, and hyperactivity was negatively associated with the second (OR=0.56, 95% CI 0.27-1.19; $p<0.05$) or third (OR=0.36, 95% CI 0.15-0.82; $p<0.05$) PFOA quartiles when compared with women in the lowest quartile. Odds ratios adjusted for parity, maternal age, pre-pregnancy BMI, pregnancy smoking and alcohol consumption, socio-occupational status, child gender, breastfeeding, birth year, home density, gestational age at blood draw, and parental behavior problem as children did not show a positive association between prenatal PFOA exposure and behavior or coordination problems. Overall, no significant association between behavioral or coordination problems in children 7 years of age and prenatal PFOA exposure was found.

Hoffman et al. (2010) examined the associations between perfluorochemicals, including PFOA, and diagnosis of attention deficit hyperactivity disorder (ADHD) using the NHANES data from 1999-2000 and 2003-2004. The study population included 571 children aged 12-15 years including those who had been diagnosed as having ADHD (n=48) and/or taking ADHD medications (n=21). Age, gender, and race/ethnicity were included as covariates, and socioecomonic status, health insurance coverage and having a routine health care provider, living with someone who smokes, birth weight, admittance to NICU, maternal smoking, and preschool attendance were confounders. Regression models were used to analyze the data. The median serum PFOA levels were 4.4 μg/L and ranged from 0.4 to 21.7 μg/L. Serum PFOA was positively associated with parental report of ADHD (OR=1.12, 95% CI 1.01-1.23). The odds

ratio for serum PFOA and parental report of ADHD and ADHD medication use was 1.19 (95% CI 0.95-1.49). Data interpretation were limited by the cross-sectional study design, random misclassification error resulting from using current PFOA levels as proxy measures of etiologically relevant exposures, and other confounders that were not included in the available data.

Postnatal Development

Lopez-Espinosa et al. (2011) examined the association of serum PFOA concentration and the age of puberty in exposed children of the Mid-Ohio Valley. Data from the C8 Health project (sex steroid hormone levels, self-reported menarche status) along with detailed date of birth information were used to determine age of puberty in males (n=3076) and females (n=2931) aged 8-18 years. Serum PFOA concentrations were divided into quartiles: <11.4, 11.4-23, >23-58, and >58 ng/mL. Confounders including age at survey, BMI, BMI *z*-score, height, family income, ethnicity, smoking status, alchohol consumption, date and time of sample collection were included in the logistic regression models used to analyze the data. The median PFOA concentrations were 26 and 20 ng/mL in boys and girls, respectively. No association between PFOA concentration and puberty was observed for boys. Reduced odds of having reached puberty was associated with higher PFOA exposure in girls (OR=0.57, 95% CI 0.37-0.89). There were 130 days of delay between the highest and lowest quartile. Reduced odds of experiencing menarche at a younger age (10-15 years) was also observed (OR 0.83, 95% CI 0.74-0.93). The results suggested that PFOA was associated with a later age of menarche. The authors expressed caution in interpretion of the data because of lack of serum PFOA concentration prior to puberty, PFOA concentration was measured after the attainment of puberty, and lack of secondary sexual maturation data (physical, Tanner criteria, and biomarker measurements).

Christensen et al. (2011) used data from a prospective cohort study in the United Kingdom to perform a nested case-control study examining the association between age at menarche and gestational exposure to perfluorinated chemicals including PFOA. The study population from the Avon Longitudinal Study of Parents and Children included single-birth female subjects who had completed at least 2 puberty staging questionnaires between the ages of 8 and 13 years and whose mothers provided at least one analyzable prenatal serum sample. If more than one serum sample were available, the earliest sample provided was used for analysis. The study does not provide information as to when samples were collected. The females were divided into two groups including a those who experienced menarched prior to age 11.5 years (n=218) and a random sample of those who experienced menarche after age 11.5 (n=230). Confounders including the mother's pre-pregnancy BMI, age at delivery, age at menarche, educational level, and the child's birth order and ethnic background were included in linear and logistic regression models used to analyze the data. The median maternal serum PFOA concentrations were 3.9 and 3.6 ng/mL for the early menarche and non-early menarche groups, respectively. There was no association between age at menarche and prenatal PFOA exposure. The authors noted that a modest non-significant association between the odds of earlier menarche and prenatal serum PFOA concentrations above the median. For all models, the confidence intervals included the null value of 1.0. The limitations of the study included having a small sample size, using a single maternal gestational serum sample for perfluorinated chemical measurement, and missing information from the controls and cases.

Halldorsson et al. (2012) examined prenatal exposure to PFOA and the risk of being overweight at 20 years of age in a prospective study. A birth cohort consisting of 665 mother-offspring pairs was recruited from a midwife center in Aarhus, Denmark. Maternal PFOA levels were measured in serum samples collected during week 30 of gestation for assessment of *in utero* PFOA exposure and offspring anthropometry at 20 years. The median PFOA concentration was 3.7±2.0 ng/mL with quartiles of 2.4±0.6, 3.3±0.4, 4.2±0.5, and 5.8±1.9 ng/mL. In covariate adjusted analyses, female offspring whose mothers were in the highest quartile had 1.6 kg/m^2 (95% CI: 0.6, 2.6) higher BMI and 4.3 cm (95% CI: 1.4, 7.3) higher waist circumference compared to offspring whose mothers were in the lowest quartile. Female offspring of mothers in the highest versus lowest PFOA quartile were also more likely to be overweight [relative risk (RR) 3.1 (95%CI: 1.4, 6.9)] and to have a waist circumference >88 cm at 20 years of age [3.0 (95%CI: 1.3, 6.8)]. Among female participants who provided blood sample at clinical examination (n = 252), maternal PFOA concentration was positively associated with insulin, leptin and the leptin-adiponectin ratio; and inversely associated with adiponectin levels. PFOA was not associated with overweight or obesity in male offspring.

4.1.2 Cancer

Occupational Population Studies

Several occupational studies examining cancer mortality have been conducted at 3M's Cottage Grove facility in Minnesota. These studies have reported associations with prostate cancer mortality and cerebrovascular disease mortality (Alexander 2001a,b; Gilliland and Mandel, 1993).

Lundin et al. (2009, previously described) further examined occupational exposure to PFOA and death from cancer in Cottage Grove employees explored by Gilliland and Mandel (1993) using a longer period of enrolment and later follow-up. Cancers outcomes including liver, bladder, pancreas, testes, and prostate were examined in the 3,993 workers. Mortality from liver, pancreatic, prostate, and testicular cancer in PFOA workers was not elevated compared to the general population of Minnesota. Within the cohort, the SMR for prostate cancer, SMR=2.1, 95% CI 0.4-6.1, was elevated in workers who had ever worked a job in which they were exposed to PFOA.

Researchers suggested that pancreas acinar cell adenomas seen in rats exposed to PFOA may be the result of increased cholecystokinin (CCK) levels secondary to blocked bile flow (Obourn et al., 1997). CCK is a peptide hormone that stimulates the digestion of fat and protein, causes the increased production of hepatic bile, and stimulates contraction of the gall bladder. As a result, CCK was measured in male workers (n=74 males) at the 3M's Cottage Grove plant in 1997 as part of the medical surveillance program (Olsen et al., 1998, 2000). Employees' serum PFOA levels were stratified into 3 categories (<1, 1- <10, and ≥10 ppm). The mean CCK values for the 3 PFOA categories were 33.4, 28.0, and 17.4 pg/mL, respectively. The means in the 2 serum categories < 10 ppm were at least 50% higher than in the ≥ 10 ppm category. A statistically significant (p = 0.03) negative association between mean CCK levels and the three PFOA categories was observed. A multiple regression model of the natural log of CCK and serum PFOA levels continued to display a negative association after adjusting for potential confounders. As stated previously (Olsen et al., 2000), no abnormal liver function tests,

hypolipidemia, or cholestasis were observed in the workers. The authors suggested that the lack of a positive association between PFOA and CCK in workers may have resulted from serum PFOA levels too low to cause an increase in CCK provided that the same mechanism that increases CCK levels in rodents exists in humans.

Limited data are available on mortality and cancer incidence at DuPont's Washington Works Plant in Parkersburg, WV. Several studies of this population have been published (Walrath and Burke, 1989; Karns and Fayerweather, 1991; DuPont, 2003). In the most recent report (DuPont, 2003) cancer incidence for active employees was reported for the 1959-2001 time period and mortality data were reported for active and retired employees for 1957 through 2000 (DuPont, 2003). No information on employee exposure, lifestyle factors, employee demographics, or information on other chemicals used at the plant, is presented in the DuPont (2003) report.

Cancer cases were identified through a combination of company health and life insurance claims and company cancer and mortality registries. Standardized incidence ratios (SIRs) were only calculated for those cancers for which 5 or more cases were observed, which included 14 types of cancer. Two of those cancer types were elevated and statistically significant ($p = 0.05$): bladder [standardized incidence ratio (SIR) = 1.9; 95% CI (1.15-3.07)] and kidney and urinary organs [SIR = 2.3 (95% CI = 1.36-3.65)]. All of the reported cases were male. Some other cancer types with elevated SIRs (not statistically significant at $p = 0.05$) included myeloid leukemia (2.02), cancer of the larynx (1.77), multiple myeloma and immunoproliferative (1.72), malignant melanoma of skin (1.3), testicular cancer (1.46), and brain cancer (1.2).

Two separate analyses of leukemia incidence were conducted prior to the DuPont (2003) effort (Walrath and Burke, 1989; Karns and Fayerweather, 1991). Walrath and Burke (1989) reported a statistically significant ($p = 0.10$, significance level chosen by researchers) elevated odds ratio (OR) of 2.1 for leukemia incidence for male employees working at the plant from 1956-1989. Eight cases (all male) of different types of leukemia were identified. The OR remained elevated when the workers were divided into wage and salaried employees (2.2 and 2.0, respectively). In the follow-up case-control study by Karns and Fayerweather (1991), four controls were selected from the plant for each case, matched on gender, age and payroll status. Matched odds ratios were significantly elevated ($p = 0.10$) for employees who had previously worked as custodians and engineers, 8.0 (90% CI, 1.1-60.0) and 7.9 (90% CI, 1.0 - 76.0), respectively and remained elevated (although not statistically significant) for these same job categories within the plant (OR= 4.0 and 5.1, respectively). Matched ORs were also reported based on the area of the plant where the cases worked; however, no statistically significant association ($p = 0.10$) was identified.

The data reported above do not show any statistically significant ($p = 0.10$) elevations in leukemia deaths, possibly because the number of cases was very small and divided among different types of leukemia. The Washington Works data provide some insight as to where more medical surveillance should be concentrated at this plant but provide little information about the relationship of PFOA to mortality or cancer incidence since no exposure information, use of other chemicals, or lifestyle information was collected on these employees.

General Population Studies

Eriksen et al. (2009) examined the association between plasma PFOA concentration and the risk of cancer in the general Danish population. The study population was chosen from individuals (50-65 years of age) who had enrolled in the prospective Danish cohort Diet, Cancer, and Health study between December 1, 1993 and May 31, 1997. The Danish Cancer Registry and Danish Pathology Data Bank were used to identify cancer patients diagnosed between December 1, 1993, and July 1, 2006. The cancer patients (n=1240) consisted of 1,111 men and 129 women whose median age was 59 years having prostate cancer (n=713), bladder cancer (n=332), pancreatic cancer (n=128), and liver cancer (n=67). The individuals (n=772) in the subcohort comparison group were randomly chosen from the cohort study and consisted of 680 men and 92 women whose median age was 56 years. The participants answered a questionnaire upon enrollment in the cohort study, and data on known confounders were obtained from the questionnaires. Study participants provided plasma samples which were analyzed for PFOA concentration by high pressure liquid chromatography coupled to tandem mass spectrometry.

The plasma PFOA concentrations for cancer patients were as follows: men 6.8 ng/ml, women 6.0 ng/ml, prostate cancer 6.9 ng/ml, bladder cancer 6.5 ng/ml, pancreatic cancer 6.7 ng/ml, and liver cancer 5.4 ng/ml. The plasma PFOA concentrations for the subcohort comparison group were 6.9, 5.4, and 6.6 ng/mL for the men, women, and combined, respectively. Incidence rate ratios, crude and adjusted for confounders, did not indicate an association between plasma PFOA concentration and prostate, bladder, pancreatic, or liver cancer. The plasma PFOA levels in the population were lower than those observed in occupational cohorts. This study is novel in that it is the first to examine PFOA levels and cancer in the general population.

More recently, Vieira et al. (2013) investigated the relationship between PFOA exposure and cancer among the residents living near the DuPont plant in Parkersburg, WV. This analysis included incident cases of 18 cancers diagnosed from 1996-2005 in five Ohio counties and eight West Virginia counties which included public water districts contaminated with PFOA. The dataset included 7,869 cases from Ohio geocoded to residence and 17,238 cases from West Virginia linked to water district. Exposure levels and serum PFOA concentrations were estimated from data collected previously from participants in the C8 Health Project and linked to the residence or water district of the cancer cases. Indivudual-level exposure was categorized as very high, high, medium, low, or unexposed based on serum concentrations of >110 μg/L, 30.8-109 μg/L, 12.9-30.7 μg/L, 3.7-12.9 μg/L, and unexposed (background levels not given), respectively. Logistic regression was applied to individual-level data to calculate odds ratios (OR) and confidence intervals (CI) for each cancer category.

Data were first analyzed by water district. The adjusted odds ratios were increased for testicular cancer (OR: 5.1, 95% CI: 1.6, 15.6; n=8), and for kidney cancer (OR: 1.7, 95% CI: 0.4, 3.3; n=10) in Little Hocking water district, and for kidney cancer (OR: 2.0, 95% CI: 1.3, 3.1; n=23) in the Tuppers Plains water district. Residents of Little Hocking also had increased odds ratio for non-Hodgkin lymphoma (OR: 1.6, 95% CI: 0.9, 2.8; n=14) and prostate cancer (OR: 1.4, 95% CI: 0.9, 2.3; n=36).

Analysis of exposure category and cancer type showed an association between the very high PFOA exposure category and several cancers, but ORs for lower exposure categories generally did not support a positive dose-response relationship. Kidney cancer was positively associated with very high and high exposure categories (OR: 2.0, 95% CI: 1.0, 3.9; n=9 and OR: 2.0, 95% CI: 1.3, 3.2; n=22, respectively) while ORs for medium and low exposure categories were close to the null compared to the unexposed. The largest OR was for testicular cancer with the very high exposure category (OR: 2.8, 95% CI: 0.8, 9.2; n=6) but the estimate was imprecise due to small numbers. ORs for the other cancer categories were all <1.0. Ovarian cancer, non-Hodgkin's lymphoma, and prostrate cancer were positively associated with the very high exposure category, but showed weaker or negative associations for the other exposure categories (Vieira et al., 2013).

4.2 Animal Studies

4.2.1 Acute Toxicity

Oral Exposure

Dean and Jessup (1978) reported an oral LD_{50} of 680 mg/kg and 430 mg/kg for male and female CD rats, respectively. Glaza (1997) reported an oral LD_{50} of greater than 500 mg/kg in male Sprague-Dawley rats and between 250 and 500 mg/kg in females. Gabriel (1976c) reported an oral LD_{50} of less than 1000 mg/kg for male and female Sherman-Wistar rats. According to the Hodge Sterner Scale, these LD_{50} values suggest that PFOA can be classified as moderately toxic after acute oral exposures.

Inhalation Exposure

Rusch (1979) reported no mortality in male or female Sprague-Dawley rats following inhalation exposure to 186,000 mg/m^3 for one hour. Kennedy et al. (1986) reported a 4-hour LC_{50} of 980 mg/m^3 for groups of 6 male rats exposed to PFOA as a dust in air. As reported in a later publication (Kennedy et al., 2004) body weight loss, irregular breathing, and red discharge around the nose and eyes were observed. Corneal opacity and corrosion were seen at concentrations greater than or equal to 810 mg/m^3.

Dermal/Ocular Exposure

The dermal LD_{50} in New Zealand White rabbits was determined to be greater than 2000 mg/kg (Glaza, 1995). Kennedy (1985) determined a dermal LD_{50} of 4300 mg/kg for rabbits, 7000 mg/kg for male rats, and 7500 mg/kg for female rats. The animals lost body weight, exhibited lethargy, labored breathing, diarrhea, and severe skin irritation (Kennedy et al., 2004). PFOA is an ocular irritant in rabbits when the compound is not washed from the eyes (Gabriel, 1976d), but is not an irritant in rabbits when washed from the eye (Gabriel, 1976a). Markoe (1983) found PFOA to be a skin irritant in rabbits, while Gabriel (1976b) did not.

4.2.2 Short-Term Studies

Oral Exposure

Monkey. In a range-finding study, Thomford (2001a) administered PFOA to male Cynomolgus monkeys as an oral capsule containing 0, 2, or 20 mg/kg-day PFOA for 4 weeks. There were 3 monkeys in the 2 and 20 mg/kg-day groups and one monkey in the control group. Animals were

observed twice daily for mortality and moribundity and were examined at least once daily for signs of poor health or abnormal behavior. Body weights were recorded weekly and food consumption was assessed qualitatively. The monkeys were fasted overnight and blood samples were collected one week prior to the start of the study and on day 30 for measurement of serum PFOA, clinical hematology, and clinical chemistry, plus analysis for hormones (estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin). Blood samples were also collected from each animal on day 2 (approximately 24 hours after the first dose) for clinical chemistry measurements.

At scheduled necropsy, liver samples were collected for palmitoyl coenzyme A (CoA) oxidase activity (a biomarker for peroxisome proliferation) and serum PFOA. Liver, testes, and pancreas were collected and assayed for cell proliferation using proliferation cell nuclear antigen. Bile was collected from each animal for measurement of bile acid. The adrenals, liver, pancreas, spleen, and testes from each animal were examined microscopically.

All animals survived to scheduled sacrifice. There were no clinical signs of toxicity in the treated groups and there was no effect on body weight. Low or no food consumption was observed for one animal given 20 mg/kg-day. There were no effects on the hormones measured with the exception of estrone, which was notably lower in the 2 and 20 mg/kg-day PFOA groups. There was no evidence of peroxisome proliferation or cell proliferation in the liver, testes or pancreas of treated monkeys. No adverse effects were noted in either gross or clinical pathology studies. Under the conditions of this study, the no observed adverse effect level (NOAEL) was 20 mg/kg and no lowest observed adverse effect level (LOAEL) was established.

Rat. Pastoor et al. (1987) dosed male Crl:CD (SD) BR rats (n=6/group) for 1, 3, or 7 days with 0 or 50 mg PFOA/kg. Liver sections were collected at necropsy and stained with hematoxylin and eosin. Sections were also examined by electron microscopy. DNA content was also determined from the livers of rats dosed for 7 days. Treatment with 50 mg PFOA/kg for 7 days caused a 17% decrease ($p<0.05$) in mean body weight. Pair fed control rats had a 24% decrease in body weight. Body weight was not different in rats treated for 1 or 3 days compared to control rats. Liver weight of rats treated for 1 day was not different from control liver weight. The relative liver weight of rats treated for 3 days was significantly increased ($p<0.05$) compared to control relative liver weight. Absolute and relative liver weights were significantly increased ($p<0.05$) after the 7 day treatment with PFOA. A 57% decreased ($p<0.05$) was observed in relative hepatic DNA/g liver, but no difference was observed between total amount of hepatic DNA/liver when compared to the total amount of DNA/liver in control rats.

The hepatocytes of rats treated with PFOA for three days were enlarged with partially occluded sinusoids, had numerous basophilic granules, eosinophillic granular material in the cytoplasm, and fewer perinuclear glycogen vacuols compared to control hepatocytes. Enlarged with hyperplastic smooth endoplasmic reticulum, increased mitochondria, increased peroxisomes, decreased rough endoplasmic reticulum, and increased autophagosomes with electron-dense material were also observed in the hepatocytes.

Loveless et al. (2008) administered 0, 0.3, 1, 10, or 30 mg linear PFOA/kg by oral gavage to groups of male CD rats (n=10/group) for 29 days. Body weight was recorded on days 0, 3, and 6-28. At necropsy, blood was collected for hematology, clinical chemistry, and corticosterone measurements. Tissues were collected for weight and microscopic examination.

Body weight, weight gain, hematocrit, and hemoglobin were reduced at ≥10 mg PFOA/kg/day. Increased reticulocytes and hematopoieses were observed in the rats dosed with 30 mg PFOA/kg/day. Total and non-HDL cholesterol were significantly reduced at 0.3 and 1 mg/kg/day compared to control. HDL cholesterol was significantly decreased at 0.3, 1, and 10 mg/kg/day. Triglyceride levels were significantly decreased at all doses except 1 mg/kg. Absolute liver weight (≥1 mg/kg/day) and relative liver weight (≥10 mg/kg/day) were significantly increased, minimum to mild (0.3, 1 mg/kg/day) and moderate (≥10 mg/kg/day) hepatocellular hypertrophy, and focal necrosis (≥10 mg/kg/day) were observed. Although not statistically significant, serum corticosterone was increased at ≥ 10 mg/kg/day. Data on several immunological endpoints were reported as part of the Loveless et al. (2008) publication. The immunological data are included in Section 4.3.2 of this report.

Cui et al. (2009) exposed male Sprague-Dawley rats (10/group) to PFOA (96% a.i.) at 0, 5, or 20 mg/kg/day for 28 days by gavage once daily. The activity of the rats was observed over the course of the study. All rats were sacrificed after the final exposure. The rats dosed with 5 mg/kg/day exhibited hypoactivity, decreased food consumption, cachexia, and lethargy during the third week of the study. Rats dosed with 20 mg/kg/day also exhibited sensitivity to external stimuli. The visceral index (hepatic, renal, gonal weight/animal's body weight) used to evaluate hyperplasia, swelling, or atrophy was significantly increased in the treated animals compared to control animals. In the liver, treatment with 5 or 20 mg PFOA/kg caused hepatic hypertrophy, fatty degeneration, acidophilic lesions as well as angiectasis (gross dilation) and congestion in the hepatic sinusoid or central vein. In the lung, treatment with 5 or 20 mg PFOA/kg caused pulmonary congestion and focal or diffuse thickened epithelial walls. No effects were observed in the kidneys of the low dose animals, but turbidness and tumefaction (swelling) in the epithelium of the proximal convoluted tubule was observed at 20 mg PFOA/kg. Under the conditions of this study, the LOAEL was 5 mg/kg/day based on liver and pulmonary effects, and no NOAEL was established.

Male Sprague-Dawley rats (n=10/group) were fed diets containing 0 or 300 pm PFOA for 1, 7, or 28 days in two studies (Elcombe et al., 2010). The mean daily intake for study 1 and study 2 were 19 and 23 mg/kg/d, respectively. A group of rats was fed diets containing 50 ppm Wyeth 14,643, a PPARα agonist, as a positive control. The animals were observed daily and body weights and food consumption were recorded. At necropsy, day 2, day 8, or day 29, the organs were weighed, examined for gross pathology and preserved for histopathology. Liver DNA content and concentration were determined, and plasma was collected for analysis (study 1 only) of liver enzymes, cholesterol, triglycerides, and glucose. Hepatic cell proliferation and apoptosis were also determined.

In both studies, body weight significantly decreased ($p<0.05$) after 7 and 28 days on the PFOA diet. Body weight was not affected by Wyeth 14,643. Absolute liver weight was significantly increased ($p<0.05$) in rats fed PFOA diets for 7 days in the first study and in rats treated for 7 and 28 days in the second study (Table 4-4). The liver-to-body-weight ratio was significantly higher in rats fed PFOA diets for 7 and 28 days in both studies. Absolute liver weight and liver-to-body-weight ratio were significantly increased in Wyeth 14,643 diet fed rats in both studies.

After 1 day of eating the PFOA diet, plasma AST was significantly decreased and triglycerides were significantly increased. After 7 and 28 days on the PFOA diet, total cholesterol, triglycerides, and glucose levels were significantly decreased. AST was also significantly decreased after 28 days on the PFOA diet. After 1 day on the Wyeth 14,463 diet, AST and total cholesterol were significantly decreased. After 7 and 28 days on the Wyeth 14,643 diet, ALT, total cholesterol, triglycerides, and glucose levels were significantly decreased. AST was not significantly decreased in rats fed Wyeth 14,643 diets for 28 days, but it was after 7 days. Liver DNA concentration was significantly decreased ($p<0.05$) in all PFOA-exposed rats except those treated for 1 day in the second study, but liver DNA content was not altered by PFOA. In the Wyeth 14,643 rats, liver DNA concentration was significantly decreased after 1 and 7 days in the first study and 7 and 28 days in the second study. Liver DNA content in the Wyeth 14,643 treated rats was significantly increased after 7 and 28 days in both studies.

Labelling indices for hepatic cell proliferation, as measured by BrdU incorporation, was significantly increased after day 1 and 7 in study 1 in both PFOA ($p<0.05$) and Wyeth 14,643 ($p<0.01$) diet fed rats. Samples from control livers at day 29 were not available for comparisons. In study 2, labeling was significantly increased ($p<0.05$) at all time points in both groups of rats compared to labeling in control rats (Table 4-5). Apoptosis of hepatic cells was not altered by treatment with PFOA at any time point. In rats fed diets containing Wyeth 14,643 for 28 days, hepatic apoptosis was significantly decreased ($p<0.01$) compared to apoptosis observed in control livers.

TABLE 4-5. Hepatic Effects of Rats Exposed to PFOA

	Day	Study 1			Study 2		
		Control	300 ppm PFOA	50 ppm Wyeth 14,643	Control	300 ppm PFOA	50 ppm Wyeth 14,643
Liver weight (g)	1	13.6 ± 1.3	14.1 ± 2.4	15.7 ± 1.2	15.2 ± 1.9	14.4 ± 0.9	15.8 ± 1.4
	7	15.3 ± 1.3	19.2 ± 3.1*	23.1 ± 3.1*	16.6 ± 1.7	22.8 ± 2.6*	23.4 ± 2.5*
	28	18.3 ± 2.5	20.8 ± 3.2	30.6 ± 3.2*	17.2 ± 2.0	24.6 ± 2.2*	29.2 ± 4.0*
Liver-to-bw (g/kg)	1	4.25 ± .34	4.39 ± 0.44	4.64± 0.17*	4.39 ± 0.36	4.27 ± 0.14	4.49 ± 0.23
	7	4.10 ± 0.26	5.83 ± 0.55*	6.26 ± 0.48*	4.28 ± 0.24	6.56 ± 0.38*	6.34 ± 0.33*
	28	3.96 ± 0.36	5.83 ± 0.56*	7.09 ± 0.42*	3.70 ± 0.21	6.13 ± 0.53*	6.65 ± 0.59*
Labeling index (%)	1	0.22 ± 0.17	0.74 ± 0.55*	2.10 ± 1.10*	1.02 ± 0.37	2.18 ± 0.73*	4.54 ± 1.03*
	7	1.42 ± 0.65	5.94 ± 2.12*	12.56 ± 6.42*	2.57 ± 1.31	13.18 ± 3.18*	23.85 ± 7.02*
	28	ND	2.08 ± 1.03	10.15 ± 2.69	0.66 ± 0.45	1.74 ± 0.96*	5.34 ± 2.79*

From Elcombe et al., 2010
*Significantly different from control ($p<0.05$); ND=No Data

Histological examination of the livers of PFOA and Wyeth 14,643 diet fed rats showed decreased glycogen after 1, 7, and 28 days. An increase in hepatocellular hypertrophy was observed after 7 and 28 days on the diets, fatty vacuolation after 7 days, and increased hepatocellular hyperplasia was observed after 28 days on the diets. The hepatic observations were similar in both studies, and findings in Wyeth 14,643-diet fed rats were generally more pronounced or severe compared to those in PFOA-diet fed rats.

Mouse. Kennedy (1987) fed male and female CRL:CD-1 mice diets containing 0, 30, 300, or 3000 ppm PFOA for 14 days. At necropsy body weight and liver weight were recorded and analyzed. No histological evaluations were conducted. All mice died at 3000 ppm. Body

weight was decreased at 300 ppm and one female died at that dose. Both male and female mice had significantly increased ($p<0.05$) absolute and relative liver weights at all doses compared to the control. The LOAEL was 30 ppm based on increased liver weight, and no NOAEL was established. Kennedy (1987) used lower doses in a follow-up study lasting 21 days. Male and female mice were fed diets containing 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30 ppm PFOA. Absolute and relative liver weights for male and female mice were significantly increased ($p<0.05$) at $\geq$ 3 ppm PFOA. The LOAEL was 3 ppm based on increased liver weight, and the NOAEL was 1 ppm.

Loveless et al. (2008) administered 0, 0.3, 1, 10, or 30 mg linear PFOA/kg by oral gavage to groups of male CD-1 mice (n=20/group) for 29 days. Body weight was recorded on days 0, 3, and 6-28. At necropsy, blood was collected for hematology, clinical chemistry, and corticosterone measurements. Tissues were collected for weight and microscopic examination. Body weight was significantly reduced at 10 and 30 mg/kg/day. An increase in neutrophils and monocytes was observed at $\geq$10 mg/kg/day along with a decrease in eosinophils. Serum corticosterone levels were significantly increased in mice dosed with 10 mg/kg and elevated in those dosed with 30 mg/kg/day. Total cholesterol and triglycerides were significantly decreased at $\geq$10 mg/kg/day. HDL was significantly reduced at $\geq$1 mg/kg/day. In mice treated with 30 mg/kg and water, triglycerides and HDL levels were significantly decreased compared to control levels. Absolute and relative liver weights were significantly increased at $\geq$1 mg PFOA/kg/day. Increased incidences of microscopic lesions in the liver included mild hepatocellular hypertrophy at 0.3 mg/kg/day, moderate to severe hypertrophy and individual cell necrosis at $\geq$1 mg/kg/day, and increased hepatocellular mitotic figures, fatty changes, and bile duct hyperplasia at $\geq$10 mg/kg/day. Data on several immunological endpoints were reported as part of the Loveless et al. (2008) publication. The immunological data are included in Section 4.3.2 of this report.

Son et al. (2008) administered 0, 2, 10, 50, or 250 mg/L PFOA (0, 0.49, 2.64, 17.63, or 47.21 mg/kg PFOA) in the drinking water to 4 week old male ICR mice for 21 days. Food and water consumption, and body weight were recorded daily. At sacrifice, blood was collected and the liver and kidneys were removed and weighed. Plasma from the blood was used to determine levels of ALT, AST, BUN, and creatinine. Sections of the liver and kidney were processed and stained with hematoxylin and eosin or stained for caspase 3 (a biomarker for apoptosis). Expression of mRNA for tumor necrosis factor-α, interleukin-1β, and transforming growth factor-β were determined using reverse transcriptase-polymerase chain reaction (RT-PCR).

The mice exposed to 250 mg/L PFOA had significantly reduced ($p<0.05$) food and water consumption, and body weight gain ($p<0.05$) compared to control mice. Body weight gain was also significantly reduced ($p<0.05$) in mice receiving 50 mg/L PFOA in the drinking water. In all PFOA-exposed mice, relative liver weight was significantly increased in a dose dependent manner ($p<0.05$) compared to liver weight of control mice. Relative kidney weight was not affected by PFOA exposure. At $\geq$ 10 mg/L PFOA, plasma ALT activity was significantly increased, and at $\geq$ 50 mg/L PFOA, plasma AST activity was significantly elevated compared to the activity level in control mice. Exposure to PFOA did not affect BUN or creatinine.

The livers of mice exposed to $\geq$50 mg/L PFOA were characterized by enlarged hepatocytes with acidophilic cytoplasm and the presence of eosinophils. No apoptotic bodies in the liver were observed with staining for caspase 3. Exposure to PFOA did not affect kidney

morphology and did not cause toxic damage or necrosis in the kidney. In the liver, tumor necrosis factor-α expression was significantly reduced at ≥ 50 mg/L PFOA, interleukin-1β expression was significantly reduced at 250 mg/L PFOA, and transforming growth factor-β expression was significantly elevated at ≥ 50 mg/L PFOA. Under the conditions of this study, the LOAEL was 2 mg/L (0.49 mg/kg/day) based on increased liver weight, and no NOAEL was established.

Wolf et al. (2008a) gavage dosed wild-type 129S1/SvlmJ mice (n=7-8/group) and PPARα-null mice (129S4/SvJae-PPARα^{tm1Gonz}/J, n=6-8/group) with 0, 1, 3, or 10 mg PFOA/kg or 50 mg Wyeth 14,643, a PPARα agonist, and wild-type CD-1 (n=7-8/group) with 0, 1, or 10 mg PFOA/kg for 7 days to characterize hepatic effects resulting from exposure. The mice were sacrificed 24 hours following the last dosing. Blood was collected for serum and the livers were removed and weighed. Liver sections were stained with hematoxylin and eosin for examination by light microscopy and with uranyl acetate for transmission electron microscopy. Liver sections were also processed for immunohistochemistry of proliferating cell nuclear antigen (PCNA). Hepatocyte hypertrophy and vacuolation, observed in both strains of wild-type mice, were assigned a score from 0-4 based on severity with 0 being no lesions observed and 4 being panlobular hypertrophy with cytoplasmic vacuolation. Hepatic lesions in PPARα-null were assigned a score (0-4) based on cytoplasmic vacuolation as no hypertrophy was observed. The percentage labeling index was obtained by counting the number of positive PCNA cells in 900-1000 hepatocyte nuclei/animal. Slides were read blind to treatment but with knowledge of genetic status.

Compared to control values, the absolute and relative liver weights, lesion score, and labeling index were significantly increased ($p<0.05$) in a dose dependent manner in both strains of wild-type mice exposed to PFOA and were also significantly increased ($p<0.05$) in the wild-type 129S1/SvlmJ mice exposed to Wyeth 14,643 (Table 4-6). The absolute and relative liver weights and lesion score were significantly increased ($p\leq0.05$) in a dose dependent manner in all PFOA-exposed PPARα-null mice. The labeling index was significantly increased ($p<0.05$) in PPARα-null mice exposed to 10 mg PFOA/kg. Absolute and relative liver weights, lesion score, and labeling index of PPARα-null mice exposed to Wyeth 14,643 were not different from control values.

TABLE 4-6. Hepatic Effects in PFOA-treated Mice				
Group	**Liver weight (g)**	**Relative liver weight (%)**	**Lesion score**	**Labeling Index**
Wild-type CD-1 mice				
Control	1.53±0.14	4.5±0.4	0.3±0.5	0.6±0.4
1 mg/kg PFOA	2.26±0.24*	6.5±0.5*	2.1±0.9	0.7±0.5
10 mg/kg PFOA	3.48±0.54*	10.5±0.8*	3.0±0*	7.7±3.0*
Wild-type 129S1/SvlmJ mice				
Control	0.87±0.08	3.3±0.4	0.3±0.5	0.3±0.2
1 mg/kg PFOA	1.22±0.22*	1.6±0.2*	2.0±0.0*	0.7±0.6
3 mg/kg PFOA	1.70 ±0.12*	6.4±0.4*	2.0±0.0*	1.0±0.4
10 mg/kg PFOA	2.20±0.23*	8.3±0.2*	4.0±0.0*	2.4±0.9*
50 mg/kg Wyeth 14,643	1.5±0.13*	5.6±0.1*	3.3±0.5*	2.1±1.2*
PPARα-null Mice				
Control	0.92±0.08	3.4±0.4	1.1±0.4	0.2±0.2
1 mg/kg PFOA	1.2±0.14*	4.5±0.2*	1.9±0.6*	0.6±0.4
3 mg/kg PFOA	1.46±0.21*	5. 8±0.3*	3.0±0.0*	0.6±0.3
10 mg/kg PFOA	2.8±0.18*	9.4±0.6*	4.0±0.0*	7.7±3.0*
50 mg/kg Wyeth 14,643	1.07±0.24	3.9±0.5	1.4±0.5	0.6±0.5

From Wolf et al., 2008a
* Statistically different from control, $p < 0.05$

Ultrastructure evaluations were done on liver sections from wild-type 129S1/SvlmJ mice and PPARα-null mice, but not from CD-1 mice. There were the expected differences in the characteristics of hepatocytes from the control wild-type mice when compared to both the PFOA-treated and Wyeth 14,643 wild-type mice. In the PPARα-null mice, the responses of the control and Wyeth 14,643 were similar, but treatment with PFOA resulted in a different response. Table 4-7 summarizes the cellular characteristics of the hepatocytes for the control, POFA-treated, and Wyeth 14,643-treated wild-type and PPARα-null mice on the basis of their glycogen content, golgi-bodies and associated rough endoplasmic reticulum (ER), mitochondrial, peroxisomes, and lipid-like cytoplasmic vacuoles.

TABLE 4-7. Mouse Hepatocyte Ultrastructure After PFOA or Wythe 14,643 Treatment					
Mouse/treatment	**Characteristics**				
	Glycogen	**Golgi/ rough ER**	**Mitochondria**	**Peroxisomes**	**Lipid-like Vacuoles**
Wild-type/ Control	Prominent	Prominent	Numerous	Few	Rare
Wild-type/ PFOA	Negative	Nominal/ scarce ER	Numerous	Numerous	Scattered
Wild-type/ Wyeth	Negative	Nominal/ scarce ER	Numerous	Numerous	Scattered
PPARα-null/ Control	Prominent	Prominent	Numerous	Absent	Scattered
PPARα-null/ PFOA	Limited	Limited	Not reported	Not reported	Numerous*
PPARα-null/ Wyeth	Prominent	Prominent	Numerous	Absent	Scattered

From Wolf et al., 2008a.
*Described as electron-dense, nonmembrane-bound spaces morphologically consistent with lipids ranging from the size of mitochondria to the size of nuclei. The vacuoles were believed to be an accumulation of PFOA.

It is apparent from the data in Table 4-7 that both PFOA and Wyeth 14,643 behaved similarly in the wild-type strains but differently in the PPARα-null mice. The hepatocytes of PFOA-dosed PPARα-null mice exhibited lower glycogen content, golgi-bodies and associated rough ER than both the control and Wyeth 14,643 PPARα-null mice. In addition, the PFOA-dosed PPARα-null mice had numerous large non-membrane bound lipid-like vacuoles throughout the cytoplasm. At the high dose (10 mg/kg/day), there was an increase in the labeling index that was not observed with Wyeth 14,643. The authors concluded that the large lipid-like vacuoles in the hepatocytes of PFOA-dosed PPARα-null mice were likely accumulations of PFOA. Under the conditions of this study, the LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight and hepatic morphology changes, and no NOAEL was established.

Nakamura et al. (2009) investigated the functional difference in PFOA response between mice and humans using a humanized PPARα transgenic mouse strain (hPPARα). Humanized PPARα mice express a high level of human PPARα protein in the liver. Male, 8-week old wild-type (mPPARα) mice, PPARα-null mice, and hPPARα mice were gavage dosed with 0, 0.1, or 0.3 mg/kg/day PFOA (n=4-6/group) for 2 weeks and sacrificed 18-20 hours following the last dose. Blood was collected and analyzed for triglycerides and cholesterol concentrations and ALT measurements. Livers were collected and analyzed for triglycerides and cholesterol concentration, and histopathological changes. The differences in the observations for the three strains of mice are summarized in Table 4-8.

TABLE 4-8. Relative Response of hPPARα, mPPARα, and PPARα-null mice to PFOA			
Parameter	**hPPARα**	**mPPARα**	**PPARα-null**
Liver weight	ND	↑ compared to control (0.3 mg/kg/day)	↓ compared to control (0.1 mg/kg/day)
Liver/body weight ratio	ND	↑ compared to control (0.3 mg/kg/day)	ND
Hepatocyte hypertrophy	Mild (0.3 mg/kg/day)	Mild (0.3 mg/kg/day)	ND
ALT	ND	ND	ND
Plasma cholesterol	↑ compared to mPPARα (all doses)	ND	ND
Liver cholesterol	↓ compared to PPARα-null (0.1, 0.3 mg/kg/day), mPPARα (0.3 mg/kg/day)	↑ compared to control (0.3 mg/kg/day)	ND
Plasma triglyceride	ND	ND	ND
Liver triglyceride	↓ compared to PPARα-null (0.3 mg/kg/day)	↓ compared to PPARα-null (0.1, 0.3 mg/kg/day; ↑ compared to control (0.3 mg/kg/day)	↑ compared to mPPARα (all doses)

From Nakamura et al., 2009
Mice were dosed with with 0, 0.1, or 0.3 mg/kg/day PFOA
↑=significant increase ($p<0.05$); ↓= significant decrease ($p<0.05$); ND= no differences

Body weight of the hPPARα mice was slightly lower than mPPAR and PPA-null mice prior to PFOA treatment and remained lower throughout the dosing regimen. Treatment with PFOA did not affect plasma ALT or triglyceride concentrations in any group. The hPPARα mice differed from the wild-type mice in that their plasma cholesterol was significantly increased and their liver cholesterol and triglycerides significantly decreased at the highest dose (Table 4-7). In addition, the increases in absolute and relative liver weights were less than those observed in the wild-type mice. The PPARα-null mice differed from the wild-type in that liver triglycerides were significantly increased. Comparable to the Wolf et al. (2008a) report, the cytoplasmic vacuoles were larger in the PPARα-null mice than in the wild-type and hPPARα mice. There were no other significant differences between PPARα-null mice and wild-type mice.

Under the conditions of the study, the LOAEL for mPPARα mice was 0.3 mg/kg PFOA based on increased liver weight and increased liver triglycerides and cholesterol concentrations. The NOAEL for mPPARα mice was 0.1 mg/kg PFOA. The NOAEL for PPARα-null mice was 0.3 mg/kg because the changes in absolute liver weight were not dose related and the increase in relative liver weight was not significantly different from the control. The NOAEL for hPPARα mice was 0.3 mg/kg PFOA, the highest dose tesed. However, a nonsignificant, dose related increase was observed in plasma cholesterol.

Minata et al. (2010) examined hepatobiliary injury in mice treated with PFOA. Male wild-type 129S4/SvlmJ mice (n=39) and PPARα-null (129S4/SvJae-Pparα^{tm1Gonz}/J mice (n=40) were orally dosed with 0, 12.5, 25, or 50 μmol/kg PFOA (~0, 5.4, 10.8, or 21.6 mg/kg PFOA) for 4 weeks. At the end of 4 weeks, animals were sacrificed and blood, liver, and bile were collected for clinical chemistry analysis and determination of PFOA concentration. Sections of the liver were processed for histological examination, oxidative DNA damage, and multidrug resistance

protein 2 (Mdr2) and tumor necrosis factor α (TNF-α) mRNA expression. Bile acid and phospholipid contents in bile were determined as well as the protein expression of canalicular bile salt export pump (BSEP) and canalicular multidrug resistance-associated protein 2 (MRPs).

Absolute and relative liver weights in all PFOA treated wild-type and PPARα-null mice were significantly increased ($p<0.05$) at sacrifice compared to control liver weight. Plasma AST was significantly increased in wild-type mice at 25 and 50 μmol/kg and in PPARα-null mice at 50 μmol/kg compared to the concentrations of their respective controls. Plasma ALT was not different from control in the treated mice. In wild-type mice, total bilirubin was significantly decreased at 12.5 μmol/kg and significantly increased at 50 μmol/kg. In PPARα-null mice, total bilibrubin was significantly increased at 50 μmol/kg. Total bile acid was significantly increased at 50 μmol/kg in PPARα-null mice. Total cholesterol was significantly decreased in wild-type mice at 25 and 50 μmol/kg and total triglyceride was significantly increased at 12.5 and 25 μmol/kg. Total cholesterol was significantly decreased at 12.5 and 25 μmol/kg and significantly increased at 50 μmol/kg in PPARα-null mice. In PPARα-null mice, total triglycerides were significantly increased at all doses.

Hepatocellular hypertrophy was observed in wild-type mice treated with 12.5, 25, or 50 μmol/kg. A dose dependent increase in eosinophilic cytoplasmic changes consistent with peroxisome proliferation was observed in liver parenchyma, but no fat droplets or focal necrosis were observed in wild-type mice. An increase in bile duct epithelium thickness suggested slight cholangiopathy in wild-type mice at 25 and 50 μmol/kg. Increased apoptosis in hepatic cells, hepatic arterial walls, and bile duct epithelium was observed at 25 and 50 μmol/kg in wild-type mice. Ultrastructure examination of livers from PFOA treated wild-type mice showed decreased glycogen granules, degranulated or disrupted rough endoplasmic reticulum, nuclear vacuoles, extensive peroxisome proliferation, and slight mitochondria proliferation.

In PPARα-null mice treated with 12.5, 25, or 50 μmol/kg PFOA, hypatocellular hypertrophy, cytoplasmic vacuolation, and increased microvesicular steatosis were observed. These observations are consistent with Wolf et al. (2008a). At 50 μmol/kg, focal necrosis was observed. Areas of bile fibrosis and bile plaque and few inflammatory cells were observed in the bile ducts of PPARα-null mice at 25 and 50 μmol/kg. Increased apoptosis was observed in bile duct epithelium at 25 and 50 μmol/kg in PPARα-null mice. Ultrastructure examination of livers from PFOA treated PPARα-null mice showed decreased glycogen granules, degranulated or disrupted rough endoplasmic reticulum, increased cytoplasmic lipid accumulation, mitochondria proliferation, and mitochondrial changes including swelling and decreased matrix density. Peroxisome proliferation was not observed. Ultrastructure of bile duct showed degradation of cytoplasmic structure, vacuolization, disintegrations of nuclei and organelles, and periductal infiltration of fibroblasts and macrophages as well as fibrosis.

The marker for oxidative damage, 8-hydroxydeoxyguanosine, and TNF-α were not elevated or upregulated in wild-type mice. In PPARα-null mice, 8-hydroxydeoxyguanosine was elevated in the liver at 50 μmol/kg and TNF-α mRNA was significantly increased at 25 and 50 μmol/kg. The transporter Mdr2 moves biliary phospholipids from hepatocytes to bile and was significantly upregulated in wild-type mice at all doses, but only at 12.5 μmol/kg in PPARα-null mice. The BSEP transports bile acid from hepatocytes to bile and was significantly decreased in wild-type mice at 50 μmol/kg, significantly increased in PPARα-null mice at 12.5 μmol/kg, and

significantly decreased at 50 μmol/kg. The transporter MRP2 also transports bile acid and was significantly decreased at 50 μmol/kg in both groups of rats.

Under the conditions of the study, the LOAEL for male wild-type and PPARα-null mice was 12.5 μmol/kg PFOA (~5.4 mg/kg) based on increased liver weight. A NOAEL was not established.

Li et al. (2011) investigated the involvement of mouse and human PPARα in PFOA-induce testicular toxicity. Wild-type, PPARα-null, and humanized PPARα male 129/Sv mice were given PFOA daily by gavage at doses of 0, 1, or 5 mg/kg/d for six weeks. Body weight and testis weight were not affected by treated in any group. Absolute and relative-to-body weights of the epididymis and seminal vesicle plus prostate gland were decreased only in high-dose wild-type mice compared to the wild-type controls. No effects on sperm count and motility were seen in any group. Sperm abnormalities were significantly increased in both treated groups of wild-typ and humanized PPARα mice, but not in the PPARα-null mice. Plasma testosterone levels were slightly decreased in low-dose wild-type mice, and significantly decreased in high-dose wild-type and low- and high-dose humanized PPARα mice compared to the control groups. Testosterone levels were slightly reduced in a dose-related manner in the PPARα-null mice, but statistical significane was not attained.

Using real-time quantitative PCR, the mRNA levels for several genes associated with testicular cholesterol synthesis, transport, and testosterone biosynthesis were examined. Levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, and aromatase were not changed after treatment in any group. Expression of steriodogenic acute regulatory protein (which transports cholesterol into mitochondria) was inhibited in wild-type mice at the high dose and in humanized PPARα mice at both doses; peripherial benzodiazepine receptor level was decreased only in high-dose humanized PPARα mice; cytochrome P450 side-chain cleavage enzyme was decreased in both groups of wild-type mice; cytochrome P450 17α-hydroxylase/C17-20 lyase was inhibited at the high dose of both wild-type and humanized PPARα mice; and 3β-hydroxysteroid dehydrogenase was decreased in both treated groups of humanized PPARα mice. Decreased expression of 17β-hydroxysteroid dehydrogenase was the only change found in treated PPARα-null mice. In the mitochondria, carnitine palmitoyltransferase was decreased in both groups of wild-type and high-dose humanized PPARα mice and superoxide dismutase levels were reduced in all treated wild-type and humanized PPARα mice. Histopathological lesions of the testes, including abnormal seminiferous tubules, lack of germ cells, or necrotic cells were observed in high-dose wild-type and humanized PPARα mice. No morphological changes were observed in the testes from PFOA treatment in PPARα-null mice.

Inhalation Exposure

No data on the effects of short term inhalation exposures to PFOA were identified in the literature.

Dermal Exposure

Fairley et al. (2007) investigated the role of dermal exposure to PFOA in an experiment to evaluate toxicity in BALB/c mice. The mice were exposed to 0, 0.01%, 0.1%, 0.25%, 0.5%, 1.0%, or 1.5% PFOA (equivalent to 0, 0.25, 2.5, 6.25, 12.5, 25, or 50 mg/kg PFOA). It was applied to the dorsal surface of both ears daily for 4 days. The mice were sacrificed 6 days later. Dermal PFOA exposure did not cause reductions in body weight or signs of inflammation at the application site. A significant increase in liver weight was observed in mice dosed with ≥ 6.25 mg/kg PFOA ($p<0.01$) compared to control liver weight. Under the conditions of the study, the LOAEL was 6.25 mg/kg PFOA based on increased liver weight, and the NOAEL was 2.25 mg/kg PFOA.

4.2.3 Subchronic Studies

Oral Exposure

Monkey. Goldenthal (1978b) administered rhesus monkeys (2/sex/group) doses of 0, 3, 10, 30 or 100 mg/kg-day PFOA by gavage for 90 days. Animals were observed twice daily and body weights were recorded weekly. Blood and urine samples were collected once during a control period, and at 1 and 3 months for hematology, clinical chemistry and urinalysis. Organs and tissues from animals that were sacrificed at the end of the study and from animals that died during the treatment period were weighed, examined for gross pathology, and processed for histopathology.

All monkeys in the 100 mg/kg-day group died between weeks 2-5 of the study. Signs and symptoms which first appeared during week 1 included anorexia, frothy emesis, swollen face and eyes, decreased activity, prostration and body trembling. Three monkeys from the 30 mg/kg-day group died during the study. Beginning in week 4, all four animals showed slight to moderate, and sometimes severe, decreased activity. One monkey had emesis and ataxia, swollen face, eyes and vulva. Beginning in week 6, two monkeys had black stools and one monkey had slight to moderate dehydration. No monkeys in the 3 or 10 mg/kg-day groups died during the study. One monkey in the 10 mg/kg-day group was anorexic during week 4, had a pale and swollen face in week 7 and had black stools for several days in week 12. Animals in the 3 mg/kg-day group occasionally had soft stools or moderate to marked diarrhea and frothy emesis.

Changes in body weight were similar to the controls for animals from the 3 and 10 mg/kg-day groups. Monkeys from the 30 and 100 mg/kg-day groups lost body weight after week 1. At the end of the study, this loss was statistically significant for the one surviving male in the 30 mg/kg-day group and reflected in body weight (2.30 kg vs. 3.78 kg for the control). The results of the urinalysis, and hematological and clinical chemistry analyses were comparable for the control and the 3 and 10 mg/kg-day groups at one and three months.

At necropsy, there were significant decreases in the absolute heart and brain weight and relative liver weight in 10 mg/kg-day females. At 3 mg/kg-day the relative pituitary weight in males was significantly increased. The biological significance of these weight changes is difficult to assess, as they were not accompanied by morphologic changes.

In animals that died, one male and two females from the 30 mg/kg-day group and all animals from the 100 mg/kg-day group had marked diffuse lipid depletion in the adrenal glands.

All males and females from the 30 and 100 mg/kg-day groups also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. One female from the 30 mg/kg-day group and all animals in the 100 mg/kg-day group had moderate atrophy of the lymphoid follicles in the lymph nodes.

The one male in the 30 mg/kg-day group that survived until terminal sacrifice had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. Under the conditions of this study, the male LOAEL was 3 mg/kg-day based on increased relative pituitary weight, and no NOAEL was established. The female LOAEL was 10 mg/kg based on decreased heart and brain weight, and the NOAEL was 3 mg/kg.

Rat. Goldenthal (1978a) administered ChR-CD rats (5/sex/group) dietary levels of 0, 10, 30, 100, 300, and 1000 ppm PFOA for 90 days. These dose levels are equivalent to 0, 0.56, 1.72, 5.64, 17.9, and 63.5 mg/kg-day in males, and 0.74, 2.3, 7.7, 22.36 and 76.47 mg/kg-day in females. Animals were observed twice daily and body weight and food consumption were recorded weekly. Blood and urine samples were collected during the pretest period and at 1 and 3 months of the study for hematology, clinical chemistry and urinalysis. At necropsy, the organs from the control, 100, 300, and 1000 ppm groups were weighed and examined for histopathologic lesions; livers from the 10 and 30 ppm groups were also examined microscopically.

There were no treatment-related changes in behavior or appearance and no treatment related deaths. There was a decrease in body weight gain for male rats at the 300 and 1000 ppm dose levels. At 13 weeks, mean body weight of males in the 1000 ppm group was significantly less than that of controls. There were no treatment related effects on the hematologic, biochemical or urinary parameters.

Relative kidney weights were significantly increased in males in the 100, 300, and 1000 ppm groups. However, absolute kidney weights were comparable among groups, and there were no histopathological lesions. Absolute liver weights were significantly increased in males in the 30, 300 and 1000 ppm groups and in females in the 1000 ppm group. The mean absolute liver weight for the 0, 10, 30, 100, 300, and 1000 ppm groups was 13.4, 14.3, 19.1, 18.6, 20.1, and 19.2 g, respectively. Relative liver weights were significantly increased in males in the 300 and 1000 ppm groups and in females in the 1000 ppm group. Discoloration on the surface of the liver was observed in male rats in the 1000 ppm group. Hepatocellular hypertrophy (focal to multifocal in the centrilobular to midzonal regions) was observed in 4/5, 5/5, and 5/5 males in the 100, 300, and 1000 ppm groups, respectively. Hepatocyte necrosis was observed in 2/5, 2/5, 1/5, and 2/5 males in the 30, 100, 300, and 1000 ppm groups, respectively. Under the conditions of this study, the LOAEL for males is 30 ppm (1.72 mg/kg-day) based on liver effects and the NOAEL is 10 ppm (0.56 mg/kg-day); the LOAEL for females is 1000 ppm (76.5 mg/kg-day) based on increased liver weight, and the NOAEL is 300 ppm (22.4 mg/kg-day).

In a dietary study, reported in Palazzolo (1993) and Perkins et al. (2004), male ChR-CD rats (45-55 per group) were administered concentrations of 1, 10, 30, or 100 ppm PFOA for 13 weeks. These doses are equivalent to 0.06, 0.64, 1.94, and 6.50 mg/kg-day. There were two control groups (a nonpair-fed control group and a control group pair-fed to the 100 ppm dose group); both fed the basal diet. Following the 13-week exposure period, 10 animals per group

were fed basal diet for an 8-week recovery period. The animals were observed twice daily for clinical signs of toxicity, and body weights and food consumption were recorded weekly. Food consumption was recorded daily for the pair-fed animals.

A total of 15 animals per group were sacrificed following 4, 7, or 13 weeks of treatment; 10 animals per group were sacrificed after 13 weeks of treatment and following the 8 week recovery period. Serum samples collected from 10 animals per group at each scheduled sacrifice during treatment and from 5 animals per group during recovery were analyzed for estradiol, total testosterone, luteinizing hormone, and PFOA. The level of palmitoyl CoA oxidase was analyzed from a section of liver that was obtained from 5 animals per group at each scheduled sacrifice. Weights of the brain, liver, lungs, testis, seminal vesicle, prostate, coagulating gland, and urethra were recorded, and these tissues were examined histologically. In addition, the brain, liver, lungs, testis, seminal vesicle, and prostate were preserved in glutaraldehyde for electron microscopic examination.

In the analysis of the data, animals in groups exposed to 1, 10, 30, and 100 ppm PFOA were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to 100 ppm PFOA. At 100 ppm, significant reductions in body weight and body weight gain were seen compared to the pair-fed control group during week 1 and the nonpair-fed control group during weeks 1-13. Body weight data in the other dosed-groups were comparable to controls. At 10 and 30 ppm, mean body weight gains were significantly lower than the nonpair-fed control group at week 2. These differences in body weight and body weight gains were not observed during the recovery period. Animals fed 100 ppm consumed significantly less food during weeks 1 and 2, when compared to the nonpair-fed control group. Overall, there was no significant difference in food consumption. There were no significant differences among the groups for any of the hormones evaluated although there appeared to be some indication of elevated estradiol for the 100 ppm group at week 5.

Significant increases in absolute and relative liver weights and hepatocellular hypertrophy were observed at weeks 4, 7, or 13 in the 10, 30 and 100 ppm groups (Table 4-9). There was no evidence of any degenerative changes. Hepatic palmitoyl CoA oxidase activity was significantly increased at weeks 4, 7, and 13 in the 30 and 100 ppm groups. At 10 ppm, hepatic palmitoyl CoA oxidase activity was significantly increased at week 4 only. During recovery, however, no liver effects were observed, indicating that the treatment-related liver effects were reversible. Under the conditions of this study, the LOAEL is 10 ppm (0.64 mg/kg-day) based on increases in absolute and relative liver weights with hepatocellular hypertrophy. The NOAEL is 1.0 ppm (0.06 mg/kg-day).

TABLE 4-9. Liver Effects In Male Rats							
Parameter	**Week**	**Dose (mg/kg-day)**					
		0 [a]	**0** [b]	**0.06 (1 ppm)**	**0.64 (10 ppm)**	**1.94 (30 ppm)**	**6.5 (100 ppm)**
Palmitoyl CoA Oxidase (IU/g)	4	8± 0.5	5± 0.4	9± 1.7	14± 3.8[c]	24 ± 11.4[c]	37± 14.8[cd]
	7	7± 1.5	7 ± 1.5	7± 0.8	18 ±5.5	32 ± 12.2[c]	54± 35.3[cd]
	13	8 ± 0.9	5± 1.1	8 ±1.9	10 ±2.1	14 ± 3.4[c]	17 ±4.5[cd]
Hepatocellular Hypertrophy	4	0/15	0/15	0/15	12/15	15/15	14/15
	7	0/15	0/15	0/15	12/15	15/15	15/15
	13	0/15	0/15	0/15	13/15	14/15	15/15
Hepatocellular Necrosis, Coagulative	4	0/15	1/15	0/15	0/15	1/15	2/15
	7	0/15	0/15	0/15	0/15	0/15	1/15
	13	0/15	0/15	1/15	0/15	1/15	0/15
Absolute Liver Weight (g)	4	16.34±2.14	15.83±1.13	15.45±1.71	17.89 ±2.13	23.23 ±2.83[c]	25.44 ±1.89[c]
	7	17.78±2.12	16.91±2.22	17.68±NA	19.42±2.10	27.76±3.51[c]	27.76±3.51[c]
	13	19.73±2.01	16.30±1.62	18.03±2.81	20.44±2.87	22.74±4.21	26.78±5.47[c]
Liver/Body Weight (%)	4	3.97± 0.37	4.07 ±0.27	3.73 ±0.23	4.49 ±0.32[c]	5.77 ±0.60[c]	6.73 ±0.49[c]
	7	3.75±0.29	3.76±0.37	3.64±0.33	4.12±0.37	5.14±0.53[c]	6.06±0.59[c]
	13	3.53±0.28	3.23±0.23	3.24±0.30[c]	3.69±0.32	4.21±0.56[c]	5.49±0.84[c]

From Palazzolo 1993; Perkins et al. 2004
Mean ±SD; NA= not available
[a] non-pair-fed controls; [b] pair-fed controls; [c] statistically significant ($p < 0.05$) with non-pair-fed control; [d] statistically significant ($p < 0.05$) with pair-fed control

Inhalation and Dermal Exposure

No data on the effects of subchronic inhalation or dermal exposures to PFOA were identified in the literature.

4.2.4 Neurotoxicity

Johansson et al. (2008) gave male neonatal Naval Medical Research Institute mice (NMRI, 3-4 litters, ~5-6 male pups/litter) a single gavage dose of 0, 0.58, or 8.7 mg PFOA/kg in a lecithin/peanut oil emulsion on postnatal day 10, the approximate peak time of rapid brain growth in mice. Spontaneous behavior (locomotion, rearing, and total activity) and habituation in response to a placement in unfamiliar environment were tested in 10 mice in each group at ages 2 and 4 months. Each test period was divided into three 20 minute periods. The habituation ratio was determined by dividing the activity for the third 20 minute period by the activity for the first period. A high habituation ratio indicated that movement patterns of the exposed animals when placed in an unfamiliar test chamber differed from control by displaying comparatively low activity for the first 20 minutes and comparatively higher activity for the last 20 minutes.

Exposure to PFOA did not affect body weight or body weight gain in male NMRI mice following treatment. Compared to controls, the habituation ratio for rearing and locomotion in the high dose animals was elevated compared to controls at 2 and 4 months with a significantly higher ratio ($p<0.01$) at 4 months when compared to 2 months. At 4 months, the changes in activity patterns for the high dose were significant ($p<0.01$) compared to controls for locomotion, rearing, and total activity. The results at the low dose were less pronounced, with a significant impact on locomotion and slight changes in on rearing behavior.

At 4 months of age, mice were tested for nicotine-induced behavior and behavior in the

elevated plus-maze. Increased activity is the expected response to nicotine injection (80 μg) as a result of stimulation of the cholinergic receptors in the brain. The activity responses of the PFOA exposed animals to nicotine stimulation were significantly less than the response of the controls, but the differences were most pronounced in the high dose animals.

The mice were also tested in an elevated plus-maze which determined whether they would select an enclosed environment (the expected response) over an open environment. No significant differences were observed in the PFOA exposed mice in this test. Under the conditions of this study, the clear LOAEL was 8.7 mg/kg based on locomotion, rearing, and total activity; habituation ratio; and response to nicotine at 2 and 4 months. There were significant differences in locomotion and total activity at 4 months in the low dose animals which support identifying the 0.58 mg/kg dose as a marginal LOAEL. However, the data at the low dose are less compelling as those for the high dose.

In a follow-up to their original study, Johansson et al. (2009) gave male neonatal NMRI mice (3-4 litters, ~5-6 male pups/litter) a single gavage dose of 0 or 8.7 mg PFOA/kg on postnatal day 10. Protein levels of calcium/calmodulin-dependent protein kinase II (CaMKII), growth-associated protein-43 (GAP-43), synaptophysin and tau protein were determined in the cerebral cortex and hippocampus. CaMKII regulates synapotogenesis and synaptic plasticity, GAP-43 modulates axon sprouting and growth, synaptophysin is a membrane glycoprotein in presynaptic vesicles, and tau protein is responsible for outgrowth of neuronal processes and microtubule assembly and maintenance.

Levels of CaMKII protein in the hippocampus were significantly higher (58%, $p \leq 0.05$) in mice exposed to PFOA than levels in control mice, but not changed in the cerebral cortex. Levels of GAP-43 protein in the hippocampus were significantly higher (17%, $p \leq 0.05$) in PFOA-exposed mice than levels in control mice, but not changed in the cerebral cortex. Synaptophysin levels in mice exposed to PFOA were significantly increased in the hippocampus (52%) and cerebral cortex (82%). Tau protein levels in PFOA-exposed mice were increased 92% and 142% ($p \leq 0.001$) in the hippocampus and cerebral cortex, respectively, above levels in control mice. The authors concluded that alterations of these proteins could be a factor in the altered behavior of adult mice that were exposed to PFOA as neonates because they are required for normal brain development.

Onishchenko et al. (2011) exposed pregnant C57BL/6/Bkl mice (n=6/group) to 0 or 0.3 mg PFOA/kg/day in the diet from GD1-end of pregnancy. The behavior of the weaned offspring was analyzed in locomotor, circadian activity, elevated plus-maze, and forced swim tests at 5-8 weeks of age. Muscle strength and motor coordination tests were given at 3-4 months of age. The distance traveled over 30 minutes was registered in 5 minute intervals in the locomotor test. For the circadian activity test, the activity of the mice in social groups was monitored for 48 hours after placement in new cages. Anxiety-like behavior was determined using the elevated plus maze. Depression-like behavior was determined in the forced swim test by tracking the time spent floating passively for 2 seconds or longer. Muscle strength (3 trials) was measured by how long (within 60 seconds) it took the mouse to fall off an upside down lid onto the cage floor. Motor coordination (4 trials) was measured by how long the mice remained on a rotating drum as a rotarod accelerated from 4 to 40 rpm over 5 minutes.

Prenatal exposure to PFOA did not alter offspring locomotor activity, anxiety-related behavior, depression-like behavior, or muscle strength. In the circadian activity tests, male offspring exposed to PFOA were significantly more active ($p=0.013$) and the female offspring were significantly less active ($p=0.036$) than than control offspring during the first hour of the test. PFOA-exposed male offspring were significantly more active ($p<0.05$) than control males from the dark phase of Day1 through the dark phase Day 2. Both male and female offspring exposed to PFOA had significantly ($p<0.05$) less inactive periods during the light phase compared to their respective controls. In the accelerating rotarod test, female offspring exposed to PFOA exhibited decreased fall latency over the 4 trials compared to control females, but no effect of treatment was observed in male offspring. The authors concluded that prenatal exposure to PFOA resulted in sex-related alterations in offspring behavior and motor function.

4.2.5 Developmental/Reproductive Toxicity

Reproductive Effects

A standard oral two-generation reproductive toxicity study of PFOA in Sprague-Dawley rats was conducted (York, 2002; Butenhoff et al., 2004a; York et al., 2010). Five groups of male and female SD rats (30/sex/group) were administered PFOA by gavage at doses of 0, 1, 3, 10, and 30 mg/kg-day. The parental generation (F0) rats (n=30/sex/group) were dosed for 10 weeks prior to mating and until sacrificed (after mating for males; after weaning for females). F1 generation rats (n=60/sex/group) were dosed similarly, beginning at weaning. The F2 generation rats were maintained through lactation day 22. Reproductive parameters evaluated in the F0 and F1 generations included estrus cyclicity, sperm number and quality, mating, fertility, natural delivery, and litter viability and growth. Age at sexual maturation in F1 pups, anogenital distance in F2 pups, and presence of nipples (males) in F2 pups were also determined. Food consumption, body-weight gain, selected organ weights, gross pathology, and appropriate histopathology were evaluated.

F0 Males. One F0 male rat in the 30 mg/kg-day dose group was sacrificed on day 45 of the study due to adverse clinical signs. Statistically significant increases in clinical signs were also observed in male rats in the high-dose group that included dehydration, urine-stained abdominal fur, and ungroomed coat. Significant reductions in body weight and body weight gain were reported for most of the dosage period and continuing until termination of the study in the 3, 10, and 30 mg/kg-day dose groups. Absolute food consumption values were also significantly reduced during these periods in the 30 mg/kg-day dose group, while significant increases in relative food consumption values were observed in the 3, 10, and 30 mg/kg-day within those same periods.

No treatment-related effects were reported at any dose level for any of the reproductive parameters assessed. No treatment-related effects were seen at necropsy or upon microscopic examination of the reproductive organs.

At necropsy, statistically significant reductions in terminal body weights were seen at 3, 10, and 30 mg/kg-day (6%, 11%, and 25% decrease from controls, respectively; $p\leq0.05$). The absolute weight of the liver was statistically significantly increased in all dose-groups (Table 4-10). Absolute kidney weights were statistically significantly increased in the 1, 3, and 10 mg/kg-

day dose groups, but significantly decreased in the 30 mg/kg-day group. Organ weight-to-terminal body weight ratios for the liver and for the left and right kidney were statistically significantly increased in all treated groups. Organ weight-to-terminal body weight ratios for the brain were statistically significantly increased for the 3, 10, and 30 mg/kg-day dose groups; organ weight-to-brain weight ratios were significantly reduced for some organs at the high dose group, and significantly increased for other organs among all treated groups; these changes occurred in a pattern typically associated with decrements in body weight. The only histologic finding was increased thickness and prominence of the zona glomerulosa and vacuolation in the cells of the adrenal cortex observed in 2/10 males in the 10 mg/kg-day group and 7/10 males in the 30 mg/kg-day dose group.

TABLE 4-10. Organ weight data from F0 males

	0 mg/kg-day	1 mg/kg-day	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
Body weight (g)	581±40	575±48	542±47**	513±54**	432±64**
Brain weight (g)	2.26±0.17	2.28±0.10	2.26±0.12	2.24±0.12	2.20±0.14
Liver weight (g)	20.3±2.5	24.3±3.2**	27.7±2.7**	28.7±3.9**	27.5±3.7**
Liver/body (%)	3.49±0.29	4.22±0.50**	5.13±0.47**	5.61±0.51**	6.42±0.73**
Liver/brain (%)	903±119	1066±154**	1230±120**	1285±183**	1248±144**
Rt. kidney (g)	2.19±0.18	2.54±0.30**	2.50±0.18**	2.36±0.25**	2.06±0.20*
Rt. kidney/body (%)	0.379±0.030	0.443±0.048**	0.463±0.039**	0.462±0.034**	0.481±0.051**
Rt. kidney/brain (%)	97.5±9.9	111.6±13.5**	111.0±9.5**	105.6±12.4**	93.5±8.7
Lt. kidney (g)	2.19±0.20	2.51±0.28**	2.51±0.21**	2.34±0.24*	1.99±0.19**
Lt. kidney/body (%)	0.378±0.036	0.437±0.047**	0.465±0.043**	0.457±0.040**	0.466±0.054**
Lt. kidney/brain (%)	97.5±10.7	110.1±12.6**	111.7±10.5**	104.6±11.7*	90.4±8.7*

From Butenhoff et al. 2004a.
Mean±S.D.; n = 29-30; significantly different from control: *p<0.05, **p<0.01.

Under the conditions of the study, the LOAEL for F0 parental males is 1 mg/kg-day, the lowest dose tested, based on significant increases in absolute and relative liver and kidney weight. A NOAEL for the F0 parental males could not be determined.

F0 Females. There were no treatment-related effects on clinical signs, body weight, food consumption, organ weights, or histology of the organs. There were no treatment-related effects on any of the reproductive parameters assessed, and no treatment-related effects were seen at necropsy or upon microscopic examination of the reproductive organs. The NOAEL for F0 parental females is 30 mg/kg-day, the highest dose tested.

F1 Generation. Pup body weight on a per litter basis (sexes combined) was significantly reduced ($p \leq 0.01$) by 8-10% throughout the first two weeks of lactation in the 30 mg/kg-day group; at weaning the mean body weights were reduced 4.5%, but the difference was not statistically significance. Although there were no effects on the viability and lactation indices, the total number of dead pups during lactation was increased in the 30 mg/kg-day groups; the difference was statistically significant on lactation days 6-8. No other effects were noted, and there were no treatment-related findings for the pups necropsied at weaning.

F1 Males. Significant increases in treatment-related deaths (5/60 animals) were reported in F1

males in the high dose group between days 2-4 postweaning. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning. Clinical signs included a significant increase in emaciation at 10 and 30 mg/kg-day, and in urine-stained abdominal fur, decreased motor activity, and abdominal distention at 30 mg/kg-day.

Mean body weight was significantly reduced in the in the 30 mg/kg-day group beginning on postweaning day 8, in the 10 mg/kg-day group beginning on postweaning day 36, and towards the end of the study in the 1 and 3 mg/kg-day groups. Terminal mean body weight was reduced in all treated groups. For all groups, there was a significant, dose-related reduction in mean body weight gain for the entire dosing period (days 1-113). Absolute food consumption values were significantly reduced at 10 and 30 mg/kg-day during the entire precohabitation period (days 1-70 postweaning), while relative food consumption values were significantly increased.

Statistically significant delays ($p \leq 0.01$) in the average day of preputial separation were observed in high-dose animals versus concurrent controls (52.2 days of age versus 48.5 days of age, respectively). There were no other effects on any of the reproductive parameters assessed, and at necropsy no effects on reproductive organs were noted.

The absolute and relative weights of the liver were statistically significantly increased in all treated groups ($p \leq 0.01$). The absolute weights of the left and/or right kidneys were statistically significantly increased in the 1 and 3 mg/kg-day dose groups and statistically significantly decreased in the 30 mg/kg-day dose group (Table 4-11). Organ weight-to-terminal body weight and brain weight ratios for the kidney were statistically significantly increased in all treated groups. Histopathology was not conducted on the kidney. All other organ weight changes observed (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes, and epididymis) were probably due to decrements in body weight and not a reflection of target organ toxicity. Treatment-related microscopic changes were observed in the adrenal glands of high-dose animals (cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex) and in the liver of animals treated with 3, 10, and 30 mg/kg-day (hepatocellular hypertrophy).

TABLE 4-11. Organ weight data from F1 males					
	0 mg/kg-day	**1 mg/kg-day**	**3 mg/kg-day**	**10 mg/kg-day**	**30 mg/kg-day**
Body weight (g)	560±60	527±55*	524±48*	499±64**	438±42**
Brain weight (g)	2.34±0.13	2.28±0.16	2.31±0.12	2.28±0.10	2.18±0.14**
Liver weight (g)	21.7±3.2	24.6±4.0**	28.2±4.2**	29.3±4.1**	29.7±4.0**
Liver/body (%)	3.86±0.32	4.65±0.51**	5.41±0.75**	5.90±0.70**	6.79±0.55**
Liver/brain (%)	927±136	1075±150**	1224±179**	1285±159**	1364±166**
Rt. kidney (g)	2.24±0.21	2.34±0.28	2.48±0.24**	2.33±0.25	2.04±0.21**
Rt. kidney/body (%)	0.402±0.034	0.446±0.041**	0.474±0.041**	0.469±0.050**	0.467±0.036**
Rt. kidney/brain (%)	95.9±9.1	102.6±7.7**	107.4±10.2**	102.3±9.8*	93.6±7.9
Lt. kidney (g)	2.21±0.20	2.35±0.26*	2.46±0.20**	2.30±0.22	2.03±0.22**
Lt. kidney/body (%)	0.396±0.031	0.446±0.042**	0.472±0.045**	0.464±0.046**	0.465±0.038**
Lt. kidney/brain (%)	94.8±7.9	102.8±7.6**	106.6±9.1**	101.0±7.9*	93.3±10.0

From Butenhoff et al. 2004a.
Mean±S.D.; n = 29-30; significantly different from control: *p<0.05, **p<0.01.

For F1 males, the LOAEL for developmental/reproductive toxicity was 10 mg/kg-day based on significantly reduced body weight and body weight gain during the lactation period, and the NOAEL was 3 mg/kg-day. The LOAEL for adult systemic toxicity in the F1 males is 1 mg/kg-day based on significant, dose-related decreases in body weights and body weight gains (observed prior to and during cohabitation and during the entire dosing period), and in terminal body weights; and significant changes in absolute and relative liver weights. A NOAEL for adult systemic toxicity in the F1 males could not be determined.

F1 Females. A statistically significant increase in treatment-related mortality (6/60 animals) was observed in F1 females on postweaning days 2-8 at the highest dose of 30 mg/kg-day. No adverse clinical signs of treatment-related toxicity were reported. Statistically significant decreases in body weights were observed in high-dose animals on days 8, 15, 22, 29, 50, and 57 postweaning, during precohabitation (recorded on the day cohabitation began, when F1 generation rats were 92-106 days of age), and during gestation and lactation. Body weight gain was significantly reduced during days 1-8 and 8-15 postweaning. Statistically significant decreases in absolute food consumption were observed during days 1-8, 8-15, and 15-22 postweaning, during precohabitation and during gestation and lactation in animals treated with 30 mg/kg-day. Relative food consumption values were comparable across all treated groups.

Statistically significant ($p \leq 0.01$) delays in sexual maturation (the average day of vaginal patency) were observed in high-dose animals versus concurrent controls (36.6 days of age versus 34.9 days of age, respectively). Prior to mating, the study authors noted a statistically significant increase in the average numbers of estrous stages per 21 days in high-dose animals (5.4 versus 4.7 in controls). For this calculation, the number of independent occurrences of estrus in the 21 days of observation was determined. This calculation can be used as a screen for effects on the estrous cycle, but should be followed by a more detailed analysis.

Both 3M (2002) and the U.S. EPA (2002b) conducted a more detailed analysis of the estrus cycle data. The 3M analysis of the data concluded that there were no differences in the

number of females with ≥3 days of estrus or with ≥4 days of diestrus in the control and high dose groups. This conclusion is consistent with that of the U.S. EPA (2002b). The cycles were evaluated as having either regular 4-5 day cycles, uneven cycling (defined as brief periods with irregular pattern) or periods of prolonged diestrus (defined as 4-6 day diestrus periods) extended estrus (defined as 3 or 4 days of cornified smears), possibly pseudopregnant, (defined as 6-greater days of leukocytes) or persistent estrus (defined as 5-or greater days of cornified smears). The two groups were not different in any of the parameters measured. Thus, the increase in the number of estrous stages per 21 days that was noted by the study authors was an outcome of the approach used for the calculations and is not biologically meaningful. There were no effects on the other reproductive parameters assessed, and at necropsy no effects on reproductive organs were noted.

No treatment-related effects were observed in the terminal body weights of the F1 females. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio, and the pituitary weight-to-brain weight ratio were statistically significantly decreased at 3 mg/kg-day and higher. Since there is not a clear dose-response relationship and histologic examination did not reveal any lesions, the biological significance of the pituitary weight data is problematic. No other differences were reported for the absolute weights or ratios for other organs evaluated. No treatment-related effects were reported following macroscopic and histopathologic examinations.

For F1 females, the LOAEL for developmental/reproductive toxicity was considered to be 30 mg/kg-day based on significantly reduced body weight and body weight gain during lactation, a delay in sexual maturation, and increased mortality during postweaning days 2-8; the NOAEL was 10 mg/kg-day. The NOAEL and LOAEL for adult systemic toxicity in F1 females are 10 and 30 mg/kg-day, respectively, based on statistically significant decreases in body weight and body weight gains.

F2 Generation. There was a statistically significant increase ($p \leq 0.01$) in the number of pups found dead on lactation day 1 in the 3 and 10 mg/kg-day groups. An independent statistical analysis was conducted by U.S. EPA (2002c), and no significant differences were observed between dose groups and the response did not have any trend in dose. There were no treatment-related effects on any of the developmental parameters assessed, and at necropsy no treatment-related effects were noted. The NOAEL for developmental/reproductive toxicity in the F2 offspring was 30 mg/kg-day.

Developmental Effects

Prenatal Development. Prenatal developmental toxicity studies of PFOA have been conducted in rats, and mice and are summarized below. A greater number of studies have been conducted using mice as experimental animals than rats. Most studies have used the oral route of exposure.

Rat. Pregnant Sprague-Dawley rats were gavage dosed with 0, 3, 10 or 30 mg/kg-day PFOA during days 4-10, 4-15, or 4-21 of gestation, or from gestation day 4 to lactation day 21 (Hinderliter et al., 2005; Mylchreest, 2003). Clinical observations and body weights were recorded daily. On gestation days 10, 15, and 21, 5 rats/group/time-point were sacrificed and the

number, location and type of implantation sites were recorded. Embryos were collected on day 10, and placentas, amniotic fluid, and embryos/fetuses were collected on days 15 and 21. Maternal blood samples were collected at 2 hours ± 30 minutes post-dose. The remaining 5 rats per group were allowed to deliver. On lactation days 0, 3, 7, 14, and 21, the pups were counted, weighed (sexes separate), and examined for abnormal appearance and behavior. Randomly selected pups were sacrificed and blood samples were collected. On lactation days 3, 7, 14, and 21, the dams were anesthetized and milk and blood samples were collected; dams were removed from their litters 1-2 hours prior to collection. Plasma, milk, amniotic fluid extract, and tissue homogenate (placenta, embryo, and fetus) supernatants were analyzed for PFOA concentrations by HPLC-MS.

All dams survived and there were no clinical signs of toxicity. In the 30 mg/kg-day group, mean body weight gain was approximately 10% lower than the control group during gestation, and mean body weights were approximately 4% lower than controls throughout gestation and lactation. The number of implantation sites, resorptions, and live fetuses were comparable among groups on days 10, 15, and 21 of gestation. One dam in the 3 mg/kg-day group and two dams in the 30 mg/kg-day group delivered small litters (litter size of 3-6 pups as compared to 12-19 pups/litter in the control group); however, given the small sample size the biological significance of this finding is unclear. There were no clinical signs of toxicity in the pups, and pup survival and pup body weights were comparable among groups. Under the conditions of this study, the maternal LOAEL was 30 mg/kg-day for decreased body weight gain during gestation, and the NOAEL was 10 mg/kg-day. The developmental NOAEL was 30 mg/kg-day.

Mouse. Lau et al. (2006) conducted a developmental toxicity study of PFOA in mice in order to ascertain whether there was a sex difference in the bioaccumulation of PFOA in the mouse, and to evaluate the effects of PFOA on pre- and postnatal development in offspring exposed during pregnancy. In that study, groups averaging 9-45 timed-pregnant CD-1 mice were given 0, 1, 3, 5, 10, 20, or 40 mg/kg PFOA daily by oral gavage on GD1-17. Maternal weight was monitored during gestation. Dams were divided into two groups. In the first group, dams were sacrificed on GD18 and underwent maternal and fetal examinations that included measure of maternal liver weight, and examination of the gravid uterus for numbers of live and dead fetuses and resorptions. Maternal blood was collected and analyzed for PFOA serum concentration. PFOA levels in the fetuses were not examined. Live fetuses were weighed and subjected to external gross necropsy and skeletal and visceral examinations. In the second group of dams, an additional dose of PFOA was administered on GD18. Dams were allowed to give birth on GD19.

The day following parturition was designated as PND 1. Time of parturition, condition of newborns, and number of live offspring were recorded. The number of live pups in each litter and pup body weight were noted for the first four days after birth and then at corresponding intervals thereafter. Eye opening was recorded beginning at PND 12. Pups were weaned on PND 23 and separated by sex. The time to sexual maturity was determined by monitoring vaginal opening and preputial separation beginning on PND 24. Two to four pups per sex per litter were randomly selected for observation of postnatal survival, growth, and development. Estrous cyclicity was determined daily by vaginal cytology. After weaning, dams were sacrificed and the contents of the uteri examined for implantation sites. Postnatal survival was calculated based on the number of implantations for each dam. Benchmark dose values (BMD_5 and $BMDL_5$) were

calculated for the maternal and developmental toxic endpoints.

Signs of maternal toxicity were observed following exposure to PFOA during pregnancy. Statistically significant ($p \leq 0.05$) dose-related increases in maternal liver weight were also observed, beginning at 1 mg/kg/day. Dose-related decreases in maternal weight gain during pregnancy were observed beginning at 5 mg/kg/day, with statistical significance ($p \leq 0.05$) seen in the 20 and 40 mg/kg/day dose groups. Under the conditions of the study, a maternal LOAEL of 1 mg/kg was indicated based on increased liver weight, and a NOAEL was not established. Signs of developmental toxicity were observed following *in utero* exposure to PFOA. The number of implantations in treated mice was comparable to control mice. Statistically significant ($p \leq 0.05$) increases in full-litter resorptions were reported at doses of $\geq$ 5 mg/kg/day, with complete loss of pregnancies at the highest dose group of 40 mg/kg/day. A 20% reduction ($p \leq 0.05$) in live fetal body weight at term was reported at 20 mg/kg/day. A statistically significant increase in prenatal loss was observed in the 20 mg/kg/day dose group. Ossification (number of sites) of the forelimb proximal phalanges was significantly decreased at all doses except 5 mg/kg. Ossification of hindlimb proximal phalanges was significantly decreased at all doses except 3 and 5 mg/kg. Reduced ossification ($p \leq 0.05$) of the calvaria and enlarged fontanel was observed at 1, 3, and 20 mg/kg and at $\geq$ 10 mg/kg in the supraoccipital bone. Statistically significant ($p \leq 0.05$) increases in minor limb and tail defects were observed in the fetuses at doses $\geq$ 5 mg/kg/day. Under the conditions of the study, a prenatal developmental LOAEL of 1 mg/kg was indicated based on increased skeletal defects, and the NOAEL was not established.

Slight, but statistically significant ($p \leq 0.05$), increases in the average time to parturition were observed at 10 and 20 mg/kg/day. Increases ($p \leq 0.05$) in stillbirths and neonatal mortality (or decreases in postnatal survival) were observed at doses $\geq$ 5 mg/kg/day, with as much as a 30% increase in these effects seen at 10 and 20 mg/kg/day dose group. Postnatal survival and viability at the 1 and 3 mg/kg/day dose groups was comparable to controls. At doses $\geq$ 3 mg/kg/day, a trend in growth retardation (body weight reductions of 25-30%; $p \leq 0.05$), was observed in the neonates at weaning. Body weights were at control levels by 6 weeks of age for females, and by 13 weeks of age for males. A trend for increasing body weight (~6-10% greater than controls) was observed in animals dosed with 5 mg/kg at 13 weeks and in animals dosed with 1 and 3 mg/kg at 48 weeks. Deficits in early postnatal growth and development were also manifested by significant ($p \leq 0.05$) delays in eye opening at doses $\geq$ 5 mg/kg/day. Slight delays ($p \leq 0.05$) in vaginal opening and in time to estrous were observed at 20 mg/kg/day in females; in contrast significant accelerations ($p \leq 0.05$) in sexual maturation were observed in males, with preputial separation occurring 4 days earlier than controls at the 1 mg/kg/day dose and 2-3 days earlier in the 3-10 mg/kg/day dose groups, but the 20 mg/kg/day dose group was only slightly delayed compared to controls. Under the conditions of the study, a LOAEL for developmental toxicity of 1 mg/kg for males was indicated based on accelerated pubertal development, and a NOAEL was not established. For females, the developmental LOAEL was 3 mg/kg based on growth retardation, and the NOAEL was 1 mg/kg.

For maternal toxicity, BMD_5 and $BMDL_5$ estimates for decreases in maternal weight gain during pregnancy were 6.76 and 3.58 mg/kg, respectively. For increases in maternal liver weight at term, BMD_5 and $BMDL_5$ estimates were 0.20 mg/kg and 0.17 mg/kg, respectively. BMD_5 and $BMDL_5$ estimates for the incidence of neonatal mortality (determined by survival to weaning) at 5 mg/kg/day were 2.84 and 1.09 mg/kg, respectively. Significant alterations in postnatal growth

and development were observed at 1 and 3 mg/kg/day, with BMD_5 and $BMDL_5$ estimates of 1.07 and 0.86 mg/kg, respectively, for decreased pup weight at weaning; and 2.64 and 2.10 mg/kg, respectively, for delays in eye opening. The BMD_5 and $BMDL_5$ estimates for reduced phalangeal ossification were < 1 mg/kg. BMD_5 and $BMDL_5$ estimates for reduced fetal weight at term were estimated to be 10.3 and 4.3 mg/kg, respectively.

Male and female 129S1/SvImJ and PPARα-null mice were used in studies to determine if PFOA-induced developmental toxicity was mediated by PPARα (Abbott et al., 2007). Pregnant 129S1/SvlmJ [wild type (WT)] and PPARα-null mice were orally dosed from GD1-17 with 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, or 20 mg PFOA/kg. Heterozygous (HET) litters were also produced by mating WT and PPARα-null males with WT and PPARα-null dams to determine if genetic background affected survival. The HET litters were sacrificed on PND15. Survival at birth was recorded and live offspring counted and weighed by sex. Litters were counted and offspring weighed on PND1-10, 14, 17, and 22. Weaning occurred on PND 22, and all dams and one pup/litter were sacrificed. Blood was collected and the uteri were stained for implantation counts.

There was no effect of treatment on maternal weight or maternal weight gain (excluding non-pregnant females and those with full litter resorptions), number of implants, or pup weight at birth. WT dams exposed to ≥0.6 mg/kg/day and PPARα-null dams exposed to ≥5 mg/kg/day had a significantly greater percentage of litter loss compared to their respective controls. At ≥5 mg/kg/day in WT dams and 20 mg/kg/day in PPARα-null dams, 100% litter loss occurred. Relative liver weight was significantly increased in WT adult females dosed with ≥1 mg/kg and in PPARα-null adult females dosed with ≥3 mg/kg.

Body weight in WT offspring born of dams dosed with 1.0 mg/kg was significantly reduced ($p<0.05$) compared to control offspring body weight gain on PND9, 10, and 22 (males) and PND7 to10 and PND22 (females). No differences were observed between PPARα-null offspring body weight and control offspring body weight. Survival of pups from birth to weaning was significantly reduced ($p<0.05$) in WT litters exposed to ≥0.6 mg/kg, but was not affected in PPARα-null litters. Survival was significantly decreased ($p<0.05$) for WT and HET pups born to WT dams dosed with 1 mg/kg and for HET pups born to PPARα-null dams dosed with 3 mg/kg. Offspring born of WT dams showed a dose-related trend for delayed eye opening compared to control offspring (significantly delayed at 1 mg/kg/day, $p<0.05$), but no difference in day of eye opening was observed in the offspring born of PPARα-null dams. At weaning, relative liver weight was significantly increased ($p<0.05$) in WT offspring gestationally exposed to ≥0.1 mg/kg and in PPARα-null offspring gestationally exposed to 3 mg/kg/day.

The authors concluded that survival of PPARα-null pups and deaths of HET pups born to PPARα-null dams indicates that expression of PPARα is required for PFOA-induced postnatal lethality; however, early prenatal lethality was independent of PPARα. Delayed eye opening and reduced postnatal weight gain appeared to be mediated by PPARα, but other mechanisms may also contribute. Under the conditions of the study, the maternal/reproductive LOAEL for WT mice was 0.6 mg/kg/day based on increased percentage of litter loss, and the NOAEL was 0.3 mg/kg/day. The developmental LOAEL for WT offspring was 0.1 mg/kg/day based on increased liver weight, and the NOAEL was not established. The maternal LOAEL for PPARα-null mice was 3 mg/kg/day based on increased liver weight, and the NOAEL was 1 mg/kg/day.

The developmental LOAEL for PPARα-null offspring was 3 mg/kg/day based on increased liver weight, and the NOAEL was 1 mg/kg/day.

To further evaluate the developmental effects potentially mediated by PPARα, groups of female wild-type, PPARα-null, and PPARα-humanized mice were given 0 or 3 mg PFOA/kg on GDs 1-17 by oral gavage (Albrecht et al., 2013). Controls received the water vehicle. Females were either sacrificed on GD18 (n = 5-8/group) or allowed to give birth and then sacrificed, along with their litters (n = 8-14), on PND20. Livers from dams, fetuses, and pups were weighed and collected for histopathological evaluation and RNA analysis. Gene expression results are given in section 4.3.4. Mammary gland whole mounts were prepared from female pups on PND20 for quantification of ductal length and number of terminal end buds.

Evaluation on GD 18 showed no effects of PFOA administration on maternal body weight, body weight gain, gravid uterine weight, number of implantations per dam, or number of resorptions per litter in dams of any genotype. For animals allowed to litter, the average day of parturition was slightly later in PFOA treated humanized mice compared with the controls. Body weight of dams during lactation, the number of pups born per litter, pup body weight during lactation, and the onset of pup eye opening were similar between treated and control groups for all genotypes. Offspring survival during PNDs 1-5 was significantly reduced in the wild-type PFOA treated group, but not the other genotypes.

Maternal liver weight was significantly increased in the treated groups of all genotypes on GD18 and in wild-type animals on PND20. Maternal liver weight was not affected on PND20 in the PPARα-null or PPARα-humanized mice. Relative fetal liver weight on GD18 was significantly increased in fetuses from treated wild-type and humanized dams. On PND20, relative liver weight was increased only in pups from treated wild-type dams. Microscopic evaluation of the maternal liver showed centrilobular hepatocellular hypertrophy in all PFOA treated groups on GD18 and PND20, with decreased incidence and severity by PND20. On GD18 the liver lesions were graded as mild in the wild-type mice, minimal-to-mild in the humanized mice, and minimal in the null mice. The morphological features of the liver lesions differed slightly between genotypes and are described in more detail in section 4.4.1. Only wild-type fetuses and pups from treated dams showed similar liver lesions.

The average length of mammary gland ducts and the average number of terminal end buds per mammary gland per litter were not different between control and treatment groups from all genotypes.

Yahia et al. (2010) gavage dosed pregnant ICR mice (n=5/group) with 0, 1, 5, or 10 mg PFOA/kg from GD0-17 or 18. The dams dosed from GD0-17 were sacrificed on GD18, and the fetal skeletal morphology was evaluated. Dams dosed from GD0-18 were allowed to give birth and their offspring were either processed for pathological examination or observed for 4 days for neonatal mortality. Maternal liver, kidney, brain, and lungs were histologically examined after necropsy. Serum was collected for clinical chemistry and lipid analysis. Body weight was significantly decreased in dams receiving 10 mg/kg. Maternal absolute liver weight was significantly increased ($p<0.05$) at doses ≥ 5 mg/kg and relative liver and kidney weights were significantly increased at all doses. Hepatic hypertrophy was localized to the centrilobular region at the two lower doses and was diffuse at the highest dose. Renal cells in the outer

medullar and proximal tubule were slightly hypertrophic at all doses. Treatment with 10 mg/kg caused a significant increase in AST, ALT, GGT, and ALP and a significant decrease in total serum protein, albumin, globulin, triglycerides, phospholipids, total cholesterol, and free fatty acids. At 5 mg/kg, total serum protein and globulin were significantly decreased, and phosphorus was increased. At 1 mg/kg, BUN and phosphorus were significantly increased. The maternal LOAEL was 1 mg/kg based on significantly increased relative liver and kidney weight, and no NOAEL was established.

Live fetal birth weight was significantly decreased at the two highest doses. There was no difference in the percentage of live fetuses between treated and control groups. At 10 mg/kg, increased incidence of cleft sternum, delayed phalanges ossification, and delayed eruption of incisors was observed. Delayed parturition was observed in dams treated with 10 mg/kg, and ~58% of all pups born to those dams were stillborn. Death occurred within 6 hours of delivery in the remaining pups, and whole body edema was observed in some of the pups. The body weight of the live pups born to dams treated with 5 or 10 mg/kg was significantly reduced compared to control pup body weight. By PND4, 16% of offspring born to dams dosed with 5 mg/kg had died. No pathological differences were observed in the lungs or brains of treated and control offspring. The developmental LOAEL was 5 mg/kg based on decreased body weight and decreased survival rate, and the NOAEL was 1 mg/kg.

Suh et al. (2011) examined placental prolactin-family hormone and fetal growth retardation in mice treated with PFOA. Pregnant CD-1 mice (n=10/group) were treated with 0, 2, 10, or 25 mg/kg PFOA from GD11 to GD16. Dams were sacrificed on GD16 and uteri were removed and examined. Three placentas/group were analyzed using histochemistry and the numbers of glycogen tropholblast cell (GlyT) in the junctional zone and sinusoidal trophoblast giant cells (S-TGC) in the labyrinth zone were counted and compared. Trophoblast cells express prolactin-family genes. mRNA from three placentas/group were analyzed using situ hybridization, northern blot hybridization, and RT-PCR for mouse placental lactogen (mPL)-II, prolactin like protein (mPLP)-E and F, Pit-1α and β isotype (transacting factors of mPL and mPLP genes).

A significant difference in maternal body weight was observed from GD13-16 in dams treated with 25 mg/kg PFOA compared to controls. At ≥2 mg/kg PFOA, placental weight was significantly decreased and the number of resorptions and dead fetuses was significantly increased. At ≥10 mg/kg PFOA, fetal weight and the number of live fetuses were significantly decreased. There were no differences in the number of implantation sites among the groups, and post-implantation loss was 3.87, 8.83, 30.98, and 55.41% for the 0, 2, 10, and 25 mg/kg PFOA groups, respectively.

The placentas of dams dosed with ≥10 mg/kg PFOA displayed necrotic changes. Parietal and sinusoidal TGC and GlyT cell frequency in the placental junctional and labyrinth zones was significantly decreased (>0.05) in a dose-dependent manner in treated dams. At 25 mg/kg PFOA, S-TGCs showed signs of atrophy with crushed cell nucleus. A significant dose-dependent decrease in mPL-II, mPLP-E, mPLP-F, and Pit-1α and β isotype mRNA and expression was observed. Correlation coefficients between fetal weight and maternal mPL-II, mPLP-E, and mPLP-F mRNA levels were positive (p>0.001). Based on the results, the authors suggested that inhibited prolactin-family gene express may be secondary to insufficient

trophoblast cell differentiation and increased cell necrosis. These effects reduced placental efficiency and contributed in part to fetal growth retardation.

Other Developmental Studies/Mammary Gland Development. The following studies used study designs and/or examined endpoints not usually included in developmental studies. The studies were conducted to determine critical periods of exposure focusing on mammary gland development in dams and female offspring. Researchers focused on effects resulting from indirect exposure of offspring via treatment of pregnant animals and direct exposure of peripubertal animals starting about the time of weaning.

Indirect gestational and/or lactational exposures

An overview of experiments designed to assess the developmental effects of PFOA exposure to CD-1 mice during gestation is given in Table 4-12. Additional details of the studies are described in the text below. All studies included measures of maternal and offspring survival and body weight.

TABLE 4-12. Overview of studies in which pregnant CD-1 mice were administered PFOA

Dose (mg PFOA /kg/day)	Timing	Endpoints	Reference
0, 5	GD 1-17, 8-17, or 12-17	Body weight; mammary gland GD 18 (dams) and PND 10 and 20 (dams, female pups)	White et al., 2007
0, 5 20	GD 7-17, 10-17, 13-17, 15-17 GD 15-17	Body weight; developmental landmarks and growth to PND 189; mammary gland of female pups up to 18 months	Wolf et al., 2007; White et al., 2009
0, 3, 5	GD 1-17 Cross-fostered at birth	Body weight; developmental landmarks and growth to PND 245; mammary gland of female pups up to 18 months	Wolf et al., 2007; White et al., 2009
0, 5	GD 8-17 Cross-fostered at birth	Mammary gland of dams and female pups on PND 1, 3, 5, 10	White et al., 2009
0, 0.3, 1.0, 3	GD 1-17	Liver weight; mammary gland of female pups PND 7, 14, 21, 28, 42, 63, 84	Macon et al., 2011
0, 0.01, 0.1, 1	GD 10-17		
0, 1, 5	GD 1-17	Body weight; reproductive parameters; mammary gland of F0, F1, and F2 females	White et al., 2011
0, 1 + 5 ppb in drinking water	GD 1-17; drinking water started GD 7 and continued to F2 generation		

White et al. (2007) orally dosed pregnant CD-1 mice with 0 or 5 mg PFOA/kg on gestation days (GD) 1-17 (n=14), 8-17 (n=16), or 12-17 (n=16) to determine if decreased neonatal body weights and survival were linked to gestational exposure or lactational changes in milk quantity or quality. The control mice (n=14) were dosed with vehicle on GD1-17. A subset of dams was sacrificed on GD 18. The remaining dams were allowed to give birth, and pups were pooled and randomly redistributed among the dams of the respective treatment groups. Litters were equalized to 10 pups per litter. Half of dams and litters were sacrificed on PND10

and remaining dams and litters were sacrificed on PND20. Mammary glands were collected from dams and female pups at time of sacrifice.

Treatment with PFOA did not affect maternal weight gain, number of implants, or the number of live fetuses. There was a significant increase ($p<0.05$) in prenatal loss in dams exposed during GD1-17. Body weight of pups exposed gestationally to PFOA was significantly decreased ($p<0.05$) at all time points measured. On GD18, stunted alveolar development was observed in the mammary gland of dams treated with PFOA on GD1-17 compared to the mammary glands of the control dams which were saturated with milk-filled alveoli. Dams treated with PFOA on GD 1-7 or 8-17 exhibited significant mammary gland epithelial differentiation delays on PND10 as evidenced by an excessive amount of adipose tissue. In comparison, mammary glands from control dams on PND10 were well differentiated, full of alveoli filled with milk, and contained few apoptotic bodies and little adipose tissue. At PND20, the mammary glands of all PFOA-treated dams were similar in appearance to the mammary glands of control dams at PND10. Mammary gland differentiation in dams treated on GD12-17 was not statistically different from control dams.

The pups were also impacted by their in utero PFOA exposure. Their mammary glands exhibited significantly stunted epithelial branching and longitudinal growth at PND10 and 20. Very little mammary gland development occurred between PND 10-20 in the offspring of dams exposed to PFOA even though postnatal growth and body weight gain increased in parallel to that of the controls. Thus, at the only dose tested, 5 mg/kg, effects were observed on the dam and pup mammary gland, decreased pup body weights and decreased survival for the pups exposed diring GD 1-17.

In the study by Wolf et al. (2007), CD-1 mice were orally dosed with 0 or 5 mg PFOA/kg on GD7-17 (n=14), 10-17 (n=14), 13-17 (n=12), and 15-17 (n=12) or with 20 mg/kg on GD15-17 (n=6) to determine if there was a specific window during which PFOA exposure produced developmental effects. The developmental results from this study were published by Wolf et al. (2007) and the mammary gland effects were published by White et al. (2009). On PND22, all dams and one male and female pup from each litter were necropsied. Blood samples were collected and livers from dams and offspring were removed and weighed. Uterine implantation sites were counted. The 4th and 5th inguinal mammary glands were removed from female offspring and analyzed at various intervals up to 18 months of age (White et al., 2009). The remaining offspring were observed until PND189.

Maternal weight gain was increased in dams exposed to PFOA beginning on GD7 and 10, but there was no affect on number of uterine implantation sites, litter loss, or number of pups per litter at birth. Male pup weight at birth was significantly decreased ($p<0.05$) in dams dosed with 5 mg/kg on GD7-17 or 10-17 or with 20 mg/kg on GD15-17. By PND78, all male offspring had recovered to control body weight levels. On PND161, the offspring of dams dosed during GD13-17 weighed significantly more than control. Litters exposed to 20 mg/kg on GD15-17 experienced decreased survival ($p<0.01$) during postnatal days 1 through 22. Maternal relative liver weight was significantly increased in all PFOA-treated dams except those treated during GD15-17. Relative liver weight in all male and female pups was significantly increased ($p<0.01$). Eye opening and growth of body hair were delayed in pups exposed GD7-17 and 10-17 (Wolf et al., 2007).

Mammary gland developmental scores for all offspring of dams exposed to PFOA were significantly lower compared to scores from offspring of control dams at PND29 and 32. Delayed ductal elongation and branching and delayed appearance of terminal end buds were characteristics of delayed mammary gland development at PND32. At 18 months of age, increases in epithelial density and in density of darkly staining foci were observed in offspring of PFOA-exposed dams. Based on histopathologic observation the darkly staining foci were hypothesized to be hyperplasia of ductal epithelium, inflammatory cell influx into ductal regions, increased stromal density, or altered differentiation of ductal epithelia. The average number of foci per mammary gland was 1.5, 29.8, 17.9, 32.8, and 25.5 for the control, GD15-17, GD13-17, GD10-17, and GD7-17 groups, respectively (White et al., 2009). The 5 mg/kg dose was associated with increased maternal and pup liver weight, altered pup mammary gland development, and delayed pup eye opening and growth of body hair. The 20 mg/kg dose was associated with decrease postnatal pup survival.

The objective of a second component of the Wolf et al. (2007)/White et al. (2009) study was to determine if postnatal body weight deficits, neonatal lethality, and developmental delays caused by PFOA exposure were the result of gestational exposure, lactational exposure, or a combination of gestational and lactational exposure. Pregnant CD-1 mice were orally dosed with 0 (n=48), 3 (n=28), or 5 (n=36) mg PFOA/kg on GD1 through 17 and their offspring cross-fostered at birth to create 7 treatment groups: control, *in utero* exposure only (3U and 5U), lactation exposure only (PFOA stored in milk during gestation and released during lactation; 3L and 5L), and *in utero* and lactation exposure (3U+L and 5U+L). On PND22, all dams and one male and female pup from each litter were necropsied. Blood samples were collected and the liver was removed from dams and offspring and weighed. Implantation sites were counted from the uteri of dams. The 4th and 5th inguinal mammary glands were removed from female offspring and analyzed at various intervals up to 18 months of age (White et al., 2009). The remaining offspring were observed until PND245.

Maternal weight and weight gain were higher in PFOA treated dams compared to control dams. Whole litter loss was significantly increased ($p<0.05$) at 5 mg/kg, but no differences in the number of implantation sites were observed between the treated and control mice. Absolute and relative livers weights of PFOA treated dams from both dose groups were significantly increased ($p<0.001$) compared to absolute and relative liver weights of control dams 23 days after the last dose (PND22). No difference in the number of live pups born per litter were found between treated and control mice, but male and female pup birth weight was reduced ($p<0.01$) in dams receiving 5 mg/kg (Wolf et al., 2007). The 3 mg/kg dose was a LOAEL for increases in liver weight in the dams while 5 mg/kg was a LOAEL for the pups based on whole litter loss and significantly reduced male and female birth weight.

A dose-dependent increase of PFOA was observed in the serum of dams treated with PFOA providing a reservoir for lactational transfer. The control dams that nursed offspring exposed *in utero* (3U and 5U) had low concentrations of PFOA in their serum from maternal grooming behavior which allowed for low level lactational transfer.

Body weight of male and female pups (3U+L, 5U, and 5U+L) was significantly reduced as early as PND2 and 1, respectively, and remained reduced throughout the lactation period.

Body weight recovery to control levels was reached by male offspring within two weeks of weaning, but recovery in female offspring in the 5U and 5U+L groups did not occur until after PND85.

Postnatal survival in 5U+L pups was significantly decreased compared to control survival beginning at PND4 and continuing throughout lactation. Survival in the other groups was not different from control survival. Eye opening and body hair growth were significantly delayed in the 3U+L, 5U, and 5U+L offspring. The relative liver weight was significantly increased in all offspring regardless of exposure scenario (Wolf et al., 2007).

All female offspring of PFOA-exposed dams had reduced mammary gland developmental scores at PND22 except for females in the 3L group. At PND42, mammary gland scores from females in the 3U+L group were the only ones not statistically different from control scores. This may have been due to inter-individual variance and multiple criteria used to calculate mammary gland development scores. Despite the fact that the mammary gland scores from the 3U+L group were not significantly different from scores of the control animals, they did exhibit reduced branching and mammary fat pads with reduced parenchymal density. All offspring of dams exposed to PFOA exhibited delayed mammary gland development at PND63 including those exposed only through lactation (3L and 5L). A higher density of dark staining foci was observed in the mammary glands of these animals at 18 months of age (White et al., 2009).

White et al. (2009) also reported the results from pregnant CD-1 mice orally dosed with 0 (n=56) or 5 (n=56) mg PFOA/kg from GD8 through 17 to determine the timing of the mammary gland development deficits observed following gestational or lactational exposure to PFOA. The groups were cross-fostered at birth to create 4 treatment groups: control, *in utero* exposure only (5U), lactation exposure only (5L), and *in utero* and lactation exposure (5U+L). Dams and litters were sacrificed on PND1, 3, 5, and 10. Blood and liver samples were collected for PFOA analysis. The 4th and 5th inguinal mammary glands were collected from dams and female offspring and analyzed.

Maternal weight gain in treated dams was significantly higher compared to control weight gain, but there were no effects of treatment on litter size or pup birth weight or weight at PND1. Significantly decreased body weight occurred in the pups of the 5U+L group on PND3 and in all PFOA-exposed pups on PND 5 and 10. Relative liver weight of the treated dams was significantly increased ($p<0.05$) compared to relative liver weight of control dams. On PND1, liver to body weight ratios were significantly increased ($p<0.05$) in pups exposed *in utero* (5U, 5U+L); serum PFOA levels were 65,000-70,000 ng/mL. The liver to body weight ratio was increased in pups exposed lactationally by PND5; serum PFOA levels were approximately 15,000 ng/mL (White et al., 2009).

On PND1, the mammary glands of PFOA-exposed dams were similar to glands seen in late pregnancy, prior to parturition. In control dams nursing offspring from PFOA-exposed dams, reduced alveolar filling was noted as early as PND3 presumably a result of exposure of the dam from maternal grooming behavior. The delayed lactational morphology in dams treated with PFOA and control dams nursing offspring from PFOA-treated dams was persistent up to PND10 (terminal sacrifice). Delayed mammary gland development was observed as early as

PND1 in all female offspring from PFOA-exposed dams including those exposed through lactation only (5L). Delayed mammary gland development persisted through the study duration (White et al., 2009).

Macon et al. (2011) gavage dosed CD-1 mice with 0, 0.3, 1.0, or 3.0 mg PFOA/kg from GD1 to GD17 (n=13 dams/group). Offspring were sacrificed on PND 7, 14, 21, 28, 42, 63, and 84, and blood, liver, brain, and the fourth and fifth mammary glands were collected from female pups.

Body weight in male and female offspring was not affected through PND 84. Absolute liver weight was significantly increased at ≥0.3 mg/kg in females and at ≥1.0 mg/kg in males on PND 7, and at 3.0 mg/kg in females at PND14. Relative liver weight was significantly increased at ≥0.3 mg/kg in males and females on PND7, at ≥1.0 mg/kg in females on PND14, and at 3.0 mg/kg in males and females on PNDs 14, 21, and 28. No dose-related differences were observed absolute and relative brain weights.

Delayed mammary gland development of female pups was observed as early as PND7 at ≥1.0 mg/kg and PND14 at ≥0.3 mg/kg and persisted until the end of the study. The delayed development was characterized by reduced epithelial growth and the presence of numerous terminal end buds at PND 63 and 84. The LOAEL was 0.30 mg/kg based on significantly increase liver weight and delayed mammary gland development. No NOAEL was established.

Macon et al. (2011) also gavage dosed CD-1 mice with 0, 0.01, 0.1, or 1.0 mg PFOA/kg from GD10 to GD17 (n=5-8 dams/group) to examine the effects of low doses of PFOA on mammary gland development. Female offspring were sacrificed on PND1, 4, 7, 14 and 21, and blood, liver, and the fourth and fifth mammary glands were collected.

No differences in body weight or brain weight were observed for male or female offspring. At 1 mg/kg, absolute and relative liver weights were significantly increased at PNDs 4 and 7. Relative liver weight was also significantly increased at PND14. Mammary gland development was delayed by exposure to PFOA, especially longitudinal epithelial growth. At PND21, all treatment groups had significantly lower developmental scores. At the highest dose, poor longitudinal epithelial growth and decreased number of terminal end buds were observed. The LOAEL was 0.01 mg PFOA/kg based on delayed mammary gland development. No NOAEL was established.

White et al. (2011) examined the extended consequences of PFOA-induced altered mammary gland development in a multigenerational study in CD-1 mice. Pregnant mice (F0, n=10-12 dams/group) were gavage dosed with 0, 1, or 5 mg PFOA/kg from GD1-17. A separate group of pregnant mice (n=7-10 dams/group) were gavage dosed with either 0 or 1 mg PFOA/kg from GD1-17 and received drinking water containing 5 ppb PFOA beginning on GD7. F1 females and F2 offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during breeding and early gestation, to simulate a chronic low-dose exposure. F0 females were sacrificed on PND22. F1 offspring were weaned on PND22 and bred at 7-8 weeks of age. F2 litters were maintained through PND63. Groups of F1 and F2 offspring (n=1-2 offspring/litter from 5-7 litters/group) were sacrificed on PND 22, 42, and 63. A group of F2 offspring (n=6-10/group) was also sacrificed

on PND10. A lactational challenge experiment was performed on PND10 with F1 dams and F2 offspring.

F0 Females. Gestational exposure to 5 mg PFOA/kg significantly increased prenatal loss in F0 mice and significantly decreased the number of live offspring and the postnatal survival of the viable pups. Weight gain and number of implants did not differ among the groups. At PND 22, control F0 dams displayed weaning-induced mammary involution. At PND22, the mammary glands of all PFOA- exposed F0 dams, including the control dams receiving 5 ppb PFOA in drinking water, resembled glands of mice at or near the peak of lactation (~PND10).

F1 Generation. Offspring body weight at PND42 was significantly reduced for those whose dams received 5 mg/kg; at PND63, body weight was significantly reduced at 1 mg/kg + 5 ppb in the drinking water. Liver-to-body weight ratios were significantly increased at 1 mg/kg on PND22 and at 5 mg/kg on PNDs 22 and 42.

F1 Females. There was no indication of toxicity in F1 females. Exposure to PFOA did not affect prenatal loss or postnatal survival, although F1 females that had been gestationally exposed to 5 mg/kg, had significantly fewer number of implants. On PNDs 22, 42, and 63, the mammary glands of all mice exposed to PFOA were significantly delayed in development. F1 females were bred and examined on LD10 and 22. On LD10, delayed mammary gland development (lactation morphology) was observed in all groups. Although, morphological differences were observed, there were no differences among the groups in the lactational challenge experiment. In the lactational challenge experiment, dams were removed from their litters for 3 hours, then returned to their litters and allowed to nurse for 30 minutes. The time from the dam's return to the litter and nursing initiation was recorded. The litters were weighed before and after nursing to estimate volume of milk produced. By LD22, only F1 dams in the 1 mg/kg + 5 ppb PFOA and 5 mg/kg groups displayed delayed mammary gland development.

F2 Generation. A significant reduction in body weight was observed in control + 5 ppb drinking water PFOA offspring on PND 1, but there was no difference by PND 3. Offspring from the 1 mg/kg + 5 ppb drinking water PFOA group had a significant increase in weight compared to controls on PNDs 14, 17, and 22. Liver-to-body weight ratios were not different among the groups. Mammary gland development in groups with 5 ppb PFOA in the drinking water was significantly delayed at PND42. Mammary glands in the remaining groups displayed delayed development, but control F2 females had unusually low scores at PNDs 10 and 22 which may have reduced the ability to detect statistically significant effects in other treatment groups.

In summary, this study showed delay mammary gland development in dams and female pups exposed continuously to a concentration as low as 5 ppb in the drinking water for three generations. Direct dosing of dams with 1 or 5 mg/kg during gestation also resulted in delayed mammary gland development in dams and their offspring at both doses as well as reduced pup survival and decreased body weight at 5 mg/kg.

Direct peripubertal exposures

Yang et al. (2009) gavage dosed 21 day old female BALB/c mice (5/group) with 0, 1, 5, or 10 mg PFOA/kg for 5 days/week for 4 weeks to determine the effects of peripubertal PFOA

exposure on puberty and mammary gland development. At necropsy, uteri and livers were weighed and processed for histological examination. Mammary glands were collected and processed for histological and whole mount examination. A significant decrease in body weight was observed following exposure to 10 mg/kg. The mammary glands of female BALB/c mice treated with 5 or 10 mg/kg had reduced ductal length, decreased number of terminal end buds, and decreased stimulated terminal ducts compared to the mammary glands of control mice. BrdU incorporation into the mammary gland revealed a significantly lower number of proliferating cells in the ducts and terminal end buds/terminal ducts at 5 mg/kg (not tested at 10 mg/kg). Absolute and relative liver weight was significantly increased in all treated BALB/c mice. The absolute and relative uterine weight was significantly decreased in all treated mice compared to uterine weight in control mice. Vaginal opening was significantly delayed in mice dosed with 1 mg/kg, and did not occur at 5 or 10 mg/kg. Under the conditions of this study, the LOAEL was 1 mg/kg/day based on delayed vaginal opening, increased liver weight and decreased uterine weight, and no NOAEL was established.

Yang et al. (2009) also dosed 21 day old female C57BL/6 mice in the same manner as the BALB/c mice and examined the effects of PFOA on mammary gland development and vaginal opening. The body weight effects were similar in both strains with 10 mg/kg causing significantly reduced body weight. At 5 mg/kg, PFOA had a stimulatory effect on the mammary glands. There was a significant increase in the number of terminal end buds and stimulated terminal ducts. Ductal length was not affected. Mammary gland development was inhibited in mice dosed with 10 mg/kg with no terminal end buds or stimulated terminal ducts present and very little ductal growth. Absolute and relative liver weight was significantly increased in all treated mice. The absolute and relative uterine weight was significantly increased in C57BL/6 mice dosed with 1 mg/kg and significantly decreased in C57BL/6 mice dosed with 10 mg/kg. There was no difference in uterine weights between mice treated with 5 mg/kg and control mice. Vaginal opening was delayed in C57BL/6 mice dosed with 5 mg/kg and did not occur in mice dosed with 10 mg/kg. Under the conditions of this study, the LOAEL was 1 mg/kg/day based on and increased liver and uterine weights, and no NOAEL was established.

Zhao et al. (2010) conducted several experiments in C57BL/6 mice to determine the potential mechanism by which peripubertal PFOA exposure resulted in the stimulation of mammary gland development observed by Yang et al. (2009). In experiments to determine if PFOA has a hormonal effect on mammary gland development, C57BL/6 mice (n=10/group) were ovariectomized (OVX) at 3 weeks of age, allowed one week to recover, and treated with 0 or 5 mg PFOA/kg bw for 4 weeks. Abdominal and inguinal mammary glands were collected at sacrifice, prepared as wholemounts, and scored for growth and development. The mammary glands of the OVX control and PFOA-treated OVX mice were similarly stunted in growth as evidenced by no outgrowth of ducts or presence of terminal end buds. This was in contrast to the stimulatory effect of PFOA observed by Yang et al. (2009) in intact mice.

In experiments to determine if PFOA-affected mammary glands respond to hormone treatment, intact C57BL/6 mice were dosed with 0 or 5 mg/kg bw PFOA for 4 weeks starting at 21 days of age. After the last dose, the mice were ovariectomized, allowed to recover for 1 week, and injected s.c. for 5 days with 17β-estradiol (E, 1 μg/0.2 ml/mouse), progesterone (P, 1 mg/0.2 ml/mouse), or both (E+P, 1 μg+1 mg/0.2 ml/mouse). The mice were sacrificed 24 hours after the last hormone injection. Abdominal and inguinal mammary glands were collected at

sacrifice, prepared as whole mounts, and scored for growth and development. In the mammary glands of mice treated with PFOA and E, stimulated terminal ducts were observed, and in PFOA-treated mice given P or E+P stimulated terminal ducts and an increased number of side branches were observed. The results showed that PFOA increased the mouse mammary gland response to exogenous estrogen and progesterone.

In experiments to determine if PFOA-induced mammary gland development stimulation is related to PPARα expression and to determine the impact of PFOA on steroid hormones and growth factors, female C57BL/6 and PPARα-null C57BL/6 mice (n=5-10 mice/group) were gavage dosed with 0 or 5 mg/kg bw PFOA 5 days/weeks for 4 weeks starting at 21 days of age (Zhao et al., 2010). Vaginal opening was monitored daily and estrous cycle state was determined at sacrifice after 4 weeks of treatment. At necropsy, blood was collected for measurement of serum steroid hormones and binding proteins. Portions of the mammary glands, ovaries, and livers were collected and processed for histological examination. RNA was extracted from the livers for quantitative RT-PCR and PCR array for selected genes related to metabolism of drugs, toxic chemicals, hormones, and micronutrients. Portions of mammary gland were used in western blot analysis of several enzymes, local growth factors, and receptors. Among those were aromatase which aids in converting testosterone to estradiol and androstenedione to estrone, hydroxysteroid 17β dehydrogenase 1 (HSD17β1) which aids in converting estrone to estradiol, and hydroxysteroid 3β dehydrogenase 1 (HSD3β1) which aids in converting pregnenolone to progesterone and androstenediole to testosterone. Growth factors critically involved in mammary gland development including amphiregulin (Areg), insulin like growth factor I (IGF-I), and hepatocyte growth factor (HGFα), and markers of cell proliferation including cyclin D1 and proliferating cell nuclear antigen (PCNA) were analyzed by western blot. Areg mediates estrogen receptor α (ERα) function and is a ligand for epidermal growth factor receptor (EGFR). These receptors were also analyzed by western blot.

The mammary glands of PPARα-null mice treated with PFOA had an increased number of terminal end buds and stimulated terminal ducts compared to control PPARα-null mice. Protein levels of Areg, IGF-I, HGFα, ERα, and EGFR were significantly increased ($p<0.05$) in PFOA-treated C57BL/6 mice, and Areg, HGFα, ERα, and EGFR were significantly increased ($p<0.05$) in PFOA-treated PPARα-null mice. Cyclin D1 and PCNA were significantly increased ($p<0.05$) in C57BL/6 and PPARα-null mice treated with PFOA compared to levels in control mice. Immunofluorescent staining of the mammary glands for ERα and Areg showed a significant increase ($p<0.05$) in Areg positive luminal epithelial cells and Areg and ERα double positive staining cells in C57BL/6 and PPARα-null mice treated with PFOA compared to control mice. The results show that the stimulatory effect of PFOA on mammary gland development is independent of PPARα expression and suggest that PFOA increases the levels of steroid hormones, growth factors, and receptors which promote mammary gland cell proliferation.

Estradiol levels were similar between intact control and treated wild-type mice, but progesterone levels were significantly increased ($p<0.05$) in PFOA-treated mice in proestrus and estrus compared to control mice in the same stages of the estrous cycle. Serum sex hormone binding globulin and albumin levels were not significantly changed by treatment with PFOA.

The effect of PFOA on aromatase, HSD17β1, and HSD3β1 activity in the ovaries of C57BL/6 PPARα-null mice was examined. In C57BL/6 mice, HSD17β1 and HSD3β1 proteins

were significantly increased ($p<0.05$), and in PPARα-null mice, HSD17β1 protein was significantly increased. Aromatase levels were not affected by PFOA. The results suggest that PFOA may increase serum steroid hormone levels in the ovaries.

Due to the increased progesterone levels observed in PFOA-treated mice, the expression of liver metabolic enzymes was analyzed. Liver metabolic function may affect steroid hormone serum levels which play a role in mammary gland development. In PPARα-null and C57BL/6 mice treated with PFOA, detoxification enzymes in the liver including glutathione s-transferase α1, μ3, and μ4, were upregulated ($p<0.05$). Expression of liver drug metabolic enzymes including but not limited to CYP1a1, CYP1a2, and HSD17β2, was significantly down-regulated ($p<0.05$) in C57BL/6 mice treated with PFOA, but expression in PFOA-treated PPARα-null mice was comparable to control mice. Hydroxysteroid 17β dehydrogenase 4, an enzyme that converts estradiol to estrone was significantly upregulated ($p<0.05$) in C57BL/6 mice treated with PFOA. The results suggested that PFOA-induced expression changes in liver enzymes may not contribute to PFOA-induced mammay gland development stimulation.

Inhalation Exposure

There were two components (inhalation and oral) to the Staples et al. (1984) developmental toxicity study of PFOA in Sprague-Dawley rats. The oral component was presented earlier. The study design consisted of whole-body dust inhalation exposure for 6 hours/day, on GD 6-15. The MMAD ranged from 1.4 to 3.4 μm and the geometric standard deviation (GSD) ranged from 4.3 to 6.0. The study was carried out in two trials with each trial including two experiments. In experiment 1, the dams were sacrificed on GD21 prior to parturition, and in experiment 2, the dams were allowed to litter and were sacrificed on PND23; offspring were sacrificed on PND35. In the first trial (both experiment), dams (n=12) were exposed to 0, 0.1, 1, or 25 mg/m^3. In trial 2, the high dose was reduced to 10 mg/m^3. In experiment 1 of trial 2, dams numbered 12-15/group and two additional groups (6 dams/group) were added and were pair-fed to the 10 and 25 mg/m^3 groups. In experiment 2 of trial 2, only 6 control and 6 dams dosed at 10 mg/m^3 were allowed to litter.

In the first experiment, the dams were weighed on GD 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Pups (live and dead) were counted, weighed and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dosed group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes.

Treatment-related clinical signs of maternal toxicity occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m^3 (on GD 12, 13, and 17). Food consumption was significantly reduced at 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight were also observed at these concentrations, with statistical significance at the high-

concentration only. Likewise, statistically significant increases in mean liver weights ($p < 0.05$) were seen at the high-concentration group. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25 mg/m^3 PFOA group ($p = 0.002$) and in the pair-fed control group ($p = 0.001$). Interpretation of the decreased fetal body weight is difficult given the high incidence of mortality in the dams. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

In the second experiment, in which the dams were allowed to litter, the procedure was the same as that for the first experiment until GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated PND 1. Dams were weighed and examined for clinical signs on PNDs 1, 7, 14, and 22. On PND 23 all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. At birth, each pup was subsequently weighed and inspected for adverse clinical signs on PNDs 4, 7, 14, and 22. The eyes of the pups were also examined on PNDs 15 and 17. Pups were sacrificed on PND 35 and examined for visceral and skeletal alterations.

In the second experiment, clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence to those described for trial one. Maternal body weight gain during treatment at 25 mg/m^3 was less than controls, although the difference was not statistically significant. In addition, 2 out of 12 dams died during treatment at 25 mg/m^3. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on PND1 (6.1 g at 25 mg/m^3 vs. 6.8 g in controls, $p=0.02$). On PNDs 4 and 22, pup body weights continued to remain lower than controls, although the difference was not statistically significant. No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

Dermal Exposure

No data on the developmental effects of dermal exposures to PFOA were identified in the literature.

4.2.6 Chronic Toxicity

Oral Exposure

Monkey. Male Cynomolgus monkeys (n=4 or 6/dose) were administered PFOA by oral capsule containing 0, 3, 10, or 30/20 mg/kg/day for 26 weeks (Thomford, 2001b, Butenhoff et al., 2002).

Dosing of animals in the 30 mg/kg-day dose group ceased after 12 days and decreased to 20 mg/kg-day when reinstated on Day 22 because of low food consumption, decreased body weight, and decreased feces. Sacrifice of the surviving monkeys, except for two control monkeys and two monkeys from the mid dose group (recovery animals) occurred at 26 weeks. The animals in the recovery groups were sacrificed 13 weeks later.

Animals were observed twice daily for mortality and moribundity and were examined at least once daily for signs of poor health or abnormal behavior. Ophthalmic examinations were performed before treatment began and at weeks 26 and 40. Body weight, food consumption, clinical hematology, clinical chemistry, urinalysis, serum hormone levels, and PFOA levels in blood and tissue were assessed throughout the study. One animal from the 30/20 mg/kg-day dose group was sacrificed in moribund condition on day 29 with signs of dosing injury and liver lesions. One animal from the 3 mg/kg-day dose was sacrificed (day 137) with signs of hind limb paralysis, ataxia and hypoactive behavior, few feces and no food consumption. Treatment of the remaining three animals given 30/20 mg/kg-day was halted on days 43, 66, and 81, respectively, because of thin appearance, few or no feces, low or no food consumption, and weight loss, but the animals appeared to recover from compound-related effects within 3 weeks after cessation of treatment. No significant changes in mean body weight were observed at doses of 3 or 10 mg/kg/day.

Serum hormone levels (estrone, estradiol, estriol, testosterone, TSH, free T4, total T4 and CCK) were not significantly altered throughout the study. However, free and total T3 levels were significantly decreased ($p<0.05$) from weeks 5 to 10 and at week 27 in the 30/20 mg/kg/day dose group compared to controls.

At terminal sacrifice (26 weeks), mean absolute liver weight was significantly increased in all dose groups, and the relative liver-to-body weight ratio was significantly increased for the high dose group. Final body weight and liver weight data are presented in Table 4-13. The cause of the increase in liver weights was suggested to be due to hepatocellular hypertrophy (indicated by decreased hepatic DNA content) which was hypothesized to result from mitochondrial proliferation based on an increase in hepatic succinate dehydrogenase activity.

TABLE 4-13. Liver weight data in monkeys administered PFOA for 6 months

Dose	Body weight	Absolute liver wt (g)	Relative liver wt (%)
0 mg/kg (n = 4)	3947 ± 591	60.2 ± 6.9	1.5 ± 0.1
3 mg/kg (n = 3)	4486 ± 30	81.8 ± 2.8*	1.8 ± 0.1
10 mg/kg (n = 4)	4447 ± 498	83.2 ± 9.7*	1.9 ± 0.1
30/20 mg/kg (n = 2)	3925 ± 583	90.4 ± 4.2*	2.4 ± 0.5*

From Butenhoff et al. 2002
*Significantly different from control, $p<0.01$.

Because administration of PFOA to rats has been shown to result in liver, Leydig cell and pancreatic acinar cell tumors, Butenhoff et al. (2002) analyzed markers of tumor formation in the monkey study just described. In the liver, a two-fold increase in hepatic palmitoyl CoA oxidase activity was observed in the 30/20 mg/kg-day group, consistent with reports for species that are not particularly responsive to PPARα-agonists. Replicative DNA synthesis in the liver, an indication of cell proliferation, was not altered in the treated animals. It has also been proposed that changes associated with the pancreatic acinar cell tumors in rats include increased serum

CCK concentrations and indications of cholestasis, including increases in alkaline phosphatase, bilirubin, and bile acids. None of these changes were observed in the Cynomolgus monkeys. There were also no significant changes in estradiol, estriol, or testosterone in the monkeys. Each of these factors is associated with Leydig cell tumors in rats. There were no changes in replicative DNA synthesis in the pancreas or testes.

After a 13 week recovery period, there were no treatment-related effects on terminal body weights or on absolute or relative organ weights, suggesting that the treatment-related liver weight changes were reversible. There were no treatment-related macroscopic or microscopic changes at the recovery sacrifice. Under the conditions of the study, the LOAEL was 3 mg/kg-day based on increased liver weight and a NOAEL was not established.

Rat. The chronic toxicity of PFOA was investigated in a 2 year study in rats by Butenhoff et al. (2012). Sprague-Dawley (Crl:CD BR) rats (50/sex) were fed diets containing 0, 30 or 300 ppm PFOA (0, 1.3, and 14.2 mg/kg-day for males; 0, 1.6, and 16.1 mg/kg-day for females). Groups of 15 additional rats per sex were fed 0 or 300 ppm PFOA and evaluated at the one year interim sacrifice. All animals were observed daily throughout the two year dosing period. Periodic observations included body weights and feed consumption, hematology, serum chemistry, urinalysis, gross pathology, organ weights, and histopathology. Animals were sacrificed after one and two years of dosing. Organ weights were determined after each sacrifice and the tissues subjected to histological examination.

There were dose-related decreases in body weight gains in male and female rats as compared to the controls; statistical significance was attained for only the high-dose group (both sexes). The body weight changes were considered treatment related since feed consumption increased (rather than decreased) during the study. There were no significant differences in mortality between the treated and untreated groups. Significant decreases in red blood cell counts, hemoglobin concentrations and hematocrit values were observed in the high-dose male and female rats as compared to control values. Clinical chemistry changes included slight (less than 2-fold) but significant increases in ALT, AST, and AP in both treated male groups from 3-18 months, but only in the high-dose males at 24 months. No dose- or treatment-related differences in absolute and relative organ weights were found between the treated and control groups at 2 years.

Significantly increased incidences of lesions in the liver were observed in the high-dose male group. At 1 year, diffuse hepatomegalocytosis, portal mononuclear cell infiltration and hepatocellular necrosis were seen. At 2-years, significant increases in megalocytosis were observed in the males, and females in the high-dose group. Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, and hyperplastic nodules, a localized proliferation of hepatic parenchymal cells, were also significantly increased in high-dose males.

Among the high dose males histological changes were noted in tissues other than the liver. A significant increase in aspermatogenesis and vascular mineralization of the testes and the incidence of pulmonary hemorrhage (44%) and alveolar macrophates (62%) were reported.

The LOAEL for male rats is 300 ppm (14.2 mg/kg/day) based on a decrease in body

weight gain and histological changes in the liver, testes, and lungs. The LOAEL for female rats is 300 ppm (16.1 mg/kg/day) based on decreased body weight gain and hematologic effects. The NOAEL for both sexes is 30 ppm (1.3 mg/kg/day and 1.6 mg/kg/kg for females).

Biegel et al. (2001) conducted a 2-year mechanistic study in which male Crl:CD BR (CD) rats (n=156/group) were fed a diet containing 0 or 300 ppm PFOA (0 or 13.6 mg/kg/day). Interim sacrifices were conducted every three months up to 21 months for measurements of liver and testes weights, peroxisome proliferation, and cell replication. Serum samples were collected and reproductive hormones measured.

Body weight was significantly decreased from days 8 to 630 in PFOA-exposed rats. In the treated group, relative liver weights and hepatic β-oxidation activity were statistically significantly increased at all time points between 1 and 21 months when compared to the controls. Absolute testis weights were significantly increased only at 24 months. No hepatic or Leydig cell proliferation was observed at any sampling times. The incidence of Leydig cell hyperplasia was significantly increased in PFOA-exposed rats (46% vs. 14% control group) Pancreatic aceinar cell proliferation was significantly increased at 15, 18, and 21 months. The incidence of acinar cell hyperplasia was 30/76 (39%) compared to the incidence in the control group, 14/80 (18%). There were no significant differences in serum testosterone or prolactin in the PFOA-treated rats when compared to the controls. Serum FSH was significantly increased at 6 months, and LH was significantly increased at 6 and 18 months. There were significant increases in serum estradiol concentrations in the treated rats at 1, 3, 6, 9, and 12 months.

4.2.7 Carcinogenicity

Oral Exposure

Tissues from the animals in the Butenhoff et al. (2012) study were evaluated for neoplastic and preneoplastic formations. Hepatocelluar carcinomas were observed at 6, 2, and 10% in the control, low-, and high-dose male rats, respectively. None were observed in females in the control and low-dose groups, but a 2% incidence was observed for female rats in the high-dose group. The differences between the treated and control groups were not significantly different. No liver adenomas were observed.

At the one-year sacrifice, testicular masses were found in 6/50 high-dose and 1/50 low-dose rats, but not in any of the controls. A significant increase ($p<0.05$) in the incidence of testicular (Leydig) cell adenomas was observed in the high-dose male rats at the end of the study. The Leydig cell tumor incidence in the control, low-, and high-dose groups was 0/50 (0%), 2/50 (4%), and 7/50 (14%), respectively. The increase was also statistically significant when compared to the historical control incidence of 0.82% observed in 1,340 Sprague-Dawley control male rats used in 17 carcinogenicity studies (Chandra et al., 1992). The spontaneous incidence of Leydig cell tumors in 2-year old Sprague-Dawley rats in other studies was reported to be approximately 5% (cited in Clegg et al., 1997).

A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats at the 2-year sacrifice. The incidence of this lesion in the control, low-, and high-dose groups was 0%, 14%, and 32%, respectively. The biological

significance of this effect at the time of the initial evaluation was unknown, as there was no evidence of progression to tumors.

Slides of the ovaries from the Butenhoff et al. (2012) study were re-evaluated by Mann and Frame (2004) with emphasis placed on the proliferative lesions of the ovary. Using more recently published nomenclature, the ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, which corresponded to the diagnoses of tubular hyperplasia or tubular adenoma by the original study pathologist. The data are summarized in Table 4-14. No statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/ adenoma combined were seen in treated groups compared to controls. There was some evidence of an increase in size of stromal lesions observed at the 300 ppm group; however, adenomas occurred in greater incidences in the control group than in either of the treated groups. Results of this follow-up evaluation indicated that rats sacrificed at the one-year interim sacrifice, as well as rats that died prior to the interim sacrifice were not considered at risk for tumor development.

TABLE 4-14. Incidence of Ovarian Stromal Hyperplasia and Adenoma in Rats

Group	0 ppm	30 ppm	300 ppm
No. examined	45	47	46
Hyperplasia (Total)	8	16	15
Grade 1	6	7	5
Grade 2	2	3	1
Grade 3	0	5	6
Grade 4	0	1	3
Adenoma	4	0	2
Adenoma and/or Hyperplasia	12	16	17

From Mann and Frame, 2004

A significant increase ($p<0.05$) in the incidence of mammary fibroadenomas in the low and high dose groups of female rats was noted in the Butenhoff et al. (2012) study. The incidence of mammary fibroadenomas was 22% (11/50), 38% (19/50) and 42% (21/50) in the control, 30, and 300 ppm groups, respectively (Hardisty, 2010). The increase was also statistically significant when compared to the historical control incidence of 19.0% observed in 1,329 Sprague-Dawley control female rats used in 17 carcinogenicity studies as reported by Chandra et al. (1992). Butenhoff et al. (2012) did not consider the mammary fibroadenomas to be treatment related on the basis of the historical control incidence (24%) from a study of 181 female rats terminally sacrificed at 18 months (Prejean et al., 1973). However, this is an inappropriate historical reference because the study duration was 18 months instead of 2 years. When the mammary fibroadenoma incidences were compared to the historical control incidence (37%) in 947 female rats in the Haskell Laboratory, however, there did not appear to be any compound related effect (Sykes, 1987). The incidence of other mammary gland tumor types is provided in Table 4-15.

The mammary gland findings were re-examined by a Pathology Working Group (Hardisty et al., 2005, 2010; Table 4-15) using the diagnostic criteria and nomenclature of the Society of Toxicological Pathologists (Mann et al., 1996). The Pathology Working Group (PWG) concluded that there were no statistically significant differences in the incidence of fibroadenoma, adenocarcinoma, total benign neoplasms (lobular hyperplasia, fibroadenoma, and

adenoma) or total malignant neoplasms (adenocarcinoma) of the mammary glands between control and treated animals using Fischer's Exact Test for pair-wise comparision (see Table 4-15). There was also no significant difference in combined benign and malignant neoplasms between control and treated groups.

The primary difference between the original reported findings and the PWG results involved findings initially reported as lobular hyperplasia being reclassified as fibroadenomas, mostly in the control group. With the reclassification, the incidence of single mammary fibroadenoma in the control, low- and high-dose groups were: 32% (16/50), 32% (16/50), and 40% (20/50), respectively. The evidence for mammary fibroadenomas in the female rat is equivocal since the incidences were comparable to some historical background incidences, so the PWG concluded that there were no statistically significant differences in the incidence of fibroadenoma or other neoplasms of the mammary gland between control and treated animals (Hardisty et al., 2005, 2010).

TABLE 4-15. Mammary Gland Tumor Incidence Comparison

	0 ppm		30 ppm		300 ppm	
	Butenhoff	**Hardisty**	**Butenhoff**	**Hardisty**	**Butenhoff**	**Hardisty**
Number necropsied	50	50	50	50	50	50
Lobular hyperplasia (%)	6 (12%)	0 (0%)	3 (6%)	2 (4%)	2 (4%)	0 (0%)
Adenocarcinoma (%)	8 (16%)	9 (18%)	14 (28%)	16 (32%)	5 (10%)	5 (10%)
Fibroadenoma* (%)	10 (20%)	18 (36%)	19 (38%)	22 (44%)	21 (42%)	23 (46%)
Adenoma (%)	3 (6%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

From Hardisty et al., 2005, 2010 (Compares Butenhoff et al., 2012 with Hardisty et al., 2005, 2010)
*Includes fibroadenoma, multiple counts

A 2-year mechanistic study in male Crl:CD BR (CD) rats (Cook et al., 1994; Biegel et al., 2001) resulted in Leydig cell tumors supporting the observations from the Butenhoff et al. (2012) study. The rats (n=156/group) were fed diets containing 0 ppm (*ad libitum* control and control pair fed to the PFOA-exposed rats) or 300 ppm PFOA (13.6 mg/kg intake). Rats were euthanized at interim time points of 1, 3, 6, 9, 12, 15, 18, and 21 months. All rats surviving the 24-month test period were necropsied for microscopic examination of various organs: e.g., kidneys, liver, testes, brain, heart, spleen.

There was a significant increase in the incidence of Leydig cell adenomas in the treated rats 11% (8/76) when compared to the pair-fed control rats (3%, 2/78). The incidence in *ad libitum* control rats was 0% (0/80). In addition, the treated group had a significant increase in the incidences of liver adenomas and pancreatic acinar cell adenomas when compared to the pair-fed and *ad libitum* control groups. The incidence of liver adenomas in the *ad libitum* control, pair-fed control, and treated groups was 3% (2/80), 1% (1/79), and 13% (10/76), respectively. The incidence for liver carcinomas was 0% (0/80), 3% (2/79), and 0% (0/76) in the *ad libitum* control, pair-fed control, and treated groups, respectively. The incidence for the pancreatic acinar cell adenomas was 0% (0/80), 1% (1/79), and 9% (7/76). The incidence of pancreatic acinar cell carcinoma was 1% (1/76) in the treated rats, 0% (0/79) in the pair-fed control rats, and

0% (0/80) in control rats.

In the first carcinogenicity study (Butenhoff et al., 2012), there was no reported increase in the incidence of pancreatic acinar cell tumors, and the incidence of pancreatic acinar hyperplasia in the male rats was 0/33, 2/34, and 1/43 in the control, 30, and 300 ppm groups, respectively. To resolve this discrepancy, the histological slides from both studies were reviewed by independent pathologists. This review of the microscopic lesions of the pancreas in the two studies indicated that PFOA produced increased incidences of proliferative acinar cell lesions of the pancreas in the rats of both studies at the dietary concentration of 300 ppm. The differences observed were quantitative rather than qualitiative; more and larger focal proliferative acinar cell lesions and greater tendency for progression of lesions to adenoma of the pancreas were observed in the Biegel et al. (2001) study compared to the Butenhoff et al. (2012) study. The difference between pancreatic acinar hyperplasia (Butenhoff et al., 2012) and adenomas (Biegel et al. 2001) in the rat was a reflection of arbitrary diagnostic criteria and nomenclature by different pathologists. The basis for the quantitative difference in the lesions observed is not known but was believed to be due most likely to difference in the diets used in the two laboratories (Frame and McConnell, 2003).

Inhalation Exposure

No data on the tumorigenic effects of chronic inhalation exposures to PFOA were identified in the literature.

Dermal Exposure

No data on the tumorigenic effects of chronic dermal exposures to PFOA were identified in the literature.

4.3 Other Key Data

4.3.1 Mutagenicity and Genotoxicity

PFOA has been tested for genotoxicity in a variety of *in vivo* and *in vitro* assays. The data from the in vitro studies are summarized in Table 4-16.

PFOA was tested in a cell transformation and cytotoxicity assay conducted in $C_3H10T_{½}$ mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation. There was no evidence of transformation at any of the dose levels tested in either the colony or foci assay methods (Garry & Nelson, 1981).

PFOA was tested twice (Lawlor, 1995; 1996) for its ability to induce mutation in the *Salmonella – E. coli*/mammalian-microsome reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen in *S. typhimurium* TA1537 when tested without metabolic activation was not reproducible. PFOA did not induce mutation in either *S. typhimurium* or *E. coli* when tested either with or without metabolic activation. PFOA did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli, 1996c; NOTOX, 2000). Sadhu (2002) reported that PFOA did not induce gene mutation when tested with or

without metabolic activation in the K-1 line of Chinese hamster ovary (CHO) cells in culture.

Murli (1996b,d) tested PFOA twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, PFOA induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, PFOA induced significant increases in chromosomal aberrations and in polyploidy (Murli, 1996b). The effects were observed only at toxic concentrations (EFSA, 2008).

PFOA did not display mutagenic activity with or without metabolic activation in *S. typhimurium* strains TA98, TA100, TA102, and TA104 (Freire et al., 2008).

TABLE 4-16. Genotoxicity of PFOA *In Vitro*

Test System	End-point	With Activation	Without Activation	Reference
$C_3H10T_{½}$ mouse embryo fibroblasts	Cell Transformation	NA	-	Garry & Nelson, 1981
C_3H $10T_{½}$ mouse embryo fibroblasts	Cytotoxicity	NA	-	Garry & Nelson, 1981
S. typhimurium TA1537	Gene Mutation	-	+ (not reproducible)	Lawlor, 1995; 1996
E. coli	Gene Mutation	-	-	Lawlor, 1995; 1996
Chinese hamster ovary cells	Chromosomal Aberrations	+, +	+, -	Murli, 1996b, 1996d
Chinese hamster ovary cells	Polyploidy	+, +	+, -	Murli, 1996b, 1996d
Human lymphocytes	Chromosomal Aberrations	-	-	Murli, 1996c; NOTOX, 2000
K-1 Chinese hamster ovary cells	Gene Mutation	-	-	Sadhu, 2002
S. typhimurium TA98, TA100, TA102, TA104	Gene Mutation	-	-	Freire et al., 2008

NA= Not applicable

The data summarized above suggest that PFOA is not a mutagen. A single positive result in *S. typhimurium* was not reproducible by the same authors and was not replicated in other studies. Potential chromosomal effects were found in CHO cells at toxic concentrations but not in human lymphocytes.

PFOA was tested twice in the *in vivo* mouse micronucleus assay. PFOA did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay (Murli, 1995, 1996a).

Zhao et al. (2010) used A_L cells to determine the mutagenicity of PFOA to mammalian cells. A_L cells are a human-hamster hybrid containing CHO-K1 chromosomes and a single copy of human chromosome 11. The significance of human chromosome 11 is that is encodes for expression of the human cell surface protein CD59. A_L and mitochondria-deficient A_L cells were incubated with 0, 1, 10, 100, or 200 μM PFOA for up to 16 days and used in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability, mutation, or caspace assays. Reactive

oxygen species, nitric oxide, and superoxide anion production were measured in the cells, and the effects of reactive oxygen species/reactive nitrogen species quenchers [0.5% dimethyl sulfoxide (DMSO) and 0.2 mM NG-methyl-$_L$-arginine, respectively] on mutagenicity and caspace acitivities were determined. At 100 and 200 μM PFOA, A_L cell viability was significantly decreased after incubation for 1, 4, 8, and 16 days. CD59 mutation frequencies were increased in A_L cells after a 16-day incubation with 200 μM PFOA. There was no increase in mutations in mitochondria-deficient A_L cells after incubation with 100 or 200 μM PFOA.

Production of reactive oxygen species, nitric oxide, and superoxide anion was significantly increased at 100 and 200 μM PFOA after incubation for 1, 4, and 16 days. Inhibition of reactive oxygen species through incubation with DMSO significantly decreased the CD59 mutation frequency caused by 200 μM PFOA after a 16 day incubation. Incubation with 100 or 200 μM PFOA did not increase reactive oxygen species or superoxide anion production in mitochondria-deficient A_L cells. The highest dose significantly increased caspase 3/7 and 9 activities after 1 and 4 day incubations. Incubation with 0.5% DMSO and 0.2 mM NG-methyl-$_L$-arginine significantly decreased the increased caspace activity induced by 200 μM PFOA. The results led the authors to suggest that mitochondrial-dependent reactive oxygen species may play an important role in PFOA-induced mutagenicity and induction of caspase activities may be mediated by reactive oxygen and nitrogen species.

4.3.2 Immunotoxicity

Yang et al. (2000, 2001, 2002a,b) completed a series of studies investigating the immunotoxic effects of PFOA. In the first study, Yang et al. (2000) examined the liver, spleen, and thymus effects of several known PPARα activators, including PFOA. The researchers administered 0.02% PFOA (~40 mg/kg/day) to male C57BL/6 mice in the diet for 2, 5, 7, or 10 days. At the end of the feeding period, mice were sacrificed and the liver, spleen, and thymus were weighed. Administration of PFOA resulted in a significant increase in liver weight relative to control even at day two. Following five days of administration, significant decreases in thymus and spleen weight were noted.

A second component of the Yang et al. (2000) study examined the effect of 0.02% PFOA in the diet on the cellularity, cell surface phenotype, and cell cycle of thymocytes and splenocytes. After 7 days, significant decreases in the total number of thymocytes (↓85%) and splenocytes (↓80%) were observed. There is a pattern to the development of thymocytes that must be considered when evaluating the impact of chemicals on their differentiation. Early thymocytes formed in the bone marrow do not express CD4 or CD8 ($CD4^-CD8^-$). In the thymus they differentiate and express both CD4 and CD8 ($CD4^+CD8^+$). They also undergo proliferation and down regulation of either the CD4 or CD8 protein expression to become either a CD4 or CD8 thymocyte (Yang et al., 2000). Following exposure to PFOA, the number of thymocytes expressing neither CD4 nor CD8 decreased by 57%; the number expressing both CD4 and CD8 decreased by 95%; the number expressing only CD4 decreased by 64% while those expressing only CD8 decreased by 72%. As detected by cell cycle flow cytometry analyses, thymocyte proliferation was inhibited based on the numbers of cells in each stage of the cell cycle.

T cells ($CD3^{\pm}$) and B cells ($CD19^{\pm}$) in the spleen decreased by 75% and 86%, respectively. Splenic T-cells are lymphocytes produced in the thymus that carry the $CD3^+$

surface protein marking them as T-cells for exportation to the spleen. There are several classes of the T-cells that are characterized by surface proteins. Yang et al. (2000) found significant decreases in helper $CD3^+$T-cells with $CD4^+$ surface proteins (78%) and cytotoxic $CD3^+$T-cells with $CD8^+$ surface proteins (74%). The authors suggested that, unlike the $CD3^+$T-cells that originate in the thymus, the decrease in $CD19^+$B cells of the spleen reflects decreased differentiation and maturation in the bone marrow where they are formed.

In the final phase of the Yang et al. (2000) study, the effects of *in vitro* exposure of thymocytes and splenocytes to PFOA were examined. The *in vitro* studies showed spontaneous apoptosis occurring in splenocytes and thymocytes after 8 or 24 hours of culturing in the presence of varying concentrations (50, 100, or 200 μM) of PFOA. However, under the exposure conditions PFOA did not appear to significantly alter the cell cycle.

In a later publication Yang et al. (2001) reported on their examination of the immunosuppressive effects of PFOA. As was the case with their earlier publication (Yang et al., 2000), the 2001 report includes several components. A diet of 0.02% PFOA (~40 mg/kg/day) was fed to C57BL/6 mice for 2 to 10 days. One group of animals was exposed to PFOA each day until day of sacrifice on days 2, 5, 7, or 10. At sacrifice body, liver, and spleen weights were recorded. A second group of animals was dosed according to the same schedule, but dosing ceased after day 7, and the animals were fed normal diets for 2 to 10 days in order to monitor recovery from the effects of exposure. In the recovery group, animals were sacrificed after 2, 5, or 10-day recovery periods.

The mice that received 0.02% PFOA for up to 10 days experienced significant increases in liver weight compared to controls beginning at day 2. Significant decreases in thymus and spleen weights were observed starting on day 5. Body weight increased for the first two days of the study and decreased continuously for the remainder of the exposure period. The activity of palmitoyl-CoA and lauroyl-CoA, biomarkers for PPARα activation and and peroxisome proliferation, were also increased significantly and increasingly across the exposure period. The impact of PFOA exposure was similar to that observed in the Yang et al. (2000) study. After administration for 7 days, the number of thymocytes expressing neither CD4 nor CD8 decreased by 65% following exposure to PFOA; the number expressing both CD4 and CD8 decreased by 95%; and the number expressing either CD4 or CD8 decreased by 65% and 75%, respectively. T cell ($CD3^+$) splenocytes and B cell ($CD19^+$) splenocytes decreased by 65% and 75%, respectively. As detected by cell cycle flow cytometry analyses, thymocyte but not splenocyte proliferation was inhibited.

The animals that participated in the recovery portion of this study rapidly regained their body weight starting on the second day after withdrawal of PFOA. However, the liver weight failed to recover even after 10 days. Thymus weight recovery started on day 2 and was completed by day 10. The spleen weights returned to normal by day 2 post-withdrawal. The increases in thymus and spleen weight during recovery were paralleled by increases in total thymocyte and splenocyte counts. Thymocyte recovery was apparent on day 5 and complete by day 10, although during the first two days of the recovery period further decreases in the $CD4^+CD8^+$, $CD4^+$ and $CD8^+$ cells were observed. Flow cytometry evaluation of the distribution of the cells across the cell cycle in the recovery group animals demonstrated increases in cell proliferation following removal of PFOA from the diet. However, final cell counts did not reach

the control values for the thymocyte ($CD4^+$ and $CD8^+$) or splenocyte ($CD3^+$ and $CD19^+$) populations evaluated.

In the second component of the Yang et al. (2001) study, C57BL/6 mice were administered diets consisting of 0.001%-0.05% PFOA (w/w) for 10 days. These doses are equivalent to approximately 2.0-100 mg/kg/day. There was a dose-related decrease in spleen and thymus weights and dose-related increase in liver weights accompanied by a corresponding increase of pamitoyl Co-A and lauroyl Co-A activity. Enzyme activity was significantly increased for all doses. Spleen and thymus weights were significantly decreased at doses ≥ 0.01% and higher but not for the lower doses; the increases in liver weights were significantly increased for the 0.02% and 0.05% doses.

A Yang et al. (2002a) study was designed to examine the possible involvement of PPARα in the immunomodulation exerted by PFOA. This study made use of transgenic PPARα-null mice (Sv/129), which are homozygous with regards to a functional mutation in the PPARα gene. These mice do not exhibit peroxisome proliferation or hepatomegaly and hepatocarcinogenesis even after exposure to peroxisome proliferators. The mice were fed a diet consisting of 0.02% PFOA (w/w) (~40 mg/kg/day) for 7 days. At the end of the feeding period, mice were sacrificed and the liver, spleen, and thymus were removed and weighed. The effect of PFOA on peroxisome proliferation, cell cycle, and lymphoproliferation was ascertained.

The results showed that, in contrast to wild-type mice, feeding PPARα-null mice PFOA resulted in no significant decrease in body weight. Liver weight in PPARα-null mice fed the PFOA diet was significantly increased compared to control PPARα null mice, but not when compared to wild type PFOA-exposed mice. Peroxisome proliferation, as measured by fatty acid oxidation, was totally lacking in PPARα-null mice. Also, in contrast to wild type mice, feeding PPARα-null mice PFOA resulted in no significant decrease in the weight of the spleen or the number of splenocytes.

There was a decrease in weight and cellularity of the thymus in the PPARα-null mice compared to PPARα-null control mice, but it was not as dramatic as that in the PFOA-exposed wild-type mice. In addition, the decreases in the size of the CD4+CD8+ population of thymus cells and the number of thymus cells in the S and G2/M phases of the cell cycle were lower in PPARα-null mice than they were in the PFOA-exposed wild-type mice but higher than in the PPARα-null control mice. PFOA treatment caused no significant change in splenocyte proliferation in PPARα-null mice in response to mitogen exposure but did show a response in the PFOA-exposed wild-type mice as described above.

The series of studies published by Yang et al. (2000, 2001, 2002a) link many of the effects of the liver, thymus and spleen in PFOA exposed mice to the activation of PPARα. However, there were some impacts on the thymus and liver that were independent of PPARα receptor activation. PPARα-null mice still showed increases in liver weight and effects on the thymus (small decrements in organ weight, thymocyte cellularity, and proliferative cell cycle) following a 7 day exposure to approximately 40 mg/kg/day PFOA.

Yang and colleagues extended their studies of the immunotoxicity of PFOA in a feeding study designed to examine the effects of PFOA on specific humoral immune responses in mice (Yang et al. 2002b). For this study, 0.02 % PFOA was administered to male C57BL/6 mice for

10 days. The the animals were then evaluated via plaque forming cell (PFC) and serum antibody assays, for their ability to generate an immune response to horse red blood cells (HRBCs). Ex vivo and in vitro splenic lymphocyte proliferation assays were also performed. The results showed that mice fed normal chow responded to challenge with HRBCs with a strong humoral response, as measured by the PFC assay. In contrast, mice fed PFOA responded to HRBC immunization with no increase in HRBC-specific PFCs, relative to unimmunized controls. However, in experiments where PFOA-treated mice received normal chow following HRBC immunization, there was a significant recovery of the numbers of specific PFCs stimulated. The suppression of the humoral immune response by PFOA was confirmed by analysis of the serum anti-HRBC response.

In ex vivo experiments, splenocytes isolated from control mice responded to both ConA and LPS with lymphocyte proliferation, as measured by thymidine incorporation. However, treating mice with PFOA (0.02% for 7 days) attenuated the proliferation. In a set of in vitro experiments, PFOA (1- 200 μM) added to the culture medium of splenocytes cultured from untreated mice did not cause an alteration of lymphocyte proliferation in response to LPS or ConA.

DeWitt et al. (2008) expanded the repertoire of studies of the immunological effects of PFOA by examining various aspects of humoral and cellular immunity. The first component of their publication had many similarities with the Yang et al. (2001) study. Adult female C57BL/6J mice (n=40/endpoint and 8/group) were exposed to a single daily dose of 30 mg PFOA/kg/day in distilled water by gavage for 10 continuous days. After 10 continuous days of exposure, half of the mice continued receiving PFOA from Day 11 through Day 15 (constant group) while the other half received distilled water from Day 11 through Day 15 (recovery group). On Day 11, the mice were immunized with sheep red blood cells (SRBC). Sacrifices took place on Day 16 (1 day post-exposure period) or Day 31 (15 days post-exposure period). Vehicle and cage controls were also included in the study. All groups were monitored for the following effects:

- Body weight and organ weights (Day 16, Day 31)
- Serum IgM levels (Day 16)
- Delayed-type hypersensitivity (DTH) foot-pad response to bovine serum album (BSA) (Day 26)
- Serum IgG levels after booster immunization with SRBC on Day 20 (Day 31)

The results for body and organ weights were comparable to those in the Yang et al. (2001) study. Body weight was significantly decreased from Days 8-11 for both PFOA-treated groups and on Day 16 for mice in the constant exposure group. By Day 31, there were no body weight differences between the groups. Relative liver weight was significantly elevated in both PFOA-treated groups on Days 16 and 31. Absolute and relative spleen and thymus weights of animals in both PFOA groups were significantly decreased compared to control groups on Day 16. By Day 31, thymus and spleen weights were not statistically different between control and treated mice. IgM levels following immunization with SRBC were reduced by up to 20% compared to controls on post exposure day 1 in both the recovery and constant exposure groups. There were no significant differences from controls for IgG levels and DTH foot-pad responses to the BSA challenge.

The C57BL/6 mice used for the continuous-dosing vs. recovery component of the DeWitt et al. (2008) study were found to develop ulcerative dermatitis following the PFOA exposure. It was determined that this effect was a genetic susceptibility in the strain and they were not used for the dose-response component of the study; the C57BL/6N strain was used in its place.

Two studies of dose-response were included in the DeWitt et al. (2008) publication. Groups of 16 female C57BL/6N mice were given 0, 3.75, 7.5, 15, or 30 mg PFOA/kg/day in the drinking water for 15 days during the first experiment. In the second experiment, the doses were 0, 0.94, 1.88, 3.75, or 7.5 mg PFOA/kg/day administered for 15 days in the drinking water. The immunological sensitization and post-dose monitoring were identical to that used in the constant-dosing vs. recovery experiment.

In the first experiment, body weight was significantly decreased from Day 8-16 at 30 mg/kg PFOA and on Day 16 at 15 mg/kg PFOA. As observed previously, liver weights were significantly elevated at Day 16 and Day 31 at all doses. Absolute and relative spleen and thymus weights were significantly decreased at ≥15 mg/kg PFOA on Day 16. With the exception of the absolute thymus weight at 15 mg/kg PFOA, all spleen and thymus weights were similar to weights in controls 15 days after dosing. The IgM response was significantly reduced at ≥3.75 mg/kg PFOA in a direct dose-related manner. The IgG response was slightly but significantly elevated at 3.75 and 7.5 mg/kg PFOA but similar to that of the control level at the higher doses. Thus, there was a direct response of IgM but not IgG to dose across the dose levels. There was no significant change in the DTH response at any dose. The LOAEL from the first experiment was 3.75 mg/kg/day dose based on decreased IgM and increased IgG response to SRBC immunization and increased liver weights ($p<0.05$).

The second dose-response experiment confirmed the 3.75 mg/kg/day dose as the immunological LOAEL on the basis of significantly decreased spleen weight, decreased IgM levels on Day 16, and increased IgG levels on Day 31. The immunological NOAEL was 1.88 mg/kg/day. Benchmark dose analysis of IgM serum titer data gave a lower bound 95% confidence limit of 1.75 mg/kg/day on a benchmark dose (one standard deviation) of 3.06 mg/kg/day. Liver weight was significantly increased at all doses on Days 16 and 31. The LOAEL for increased liver weight was 0.94 mg/kg PFOA.

Loveless et al. (2008) administered 0, 0.3, 1, 10, or 30 mg linear PFOA/kg by oral gavage to groups of male CD rats (n=10/group) and CD-1 mice (n=20/group) for 29 days. Both species received a dose of SRBC on day 23 (rat) or 24 (mice). A separate group of high dose rats and mice were injected with water instead of SRBC. Rat body weight was recorded on days 0, 3, and 6-28, while mice were weighed daily. At necropsy blood was collected for immune parameters. Cell counts were determined for the thymus and spleen. Total spleen and thymocyte cell counts and organ weights in exposed rats were comparable to control. Microscopic examination of the thymus, mesenteric lymph nodes, and popliteal lymph nodes revealed no effects in treated rats resulting from PFOA exposure. There was no difference observed in IgM titers between treated and control rats. The immunological NOAEL was 30 mg/kg/day.

In mice, absolute and relative spleen and thymus weights were significantly decreased at ≥10 mg/kg/day. The relative spleen weight of mice dosed with 1 mg/kg/day was also

significantly decreased compared to control animals. Spleen and thymus cell counts were significantly decreased and minimal to severe lymphoid depletion/atrophy of the thymus was observed at ≥10 mg/kg/day. IgM titers were significantly decreased at ≥10 mg/kg/day. Serum corticosterone was significantly increased at 10 mg/kg/day and elevated (not statistically significant) at 30 mg/kg/day. When IgM and corticosterone were plotted against each other, a negative correlation coefficient suggested that increasing corticosterone levels decreased the ability to make SRBC antibody. The LOAEL was 1 mg/kg/day based on decreased spleen weight, and the NOAEL was 0.3 mg/kg/day.

Loveless et al. (2008) hypothesized that at least a portion of the thymic response to PFOA might be related to physiological stress and increased levels of corticosterone hormones. DeWitt et al. (2009) investigated this hypothesis by comparing the immunological response of adrenalectomized (ADX) C57BL/6N female mice to that of sham operated female mice from the same strain. The animals were dosed with 0, 3.75, 7.5, or 15 mg PFOA/kg/day in the drinking water for 10 days. Body weight was recorded on dosing days 0, 4, and 8, plus 2 and 5 days post dosing. On exposure days 5 and 10, blood and serum were collected for analysis of a broad array of clinical chemistry parameters including activity of liver enzymes indicative of cellular damage (AP, AST, ALT, LDH, GGT, and SDH), serum lipids (cholesterol and triglycerides) and corticosterone. A baseline measure of corticosterone was determined from serum samples collected before dosing began. One day after cessation of exposure, the mice were immunized with SRBC. Four days later, serum was collected and the levels of corticosterone and IgM were determined.

Body weight in the sham-operated mice declined during dosing in the highest dose group but recovered by 5 days post dosing. In the ADX mice, body weight declined during dosing at 7.5 and 15 mg PFOA/kg/day, but recovered in mice receiving 7.5 mg PFOA/kg/day by 5 days post dosing. There were significant increases in ALT, AST, LDH, and SDH at the highest dose for the ADX mice indicative of damage to hepatic cell membranes (Table 4-17).

TABLE 4-17. Selected Clinical Chemistry Parameters in Mice Treated with PFOA for 5 Days

Dose (mg/kg/day)	ALT	AST	LDH	SDH
Sham-Operated				
0	39.52±2.50	121.56±17.96	320.57±29.84	46.43±1.03
3.75	43.88±0.93	104.07±10.24	293.92±68.65	39.31±3.32
7.5	56.96±6.78	95.55±10.22	262.71±35.60	39.02±7.77
15	62.57±3.15	89.07±1.30	191.76±22.25	46.87±1.46
ADX				
0	26.96±1.78	73.53±4.70	176.50±19.32	33.05±1.58
3.75	29.67±1.62	76.58±3.38	222.69±19.18	37.95±2.35
7.5	39.04±2.59	83.79±8.94	320.45±53.34	46.35±1.42
15	94.23±31.66*	126.47±16.39*	435.57±81.42*	77.61±19.89*

From DeWitt et al., 2009
*=p<0.05 vs corresponding sham control or ADX control group

Serum levels of triglycerides significantly decreased compared to controls with all doses for the sham operated mice on day five of dosing but only for the 7.5 and 15 mg/kg/day doses in the ADX mice. Cholesterol levels were significantly decreased ($p<0.05$) in the sham operated mice at the highest dose, but no differences in cholesterol levels were observed in the ADX mice.

After 10 days, there were no significant differences in liver enzymes for the ADX or sham mice. However, there was a dose-related trend towards increased levels of liver enzymes for the PFOA-exposed sham operated animals and for LDH in the PFOA-exposed ADX animals (Table 4-18).

TABLE 4-18. Selected Clinical Chemistry Parameters in Mice Treated with PFOA for 10 Days

Dose (mg/kg/day)	ALT	AST	LDH	SDH
Sham-Operated				
0	51.51±14.62	93.30±6.33	333.48±86.86	54.60±16.72
3.75	79.26±33.87	123.73±15.20	404.14±59.89	45.50±10.15
7.5	135.57±38.18	142.66±15.59	490.44±69.14	80.71±14.59
15	344.53±235.63	242.92±117.62	595.01±137.37	89.20±26.03
ADX				
0	128.22±24.80	106.00±8.86	236.96±30.23	61.88±8.87
3.75	282.23±193.54	217.10±3.48	379.61±80.67	68.78±24.88
7.5	89.79±21.54	99.78±12.59	574.65±236.38	52.07±11.98
15	261.14±75.95	181.40±32.94	614.05±144.95	101.93±24.00

From DeWitt et al., 2009

At the end of dosing, corticosteroid levels in the sham-operated animals were greatly elevated compared to the levels in the control animals at all doses and the difference was statistically significant at the highest dose. By 5-days post dosing the corticosterone levels had declined for all doses but were still elevated compared to controls for the 7.5 and 15 mg/kg/day groups. In the animals lacking their adrenal glands, there were no statistically significant differences in the hormone levels. IgM levels were significantly lower than controls at the highest dose for the sham-operated animals and at the two highest dose groups for the ADX mice. However, when comparing the sham mice to the ADX mice the only significant difference in IgM was found for the 7.5 mg/kg/day animals. On the basis of data it appears that stress-related corticosterone production did not have a major impact on the IgM response to the SRBC inoculation.

Son et al. (2009) administered 0, 2, 10, 50, or 250 ppm PFOA (0, 0.49, 2.64, 17.63, or 47.21 mg/kg PFOA) in the drinking water to 4 week old male ICR mice for 21 days to determine if PFOA alters T lymphocyte phenotypes and cytokine expression in mice. The spleen, thymus, and trunk blood were collected at sacrifice. Sections of the spleen and thymus were processed for histological examination. Splenic and thymic expression of mRNA from proinflammatory cytokines including tumor necrosis factor-α, interleukin-1β, and interleukin-6, and the proto-oncogene c-myc were analyzed using RT-PCR. Flow cytometry was used to phenotype the splenic and thymic lymphocyte populations.

Spleen and thymus weights were slightly decreased in mice treated with PFOA. Enlargement with marked hyperplasia of the white pulp and increased cellular density of the lymphoid follicles were observed in spleens at 250 ppm. In the thymus, decreased cortex and medulla thickness and densely arranged cortex lymphoid cells were observed at 250 ppm. Tumor necrosis factor-α, interleukin-1β, interleukin-6, and c-myc expression were significantly elevated at 250 ppm in the spleen. Interleukin-1β expression was also elevated at 50 ppm in the spleen. In the thymus, c-myc expression was significantly elevated by treatment with 50 and 25 ppm PFOA.

The splenic and thymic lymphocyte population was altered by PFOA treatment as shown in Table 4-19. A 50% decrease was observed in splenic $CD8^+$ lymphocytes at all PFOA doses, and an increase in splenic $CD4^+$ lymphocytes of 43% and 106% at 50 and 250 ppm PFOA, respectively, were observed. In the thymus, a 110% increase was observed in thymic $CD8^+$ lymphocytes at 250 ppm, but thymic $CD4^+$ lymphocyte populations were not affected by PFOA.

TABLE 4-19. Impact of PFOA on Splenic and Thymic Lymphocyte Populations

	Dose (mg/kg/day)			
Spleen	0.49	2.64	17.63	47.21
$CD4^-CD8^-$	↑	-	-	↓
$CD4^+CD8^-$	-	-	↑	↑
$CD4^-CD8^+$	↓	↓	↓	↓
$CD4^+CD8^+$	↓	↓	-	-
Thymus				
$CD4^-CD8^-$	-	-	-	↑
$CD4^+CD8^-$	-	-	-	-
$CD4^-CD8^+$	-	-	-	↑
$CD4^+CD8^+$	-	-	↓	↓

From Son et al., 2009
↑significantly increased compared to control ($p<0.05$)
↓significantly decreased compared to control ($p<0.05$)
- Not significantly different from control

Qazi et al. (2009) investigated the impact of PFOA on the innate immune system. Adult male C57BL/6 ($H\text{-}2^b$) mice were administered 0.001% or 0.02% PFOA (~2 or 40 mg/kg) in the diet (w/w) for 10 days. After the last dose all mice were sacrificed. Sacrifice was delayed for a subset of the animals until 2 hours after they had received a lipopolysaccharide (LPS) injection to stimulate an immunological response. Blood, peritoneal exudate cells, liver, epididymal fat, spleen, thymus, and bone marrow were recovered. The blood, peritoneal exudate, bone marrow, and spleen were evaluated for total and differential white blood cell counts and concentrations of tumor necrosis factor (TNF-α) and interleukin 6 (IL-6).

Consistent with other studies of the 0.02% dose, there was a significant increase in liver weight after the 10-day exposure. Body weight, thymus weight, spleen weight, and epididymal fat depots were decreased. Food consumption in these animals was reduced by 35% which may have played a role in the reduced body, organ, and tissue weights. Compared to the controls there was a significant decrease in total white cells, lymphocytes, and neutrophils at 0.02% PFOA. This same dose was associated with a decrease in total white cell count in bone marrow and spleen, and an increase in the proportion found as macrophages in the bone marrow, spleen and peritoneal cavity. Although the total number of macrophages was not reduced in the peritoneal cavity and spleen, it was reduced in the bone marrow. The increase in the proportion of macrophages reflects a decrease in other white cell populations. There was significant increase in the concentration of IL-6 in all of the 0.02 % dosed animals but only those receiving the LPS injections showed a significant increase in TNF-α. The 0.001% dose (about 2 mg/kg/day) was a NOAEL.

In a pilot study, Brieger et al. (2011) examined the effects of PFOA on human

leukocytes. Peripheral blood mononuclear cells (PBMC) were obtained from the blood of 11 voluntary donors (n=6 females, 5 males). PBMC were incubated with varying concentrations of PFOA followed by assays for cell viability, proliferation, and natural killer (NK) cell activity. The human promyelocytic leukemia cell line, HL-60, was used in cell viability and monocyte differentiation assays. The various components of the assays employed are identified as follows:

- In the cell viability assay, the PBMC were incubated with 0-500 μg/mL for 24, 48, or 72 hours, and HL-60 cells were incubated with 0-125 μg/mL PFOA for 24 hours.
- In the proliferation assay, the PBMC were incubated with 0-100 μg/mL PFOA for 24 hours, labeled with 6-carboxyfluorescein succinimidyl ester (CFSE), stimulated with concanavalinA, a T-cell mitogen (ConA, 5 μg/mL to half of all samples), and incubated for an additional 72 hours.
- For the NK assays, PBMC cells were incubated with 0-100 μg/mL PFOA for 24 hours followed in incubation of 3 hours with K562 target cells (12.5:1 ratio) labled with CFSE.
- In the monocyte differentiation assay, HL-60 cells were incubated with 0-100 μg/mL PFOA for 72 hours. Half of each sample was stimulated with 25 nM calcitrol, 1α,25-dehydroxyvitamin D3 ($1,25D_3$) 24 hours into the incubation period. Expression of CD11b and CD14 were measured as markers of differentiation.
- Whole blood was incubated with 0-100 μg/mL PFOA in the presence or absence of 25 μg/mL phytohemagglutinin (PHA), T-cell cytokine secretion stimulator, for 48 hours in quantification assays for the cytokines TNF-α and IL-6. Lipopolysaccharide (LPS, 0 or 250 ng/mL) was added to whole blood incubated with 0.1-100 μg/mL PFOA either 4 or 24 hours prior to the end of the 48 hour incubation period to determine TNF-α and IL-6 release.

The plasma concentrations of PFOA were 3.3, 1.56, and 4.19 ng/mL for all, female, and male volunteers, respectively. Exposure to 31.3 and 62.5 μg/mL PFOA significantly increased PBMC viability at the 72 hour endpoint, and 62.5 μg/mL PFOA significantly increased cell viability at 24 hours. Exposure to 250 and 500 μg/mL PFOA significantly decreased cell viability at all time endpoints. Exposure to PFOA did not affect HL-60 cell viability. A trend towards slightly augmented proliferation was observed following incubation with PFOA. Of the 9 samples used, cells from 6 donors had slightly increased proliferation and 3 had no response. In cells incubated with ConA and 100 μg/mL PFOA, a non-significant decrease in the number of proliferating cells was observed. PFOA decreased NK cell activity approximately 16% (not statistically significant). In the presence of $1,25D_3$, 100 μg/mL PFOA significantly increased the percentage of HL-60 cells expressing CD11b and CD14. There were no differences in monocyte differentiation in the absence of $1,25D_3$.

In whole blood, exposure to PFOA for 48 hours caused a slight increase in TNF-α and IL-6 levels. In the presence of PHA, a slight dose dependent decrease in TNF-α and IL-6 was observed. There was a slight dose-dependent decrease in TNF-α release when LPS was added 4 hours before the end of the incubation period and a slight dose dependent increase in IL-6 release when LPS was added 24 hours priod to the end of incubation. The authors also looked at the correlation between basal PFOA concentration and cytokine release. A significant association was observed between PFOA concentration and the release of LPS-induced TNF-α and IL-6 by peripheral monocytes. The authors suggested that the trends observed at the lower

concentrations may show an impact on human immunity with a larger population.

Inhalation Exposure

No data on the effects of inhalation exposure on immunological endpoints were identified in the literature.

Dermal Exposure

Fairley et al. (2007) carried out a complex study of toxicity and respiratory hypersensitivity to ovalbumin (OVA) as impacted by dermal exposure to PFOA dissolved in acetone compared to acetone alone. There were several phases to the study. In the first phase, a range finding study, PFOA was applied to each ear of female BALB/c mice (n=5/group) at doses of 0, 0.01%, 0.1%, 0.25%, 0.5%, 1.0%, or 1.5% PFOA (equivalent to 0, 0.25, 2.5, 6.25, 12.5, 25 or 50 mg/kg/day) for 4 days. Six days after last inoculation, the animals were sacrificed. The liver, spleen, and thymus were recovered and weighed. A significant increase in liver weight was observed at ≥6.25 mg/kd PFOA. Spleen weight was significantly decreased in mice dosed with 25 mg/kg and 50 mg/kg PFOA, and thymus weight was significantly decreased in mice at the highest dose ($p<0.05$). The cell counts in the spleen were significantly decreased compared to control at all doses and for the highest two doses in the thymus. The LOAEL was 6.25 mg/kg/day based on a statistically-significant increase in liver weight ($p<0.01$) and the NOAEL was 2.5 mg/kg/day.

In the second phase of the Fairley et al. (2007) study, groups of 5-15 animals were dosed dermally on the ears for 4 days with doses of 0, 0.5%, 0.75%, 1.0%, or 1.5% PFOA (equivalent to 0, 12.5, 18.75, 25, or 50 mg/kg/day). On days 1 and 10 they were injected ip with either 2.0 mg alum or 7.5 μg ovalbumin (OVA) and 2.0 mg alum in phosphate-buffered saline solution (100μL). Four days after the last inoculation the animals were sacrificed and blood was collected by cardiac puncture. Liver, spleen and thymus were recovered and weighed; spleen and thymus cellularities were determined. A significant ($p<0.01$) increase in liver weight and decrease in spleen weight and spleen cellularity occurred at all doses. Thymus weight and cellularity were significantly decreased ($p<0.01$) at ≥18.75 and ≥ 25 mg/kg/day, respectively. There were no significant differences in the $CD4^+$, $CD8^+$, $CD4^-8^-$ or CD3e T cells. CD3e protein is expressed by both thymocytes and mature T cells.

Levels of IgE and OVA-specific IgE were measured in the control and dosed animals by enzyme-linked immunosorbent assay (ELISA). IgE is the immunoglobulin that is best correlated with respiratory allergic responses. It functions to stimulate mast cells and basophils to release histamine and other mediators of inflammation (Saladin, 2004). The IgE response was increased in a dose-related fashion compared to the OVA control for all the PFOA treated animals; the increase was significant ($p<0.05$ or 0.01) at doses ≥18.75 mg/kg/day. The OVA-specific IgE response did not demonstrate a direct response to dose but there was a significant increase ($p<0.05$) for the 18.75 and 25 mg/kg/day groups. The OVA-specific response for the 37.5 mg/kg/day highest dose groups was practically indistinguishable from the OVA control.

The dermal LOAEL was 12.5 mg/kg/day based on increased liver weight and decreased spleen weight and spleen cellularity. No NOAEL was established.

In part three of the Fairley et al., (2007) study, mice (n=5) were dosed dermally via their ears for 4 days as described above (0, 12.5, 18.75, 25, and 50 mg/kg/day). On days 19 and 26 after the start of dosing they were challenged by pharyngeal aspiration of 250 μg OVA in the phosphate-buffered saline vehicle and sacrificed 24 hours after the last challenge. There was a dose-related decrease in number of spleenocytes carrying the $B220^+$ marker (expressed on B cells, activated B cells, subsets of T and NK cells) compared to the OVA controls. The changes were significantly different for the 25 mg/kg/day ($p<0.05$) and 37.5 mg/kg/day ($p<0.01$) groups.

After the day 19 challenge the mice (n=5) were placed in a plethysmography chamber for measurement of enhanced pause airway respiration (PenH) values. PenH values reflect volume of air in the lungs. Once in the chamber they were challenged with nebuilized methacholine for three minutes followed by two minutes of fresh air. The PenH measures were recorded every 30 seconds over the next 5 hours. The area under the curve (AUC) for the PenH measures was determined after correction for baseline (acetone control, no OVA or PFOA). An AUC of 1.6 was considered to be a positive response. Twenty-four hours later blood was drawn from the abdominal artery and the mice were sacrificed. The lungs were recovered for histological analysis. An increase in antigen-specific hyperactivity response to PFOA, in both the PenH values and the number of animals responding was observed at doses up to about 25 mg/kg/day. The PenH AUC was significantly ($p <0.05$) increased in mice treated with 25 mg/kg/day PFOA and OVA compared to the OVA control mice, but there was no significant difference between the OVA control and the animals exposed to 50 mg/kg/day PFOA and OVA. The LOAEL for the PenH response was 25 mg/kg/day, and the NOAEL was 18.75 mg/kg/day.

Histopathological evaluation of the lungs revealed macrophage and eosinophil infiltration in response to the challenge with 250 μg OVA by pharyngeal aspiration. The severity of the response increased with increasing concentrations of PFOA. Eosinophils and macrophages were found in the interstitial, peribronchiole, and perivascular areas. Neutrophils, lymphocytes, and some multinucleated giant cells were also observed. Increased secretory matter, sloughing of epithelium, and secretory cell necrosis were observed in mice exposed to all concentrations of PFOA and OVA. The response was not observed in the mice exposed to only PFOA. The authors concluded that dermal exposure to PFOA was immunotoxic and enhanced the airway hypersensitivity response to OVA suggesting that PFOA may augment the IgE response to environmental allergens.

4.3.3 Hormone Disruption

Thyroid. Martin et al. (2007) administered 20 mg PFOA/kg to adult male Sprague-Dawley rats (n = 4 or 5) for 1, 3, or 5 days by oral gavage and determined the impact of PFOA on hormone levels. Blood was collected via cardiac puncture and the serum was analyzed for cholesterol, testosterone, free and total T4, and total T3. RNA extracted from the livers was used for gene expression profiling, genomic signatures, and pathway analyses to determine a mechanism of toxicity.

Following a 1 day, 3 day, and 5 day dose, a significant decrease ($p<0.05$) was observed in serum cholesterol (~↓45-72%), total T4 (~↓83%), free T4 (~↓80%), and total T3 (~↓25-48%). Serum testosterone was significantly decreased ($p<0.05$, ~↓70%) following a 3 day and 5 day

PFOA dose. PFOA treatment was matched to hepatotoxicity-related genomic signatures, as well as signatures for hepatocellular hypertrophy, hypocholesterolemia, hypolipidemia, and peroxisome proliferation. PPARα nuclear regulated genes were induced by PFOA treatment. Genes associated with the thyroid hormone release and synthesis pathway including, *Dio3* which catalyzes the inactivation of T3 and *Dio1,* which deiodinates prohormone T4 to bioactivate T3, were affected by PFOA. Treatment with PFOA resulted in significantly upregulated expression of *Dio3* and downregulated expression of *Dio1* ($p<0.05$). Expression of HMG-CoA reductase (involved in cholesterol biosynthesis) was significantly upregulated and cholesterol biosynthesis was downregulated in a manner consistent with PPARγ agonists. The authors suggested a link between PFOA, PPAR, and thyroid hormone homeostasis based on work by Miller et al. (2001) who observed decreased serum T4 and T3 levels and increased hepatic proliferation following exposure to peroxisome proliferators. They also noted that PFOA exhibited similarities to compounds that induce xenobiotic metabolizing enzymes through PPARγ and CAR.

Reproductive Hormones. Cook et al. (1992) gavage dosed male CD rats (n=15/group) for 14 days with 0, 1, 10, 25, or 50 mg PFOA/kg to examine the possibility than an endocrine-related mechanism may explain Leydig cell adenomas observed in rats. A separate control group was pair fed to the 50 mg/kg group. Blood and testicular interstitial fluid were collected at necropsy for hormone analysis including testosterone, estradiol, and LH. A separate group of rats was dosed with 0 or 50 mg PFOA/kg for 14 days and challenged with 100 IU of human corionic gonadotropin (hCG) or 2 mg naloxone/kg 1 hour prior to necropsy to induce testosterone concentrations. Blood was collected and analyzed for testosterone and LH. Serum from rats challenged with 100 IU hCG was also anlayzed for progesterone, 17 α-hydroxyprogesterone, and androstenedione.

The relative liver weight at 10 mg PFOA/kg was significantly increased ($p<0.05$). The relative liver weight was significantly increased ($p<0.05$) and the accessory sex organ unit relative weight was significantly decreased ($p<0.05$) at 25 and 50 mg PFOA/kg compared to those weights in control rats. The results at the highest dose were also different when compared to the pair fed controls. The relative weight of the ventral prostate was significantly decreased at the highest dose compared to the pair fed control. Serum estradiol was significantly increased at ≥10 mg PFOA/kg compared to the control. No differences were observed in testosterone and LH between the treated rats and control. In the challenge experiment, serum testosterone was significantly decreased ($p<0.05$) by treatment with 50 mg PFOA/kg after challenge with 100 IU hCG. No differences in testosterone concentration were observed in the naloxone challenged rats, and no differences in LH were observed after either challenge. In the hCG challenged rats, androstenedione was significantly reduced at 50 mg PFOA/kg, but no differences in concentrations were observed in progesterone or 17 α-hydroxyprogesterone between control and treated rats. The authors suggested that the decreased serum testosterone levels observed may be due to decreased conversion of 17 α-hydroxyprogesterone to androstenedione attributable to the increased serum estradiol levels. The LOAEL was 10 mg/kg based on increased liver weight and increased serum estradiol levels, and the NOAEL was 1 mg/kg.

Biegel et al. (1995) conducted *in vitro, in vivo,* and *ex vivo* studies to determine the effects of PFOA on Leydig cell function. In the *in vitro* study, Leydig cells were cultured ± 2 IU hCG (final 3 hours) and 0, 100, 200, 250, 500, 700, or 1000 μM PFOA for a total of 5 hours and analyzed for testosterone concentration. Leydig cells were also incubated ±500 μM PFOA and

anlayzed for testosterone and estradiol at 0, 0.5, 1, 3, 6, 12, 24, and 48 hours. Male CD rats were gavage dosed for 14 days with 0, 0 pair fed, or 25 mg PFOA/kg and necropsied on day 15. Blood and testicular interstitial fluid were collected for hormone analysis. Liver samples were collected for analysis of peroxisomal β-oxidation and microsomal aromatase activities. Leydig cells from the treated rats in the *in vivo* study were isolated and cultured for analysis of testosterone concentration for the *ex vivo* study.

In the *in vitro* studies, there was no effect of PFOA treatment on testosterone in Leydig cells cultured without hCG. In cells cultured with hCG, PFOA caused a dose dependent decrease in testosterone production. At 100 μM PFOA + hCG, the testosterone concentration was significantly increased compared to cells treated with only 100 μM PFOA. Cytotoxicity occurred at ≥ 750 μM PFOA. In the time course experiment, 500 μM PFOA significantly inhibited hCG-stimulated release of testosterone at time points of at least 3 hours compared to control. Estradiol levels of PFOA-treated Leydig cells at 48 hours were statistically greater than the control.

In the *in vivo* study, serum estradiol was significantly increased ($p<0.05$) by 25 mg PFOA/kg when compared to the *ad libitum* and pair fed control rats. Testicular interstitial fluid testosterone concentration was significantly decreased ($p<0.05$) and microsomal aromatase activity, and peroxisomal β-oxidation activity were significantly increased ($p<0.05$) in PFOA-treated rats compared to the pair fed control rats. In the *ex vivo* study, an increase of 8.6-fold in testosterone production ($p<0.05$) was observed in Leydig cells isolated from PFOA-treated rats. The authors suggested that the increased serum estradiol levels resulted from liver aromatase induction by PFOA, and that PFOA may directly affect Leydig cell function.

Liu et al. (1996) treated adult male Crl:CD BR (CD) rats (n=15/group) with 0, 0 pair fed, 0.2, 2, 20, or 40 mg PFOA/kg for 14 days by oral gavage to determine the impact of PFOA on aromatase activity. A separate study examined the effect of PFOA on aromatase activity in cultured hepatocytes and is discussed below. Aromatase is a cytochrome P450 enzyme localized to the endoplasmic reticulum that catalyzes the conversion of androgens to estrogens. At necropsy on day 15, blood was collected for serum estradiol determination. Liver samples were collected for determination of microsomal aromatase activity and total P450 concentration. The testes were collected and testicular aromatase was determined.

In the *in vitro* study, hepatocytes isolated from control male CD rats were incubated with 0-1000 μM PFOA and the aromatase activity was evaluated after 18, 42, and 66 hours (Liu et al. 1996). Compared to aromatase activity in time-matched control cultures, PFOA caused a decrease in aromatase activity after 18 and 42 hours incubation with hepatocytes and an increase after the 66 hour incubation period.

In the *in vivo* study, the body weight of rats treated with ≥ 20 mg PFOA/kg was significantly decreased ($p<0.5$) compared to the control rats. Pair fed control rats also had significantly decreased body weight compared to the control rats. Body weight was not different between the pair-fed control rats and rat dosed with 40 mg/kg PFOA. Absolute and relative liver weight were significantly increased ($p<0.5$) at ≥ 2 mg PFOA/kg. Relative testes weight was significantly increased at ≥20 mg PFOA/kg, but the differences were due to decreased body weight. There were no differences observed in testicular aromatase activity. In the remaning

analysis, the pair-fed control group were similar to the *ab libitum* control group. The protein yield of hepatic microsomes was significantly increased at ≥0.2 mg PFOA/kg and hepatic aromatase activity, total hepatic aromatase activity adjusted for liver and body weight effects, and serum estradiol were significantly increased ($p<0.05$) at ≥2 mg PFOA/kg. The maximum increase in total hepatic aromatase activity was 16-fold and the increase was 2-fold for serum estradiol. A significant correlation ($p<0.0001$) was observed between total hepatic aromatase activity and serum estradiol. The aromatase activity in liver microsomes isolated from control rats and incubated for 2 hours with PFOA was significantly decreased at ≥ 100 μM. The authors estimated the EC_{50} values for the outcomes and they are shown in Table 4-20. Liu et al. (1996) concluded that the PFOA-increased protein yields suggested induction of the endoplasmic reticulum resulting in aromatase induction which led to increased serum estradiol. However, PFOA also inhibited aromatase activity which would explain why serum estradiol was only increased up to 2-fold.

TABLE 4-20. Estimated EC50 Values

Parameters	EC_{50} (mg PFOA/kg)
Hepatic microsome protein yield	0.53
Hepatic microsomal aromatase activity	0.76
Absolute liver weight	1.07
Relatvie liver weight	1.56
Serum estradiol	3.24
Terminal body weight	11.65

From Liu et al. 1996
EC_{50}= half-maximum response

In a study examining the impact of PFOA on aromatase activity, Liu et al. (1996) also examined the impact of PFOA on peroxisome β-oxidation and cytochrome P450 activities. Male Crl:CD BR (CD) rats (n=15/group) were orally dosed with 0, 0 pair fed, 0.2, 2, 20, or 40 mg PFOA/kg for 14 days. Liver samples were collected for determination of microsomal total cytochrome P450 concentration and peroxisome β-oxidation activity. Total cytochrome P450 was significantly increased ($p<0.05$) at ≥20 mg PFOA/kg and β-oxidation activity were increased at ≥2 mg PFOA/kg. The estimated EC_{50}s for total cytochrome P450 and β-oxidation were 18.18 and 2.19 mg PFOA/kg, respectively. The LOAEL was 2 mg/kg based on increased liver weight, serum estradiol, and hepatic aromatase activity, and the NOAEL was 0.2 mg/kg.

Hines et al. (2009) examined the roles that exposure to PFOA and ovarian hormones may play in animals exposed during gestation compared to during their adult years. Timed-pregnant CD-1 mice were gavage dosed in two blocks on gestation days 1-17 but not thereafter. Block 1 animals were dosed with 0, 1, 3, or 5 mg PFOA/kg, and Block 2 animals were dosed with 0, 0.01, 0.1, 0.3, 1, or 5 mg PFOA/kg/day. At birth, pups were pooled within each block and dose group and randomly redistributed among the dams (10 pups/litter). Offspring were weaned at 3 weeks, and a subset of females from each dose group (0, 0.01, 0.1, 0.3, 1, and 5 mg PFOA/kg/day) was ovariectomized (OVX) at weaning or the day after weaning. All animals were observed until they reached 18 months of age.

Body weight was recorded weekly for the first 9 months of age, followed by monthly body weight recordings over the next 9 months. As the animals matured, they were evaluated for the endpoints listed in Table 4-21. A group of naive 8-week old adult mice were dosed for 17 days with 0, 1, or 5 mg PFOA/kg/day to compare the impact of exposure in adult animals to

those occurring during gestation. At 18 months of age, the mice were sacrificed. Blood, retroperitoneal abdominal fat, interscapular brown fat, organs, and abnormal growths were collected at necropsy.

TABLE 4-21. Data Collection for Female Mice Gestationally-exposed to PFOA

Test	Age at Test	Dose (mg/kg/day)	Group
Glucose tolerance test	15-16 weeks	0, 1, 5	Intact
Serum leptin and insulin	21-33 weeks	0, 0.01, 0.1, 0.3, 1	Intact, ovx
Body mass composition	42 weeks	0, 0.01, 0.1, 0.3, 1	Intact
Glucose tolerance test	17 months	0, 0.1, 1, 5	Intact
Food consumption	17 months	0, 0.1, 1, 5	Intact
Serum estradiol	18 months	0, 0.01, 0.1, 0.3, 1, 5	Intact

From Hines et al., 2009

Body weight of offspring born to dams exposed to 5 mg PFOA/kg was significantly decreased ($p<0.05$) on PND1 and through 18 months of age compared to control offspring body weight. At weaning, the body weight of offspring born to dams exposed to 1 mg PFOA/kg/day was significantly decreased ($p<0.05$) compared to control offspring body weight. A significant increase ($p<0.05$) in body weight, due to more rapid weight gain after week 10, compared to intact control body weight was observed in intact mice exposed to 0.01-0.3 mg PFOA/kg/day during gestation. Body weight of intact mice gestationally exposed to 0.01-0.3 mg PFOA/kg/day was comparable to body weight of control mice at 18 months.

Due to the increased weight gain observed in intact mice exposed to PFOA during gestation, glucose tolerance tests were carried out along with determination of serum insulin concentration. In cases of insulin resistance, plasma glucose and insulin levels are elevated and the insulin response is lessened. Insulin resistance has also been associated with excess abdominal fat. Serum leptin levels were also determined as increased leptin levels have been associated with a leptin-resistance mechanism of action for increased weight gain in humans. Body mass composition was used to determine if there were differences in body fat between the intact groups, and feed consumption was recorded to determine if consumption played a role in body weight differences in intact control and intact gestionally-exposed mice. Serum estradiol was measured to determine if PFOA impacted hormone levels at 18 months in intact control and intact gestionally-exposed mice.

Glucose tolerance testing showed no statistically significant differences in baseline glucose or response to glucose challenge at 15-16 weeks or at 17 months. At 21 and 31 weeks of age, a significant increase in serum leptin and insulin levels was observed in intact mice exposed to 0.01 and 0.1 mg PFOA/kg/day. No statistically significant difference was observed between the fat to lean ratio of intact control and intact gestationally-exposed animals at 42 weeks of age. No significant difference was observed in food consumption between intact control and intact gestationally-exposed animals at 42 weeks of age. Serum estradiol levels were not different consumption between intact control and intact gestionally-exposed animals at 18 months.

Exposure to PFOA as an adult did not result in body weight differences among the groups at 18 months of age. The body weight of intact mice gestationally exposed to 1 mg PFOA/kd/day was significantly increased ($p<0.05$) compared to adult mice exposed to 1 mg PFOA/kg/day. No other differences in body weight among the groups were observed.

No significant differences among the groups were observed in survival during the 18 month study. At necropsy, abdominal white fat was significantly decreased ($p<0.05$) at 1 and 5 mg PFOA/kg/day in gestationally-exposed intact mice compared to intact control mice. Interscapular brown fat was significantly increased ($p<0.05$) at 1 and 3 mg PFOA/kg/day in gestationally-exposed intact mice and in gestationally-exposed ovx mice at 1 mg PFOA/kg/day. Relative spleen weight was significantly decreased ($p<0.05$) at 3 mg PFOA/kg/day in gestationally-exposed intact mice and at 1 and 5 mg PFOA/mg ($p=0.05$-0.07) in gestationally-exposed ovx mice. Relative liver weight was not different between the groups. No differences were observed at 18 months of age in tissue weight in mice exposed to PFOA as adults. At 1 mg PFOA/kg/day, white and brown fat weight was significantly increased in gestationally-exposed intact mice compared to adult-exposed mice exposed to 1 mg PFOA/kg/day.

The authors concluded that developmental exposure to low doses and high doses of PFOA resulted in different phenotypes in mice. At low doses, increased weight, increased serum insulin, and increased serum leptin were observed in adult mice, and at high doses, decreased weight in early and late life, decreased white fat, increased brown fat, and decreased spleen weight were observed. Under the conditions of the study, the developmental LOAEL was 0.01 mg PFOA/kg based on increased weight gain and increased serum insulin and leptin levels. No developmental NOAEL was established. The adult NOAEL was 5 mg PFOA/kg, and no LOAEL was established.

Adrenal Hormones. Thottassery et al. (1992) exposed intact or adrenalectomized (ADX) male Sprague-Dawley rats to a single dose of 150 mg/kg PFOA in corn oil to determine the role of adrenal hormones on liver enlargement and peroxisomal proliferation. ADX rats were dosed 2 days after surgery with PFOA (ADX PFOA), corticosterone (ADX CORT), or both (ADX CORT PFOA). A group of intact and ADX rats received only the vehicle and served as controls. The animals were sacrificed 48 hours after dosing with PFOA or vehicle. Assays were conducted to determine DNA levels and changes in peroxisomal β-oxidation, catalase, and ornithine decarboxylase activities. An increase in ornithine decarboxylase activity has been associated with proliferation of many different cell types. An increase of ornithine decarboxylase in the livers of animals exposed to PFOA would suggest that the increased liver weight observed in PFOA-exposed animals was the result of hyperplasia. Ornithine decarboxylase was determined by measuring liberated CO_2 from DL-[1-^{14}C] ornithine hydrochloride in all animals except those in the ADX CORT PFOA group.

Relative liver weight in intact rats treated with PFOA was significantly increased compared to control (36%, $p<0.05$). Relative liver weight in rats in the ADX PFOA group was significantly increased compared to rats in the ADX vehicle group (16%, $p<0.05$). Relative liver weight in rats in the ADX CORT PFOA group was significantly increased compared to rats in the ADX CORT group (32%, $p<0.05$). Hepatic DNA levels were significantly decreased $p<0.001$) in intact rats treated with PFOA and in rats in the ADX CORT PFOA group.

Ornithine decarboxylase activity was significantly increased in the rats in the ADX PFOA group compared to rats in the ADX group (170.5 pmole CO_2/hr/mg protein, vs. 30.5 pmole CO_2/hr/mg protein, $p <0.001$). Ornithine decarboxylase activity was not different between intact rats treated with PFOA and intact rats treated with the vehicle.

PFOA increased whole liver peroxisomal β-oxidation activity by a similar amount and was not different among the groups. In intact rats and rats in the ADX CORT PFOA group, exposure to PFOA increased whole liver catalase activity, but activity was not increased in rats of the ADX PFOA group. Based on the results, the authors concluded that adrenal hormones were not required to induce peroxisomal β-oxidation activity in PFOA-exposed rats, but are required to increase catalase activity. They also concluded that the enlarged livers of PFOA-exposed animals were the result of hypertrophy rather than hyperplasia based on decreased hepatic DNA content and lack of increased ornithine decarboxylase activity.

4.3.4 Physiological or Mechanistic Studies

Gene Expression. Rosen et al. (2007) examined the gene expression profile in the lung and liver of mouse fetuses exposed to PFOA. Pregnant CD-1 mice were gavage dosed with 0, 1, 3, 5, or 10 mg PFOA/kg/day on GD1-17. Dams were sacrificed on GD18, and three fetuses per litter were processed for total RNA from portions of the liver and lung. Global gene expression was analyzed using Affymetrix gene chips.

A dose-related increase was observed in the number of genes altered by PFOA exposure in both the liver and lung. A greater number of genes in the liver were altered compared the number of genes altered in the lung. Analysis of the genes by canonical pathway or biological function showed that most of the altered genes in both the liver and lung were associated with lipid homeostasis. In the fetal lung, the two highest doses of PFOA altered genes associated with fatty acid catabolism. In the fetal liver, all doses of PFOA were associated with genes involved in fatty acid catabolism, lipid transport, cholesterol biosynthesis, bile acid biosynthesis, lipoprotein metabolism, steroid metabolism, retinol metabolism, inflammation, phospholipid metabolism, glucose metabolism, proteosome activation, and ketogenesis. Although PPARα is known to regulate (at least partly) the expression of genes for the pathways or biological functions involved in lipid homeostasis, PFOA may also activate other nuclear receptors influencing the metabolic responses observed.

Rosen et al. (2008a) described the gene profiles in liver tissue from wild-type 129S1/SvlmJ mice (7-8/group) and PPARα-null mice (129S4/SvJae-PPARαt^{m1Gonz}/J, 6-8/group) dosed with 0, 1, or 3 mg PFOA/kg or 50 mg Wyeth 14,643, a PPARα agonist (Wolf et al., 2008a). RNA was isolated from the tissues and gene expression analyzed using Applied Biosystems Mouse Genome Survey Microarrays. Real-time (RT)-PCR was used to evaluate selected genes.

In both wild-type and PPARα-null mice exposed to PFOA, the number of significant and fully annotated genes used to evaluate the data for relevance to canonical pathway or biological function was less at 1 mg/kg than at 3 mg/kg PFOA. However, 85% of the altered genes at 1 mg/kg PFOA were alterd at 3 mg/kg PFOA.

PPARα target genes including Acox1, Me1, Slc27a1, Hsd17b4, Hadha, Hadhb, and Pdk4 were upregulated in PFOA and Wyeth 14,643 treated wild-type mice, but not in PPARα-null mice. Pdk4 was downregulated in PPARα-null mice exposed to PFOA but not in PPARα-null

mice exposed to Wyeth 14,643. Principle components analysis showed that PFOA-treated PPARα-null mice shared similarity with PFOA-treated wild-type mice at 3 mg PFOA/kg.

In wild-type PFOA and Wyeth 14,643 treated mice, alterations were observed in genes associated with fatty acid metabolism (mostly upregulated), inflammatory response (mostly downregulated), cell cycle control (mostly upregulated), peroxisome biogenesis (mostly upregulated), and proteasome structure and organization (mostly upregulated). In genes associated with xenobiotic metabolism, the response was different between PFOA and Wyeth 14,643 wild-type mice. Many of the Cyp2 genes were upregulated by PFOA and downregulated by Wyeth 14,643. In PPARα-null PFOA-treated mice, genes associated with fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle control were altered similar to the changes observed in PFOA-treated wild-type mice.

RT-PCR generally revealed good agreement with microarray analysis. However, expression of Ehhadh, a PPARα regulated gene, was up-regulated in PFOA-treated wild-type mice but not in PFOA-treated PPARα-null mice in microarray analysis. Expression of Ehhadh was up-regulated in all PFOA-treated mice in RT-PCR analysis. The authors concluded that PFOA induces transcriptional changes mediated through PPARα activation, and it also alters gene expression independently of PPARα. They noted that PFOA had multiple modes of action and can function as a biologically active xenobiotic in the absence of PPARα.

Rosen et al. (2008b) described the transcript profiles in the livers of adult mice exposed to PFOA. Tissues from several different studies were analyzed. The samples included liver tissue from male wild-type (strain 129S1/SvlmJ) and PPARα-null (strain 129S4/SvJae) mice dosed with 3 mg/kg/day PFOA for 7 days (from Wolf et al., 2008a); male wild-type and PPARα-null mice (strain SV129/C57BL/6) gavage dosed or fed diets containing Wyeth (WY)-14,643 (PPARα agonist); female wild-type and CAR-null (strain C57BL/6x129Sv) gavage dosed with CAR activators phenobarbital (PB) or 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP); and wild-type and Nrf-null ICR mice gavage dosed with the Nrf activator dithiol-3-thione. RNA was isolated from the tissues and gene expression was analyzed using Affymetrix full genome mouse chips. Rosetta Resolver software was used to identify significantly altered genes.

Exposure to 3 mg/kg PFOA for 7 days upregulated 641 genes and downregulated 451 genes in wild-type mice compared to 104 upregulated genes and 52 downregulated genes in PPARα-null mice. A total of 117 genes were regulated similarly in both strains, and 29 upregulated genes and 11 downregulated genes were unique to PPARα-null mice.

The gene expression profile of wild-type and PPARα-null mice exposed to PFOA for 7 days or WY-14,643 for 12 hours, or 3 or 7 days were compared. Four groups of altered genes were identified based on their expression behavior in wild-type and PPARα-null PFOA-exposed mice compared to genes from WY-14,643 treated mice. The first group consisted of genes (397) regulated by both PFOA and WY-14,643 in wild-type mice. They had a common direction and magnitude of change and were characterized as being lipid homeostasis, inflammation, cell proliferation, or proteome maintenance genes. Group II consisted of genes in wild-type mice (51) regulated solely by PFOA, and most were involved in amino acid metabolism. Of the 81 genes altered by PFOA exposure in PPARα-null mice (Group III), 62 had similar expression in wild-type mice, and many were involved in lipid metabolism. Regulation of these genes was

also observed in WY-14,643 wild type mice. Group IV genes (19) were altered by PFOA only in PPARα-null mice, and most were xenobiotic metabolizing enzymes.

By comparing the gene expression patterns between PFOA and WY-14,643, the authors concluded that:

- PPARα is required for a majority of the transcriptional changes observed in the mouse liver following PFOA or WY-14,643 exposure
- PFOA regulates genes that are also regulated by other peroxisome proliferators in a PPARα-dependent manner, and
- PFOA also regulates PPARα-independent genes.

The PPARα-independent genes included those involved in lipid homeostasis (upregulated), amino acid metabolism (down regulated), and xenobiotic metabolism (upregulated).

The transcription profiles of PFOA exposed wild-type and PPARα-null mice were compared to the transcription profile of PB or TCPOBOP exposed wild-type and CAR-null mice and dithiol-3-thione exposed wild-type and Nrf2-null mice to determine if PFOA activated CAR or Nrf2. A similar pattern was observed in the modified gene expression of PFOA exposed PPARα-null mice and PB (0.86 Pearson's correlation) or TCPOBOP (0.84 Pearson's correlation) exposed wild-type mice but no pattern was observed in gene expression of dithiol-3-thione exposed mice (≤0.06 Pearson's correlation) and PFOA exposed PPARα-null mice. These results suggested that some genes altered by PFOA exposure in PPARα-null mice are regulated by CAR but not Nrf2.

Bjork and Wallace (2009) examined the PPARα-dependent transcriptional activation potential of PFOA in rodent and human hepatic liver cells. Primary rat and human hepatocytes and HEPG2/C3A cells were incubated with 0, 5, 10, 20, 30, 50, 100, or 200 μM PFOA for 24 hours. Expression of acyl-CoA oxidase (Acox), Cyp4a1 (rat), Cyp4a11 (human), acyl-CoA thioesterase (Cte-rat, Acot1-human), and DNA damage inducible transcript (Ddit3) were determined by quantitative RT-PCR. The aforementioned genes are inducible by peroxisome proliferators except Ddit3 which is induced in the presence of direct or indirect DNA damage. Exposure to ≥ 100 μM PFOA significantly increased Ddit3 mRNA expression in primary rat hepatocytes. At the highest dose, Ddit3 was significantly increased in human hepatocytes and HepG2/C3A cells. Expression of Acox was significantly induced by 5, 10, 20, and 30 μM PFOA, and Cte/Acot1 was significantly induced at ≥20 μM PFOA in rat hepatocytes only. Expression of Cyp4a1/11 was significantly induced in rat hepatocytes at 5-50 μM and in human hepatocytes at 20-50 μM. The authors concluded that induction of peroxisome-related fatty acid oxidation gene expression is not observed in primary human liver cells or in transformed human liver cells *in vitro.*

Nakamura et al. (2009) investigated the differences in PFOA response between mice and humans using a humanized PPARα transgenic mouse line (hPPARα). The study design and whole animal toxicity data are described in section 4.2.2. Male, 8-week old wild-type (mPPARα) mice, PPARα-null mice, and hPPARα mice were gavage dosed with 0, 0.1, or 0.3 mg/kg PFOA (n=4-6/group) for 2 weeks and sacrificed 18-20 hours following the last dose. Livers were collected and analyzed for mRNA (RT-QPCR) and protein levels (Western blot analysis) of PPARα and related genes (retinoid X receptor alpha [RXRα], peroxisomal

bifunctional protein [PH], peroxisomal thiolase [PT], very long chain acyl-CoA dehydrogenase [VLCAD], medium chain acyl-CoA dehydrogenase [MCAD], and CYP4A10). RXRα forms a heterodimer with PPARα controls transcription of genes affecting lipid metabolism. CYP4A10 also plays a role in lipid metabolism. Treatment with peroxisome proliferators caused an increase in both PH and PT. MCAD and VLCAD are mitochondrial fatty acid metabolizing enzymes whose gene expression is mediated by PPARα (Aoyama et al., 1998). The results of mRNA expression impacted by PFOA exposure are shown in Table 4-22.

TABLE 4-22. mRNA Expression of Hepatic PPARα and Related Genes

	mPPARα			PPARα-null			hPPARα		
	0 mg/kg	0.1 mg/kg	0.3 mg/kg	0 mg/kg	0.1 mg/kg	0.3 mg/kg	0 mg/kg	0.1 mg/kg	0.3 mg/kg
PPARα	-	-	-	NA	NA	NA	-*↑	-*↑	-*↑
RXRα	-	-	-	-	-	-	-	-	-
PH	-	-	+↑	-	-*↓	-*↓	-	-*↓	-*↓
PT	-	-	+↑	-	-*↓	-*↓	-*↑	-	-
VLCAD	-	-	+↑	-*↓	-*↓	-*↓	-	-*↓	-*↓
MCAD	-	-	-	-	-*↓	-	-	-*↓	+↑
CYP4A10	-	+↑	+↑	-	-*↓	-*↓	-*↑	-*↓	-*↓

From Nakamura et al., 2009
- Not different from respective control
+Significantly different from respective control
*Significantly different from mPPARα mice treated with same concentration
↓-Decreased expression relative to respective control or mPPARα mice at same concentration
↑-Increased expression relative to respective control or mPPARα mice at same concentration

Treatment with PFOA did not alter mRNA expression or protein expression of PPARα, RXRα, or MCAD in mPPARα mice. At 0.1 mg/kg PFOA, mRNA expression of CYP4A10 was significantly increased ($p<0.05$) in mPPARα mice compared to control mPPARα mice. Treatment with 0.3 mg/kg PFOA resulted in significantly increased ($p<0.05$) mRNA expression of CYP4A10 and mRNA and protein expression of PH, PT, and VLCAD, in mPPARα mice when compared to control mPPARα mice.

As expected, mRNA and protein expression of PPARα was absent in PPARα-null mice. Treatment with 0.1 or 0.3 mg/kg PFOA did not alter mRNA or protein expression for any genes investigated compared to control PPARα-null mice. VLCAD mRNA expression and PT protein expression in control PPARα-null mice was significantly decreased ($p<0.05$) compared to mPPARα control mice. VLCAD mRNA and protein expression of PFOA treated PPARα-null mice was significantly decreased ($p<0.05$) compared to mPPARα mice treated with the same doses. Following treatment with 0.1 mg/kg PFOA, MCAD mRNA expression was decreased ($p<0.05$) compared to mPPARα mice treated with the same dose. When compared to mPPARα mice of the same dose, mRNA and protein expression of PH and PT was significantly decreased ($p<0.05$) in PPARα-null mice, as was CYP4A10 mRNA expression.

Treatment with PFOA did not alter mRNA or protein expression of PPARα, RXRα, PH, PT, or VLCAD in hPPARα mice compared with their respective controls. Expression of CYP4A10 mRNA was also not altered by PFOA treatment. MCAD mRNA and protein expression were significantly increased ($p<0.5$) in hPPARα mice treated with 0.3 mg/kg PFOA compared to hPPARα control mice. Expression of PPARα mRNA and protein levels were

significantly higher ($p<0.05$) in all hPPARα mice compared to mPPARα mice given the same concentration of PFOA. Treatment of hPPARα mice with 0.1 or 0.3 mg/kg PFOA caused a decrease ($p<0.05$) in mRNA expression of PH, VLCAD, and CYP4A10 compared to mPPARα mice at the same dose. Only hPPARα mice treated with 0.3 mg/kg PFOA had decreased protein expression of PH and VLCAD compared to mPPARα mice given the same treatment.

Treatment with 0.3 mg/kg PFOA caused activation of PPARα in mouse, but not in humanized PPARα mice. The results suggest that the functional activation of human PPARα may be weaker than that of mice as expression of human PPARα in mice was greater than the expression of mouse PPARα. Higher concentrations of PFOA may be needed to cause activiation of human PPARα in hPPARα mice.

To further evaluate the developmental effects potentially mediated by PPARα, groups of female wild-type, PPARα-null, and PPARα-humanized mice were given 0 or 3 mg PFOA/kg on GDs 1-17 by oral gavage (Albrecht et al., 2013). The study design and developmental toxicity data are described in section 4.2.5. Females were either sacrificed on GD18 (n = 5-8/group) or allowed to give birth and then sacrificed, along with their litters (n = 8-14), on PND20. Livers from dams, fetuses, and pups were collected for measurement of mRNAs encoding the PPARα target genes cytochrome P4504a10 (*Cyp4a10*) and acyl-CoA oxidase 1 (*Acox1*), the constitutive androstane receptor (CAR) target gene (*Cyp2b10*), and the pregnane X receptor (PXR) target gene (*Cyp3a11*).

On GD18, maternal liver samples from treated groups showed increased expression of *Acox1* in wild-type mice and *Cyp4a10* in wild-type and humanized mice. Expression of *Cyp2b10* and *Cyp3a11* were increased following PFOA administration in all three genotypes. On PND20, maternal liver samples from treated groups showed increased expression of *Acox1* in wild-type mice; expression of *Cyp2b10* was unchanged in all groups; and expression of *Cyp3a11* was increased following PFOA administration in all three genotypes.

For fetuses on GD18, liver samples from treated groups showed increased expression of *Acox1* and *Cyp4a10* in wild-type and humanized mice. Expression of *Cyp2b10* was unchangeded following maternal PFOA administration in all three genotypes, while expression of *Cyp3a11* was increased in humanized fetal liver. On PND20, pup liver samples from treated dams showed increased expression of *Acox1* and *Cyp4a10* in wild-type mice; expression of *Cyp2b10* was increased in all genotypes; and expression of *Cyp3a11* was increased following maternal PFOA administration in wild-type and humanized pups. Thus, expression of PPARα target genes that modulate lipid metabolism was increased in both wild-type and humanized mice coincident with increased liver weight and microscopic lesions, however the neonatal mortality was observed only in wild-type offspring (Albrecht et al., 2013).

Walters et al. (2009) examined the impact of PFOA on mitochondrial biogenesis and gene transcription in adult male Sprague-Dawley rats orally dosed with 0 or 30 mg/kg PFOA for 28 days. At sacrifice, a portion of the midlobe region of the livers was collected. Liver DNA and RNA were isolated for RT-PCR of genes in the Pgc-1α –mediated pathway of mitochondrial biogenesis: peroxisome proliferator-activated receptor gamma coactivator 1α (Pgc-1α), estrogen-related receptor α (Errα), nuclear respiratory factors 1 and 2 (Nrf1, Nrf2), transcription factor A (Tfam), cytochrome c oxidase subunit II and IV (Cox II, Cox IV), NADH dehydrogenase 2

(Nd2), and NADH dehydrogenase iron-sulfur protein 8 (Ndufs8). In mitochondrial biogenesis, Pgc-1α and Errα increase expression of the transcription factors Nrf1 and Nrf2. The Nrf transcription factors promote expression of Tfam which is required for mitochondrial DNA replication and transcription. Within the mitochondrial membrane, oxidative phosphorylation proteins (Cox II and IV, Nds, and Ndufs8) catalyze the transfer of electrons and/or pump protons from the matrix to the intermembrane space. Western blotting was used to analyze protein expression of Pgc-1α, Tfam, Cox II, and Cox IV.

Mitochondrial DNA in rats treated with PFOA was significantly increased ($p<0.05$) compared to control rats. In PFOA-treated rats, the expression of Pgc-1α, Errα, Nrf1, Nrf2, and Tfam was significantly increased 1.3-2.2 fold ($p<0.05$), and expression of Cox II, Cox IV, Nd2, and Ndufs8 were significantly increased 2 to 9 fold ($p<0.05$) compared to controls. Protein expression of Pgc-1α was increased, and expression of Cox II and Cox IV were decreased in PFOA-treated rats. Protein expression of Tfam was not affected by treatment with PFOA. The results suggested that PFOA induced mitochondrial biogenesis at the transcriptioinal level by activation of the Pgc-1α pathway.

Elcombe et al. (2010) examined the expression of some cytochrome P450 isoforms in the livers of male Sprague-Dawley rats fed diets containing 300 ppm PFOA or 50 ppm Wyeth 14,643 for 1, 7, or 28 days. The isoforms included those involved in activation of PPARα (CYP4A1), CAR (CYP2B1/2), and PXR (CYP3A1). All three isoforms were induced by PFOA exposure. CYP2B1/2 and CYP4A1 were induced after 1 day of exposure to PFOA. CYP3A1 was induced in all PFOA-exposed rats after 7 days of exposure to PFOA. Treatment with Wyeth 14,643 caused the induction of CYP4A1 only.

PPAR Receptor Activation. Takacs and Abbott (2007) evaluated the potential for PFOA to activate PPARs, using a transient transfection cell assay. Cos-1 cells, derived from the kidney cells of the African green monkey, were transfected with mouse or human PPARα, β/δ, or γ reporter plasmids and exposed to 0.5-100 μM PFOA or 0.5-100 μM PFOA and MK-886 (PPARα antagonist) or GW9662 (PPARγ antagonist). An antagonist for PPARβ/δ was not available. The three types, PPARα, β/δ or γ, are encoded by different genes, expressed in many tissues and have specific roles during development as well as in the adult. The results are shown in Table 4-23. PFOA activated mouse and human PPARα in a dose-dependent manner with a significant increase in activity observed at 10, 20, 30, and 40 μM in the mouse and 30 and 40 μM in the human compared to the negative control. The presence of the PPARα antagonist MK-886 prevented the activity increase resulting from PFOA exposure alone in mouse and human PPARα constructs. Activity of mouse PPARβ/δ was significantly increased after exposure to 40-80 μM PFOA compared to the negative control. Activity of human PPARβ/δ was not increased by PFOA exposure. Activity of mouse and human PPARγ were not increased by exposure to PFOA. PFOA was found to activate mouse and human PPARα and mouse PPAR β/δ under the conditions in this study.

TABLE 4-23. Activation of Mouse and Human PPAR by PFOA								
PPARα			PPARβ/δ			PPARγ		
PFOA (µm)	Mouse	Human	PFOA (µm)	Mouse	Human	PFOA (µm)	Mouse	Human
0	-	-	0	-	-	0	-	-
0.5	-	-	10	-	-	1	-	-
1	-	-	15	-	-	5	-	-
3	-	-	20	-	-	10	-	-
5	-	-	30	-	-	20	-	-
10	+	-	40	+	-	30	-	-
15	-	-	50	+	-	40	-	-
20	+	-	60	+	-	50	-	-
30	+	+	70	+	-	75	-	-
40	+	+	80	+	-	100	-	-

From Takacs and Abbott, 2007
+ Significant increase in activity between treated and control
- No difference in activity between treated and control

Biomarkers for Peroxisome Proliferation. Pastoor et al. (1987) dosed male Crl:CD (SD) BR rats for 1, 3, or 7 days with 0 or 50 mg PFOA/kg. Hepatic DNA content, cytochrome P450 content, UDP-glucuronyltransferase, glutathione S-transferase, benzphetamine N-demethylase activity (marker for smooth endoplasmic reticulum proliferation), and ethoxyresorufin O-deethylase activity (marker for cytochrome P450 induction via the aryl hydrocarbon receptor) were measured from rats dosed 1 and 3 days. Liver microsomes were prepared from rats dosed for 3 days for carnitine aceyltransferase (CAT) and carnitine palmitoyltransferase (CPT) activity assays. CAT served as a marker for peroxisome proliferation and CPT was a marker for mitochondrial proliferation. Incorporation of [^{14}C]acetate into hepatic lipids was used to determine the effect of PFOA on hepatic lipid metabolism. Plasma total cholesterol and triacylglycerides was determined from rats dosed for 7 days.

Hepatic DNA content was not increased in treated rats when compared to content in control rats. Cytochrome P450 was significantly increased ($p<0.05$) and ethoxyresorufin O-deethylase activity was significantly decreased ($p<0.05$) after treatment for 1 and 3 days. Benzphetamine N-demethylase activity was significantly increased ($p<0.05$) after treatment with PFOA for 3 days. CAT activity increased 12-fold ($p<0.05$) and CPT increased 2-fold ($p<0.05$) after a 3-day treatment with 50 mg PFOA/kg. No differences were observed among the groups for the other enzymes. No differences were observed between rats treated for 7 days and control rats in plasma total cholesterol or triacylglycerol. Although a significant increase ($p<0.05$) was observed for [^{14}C]acetate incorporation into triacylglycerols, cholesteryl esters, and polar lipids, there was no difference in the distribution of the incorporated label between control and treated rats. The authors concluded that the lack of increased DNA content, proliferation of smooth endoplasmic reticulum, and peroxisome proliferation pointed to increased liver weight due to hepatocyte hypertrophy.

Cook et al. (1992) examined peroxisome β-oxidation activity in male CD rats gavage dosed with for 14 days with 0, 1, 10, 25, or 50 mg PFOA/kg. Peroxisome β-oxidation activity was significantly increased at ≥10 mg PFOA/kg compared to the control. At the highest dose, β-

oxidation activity was significantly different from the pair fed controls.

Sohlenius et al. (1992) fed male and female Wistar rats diets containing 0 or 0.02% PFOA for 7 days and examined hepatic peroxisome proliferation and related parameters. At necropsy, liver samples were collected for analysis of catalase, peroxisomal lauroyl-CoA oxidase activity, palmitoyl-CoA oxidation, DT-diaphorase, cytosolic epoxide, glutathione transferase activity, ω-hydroxylation of lauric acid, and protein concentration. In males, an increase was observed in liver weight, liver somatic index, mitochondrial protein content, and palmitoyl CoA-oxidation. No effects were observed in the females.

Sohlenius et al. (1992) fed male and female C57Bl/6 mice diets containing 0 or 0.02% PFOA for 7 days to examine possible sex differences in hepatic response to PFOA. Two additional groups of female mice were fed a diet containing 0 or 0.05% PFOA for 5 or 10 days. At necropsy, liver samples were collected for analysis of catalase, peroxisomal lauroyl-CoA oxidase activity, palmitoyl-CoA oxidation, DT-diaphorase, cytosolic epoxide hydrolase, glutathione transferase activity, ω-hydroxylation of lauric acid, and protein concentration.

Liver weight, liver somatic index, mitochondrial protein, palmitoyl-CoA oxidation, and lauroyl-CoA oxidase activity were significantly increased ($p<0.05$) in all treated mice. Microsomal protein was significantly increased ($p<0.05$) in male mice fed diets of 0.02% PFOA for 7 days. In male and female mice fed diets of 0.02% PFOA, catalase, DT-diaphorase, ω-hydroxylation of lauric acid, cytosolic epoxide hydrolase, and glutathione transferase activities were significantly increased ($p<0.05$) compared to controls. The authors concluded that no sex differences were apparent hepatic peroxisome proliferation and related parameters in mice fed diets containing PFOA.

Elcombe et al. (2010) examined peroxisome proliferation in the livers of male Sprague-Dawley rats exposed to 300 ppm PFOA in the diet for 1, 7, or 28 days (described in 7.2.2). Analysis of palmitoyl-CoA oxidation showed significant increases ($p<0.05$) in β-oxidation at all time points in PFOA diet-fed rats compared to β-oxidation in control rats.

Cellular Differentiation. Slotkin et al. (2008) characterized the neurotoxicity of PFOA using PC12 cells. The cells were derived from a neuroendocrine tumor of the rat adrenal medulla and serve as a model for neuronal development and differentiation. Exposure to nerve growth factors causes PC12 cells to differentiate into cells expressing either dopamine or acetylcholine phenotypes. The cells were incubated with 10, 50, 100, or 250 μM PFOA. Synthesis of DNA, cell viability, cell growth, and lipid peroxidation were measured to determine if PFOA targets specific events in neural cell differentiation. Differentiation shifts towards or away from the dopamine and acetylcholine phenotypes were measured by assessing the activities of tyrosine hydroxylase (TH, dopamine) and choline acetyltransferase (ChAT, acetylcholine). The undifferentiated cells were evaluated after a 24 hour exposure, and differentiating cells were evaluated after 4-6 days of exposure to PFOA. The results are shown in Table 4-24.

Significant inhibition ($p<0.0001$) of DNA synthesis occurred in the undifferentiated cells after exposure to 250 μM PFOA with no change in DNA content. Lipid peroxidation was significantly increased ($p<0.02$) after exposure to 10 μM PFOA, and cell viability was significantly decreased ($p<0.03$) after 24 hour exposure to 100 or 250 μM PFOA.

In differentiating PC12 cells, exposure to 250 μM PFOA caused decreased DNA content with no change in total protein/DNA content ratio or the membrane/total protein ratio. The lowest and highest PFOA concentrations caused a significant increase ($p<0.007$) in lipid peroxidation, but no effect was observed in cell viability. Tyrosine hydroxylase activity was decreased ($p<0.05$) after exposure to 10 and 250 μM PFOA, and the TH/ChAT ratio was decreased ($p<0.05$) at 10 μM PFOA. The results suggest that PFOA exposure caused the differentiating cells to shift (slightly) to favor the acetylcholine phenotype.

TABLE 4-24. Impact of PFOA exposure on PC12 cells

Undifferentiated PC12 Cells	**PFOA (μM)**				
	0	**10**	**50**	**100**	**250**
DNA Synthesis	-	-	-	-	↓
DNA Content	-	-	-	-	-
Lipid Peroxidation	-	↑	-	-	-
Cell Viability	-	-	-	↓	↓
Differentiating PC12 Cells					
Lipid Peroxidation	-	↑	-	-	↑
Cell Viability	-	-	-	-	-
DNA Content	-	-	-	-	↓
Total protein/ DNA content ratio	-	-	-	-	-
Membrane/Total protein	-	-	-	-	-
TH activity	-	↓	-	-	↓
ChAT activity	-	-	-	-	-
TH/ChAT activity	-	↓	-	-	-

From Slotkin et al., 2008
↑,↓ Significantly different from control ($p<0.05$)
- Not different from control

Ahuja et al. (2009) examined the effects of PFOA on the production and activation of human monocyte-derived dendritic cells. These cells are responsible for a primary immune system response by activating lymphocytes and secreting cytokines. Peripheral monocytes and immature dendritic cells were incubated with 200 μM PFOA from day 0 to day 6 or 8 to determine the impact on phenotype and cytokine secretion. Maturation stimulus (prostaglandin E2, tumor necrosis factor, interleukin 1β, and interleukin 6) was added during the last 48 hours of incubation to induce dendritic cell maturation. Mixed leukocyte reaction assays were conducted to determine if immature dendritic cells could stimulate T cells. Cytokine (HLA-DR, CD25, CD80, CD83, and CD86) expression was measured as a determination of maturity. HLA-DR is a cytokine which presents antigens to elicit T-cell response. CD25, 80, 83, and 86 are cell receptors that act as co-receptors in T-cell activation; and interleukin 12p40 and 10 stimulate T-cells. Mature cytokine-activated dendritic cells secrete interleukin 12p40 and interleukin 10 as anti-inflammatory cytokines.

In peripheral monocytes incubated with only PFOA from day 0-6 or day 0-8, expression of HLA-DR and CD86 was increased compared to expression in control cells indicating that immature dendritic cells were present. In the mixed leukocyte reaction assay, the ability to

stimulate T cells was not different between immature dendritic cells generated in the absence or presence of PFOA.

To determine if PFOA impacted the differentiation of immature dendritic cells to mature dendritic cells, immature dendritic cells were incubated with 200 μM of PFOA for 6 days and the maturation stimulus was added for the final 2 days of incubation. There were no differences in cytokine (CD25, CD80, CD83, and CD86) expression between cells incubated with PFOA and control cells. Expression of interleukin 12p40 and interleukin 10 was significantly inhibited by PFOA in mature cytokine-activated dendritic cells, even in the presence of maturation stimulus during the last 48 hours of incubation. The resulted suggested that exposure to PFOA during generation of dendritic cells affected the phenotype and cytokine production of human dendritic cells and could lead to immunomodulation in the development of the immune response.

Intracellular Communication. Upham et al. (1998, 2009) examined the effects of perfluorinated fatty acids on gap junctional intercellular communication in male Fischer 344 rats fed diets containing 0 or 0.02% PFOA (intake 37.9 mg/kg/day) for 1 week and in WB-F344 rat liver epithelial cells. The chain lengths of the perfluorinated fatty acids ranged from 2-10, 16, and 18 carbons. Liver weight in the rats fed diets containing 0.02% PFOA was significantly increased compared to control rat liver weight. No differences were observed in serum AST, ALT, and ALP. PFOA significantly inhibited gap junctional intercellular communication in the livers of rats after treatment for 1 week. In WB-F344 cells, gap junctional intercellular communication was inhibited by perfluorinated fatty acids with 7 to10 carbons within 15 minutes of incubation. The inhibition was reversible with full recovery occurring within 30 minutes of PFOA removal from media. Extracellular receptor kinase was activated by PFOA within 5 minutes of incubation in the cells. Preincubation of cells with the phosphatidylcholine-specific phospholipase C inhibitor D609 partially prevented gap junction intercellular communication inhibition by PFOA. The authors concluded that PFOA, having an 8 carbon chain, inhibited gap junction intercellular communication by activation of extracellular receptor kinase and phosphatidylcholine-specific phospholipase C, but noted that other mechanisms may be involved.

Production of Reactive Oxygen Species. Takagi et al. (1991) fed male Fischer 344 rats diets containing 0, 10 or 20 mg PFOA/kg for two weeks to determine the formation of 8-hydroxydeoxyguanosine (8-OH-dG, marker of oxidative DNA damage). Livers and kidneys were removed at necropsy and DNA was isolated from each organ and analyzed. The relative liver and kidney weights were significantly increased ($p<0.05$) in the treated rats compared to the control. A significant increase in 8-OH-dG liver levels was observed at ≥ 10 mg PFOA/kg. There were no significant differences in 8-OH-dG kidney levels between PFOA-treated and control rats. The authors concluded that PFOA could cause organ specific oxidative DNA damage.

Hu and Hu (2009) exposed human hepatoma cells, HepG2, to PFOA to evaluate cytotoxic effects. Cells were also exposed to a mixture of PFOA and PFOS to determine antagonistic or synergic effects. The cells were exposed to 0, 50,100, 150, or 200 μM PFOA or to 0, 50, 150, or 200 μM each of PFOA and PFOS. A group of cells were also exposed to 0, 50,100, 150, or 200 μM PFOS. The cells were cultured for 24, 48, or 72 hours. Cell viability, apoptosis, reactive oxygen species, mitochondrial membrane potential, antioxidant enzymes,

glutathione content, and differential expression of apoptosis gene regulators p53, Bax, Bcl-2, caspace-3, and caspace-9 genes were evaluated.

Exposure to PFOA or PFOS caused a dose-dependent decrease in viability of HepG2 cells. A non-significant dose-dependent increase in apoptosis was observed in the cells cultured with PFOA. However, the combination of both PFOA and PFOS showed a significant dose-dependent increase ($p<0.05$) in apoptosis. Intracellular reactive oxygen species were significantly increased ($p<0.05$) in cells cultured with 100, 150, or 200 μM PFOA or PFOS. HepG2 cells exposed to the mixture of 100 or 200 μM PFOA and PFOS exhibited a decline in fluorescence intensity in the mitochondrial membrane potential assay indicating that mitochondrial pathways were involved in the apoptosis observed. Exposure to 100 μM PFOA significantly decreased ($p<0.05$) glutathione concentration and glutathione peroxidase activity, and 150 μM PFOA significantly increased ($p<0.05$) the activities of superoxide dismutase, catalase, and glutathione reductase, and significantly decreased ($p<0.05$) glutathione peroxidase activity, and glutathione concentration in HepG2 cells. The trend was the same at 200 μMol PFOA with the exception of glutathione-*S*-transferase activity being significantly decreased ($p<0.05$).

Exposure to PFOA did not change p53, Bax, or caspace-3 expression in HepG2 cells. Expression of Bcl-2 was down-regulated and caspace-9 was up-regulated in a dose-dependent manner in HepG2 cells following exposure to 50-200 μ/Mol PFOA. The authors proposed that PFOA and PFOS induced cell apoptosis by overwhelming the homeostasis of antioxidative systems, increasing reactive oxygen species, impacting mitochondria, and changing gene expression of apoptosis gene regulators.

Eriksen et al. (2010) examined ability of PFOA to generate reactive oxygen species (ROS) and induce oxidative DNA damage in human HepG2 cells. Cell were incubated with 0, 0.4, 4, 40, 200, 400, 1000, or 2000 μM PFOA and 2',7'-dichlorofluorescein diacetate (DCFH-DA). Hydrogen peroxide, H_2O_2, was used as a positive control. A fluorescence spectrophotometer was used to measure ROS production every 15 minutes during the 3 hour incubation period in all cultures. The comet assay was used to measure DNA damage in cells exposed to 0, 100, and 400 μM PFOA for 24 hours. Cytoxicity was determined by measuring the level of lactate dehydrogenase activity in the cell medium. Exposure to PFOA caused a dose-independent increase (all doses $p<0.05$) in ROS production in HepG2 cells. Compared to ROS production in negative control cells, PFOA induced a 1.52-fold increase in production. There was no difference in oxidative DNA damage and lactase dehydrogenase activity between PFOA-treated cells and negative control cells. The authors concluded that oxidative stress and DNA damage were probably not relevant for potential adverse effects of PFOA.

4.3.5 Structure-Activity Relationship

Bjork and Wallace (2009) compared the PPARα-dependent transcriptional activation potential of linear perfluorocarboxylid and sulfonic acids in rodent and human hepatic liver cells. The PFAAs tested included perfluorinated carboxylic acids with carbon chain lengths of 2 to 8 and perfluorinated sulfonic acids with chain lengths of 4 to 8. Primary rat and human hepatocytes and HEPG2/C3A cells were incubated with 0 or 25 μM perfluorinated compounds for 24 hours. Expression of acyl-CoA oxidase (Acox), Cyp4a1 (rat), Cyp4a11 (human), acyl-CoA thioesterase (Cte-rat, Acot1-human), and DNA damage inducible (Ddit3; GADD153) transcripts were determined by quantitative RT-PCR. All the genes are inducible by peroxisome proliferators except Ddit3 which is induced in the presence of direct or indirect DNA damage.

Perfluorinated compounds induced mRNA expression of either Acox or Cte/Acot1 only in rat hepatocytes, and the degree of stimulation of gene expression increased with increasing carbon number. The Cyp4a11 gene was not expressed or stimulated by any of the PFAAs in HepG2/C3A cells. However, this gene expression was stimulated by perfluorinated exposure in both rat and human hepatocytes with the perfluorocarboxylates showing a chain-length dependent structure activity relationship. The study results suggested that the PPARα related gene expression by perfluorinated compounds was induced in primary rat hepatocytes, increased with carbon chain length and appeared to be greater in the carboxylic acids (i.e. PFOA) when compared to the sulfonates (i.e. PFOS). There was no induction in expression of Acox and Cte/Acot 1 in either primary or transformed human liver cells in culture. The authors suggested that the PPARα mediated peroxisome proliferation observed in rodent liver may not be relevant as an indicator to human risk.

Wolf et al. (2008b) tested PFAAs, including PFOA, to determine if mouse and human PPARα activity could be induced in transiently transfected COS-1 cell assays. COS-1 cells were transfected with either a mouse or human PPAR-α receptor-luciferase reporter plasmid and after 24-hours were exposed to either negative controls (water or 0.1% dimethyl sulfoxide [DMSO]), a positive control (WY14,643) or PFOS at 0.5-100 μM. Other concentrations of PFAAs were used but not provided in this report. At the end of 24-hours of exposure, the luciferase activity was measured. The positive and negative controls had the expected results. A lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) was determined for each PFAA. In the study, the mouse PPARα was more responsive than the human. Also, carboxylates induced higher mouse and human PPARα activity than the sulfonates. In this study, the NOEC for PFOA was 0.5 μM in the mouse and 5 μM in humans; the LOEC was 1 μM (0.43 μg/mL) in the mouse and 10 μM (4.3 μg/mL) in humans.

A similar study as conducted above (Wolf et al., 2008b) was repeated but included additional PFAAs (Wolf et al., 2012). Transfected cells were incubated with PFAAs at concentrations of 0.5 to 100 μM, vehicle (water or 0.1% DMSO as negative control) or with 10 μM WY14,643 (WY, positive control) on each plate. Assays were performed with 3 identical plates per compound per species with 9 concentrations/plate and 8 wells/concentration. Cell viability was assessed using the Cell Titer Blue cell viability kit and read in a fluorometer. The positive and negative controls had the expected results. All human and mouse PPARα was induced and was significant in all the PFAAs. Again, similar findings were identified with the carboxylates and the mouse PPARα being more reactive. The study also provided the C_{20max}

which was the concentration at which the PFAA produced 20% of the maximal response elicited by the most active PFAA. For PFOA, this was 6 μM in mouse PPARα and 7 μM in human PPARα. For comparison, PFOS was 94 μM and 262 μM, respectively.

4.4 Hazard Characterization

4.4.1 Synthesis and Evaluation of Major Noncancer Effects

Serum Lipids. Some epidemiological studies have noted an association between PFOA exposure and increased serum lipid levels, a risk factor for cardiovascular disease in humans. Because of the structural similarities between linear perfluorinated acids and the short and medium chain fatty acids, the potential for these chemicals to cause elevated serum lipids has been an area of considerable interest. Steenland et al. (2009) and Frisbee et al. (2010) examined the relationship between serum PFOA and total cholesterol, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol in participants of the C8 Health project. Both studies reported positive associations between PFOA and total cholesterol. Steenland et al. (2009) found significant increasing trends with increasing serum PFOA decile for LDL and triglycerides but not HDL. Frisbee et al. (2010) found an association between serum PFOA and total cholesterol and LDL in children and adolescents in the C8 study. Nelson et al. (2010) found no associations between PFOA and LDL or VLDL in the general population. A study of residents in the Little Hocking water district by Emmett et al. (2006) found no association between PFOA and cholesterol. Occupational studies (Olsen et al., 2001a,b, 2003; Sakr et al., 2007a,b; Costa et al., 2009) found positive significant associations between serum PFOA and total cholesterol. These studies also found positive associations between serum PFOA and VLDL and LDL (Sakr et al., 2007a), and triglycerides (Olsen et al., 2001 a,b, 2003; Olsen and Zobel, 2007).

Taken together, the above studies show a positive association between serum PFOA and cholesterol in both worker and general populations. Although not all studies showed an association, positive results were reported in both cross-sectional and longitudinal studies. Limited evidence also showed an increase in VLDL and LDL with higher serum PFOA concentrations. The C8 Science Panel (2012) concluded that there is a probable link between exposure to PFOA and diagnosed high cholesterol which is consistent with the data reviewed by EPA.

High levels of serum lipids are a risk factor for cardiovascular disease in humans including ischemic heart disease (IHD), a condition in which blood flow to the heart is decreased through the development of atherosclerotic plaque or clots in the cardiac arteries. Sakr et al. (2009) conducted a mortality study examining workers at the Washington Works plant and IHD. The amount of time spent in relative job categories was used to estimate cumulative exposure. There were over 4,000 employees in the mostly male cohort and 239 deaths from IHD. Mortality from IHD was not associated with PFOA exposure. These results were similar to Leonard et al. (2008) who found a non-significant elevation for IHD mortality when compared to the regional worker population. Leonard et al. (2008) used 3 reference populations to derive his mortality rates for comparison; the US population, the West Virginia state population, and an 8-state regional employee population from the same company. Lundin et al. (2009) found no association between serum PFOA and risk of dying from IHD in workers of the Cottage Grove, Minnesota, plant compared to the general population of Minnesota.

Information on serum lipids from animal studies have received less attention than in the human population because of the fact that decreases in triglycerides, cholesterol, and lipoprotein complexes are an expected consequence of PPARα activation in rodents. Activation of the PPARα receptor leads to lowered serum lipids because of its induction of peroxisome proliferation. Peroxisomes are subcellular organelles that increased beta oxidation of fatty acids. PPARα activation also stimulates metabolic changes that lower hepatic cholesterol. The effects of human PPARα activation are much less pronounced than those in rats and mice.

Cholesterol and/or triglycerides have been monitored in only a few of the animal studies. Nakamura et al. (2009) and Minata et al. (2010) examined the lipid endpoints relative to the mouse strain's PPARα status and PFOA exposure. Nakamura et al. (2009) found that mice with a normal PPARα receptor had significantly increased levels of cholesterol and triglycerides in liver but not plasma at a LOAEL of 0.3 mg/kg/day. However, there were no differences in serum or liver cholesterol or triglycerides between PFOA- treated mice with a humanized PPARα receptor or PPARα null mice (NOAEL = 0.3 mg/kg/day) and their respective controls. The study by Minata et al. (2010) used higher doses than Nakamura et al. (2009) and found that total cholesterol was significantly decreased (LOAEL= 10.8 mg/kg/day) and total triglycerides significantly increased (LOAEL= 5.4 mg/kg/day) in wild type mice. In the PPARα null mice, the total cholesterol was significantly decreased for the 5.4 and 10.8 mg/kg/day doses but significantly increased for a 21.6 mg/kg//day dose while total triglycerides were significantly increased at all doses.

Martin et al. (2007) identified a 45-72% decrease in serum cholesterol after treatment of male Sprague Dawley rats with 20 mg PFOA/kg/day for up to 5 days, and Loveless et al. (2008) reported decreased total cholesterol, HDL, and non-HDL in male CD rats after doses of 0.3 and 1 mg/kg/day for 28 days. Triglycerides were decreased in the rats at ≥0.3 mg/kg/day. In male CD-1 mice, total cholesterol, HDL, and triglycerides were decreased at 10 and 30 mg/kg/day (Loveless et al., 2008). In pregnant female ICR mice, triglyceride, total cholesterol, and free fatty acids were significantly decreased at 10 mg/kg (Yahia et al., 2010). Elcombe et al. (2010) found a significant decrease in cholesterol in male Sprague-Dawley rats following a 7 or 28 day exposure to 300 ppm PFOA in the diet. Accordingly, there is not a high degree of concordance between the lipidemic effects of PFOA as noted in human epidemiology studies and those seen in animals.

Clinical Chemistry. Epidemiological studies examining clinical chemistry parameters have reported an association between PFOA exposure and increased liver enzymes and decreased bilirubin. With the exception of Olsen et al. (2000, 2001a,b, 2003), the occupational studies reported positive associations between PFOA and increased GGT (Sakr et al., 2007a; Decatur workers only, Olsen and Zobel, 2007; Costa et al., 2009), AST (Sakr et al., 2007b), ALP (Decatur workers only, Olsen and Zobel, 2007), or ALT (Decatur workers only, Olsen and Zobel, 2007, Costa et al., 2009). A negative association between serum PFOA and bilirubin was also observed in some workers (Sakr et al., 2007b; Olsen and Zobel, 2007; Costa et al., 2009). Examining the general adult population in the US, Lin et al. (2010) and Gallo et al. (2012) found a slight, but significant association with ALT and GGT in some population groups and in individuals with a higher BMI.

Changes in renal enzymes and hematology were not associated with PFOA exposure in worker populations (Olsen et al., 2000, 2001a,b, 2003; Sakr et al., 2007; Costa et al., 2009). However, one study found the odds ratio for chronic kidney disease was positively correlated with serum PFOA levels in the general population (Shankar et al., 2011). It is noted that a low glomerular filtration rate would diminish the removal of PFOA from serum for excretion by the kidney.

In animal studies, serum levels of ALT and/or AST were significantly increased indicating apoptotic or necrotic damage to liver cells (Butenhoff et al., 2012; Son et al., 2008; Minata et al., 2010). Increased levels of ALT were observed at a LOAEL of 2.65 mg/kg/day in ICR mice by Son et al. (2008). Yahia et al. (2010) reported significantly increased ALT, GGT, AST, and ALP in PFOA-exposed (10 mg/kg) pregnant ICR mice. Total protein, albumin, and globulin were significantly decreased in the same mice.

Both the human and animal studies suggest effects on the liver as indicated by increases in liver enzymes. The data from animal studies for increases in ALTand AST are more definitive than the findings in human epidemiology studies. Concurrent with the evidence in animals of damage to liver cells, levels of certain transport proteins have been shown to be altered. In mice, increased expression of MRP3 and MRP4 (Maher et al., 2008) and decreased expression of OATps (Cheng and Klaassen, 2008) favors excretion of PFOA into the bile but may interfere with normal transport of biliary components.

Hepatic Hypertrophy. In most PFOA animal studies (monkeys, rats, mice), short term and chronic exposure caused an increase in liver weight as at least one of the critical effects (Christopher and Marias, 1977; Merrick and Marias, 1977; Goldenthal, 1978a; Pastoor et al., 1987; Butenhoff et al., 2012; Palazzolo, 1993; Butenhoff et al., 2002, 2004a; York, 2002; Perkins et al., 2004; Son et al., 2008; Wolf et al., 2008a; DeWitt et al., 2009; Elcombe et al., 2010; Minata et al., 2010). Increased liver weights were observed in mice that are both active and null for PPARα activation (Wolf et al., 2008a; Minata et al., 2010; Albrecht et al., 2013). The histological characteristics of the liver differed in the mice with and without the PPARα receptor, but the liver weight increase was the same. Liver effects were seen in mice with an active PPARα receptor at doses as low as 0.3 mg/kg/day (Nakamura et al., 2009) and 1 mg/kg/day in the null mice (Wolf et al., 2008a).

Histological examination of liver tissues from PFOA exposed wild-type mice and PPARα null mice were distinctly different from their respective controls (Wolf et al., 2008a; Minata et al., 2010). In the case of the wild-type PFOA-exposed mice, there was less endoplasmic reticulum than in controls and more lipid-like vacuoles scattered throughout the cytoplasm. The PFOA-exposed PPARα null mice had limited endoplasmic reticulum and Golgi bodies compared to their controls. The PPARα null control mice had the scattered lipid-like vacuoles seen in the wild-type PFOA exposed mice, however, their lipid-like vacuoles were considerably larger than those seen in the wild-type animals and occupied a considerable volume within the cytoplasm. The vacuoles in the PPARα null PFOA-exposed mice were hypothesized to be filled with PFOA as a consequence of its uptake into the cell without dispersion or assimilation.

Similarly, Albrecht et al. (2013) observed centrilobular hepatocellular hypertrophy in mouse dams given 3 mg/kg on GDs 1-17, but the morphological features differed slightly

between wild-type, PPARα-humanized, and PPARα-null mice. In wild-type mice, hypertrophy was characterized primarily by centrilobular hepatocytes with increased amounts of densely eosinophilic and coarsely granular cytoplasm consistent with increased peroxisomes. In null mice, hypertrophy was generally less prominent than seen in wild-type mice, and affected hepatocytes had pale eosinophilic, finely granular to amorphous cytoplasm. The morphological features of centrilobular hepatocytes in humanized mice were intermediate between those observed in wild-type and null mice. The lesion was graded as mild in wild-type mice, minimal in null mice, and minimal or mild in humanized mice. An additional finding in PFOA-treated null and humanized mice, but not in wild-type mice, was the presence of few clear, discrete vacuoles within the cytoplasm of centrilobular hepatocytes.

Minata et al. (2010) reported degenerative histological changes in the bile ducts of PPARα null mice at doses ≥10.6 mg/kg/day. PPARα null mice had an increased hepatocyte proliferating cell nuclear antigen labeling index at a dose of 10 mg/kg/day (Wolf et al., 2008a). When considering the studies in animals with and without the active PPARα receptor, it is clear that PFOA has effects on the liver that appear to be independent of PPARα activation.

Other Organs. In general, effects on organs other than the liver tend to occur at doses higher than those that affect the liver. Some studies have observed kidney and lung effects including tubular epithelium swelling and pulmonary congestion in male Sprague Dawley rats (LOAEL = 20 mg/kg/day; Cui et al., 2009). In a chronic rat study, increased relative kidney weight was observed at 5.64 mg/kg/day (Goldenthanl, 1978a). In a chronic monkey study (Goldenthal, 1978b), the critical effects were increased pituitary weight in males (LOAEL= 3 mg/kg/day) and increased brain and decreased heart weight in females (LOAEL=10 mg/kg/day). The chronic study by Butenhoff et al. (2012) noted hematological effects (decreased red blood cells counts and hemoglobin concentrations) in treated male and female Sprague Dawley rats at a LOAEL of 24 mg/kg/day. Increased thickness and prominence of the zona glomerulosa and vacuolation were observed in the cells of the adrenal cortex of male rats fed 10 mg/kg/day for approximately 56 days (York, 2002; Butenhoff et al., 2004a).

Hyperglycemia. Several human epidemiology studies have examined the association between PFOA and hyperglycemia both in the general population or a subset of individuals with type II diabetes. Leonard et al. (2008) examined mortality associated with diabetes in workers of the Washington Works plant and found an association between the Washington Works employees and the regional plant worker population (SMR=1.97, 95% CI 1.23, 2.98). Lundin et al. (2009) found diabetes mortality to be associated with moderate PFOA exposure (Hazard Ratio 3.7, 95% CI: 1.4-10.1) among Cottage Grove workers whose cause of death had been identified as diabetes. No deaths from diabetes had occurred among workers with high exposure, based on job classification. MacNeil et al. (2009) examined type 2 diabetes among adults from the C8 health project and found no association found between serum PFOA concentration and elevated fasting serum glucose levels or type II diabetes among members of the community in the vicinity of the Washington Works plant. There are limitations in mortality analyses for disorders such as diabetes where adherence to diet and other treatment modalities has a major impact on longevity. Occupational studies examining glucose levels or related endpoints found no relationship to PFOA concentration (Olsen et al., 2000, 2001a,b, 2003; Sakr et al., 2007a; Costa et al., 2009). Lin et al. (2009) and Nelson et al. (2010) found no association between PFOA and metabolic

syndrome, glucose concentration, insulin levels, and/or insulin resistance in the general US population.

Hines et al. (2009) found no differences in glucose tolerance tests at 15-16 weeks and at 17 months of age in CD-1 mice, but did observe significantly increased serum leptin and insulin levels at 21 and 31 weeks of age suggesting that the insulin resistance mechanistic pathway could be affected by PFOA.

Hypertension. Serum uric acid levels were measured in two occupational populations and in the C8 Health Project population as a biomarker for hypertension. Hypertension is a complex condition related to a number of physiological conditions. Cholesterol buildup in the arteries is one factor that can lead to elevated blood pressure as are kidney function and dietary sodium. Impaired circulation can impact glomerular and tubular removal of PFOA and other substrates excreted utilizing the common transporters in the renal tubules. Both occupational studies (Sakr et al., 2007a, Costa et al., 2009) found an increased association with serum PFOA and uric acid levels. The odds ratio for high serum uric acid levels increased from the lowest to the highest quintile of PFOA serum concentrations in the C8 Health Project population (Steenland et al., 2010). The study showed that higher PFOA concentrations were associated with higher incidence of high serum uric acid, but uric acid levels remained within the normal range. A positive association between chronic kidney disease and serum PFOA levels found in the general population (Shankar et al., 2011) was independent of body mass index, hypertension and serum cholesterol levels. No epidemiology data were identified that have examined the relationship between serum PFOA levels and resting blood pressure, and none of the animal studies have examined this end point.

Neurotoxicity. There are limited data on the neurotoxicity of PFOA. Fei et al. (2008b) found no association between maternal PFOA concentration and fine motor skills, gross motor skills, and cognitive abilities at 6 and 18 months of age. An epidemiology study of children derived from the NHANES population found a weak statistical association between serum PFOA with parental reports of ADHD (OR=1.12, 95% CI 1.01-1.23). However, one animal study (Johansson et al., 2009) suggests a potential effect on habituation and activity patterns in NMRI mice treated on PND10 with a single dose of PFOA and evaluated at and 2 and 4 months of age (LOAEL=0.58 mg/kg). The *in vivo* observations were supported by changes in the expression of a variety of neurologically active brain proteins in the treated pups (Johansson et al., 2009). The offspring of C57BL/6/Bkl dams fed 0.3 mg PFOA/kg/day throughout gestation had detectable levels of PFOA in their brains at birth (Onishchenko et al., 2011). Behavioral assessments of the offspring starting at 5 weeks of age revealed sex-related exploratory behavior differences. In the social group setting, the PFOA-exposed males were more active and PFOA-exposed females were less active than their respective controls. The PFOA-exposed males also had increased activity counts than control males in circadian activity experiments. The results of an *in vitro* study of hippocampal synaptic transmission and neurite growth in the presence of long chain perfluorinated compounds showed that 50 or 100 μM PFOA increased spontaneous synaptic current and had an equivocal impact on neurite growth (Liao et al., 2009a, b). These data suggest a need for additional studies on the effects of PFAs, including PFOA, on the brain.

Thyroid Effects. A few epidemiological studies have examined the association between presence of PFOA and altered thyroid homeostasis in humans. Thyroid-related measures among

fluorochemical production workers were assessed by Olsen et al. (2001a, 2003), Sakr et al. (2007a), Olsen and Zobel (2007), and Costa et al. (2009) relative to serum PFOA levels. Most of these studies showed no associations between PFOA and any thyroid hormones. Olsen and Zobel (2007) reported a negative association for free T4 and a positive association for T3; however, the hormone levels were well within the normal reference range. The authors concluded that there was no consistent relationship between altered thyroid hormone status and serum PFOA.

Emmett et al. (2006) found no association between serum PFOA and thyroid hormones and/or disease in residents served by the Little Hocking water district. Results from a study by Bloom et al. (2010) did not identify an association between thyroid function and non-occupational PFOA exposure (mean serum concentration of 1.3 ng/ml; 95% CI: 1.15-1.53). TSH and free T4 levels were used as markers for thyroid function. Only one subject (3.2%) exceeded the laboratory reference range for normal (0.40-5.00 μIU/mL) and no subjects exceeded the laboratory reference range for normal free T4 (0.80-1.80 ng/dL). Pirali et al. (2009) reported no relationship between intrathyroidal concentrations of PFOA and underlying thyroid disease.

Chan et al. (2011) found no association between serum PFOA concentrations and hypothyroxinemia in pregnant women. More recently, Melzer et al. (2010) examined the records of the NHANES data for thyroid disease and serum PFOA levels in the U.S. general population and found that women with PFOA $\geq$ 5.7 ng/ml were more likely to report current thyroid disease compared to women with PFOA $\leq$ 4.0 ng/ml. A near significant and similar trend was observed in men. However, lack of segregating the thyroid disorders into those associated with hyperactivity versus hypoactivity was a limitation in their analysis. The physiological significance of these cross-sectional analyses remains to be determined.

Effects of PFOA on thyroid hormones in animals are generally not as well characterized as those of PFOS. Butenhoff et al. (2002) evaluated the toxicity of PFOA in male Cynomolgus monkeys during 6 months of oral administration and reported that levels of total T3 and free T3 in circulation were reduced significantly in the 30/20 mg/kg/day treatment group, an effect seen as early as 5 weeks after initiation of treatment. Recovery of T3 deficits was noted upon cessation of chemical treatment after serum level of PFOA returned to baseline 90 days later. Serum total T4, free T4 and TSH were not altered throughout the study. The preferential effects of PFOA on serum T3 and a lack of TSH compensatory response are similar to those observed with PFOS.

Martin et al. (2007) showed that serum total T4 and free T4 were markedly and abruptly (1 day after oral gavage treatment) depressed (~ 80%) by PFOA (20 mg/kg) in adult male rats; while serum T3 was also reduced (25%) though to a lesser extent. These findings were confirmed when both male and female rats were given PFOA (10 mg/kg) daily for 3 weeks and serum thyroid hormones were monitored (Lau, personal communication). Serum total T4 and free T4 were profoundly depressed (>85%) and T3 less so (~ 25%) in male rats, but serum TSH levels were not altered significantly. These hormonal changes were noted when serum PFOA level reached about 67 μg/ml. The dose-response relationship of serum total T4 and PFOA has yet to be evaluated and the lowest effective dose of the chemical remains unknown.

None of the thyroid hormones were affected by PFOA in mature female rats, primarily because these animals were able to clear the chemical effectively (with half-life estimate of 2-4 hours compared to that of 6-7 days for male rats). This suggests that the thyroid disrupting effects of PFOA are directly related to endogenous accumulation of the chemical and may be relevant to humans because of the long PFOA human half-life.

Displacement of T4 from binding to the hormone transport protein transthyretin (TTR) has been proposed as a possible mechanism to account for the hypothyroxinemia in rats. However, although PFOA binds to human TTR, its binding affinity is only one-fifteenth of that of the natural ligand T4 (Weiss et al., 2009). Based on a toxicogenomic analysis of rat liver after an acute exposure to PFOA, Martin et al. (2007) suggested a possible role of peroxisome proliferators in the thyroid hormone imbalance, although this hypothesis has yet to be explored in detail.

Reproductive and Developmental Effects. There is a wealth of data on the impact of PFOA exposure on reproductive and/or developmental effects in both humans and animals. A series of epidemiological studies have been published examining a variety of endpoint: fertility (fecundity), growth, and developmental biomarkers or milestones. The animal data have focused more extensively on developmental endpoints using traditional and non-traditional approaches.

Joensen et al. (2009) found that there was no association between serum levels of PFOA and testicular hormones among 105 Danish men who provided blood and semen for the military draft in 2003. A nonsignificant negative association was observed between serum PFOA concentration and semen volume, sperm concentration, sperm count, sperm motility, or sperm morphology. Fei et al. (2009) aimed to measure fecundity by using longer time to pregnancy as the outcome. More than 1200 women were included in a phone survey. Infertility was defined as having > 12months time to pregnancy (TTP); pregnancies were separated into: planned, partially planned, and not planned. The fecundity odds ratios for the three highest PFOA concentration quartiles were 0.72, 0.73, and 0.60. The likelihood ratio test for trend was significant ($p < .0001$). The results provide suggestive evidence that PFOA concentration may reduce fecundity, however, the results should be viewed with caution, due to potential selection bias and/or confounding biological and behavioral factors.

Several studies have looked at the association between PFOA concentration and fetal growth (birth weight, birth length, head/chest circumference, etc); whereas some researchers found no significant associations, the results for others were positive. Monroy et al. (2008) found no association between umbilical cord or serum PFOA concentration and birth weight in 105 mothers in the Family Study conducted in Canada. These findings were supported by Nolan et al. (2009) who examined mothers and babies who were serviced by the Little Hocking Water Association (LHWA). Many researchers used log-transformed values and adjusted for possible confounders, but still found no significant association between PFOA and birth weight, birth length, head, or chest circumference (Hamm et al., 2009, Washino et al., 2009). Conflicting results were seen in the early studies from Fei et al (2007, 2008a) where there was a statistically significant inverse association between birth weight and plasma PFOA concentration among "normal-weight" women. A statistically significant decrease in birth length was also observed when the second and fourth quartile measures of PFOA exposure were compared to those for the first quartile.

In studies that examined pregnancy outcome among women believed to be exposed to PFOA, there were no association seen between congenital anomalies and complications of labor and delivery such as meconium, prolonged labor, breach, cord prolapse, or fetal distress (Nolan et al., 2010). There was also no association between PFOA and birth outcomes including miscarriages and livebirths (Stein et al., 2009). Knox et al. (2011) suggested that PFOA may be associated with endocrine disruption in women after finding that the odds of having experience menopause were significantly higher in the highest PFOA quintile group relative to the lowest PFOA group.

Fei et al. (2008b) found no association between maternal PFOA concentration and fine motor skills, gross motor skills, cognitive skills, at 6 and 18 months of age. The age at which children reached developmental milestones did not show any relationship to maternal plasma PFOA concentration. Maternal plasma PFOA concentration was also not associated with child risk of hospitalization for infectious diseases (Fei et al., 2010), but was associated with decreased antibody titers in children following vaccination (Grandjean et al., 2012). In the general adolescent population in the US, a positive association was observed between serum PFOA and parent-reported ADHD (Hoffman et al., 2010). Lopez-Espinosa et al. (2011) reported reduced odds of having reached puberty in girls included in the C8 Health project. Christensen et al. (2011) found no association between puberty and PFOA exposure in children of the Avon Longitudinal Study of Parents and Children in the United Kingdom. Halldorsson et al. (2012) found that low dose developmental exposures to PFOA resulted in obesogenic effects in female offspring at 20 years.

Among the animal studies there was no effect of PFOA on reproductive or fertility parameters in rats, but some effects were observed in mice. Both species, however, showed some indications of the potential developmental toxicity of PFOA. Doses which elicited a response were much higher in rats compared with those in mice. The species differences in response and dose rate are likely related to half-life differences of hours for the female rat and days-to-weeks for the female mouse.

Body weight gain was decreased in parental male animals in a two-generation study after dietary exposure to 30 mg/kg/day PFOA (York, 2002; Butenhoff et al., 2004a, York et al., 2010). Maternal body weight gain was reduced at ≥30 mg/kg/day and death occurred at ≥100 mg/kg/day in rats given PFOA via gavage during gestation (Staples et al., 1984; Hinderliter et al., 2005; Mylchreest, 2003). In mouse gavage studies, increased time to parturition was observed at ≥10 mg/kg/day, and decreased maternal weight gain was observed at ≥20 mg/kg/day (Lau et al., 2006). Mouse maternal mammary glands displayed stunted alveolar development (GD18) and delayed epithelial differentiation (PND10) (White et al., 2009).

Decreased fetal and offspring body weight was observed in rats following an inhalation exposure to 25 mg/m^3 for 6 hours/day on GD6-15 (Staples et al., 1984). A two-generation diet study in rats resulted in significantly decreased body weight gain prior to weaning and delayed sexual maturity in the first generation males and females at 30 mg/kg/day (York, 2002; Butenhoff et al., 2004a). In mouse gavage studies, decreased body weight and decreased neonatal survival were observed at ≥ 1 mg/kg/day and increased full litter resorptions and increased stillbirths were observed at ≥ 5 mg/kg/day for exposures lasting from GD1 to 17 (Lau et al., 2006; Abbott et al., 2007; White et al., 2007; Wolf et al., 2007).

Delayed mammary gland development of female pups was also observed following maternal doses ≥ 0.01 mg PFOA/kg in one study (Macon et al., 2011) and ≥3 mg/kg in another study (White et al., 2009). However, Albrecht et al. (2013) found no differences in the average length of mammary gland ducts and the average number of terminal end buds per mammary gland per litter in female pups following a maternal dose of 3 mg/kg.

Two developmental studies with compared wild-type mice with PPARα null mice but results are inconclsive. One study revealed that the litter resorptions were independent of PPARα expression (≥5 mg/kg), while decreased neonatal survival (0.6 mg/kg) and delayed eye opening (1 mg/kg) were dependent upon PPARα expression (Abbott et al., 2007). These results are only partially supported by Albrecht et al. (2013) who used a single dose of 3 mg/kg. They found decreased pup survival only in wild-type mice but no differences in litter resorptions or eye opening between wild-type and null mice. Albrecht et al (2013) did not find effects on pup survival in PPARα-humanized mice suggesting that the mouse PPARα is involved in the etiology of PFOA-induced neonatal mortality.

Cross-fostering studies showed that gestational PFOA exposure was required to produce decreased body weight, delayed eye opening, delayed body hair growth, and decreased survival in the offspring. However, persistent (PND1-PND63) mammary gland development delays were observed in offspring that had been gestationally or lactationally exposed as well as in offspring who had been gestationally and lactationally exposed (White et al., 2009). Restricted exposure studies showed that gestational exposure to PFOA during a specific time period led to differeing offspring effects (Wolf et al., 2007). Delayed eye opening and body hair growth were observed at 5 mg/kg/day in offspring exposed GD7-17 or 10-17, but decreased postnatal survival was observed at the same dose in offspring exposed GD15-17. Delayed mammary gland development was observed irrespective of exposure duration, and delayed lactational differentiation was seen in the dams (White et al., 2009). In a three generation study, PFOA induced delays in mammary gland development and/or lactational differentiation. A chronic dose of 5 ppm PFOA in the drinking water also altered mammary morphology in mice (White et al., 2011). The mode of action for PFOA-induced delayed mammary gland development is unknown.

Delayed vaginal opening was observed in BALB/c mice who received 1 mg/kg/day by gavage for four weeks starting on PND21 (Yang et al., 2009). At higher doses (5 and 10 mg/kg/day), vaginal opening did not occur and mammary gland development was delayed. In C57BL/6 mice, vaginal opening was delayed at 5 mg/kg/day and did not occur at 10 mg/kg/day. Mammary gland development was accelerated at 5 mg/kg/day, but delayed at 10 mg/kg/day indicating strain differences in pubertal mammary gland development following a dose of 5 mg/kg/day. Zhao et al. (2010) showed that 5 mg PFOA/kg/day stimulates mammary gland development in C57BL/6 mice by promoting steroid hormone production in the ovaries and increasing mammary gland growth factor levels.

Immunotoxicity. An occupational study of the immunotoxicity of PFOA found no association between the immunoglobins IgA, IgG, and IgM and PFOA in male workers (Costa et al., 2009). No associations were found between maternal PFOA concentrations and hospitalization for infectious diseases in early childhood (Fei et al., 2010b) or infant allergies (Okada et al., 2012).

In contrast, higher PFOA concentrations in children were associated with lower antibody levels to diphtheria and tetnus (Grandjean et al., 2012) and a greater risk of having asthma (Dong et al., 2013). In a pilot study using volunteer donated blood, Brieger et al. (2011) found that PFOA altered peripheral blood mononuclear cell viability and reported a significant association was observed between PFOA concentration and the release of LPS-induced TNF-α and IL-6 by peripheral monocytes.

Several animal studies demonstrate effects on the spleen and thymus as well as their cellular products (B lymphocytes and T-helper cells) in several strains of mice. Studies by Yang et al. (2000, 2001, 2002b) and DeWitt et al. (2008) were conducted using relatively high PFOA doses (~30-40 mg/kg/day). In each study the PFOA treated animals exhibited significant decreases in spleen and thymus weights as well as splenocyte and thymocyte populations at various stages of differentiation. Recovery usually occurred within several days of cessation of PFOA dosing.

Activation of the PPARα receptor appears to be related to at least a part of the thymus response to PFOA. When Yang (2002a) dosed PPARα null mice with the same dose that caused an effect in the wild-type mice, no significant changes in spleen weight or cellularity were observed, but there was a small and significant decrease in thymus weight and cellularity compared to controls.

In one component of the Yang et al. (2002b) study, the functional impact of changes in spleen and thymus were evaluated through the response of treated mice to horse red blood cells (HRBC). The control mice responded to the HRBC exposure with an increased plaque forming response; however, the PFOA treated mice did not have an increased plaque forming response when tested (Yang et al., 2002b). In addition, when serum from PFOA treated mice was evaluated post treatment, there was no increase in lymphocyte proliferation in response to the addition of Con-A and LPS to the test media. The control mice responded with the expected lymphocyte proliferation after the addition of Con-A and LPS antigens.

DeWitt et al. (2008) used different functionality assays in their study in C57Bl/6 mice. The IgM response to sheep red blood cells (SRBC) was suppressed by 20% when mice were immunized immediately after exposure to the initial dose of 30 mg PFOA/kg/day ceased. However, there was no significant increase in the response to BSA four days post PFOA exposure, or in the IgG response to SRBC 15 days post PFOA exposure. These results are indicative of recovery once PFOA exposures have ceased.

DeWitt et al. (2008) followed their initial study of PFOA with one designed to examine the dose response for a 15 day drinking water exposure in a slightly different mouse strain, C57Bl/6N. The study design examined the spleen and thymus weights, splenocyte and thymocyte numbers, and IgM response of the immune system to the immunological challenges as described above. The LOAEL was 3.75 mg/kg/day based on a significant decrease in IgM response at the NOAEL of 1.88 mg/kg/day.

Loveless et al. (2008) looked at the IgM response to SRBC in male CD rats and CD-1 mice following a 29-day exposure to 0-30 mg PFOA/kg/day. The thymus and spleen cell counts and organ weights and the IgM titers were not altered by PFOA treatment in rats. In mice,

however, thymus and spleen weights, thymus and spleen cell counts, and IgM titers were decreased at ≥10 mg PFOA/kg/day. Corticosterone was also increased in mice at the same doses.

The data collected from the immunotoxicity studies support a mode of action through which PFOA interferes with splenocyte and thymocyte precursor cells in the bone marrow as well as maturation of the cells once they have been transported to their respective organs. Examination of cell populations at different stages of development reveals lower numbers of the $CD4^{-}CD8^{-}$ cells formed in bone marrow as well as decreased populations of splenocyte and thymocyte cells at different stages of expressing the surface proteins that mark them as functional beta lymphocytes (thymus) or T-helper cells (spleen) (Son et al., 2009). Although the studies that measured the splenocyte and thymocyte populations were carried out at doses higher than the 3.75 mg/kg/day LOAEL observed by DeWitt et al. (2008), the fact that the IgM response to an antigenic material was decreased at that dose indicates an inability to produce antibodies at adequate levels when exposed to a challenge.

Loveless et al. (2008) hypothesized that the observed effects on serum lymphocytes could be the result of adenocorticotropic steroids in a response to stress. A study by DeWitt et al. (2009) in which the immunological response of adrenalectomized mice treated with PFOA were compared to sham operated controls did not support the Loveless et al. (2008) hypothesis.

Data from PPARα null mice suggest that rodents may be more susceptible to the immunosuppressive impacts of PFOA than humans. However, the fact that there were still effects on the thymus weight and cellularity even in the null mouse strain indicate the potential for an inadequate humoral response in exposed populations.

4.4.2 Synthesis and Evaluation of Carcinogenic Effects

A number of human epidemiology studies were conducted on worker populations to examine an association between PFOA exposure and cancer. Lundin et al. (2009) saw no association between cancer mortality and exposure to PFOA when comparing workers of the Cottage Grove facility to the Minnesota general population. The mortality data for liver cancer, pancreatic cancer, prostate cancer, and testicular cancer all showed no association to exposure. The Dupont (2003) report on the West Virginia Washington Works Plant found statistically significant elevations in cancer of the bladder (SIR 1.9; 95% CI: 1.15-3.07) and kidney and urinary organs (SIR 2.3; 95%CI: 1.36-3.65). This same report found non-statistically significant elevations in leukemia incidence.

Two analyses of leukemia incidence were conducted prior to the Dupont (2003) report. Walrath and Burke (1989) reported statistically significant elevated Odds Ratio of 2.1 for leukemia incidence in male employees. Karns and Fayerweather (1991) conducted a follow up case-control study at the same Washington Works facility where they matched employees (four controls for each case). The matched Odds Ratios were significantly elevated for employees who had previously worked as custodians and engineers, 8.0 (90% CI, 1.1-60.0) and 7.9 (90% CI, 1.0-76.0), respectively. The level of significance chosen by the authors for each p=.10 were not comparable in stringency to those used in many of the other papers; also the CIs were quite wide even at the 90%level.

Two human epidemiology studies focused on general populations, one of which was exposed to PFOA through contaminatd water supplies. First, Eriksen et al. (2009) looked at risk of cancer in the Danish general population and plasma PFOA concentration. The Incidence Rate Ratios (IRRs) crude and adjusted showed no association between plasma PFOA concentration and prostate, bladder, pancreatic, and liver cancer. Vieira et al. (2013) showed a positive association between PFOA levels and kidney and testicular cancers in residents living in an area with contaminated water. Positive associations were found between kidney cancer and the very high and high serum exposure categories (OR: 2.0, 95% CI: 1.0, 3.9; n=9 and OR: 2.0, 95% CI: 1.3, 3.2; n=22, respectively) and between for testicular cancer with the very high exposure category (OR: 2.8, 95% CI: 0.8, 9.2; n=6). No associations for either cancer were found in the lower exposure categories. Ovarian cancer, non-Hodgkin's lymphoma, and prostrate cancer were also positively associated with the very high exposure category, but showed weaker or negative associations for the other exposure categories.

Two animal carcinogenicity studies have shown that PFOA exposure can lead to liver adenomas or carcinomas (Butenhoff et al., 2012; Biegel et al., 2001), Leydig cell adenomas (Butenhoff et al., 2012; Biegel et al., 2001), and pancreatic acinar cell tumors (PACT) (Biegel et al., 2001) in male Sprague-Dawley rats. Liver adenomas were observed in the Biegel et al. (2001) study at an incidence of 10/76 (13%) at 20 mg/kg/day. The incidence in the control group was 2/80 (3%). Although no liver adenomas were observed in Butenhoff et al. (2012), carcinomas were identified in both the male controls and exposed male and female rats in the high dose group and exposed males in the low-dose group. The differences from control were not significant in either case but the incidence among the high dose males (10/50) was similar to that for the adenomas in the Biegel et al. (2001) study. Liver lesions were identified in the males and females at the one- and two-year sacrifices (Butenhoff et al., 2012). Increased incidence of diffuse hepatomegalocytosis and hepatocellular necrosis were occurred at 20 mg/kg/day. At the 2-year sacrifice, hepatic cystoid degeneration was observed at 8, 14, and 56% in males of the control, 2, and 20 mg/kg/day dose groups, respectively. Hyperplastic nodules in male livers were increased in the high dose group (6% vs. 0% in control rats).

Testicular Leydig cell tumors (LCT) were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. The tumor incidence was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the control, 2.0, and 20 mg/kg/day dose groups, respectively (Butenhoff et al., 2012). The Biegel et al. (2001) study included one dose group (20 mg/kg/day), and the tumor incidence was 8/76 (11%) compared to 0/80 (0%) in the control group. LCT incidence at 20 mg/kg/day was comparable between the two studies. PACTs were only observed in the Biegel et al. (2001) study at an incidence of 8/76 (11%; 7 adenoma, 1 carcinoma) at 20 mg/kg/day while none were observed in the control animals. Although no PACTs were observed by Butenhoff et al. (2012), pancreatic acinar hyperplasia was observed at 2 and 20 mg/kg/day; 2/34 (6%) and 1/43 (2%), respectively. Re-examination of the pancreatic lesions in Butenhoff et al. (2012) and Biegel et al. (2001) resulted in the conclusion that 20 mg/kg/day increased the incidence of proliferative acinar cell lesions in both studies, and the lesions in the Biegel et al. (2001) study had progressed to adenomas.

The initial findings from the Butenhoff et al. (2012) study were equivocal for mammary fibroadenomas in female rats. However, a re-examination of the tissues by a PWG found no statistically significant differences in the incidence of fibroadenomas or other neoplasms of the

mammary gland between control and treated animals (Hardisty et al., 2010). The PWG used the diagnostic criteria and nomenclature of the Society of Toxicological Pathologists for the re-examination. Under those criteria, there was an increase in the number of tumors documented in the control group, especially fibroadenomas originally classified as lobular hyperplasia. The reclassification led to a loss of significance when the tumors in the treated animals were compared to tumors in the control animals.

Ovarian tubular hyperplasia and adenomas were also observed in female rats (Butenhoff et al., 2012). Mann and Frame (2004) re-examined the ovarian lesions using an updated nomenclature system which resulted in some of the hyperplastic lesions being reclassified. The ovarian lesions originally described as tubular hyperplasia or tubular adenomas were regarded as gonadal stromal hyperplasia and/or adenomas. After the reclassification there were no statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined in treated groups compared to controls.

Mutagenicity studies of PFOA using the *S. typhimurium* (Lawlor, 1995, 1996; Friere et al., 2008) and *E. coli* (Lawlor 1995, 1996) system have resulted in negative results in the presence and absence of activation. One mutagenicity study (Lawlor, 1995, 1996) in *S. typhimurium* gave a positive result but it was not reproducible. In clastogenicity studies in CHO by Murli (1996b, 1996d), the results were positive with activation for chromosomal abnormalities and polyploidy and equivocal in the absence of activation. Micronucleus assays by Murli (1995, 1996a) were negative.

A significant increase in 8-OH-dG liver levels, a biomarker for oxidative stress, was observed at ≥ 10 mg PFOA/kg in the liver but not the kidney of Fischer 344 male rats by Takagi et al. (1991). Work with HepG2 cells by Hu and Hu (2009) suggested that PFOA could induce apoptosis by overwhelming the homeostasis of antioxidative systems, increasing reactive oxygen species, impacting mitochondria, and changing expression of apoptosis gene regulators. Erikson et al. (2010) observed a PFOA-induced increase in reactive oxygen species production in HepG2 cells, but no PFOA-induced oxidative DNA damage or cytotoxicity.

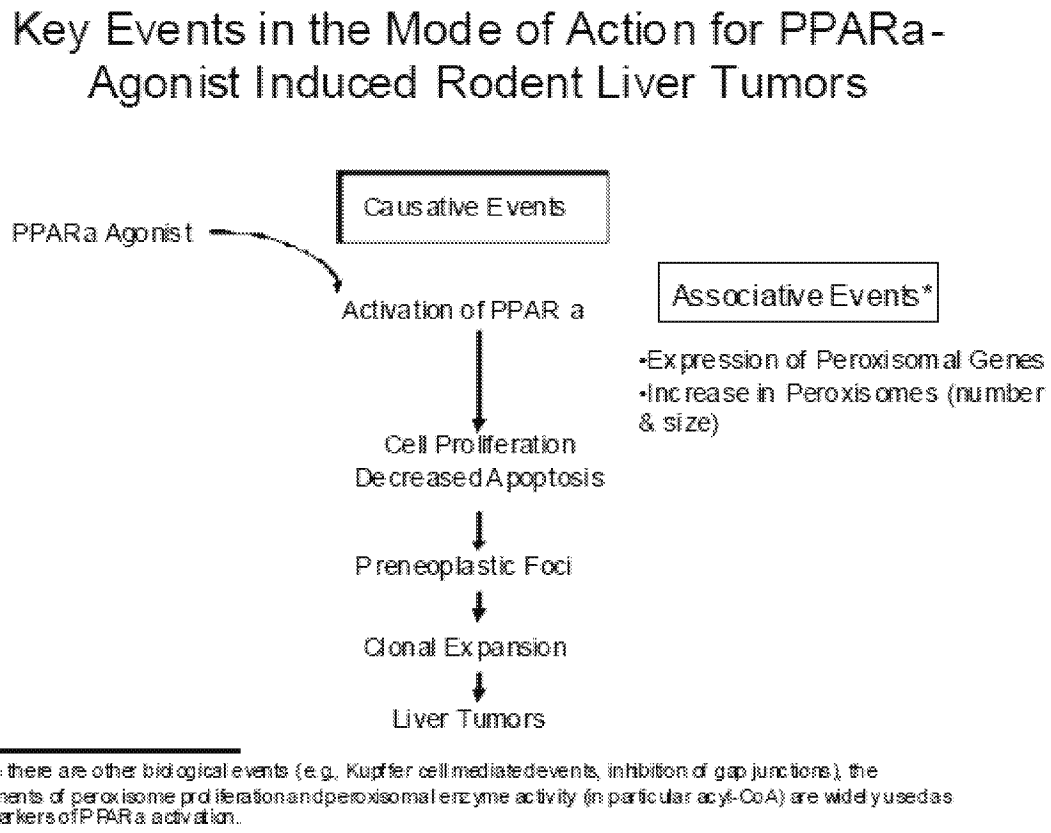
4.4.3 Mode of Action and Implications in Cancer Assessment

The modes of toxicological/carcinogenic action of PFOA are not clearly understood. However, available data suggest that the induction of tumors is likely due to non-genotoxic mechanism involving receptors and perturbations of the endocrine system.

Rat Liver Tumors. PPARα agonism is the proposed mechanism of action (MOA) for the liver carcinomas and adenomas in rats following chronic PFOA exposure (Maloney and Waxman, 1999; Klaunig et al., 2003). In the PPARα agonism MOA (Figure 4-1), binding of PFOA to the PPARα receptor results in increased peroxisome proliferation and cell replication. Peroxisome are single-membrane organelles found in a number of plant and animal cells that have the capacity to carry out oxidation of long chain fatty acids and other substrates through hydrogen peroxide generating pathways (Goodrich and Sul, 2000). Peroxisome beta oxidation produces acetyl CoA that can be utilized for anabolic reactions but is not linked to ATP production. Peroxisomes metabolize the long chain fatty acids via both beta and omega oxidation pathways (Fielding, 2000).

When a chemical binds to and activates the PPARα receptor, it forms a heterodimer with the retinoid-X receptor and binds to the peroxisome proliferator response element found in the promoter region of selected genes (Spector, 2000). In addition to a variety of xenobiotic chemicals, there are a number of endogenous substances in animals and humans that can activate the PPARα receptor including unsaturated C18 fatty acids, metabolites of arachidonic acid, and the prostaglandin metabolite PGJ2 (Spector, 2000).

The key events in the MOA of PPARα-agonist induced liver tumors involve four causal key events (Klaunig, 2003). The first key event is activation of PPARα. Other key events are cell proliferation, apoptosis, and clonal expansion. Of these key events, PPARα activation is highly specific for this MOA whereas cell proliferation, apoptosis, and clonal expansion are common for other MOAs.



From U.S. EPA, 2005b

FIGURE 4-1. PPARα-Agonism Mode of Action for Liver Tumors

The primary data that demonstrate PFOA activation of the PPARα receptor are those from Rosen et al (2008a,b) that examined the transcript profiles in the livers of wild-type and PPARα-null mice dosed with 1, 3, and 10 mg/kg day PFOA for up to 7 days. The data from the wild-type mice were compared to those from the known PPARα gene activator WYETH-14,643, and PPARα null mice. Based on the analysis of gene regulation it was clear that PPARα activation was required for a majority of the transcriptional changes observed in the mouse liver following PFOA or Wyeth 14,643 exposure. The data from this study demonstrate the ability of PFOA to act as a PPARα agonist, and evidence that PFOA also acts through PPARα independent mechanisms associated with CAR and PXR receptors. Martin et al. (2007) examined the

genomic signature from PFOA-treated Sprague-Dawley rats (up to 5-day exposure) using microarray expression profiling, pathway analysis, and quantitative-PCR. The animal responses were consistent with PPARα agonism, but there was also evidence of PPARγ agonism (down-regulation of cholesterol synthesis) and activation of CAR and PXR-related genes.

Few studies examined the occurrence of steps in the PPARα agonism MOA beyond activation of PPARα. The one study that looked at apoptosis from a MOA perspective (Elcombe et al, 2010) failed to see a significant decrease in male Sprague-Dawley rats with a 28 day exposure to a diet containing 300 ppm PFOA (comparable to the high dose in both cancer studies). The data in mice were not consistent. Minata et al. (2010) observed increased apoptosis in hepatocytes, arterial walls, and bile duct epithelium of wild type 129S4/SvlmJ mice and in the bile duct epithelium of PPARα-null mice at 10.8 or 21.6 mg/kg PFOA. On the other hand, Son et al. (2008) saw evidence of decreased apoptosis in liver and kidney cells stained for caspace3 in 4 week old male ICR mice treated for 21 days.

There are studies in male rats that examined cell proliferation. Using a BrdU labeling technique, Elcombe et al. (2010) observed significant increases in cell proliferation in male Sprague-Dawley rats after 1, 7, and 28 days of exposure to a 300 ppm dietary dose. The highest increase was observed after 7 days of treatment (5-fold increase) which had declined to a 2-fold increase after 28 days of dosing. The liver results from the Biegel et al. (2001) mechanistic study were negative for cell proliferation in male Sprague-Dawley rats exposed to the same dietary concentration (20 mg/kg/day) and sacrificed at 1, 3, 6, 9, 12, or 15 months. However, based on the Elcombe et al. (2010) observations, the timing of the interim sacrifice would have missed the peak of the proliferative response. The Butenhoff et al. (2012) study identified hyperplastic nodules in 6/50 high dose males and 4/50 high dose females at 20 mg/kg/day; 10/50 males and 2/50 females had hepatocellular carcinomas.

The study by Wolf et al. (2008) looked at the labeling index in 129S1/SvlmJ mice and PPARα-null mice and found a difference in their dose response. In the wild-type mice, the labeling index was increased at all doses ≥ 1 mg/kg/day but only at 10 mg/kg/day, the highest dose, in PPARα-null mice. This suggests PPARα involvement in increased cell division of wild-type mice at lower doses.

There were no studies identified that focused specifically on preneoplastic foci and clonal expansion of altered cells after PPAR exposures. Minata et al (2010) observed a dose dependent increase in eosinophilic cytoplasmic changes consistent with peroxisome proliferation in liver parenchyma, but found no focal necrosis at doses ≤ 21.6 mg/kg/day in wild-type 129S4/SvlmJ mice.

There are several "associative" events that are markers of PPARα agonism but are not directly involved in the etiology of liver tumors. These include peroxisome proliferation and expression of genes for enzymes associated with the peroxisomes. These associative events include (Klaunig et al., 2003):

- *In vivo* evidence of an increase in number and size of peroxisomes,
- Increases in the activity of acyl CoA oxidases such as palmitoyl CoA oxidase activity,
- Hepatocyte hypertrophy, increase in liver weights,
- Hepatic CYP4A1 (Lauric acids monooxygenase) induction, and

- Peroxisomal gene expression.

There is ample evidence that PFOA is a potent peroxisome proliferator inducing peroxisome formation in the livers of rats and mice (Ikeda et al, 1985; Pastoor et al., 1987; Sohlenius et al., 1992; Yang et al., 2001; Wof et al., 2008; Minata et al., 2010; Elcombe et al., 2010). PFOA was also found to activate mouse and human PPARα using a transient transfection cell assay (Takacs and Abbott (2007). Maloney and Waxman (1999) demonstrated that 5-10 μM PFOA activated mouse PPARα using COS1 cells (kidney fibroblast derived cells) transfected with a luciferase reporter gene.

The temporal and dose-response relationship of measures of peroxisome proliferation, hepatocellular hypertrophy, liver weight, and liver histopathology have been examined in male Sprague-Dawley rats following 4, 7 and 13 weeks of administration of dietary PFOA at doses ranging from 0-6.5 mg/kg-day (Palazzolo, 1993; Perkins et al., 2004). There was no evidence of peroxisome proliferation, hepatocellular hypertrophy or liver weight increases at 0.06 mg/kg-day. At doses ranging from 0.64 to 6.5 mg/kg-day there is a clear relationship between peroxisome proliferation (indicated by increased palmitoyl CoA oxidase activity), hepatocellular hypertrophy and increases in liver weight at all time points.

One can conclude, based on the available data, that the liver tumors in the PFOA Sprague-Dawley cancer bioassays can be attributed to its peroxisome proliferative impact since there are data that supports each of the steps in the proposed mode of action and thus are not relevant to humans (Klaunig et al., 2003).

Leydig Cell Tumors (LCT). The mode of carcinogenic action for PFOA-induced LCTs identified in Biegel et al. (2001) has not been fully elucidated. A large number of non-genotoxic compounds of diverse chemical structures have been reported to induce LCTs in rats, mice, or dogs. LCTs also occur in humans but are relatively rare (Carpino et al., 2007). The consensus conclusions of a workshop on the MOA for LCT were reported by Clegg et al. (1997) and classified the chemicals that caused LCT in animal studies into seven MOA categories. The postulated MOAs support the following hormonal steps to the process:

- A xenobiotic chemical inhibits the production of testosterone leading to low serum levels
- Low serum testosterone levels signal the hypothalamus to produce gonadotropin releasing hormone (GnRH)
- GnRH signals the pituitary to release luteinizing hormone (LH)
- LH signals the Leydig cells to produce testosterone
- LH causes Leydig cell proliferation in testosterone production

The sustained increase in circulating LH and chronic stimulation of Leydig cells by growth-stimulating mediators including IGF-1, TGF-α, leukotrienes and various free radicals could lead to DNA replication errors and LCT development (Clegg et al., 1997). TGF-α, which binds to the epidermal growth factor receptor and stimulated cell proliferation has been detected in Leydig cells (Teerds et al., 1990).

A series of mechanistic studies have been conducted by Cook and colleagues (Cook et al., 1992; Biegel et al., 1995; Liu et al., 1996) to investigate the mechanism of LCT formation in

male Sprague-Dawley rats exposed to PFOA. Administration of PFOA to adult male rats by gavage for 14 days was shown to decrease testosterone levels and increase serum estradiol levels (Cook et al., 2002). These endocrine changes correlated with its potency to induce LCTs in rats and were hypothesized to play a role in the PFOA-induction of LCTs (Biegel et al., 2001).

Subsequent experiments have shown that PFOA increased levels of estradiol by inducing cytochrome P450 CYP19 (aromatase). Aromatase converts androgens to estrogens including the conversion of testosterone to estradiol. PFOA has also been shown to directly inhibit testosterone production when incubated with isolated Leydig cells and *ex vivo* studies demonstrated that this inhibition was reversible (Biegel et al., 1995). This inhibition of testosterone biosynthesis appeared to be mediated by PPARα (Gazouli et al., 2002) and may contribute to the development of LCTs through disruption of the hypothalamus-pituitary-testicular axis. However, in the mechanistic bioassay by Biegel et al. (2001), serum testosterone and LH levels were not significantly altered at the levels of PFOA that were tested.

Support for PPARα-mediated inhibition of testosterone production is found in Li et al. (2011). Lower testosterone concentrations, reduced reproductive organ weights, and increased sperm abnormalities were found in PFOA-treated male wild-type and humanized PPARα mice but not in PPARα-null mice. Similarly, disruption of testosterone biosynthesis by lowering the delivery of cholesterol into the mitochondria and decreasing the conversion of cholesterol to pregnenolone and androstandione in the testis was shown to occur in wild-type and humanized PPARα mice. These effects were not seen in PPARα-null mice.

The induction of LCTs by PFOA can be attributed to a hormonal mechanism whereby PFOA either inhibits testosterone biosynthesis and/or lowers testosterone by increasing its conversion to estradiol by upregulating aromatase activity in the liver. Both of these mechanisms appear to be mediated by PPARα. However, the data are not currently sufficient to demonstrate that the other key steps in the postulated MOA are present in PFOA-treated animals following exposures that lead to tumor formation. Studies are needed to demonstrate the increase of GnRH and LH in concert with the changes in aromatase and estradiol.

Pancreatic Acinar Cell Tumors (PACT). As with LCT, the MOA for PACTs is not understood. These tumors are most commonly identified in rats but do occur in other animal species (mice, hamsters) and in humans (Wisnoski et al, 2008). Males are more susceptible to pancreatic tumors than females. Two hypothetical MOAs have been proposed and are as follows (Obourn et al., 1997; Klaunig et al., 2003):

- Events that stimulate increases of growth factors such as cholecystokinin (CCK) and/or gastrin activate a feedback loop resulting in proliferation of the secretory pancreatic acinar cells. A high fat diet, trypsin inhibition, cholestasis, or changes in bile composition are proposed initiators of this sequence of events.

- Increased levels of testosterone support the growth of acinar cell preneoplastic foci and carcinomas and estrogens inhibit the cellular proliferation.

There is minimal information on the relationship of PFOA exposure to either of the proposed MOAs. Obourn et al. (1997) studied the impact of PFOA on CCK and trypsin using *in*

vitro assays and found that PFOA was not an agonist for the CCKA receptor that activated CCK release. PFOA also had no inhibitory action on trypsin at levels 1000 times greater (0.31 μg/mL) than the positive control.

The Obourn et al. (1997) study also looked at Wyeth-14,643, a peroxisome proliferator, in these same assays and found results similar to those for PFOA. When they conducted an *in vivo* study with 100 ppmWyeth-14,643, they found a small but significant increase ($p<0.05$) in bile flow per unit body weight, a decrease ($p<0.05$) in bile flow per unit liver weight, and a small decrease ($p<0.05$) in the total bile acid concentration following a 6 month dietary exposure. Plummer et al. (2007) reported on gene expression changes induced in pancreatic acinar cells isolated from Sprague-Dawley rats fed diets containing 300 ppm (~20 mg/kg/day) PFOA for 28 days. Expression of genes regulated by PPARα, γ, δ in pancreatic acinar cells was directly opposite of the expression of those same genes in liver tissue.

As indicated in the discussion of the LCTs above, PFOA appears to suppress testosterone production through the induction of aromatase. Accordingly, the second proposed MOA for PACTs is not applicable to PFOA. At the present time, there are insufficient data to demonstrate a MOA that can account for the PACTs identified in the chronic study by Biegel et al. (2001).

Other Potential Modes of Action. There are other potential MOAs of actions that may apply to PFOA. They include interruption of intercellular communication, mitochondrial effects, and hormonal effects. None of these mechanisms are considered to be key steps in the MOAs discussed above.

Gap junction intercellular communication (GJIC), a process by which cells exchange ions, second messages, and other small molecules, is important for normal growth, development, and differentiation, as well as maintenance of homeostasis in muticellular organisms. Because tumor formation requires loss of homeostasis and many tumor promoters inhibit GJIC, it has been hypothesized that GJIC may play a role in carcinogenesis (Trosko et al, 1998). PFOA has been demonstrated to inhibit GJIC in liver cells in vitro and in vivo (Upham et al., 1998. 2009). However, inhibition of GJIC is a widespread phenomenon, and the effect by PFOA was neither species nor tissue specific. In addition it was reversible. Thus, the significance of GJIC inhibition in regard to the mode of carcinogenic action of PFOA is unknown.

Several chemicals structurally related to PFOA have been shown to manifest their toxicity by interfering with mitochondria biogenesis and bioenergetics. Berthiaume and Wallace (2002) noted an increase in mitochondrial biogenesis in liver following treatment of rats with PFOA. PFOA has also been demonstrated to uncouple oxidative phosphorylation in mitochondria of the liver from rats exposed via their diet (Keller et al., 1992). At high concentrations, PFOA caused a small increase in resting respiration rate and slightly decreases the membrane potential. The observed effects are believed to be attributed to a slight increase in nonselective permeability of the mitochondria membranes caused by PFOA's surface-active properties (Starkov and Wallace, 2002).

Estrogen has been shown to promote hepatocarcinogenesis in rats (Yager and Yager, 1980; Cameron et al., 1982). Thus, the increase in estrogen levels after PFOA exposure may play a role in hepatocarcinogenesis in rats as well as influencing LCTs. More research is needed

to support the involvement of this MOA to the hepatocarcinogenesis of PFOA.

4.4.4 Weight of Evidence Evaluation for Carcinogenicity

The findings for cancer in humans are equivocal; some epidemiological studies show a positive association with exposures and others are negative. The Dupont (2003b) report on the West Virginia Washington Works Plant found statistically significant elevations in cancer of the bladder, kidney, and urinary organs and a nonstatistically significant elevation in leukemia incidence. Two earlier worker studies had statistically significant findings for leukemia but both had methodological limitations (Walrath and Burke, 1989; Karns and Fayerweather, 1991). The mortality data for liver cancer, pancreatic cancer, prostate cancer, and testicular cancer all showed no association to exposure. In contrast, the highest serum PFOA levels were associated with testicular, kidney, prostate, and ovarian cancers and non-Hodgkin's lymphoma among the general population exposed through contaminated water (Vieira et al., 2013). These findings are limited by small numbers of cases or a positive association with the very high exposure category, but weaker or negative associations for the other exposure categories.

The only chronic bioassays of PFOA were conducted in rats (Butenhoff et al., 2012; Biegel et al, 2001). The two studies support a positive finding for the ability of PFOA to be tumorigenic in one or more organs of male but not female rats. There are no carcinogenicity data from a second animal species. There are some data that provide support for the hypothesis that the PPARα agonism MOA is wholly or partially linked to each of the observed tumor types. The data support a PPARα MOA for the liver tumors and thus are indicative of lack of relevance to humans. PPARα activation may also play a role in the other tumor types observed, but more data that will support intermediate steps in the proposed MOAs are needed.

The mutagenicity data on PFOA are largely negative although there is some evidence for clastogenicity in the presence of microsomal activation and at cytotoxic concentrations. Given the fact that PFOA is not metabolized and would not be activated by the microsomal enzymes, the clastogenic effects were likely the result of an indirect mechanism. Involvement of ROS in the MOA as a result of PFOA alone is unlikely because of its metabolic stability. Conditions leading to ROS would be a function of metabolic conditions perturbed by PFOA rather than PFOA alone. A compound that is not metabolized will not be able to covalently introduce any of its atoms into the structure of DNA.

Despite the limitations in the data for the Leydig cell and pancreatic tumors, a finding of *Suggested evidence of carcinogenicity* is justified and quantification of the dose response is not recommended. The tumor dose-response data are not indicative of a high potency, and the dose-response information from the noncancer studies indicate that protecting for several of the noncancer endpoints (e.g. increases in liver weights, hepatocyte cellular abnormalities, immunotoxicity, developmental effects and delays) will result in an RfD that will be protective for any tumorigenic effects.

4.4.5 Potentially Sensitive Populations

Human biomonitoring studies do not suggest major differences between serum PFOA levels in males and females. However, the worker populations that would be those most likely to demonstrate such differences because of their higher exposures were predominantly male.

Some animal models have gender differences which affect toxicity of PFOA. Sexually mature female rats excreted almost all of a 10 mg/kg dose of PFOA within 48 hours compared to only 19% excreted by male rats. Male hamsters excrete PFOA faster than female hamsters, and female rabbits excrete PFOA slightly faster than male rabbits. Male and female mice excrete PFOA at approximately the same rate (Hundly et al., 2006). Studies of the transporters involved in the toxicokentics of PFOA demonstrate that they are differentially impacted by the presence of male and female sex hormones (Kudo et al., 2002; Cheng et al., 2006) influencing tissue persistence. As studied in rats (Kudo et al., 2002), the male sex hormones increased half-life (decreased excretion) of PFOA while the female hormones were associated with shorter half lives (increased excretion). The gender differences in mice are not as pronounced as those in rats. Work by Cheng et al. (2002) and Cheng and Klaassen et al. (2009) demonstrated that hormones impact transporters in liver and kidney.

In studies in which male and female rats were used, the males were more sensitive to toxicity than female rats (Goldenthal, 1978a; York, 2002; Butenhoff et al., 2004a; Sohlenius et al., 1992). Monkeys and mice displayed similar sensitivities following PFOA exposure (Goldenthal, 1978b; Christopher and Marias, 1977; Sohlenius et al, 1992). In the monkey studies the number of animas/sex/dose group was too small reveal a difference related to gender.

Unfortunately much work remains to be done in order to determine whether the gender difference seen in rats is relevant to humans. Similarities are possible because the long half-life in humans suggests that they may be more like the male rat rather than the female rat. There is a broad range of half-lives in human epidemiology studies suggesting a variability in human transport capabilities resulting from genetic variations in structures and consequently in function. Genetic variation in human OATs and OATps has been identified as described in a review by Zaïr et al. (2008).

Neonates, Infants, and Fetuses.

An epidemiological study examining postnatal outcomes including developmental milestones at 6 and 18 months of age did not find any differences in fine motor, gross motor, or cognitive skills relative to maternal PFOA concentration (Fei et al., 2008b).

Several animal studies have examined potential modes of action for developmental effects following prenatal exposure to PFOA. PFOA-exposure during development in rabbits, rats, and mice resulted in increased resorptions (mouse) increased fetal skeletal variation (rabbits, rats, mouse), decreased neonatal survival (rat, mouse), decreased postnatal body weight (mouse), delayed eye opening and body hair growth (mouse), delayed vaginal opening (mouse), accelerated preputial separation (mouse), delayed mammary gland development (mouse dam and offspring) (York, 2002; Butenhoff et al., 2004a; Lau et al., 2006; Abbott et al., 2007; Wolf et al., 2007; White et al., 2007, 2009, 2011; Macon et al., 2011). Other long term effects observed in the surviving offspring included increased body weight gain, serum leptin, and serum insulin levels along with changes in adipose tissue (Hines et al., 2009). The mechanisms of action for these developmental effects are unknown, but several potential modes of action have been investigated.

Wolf et al. (2007) restricted mouse prenatal PFOA-exposures to 3-11 day periods during gestation to determine if PFOA was affecting a certain stage of organogenesis that resulted in the observed developmental effects. Decreased postnatal survival was observed at the highest dose used (20 mg/kg/day). Eye opening and body hair growth were delayed in offspring exposed for the longest periods of time (GD7-17 and GD10-17) and may have been the result of a higher cumulative dose or greater sensitivity during early gestation. A cross-fostering paradigm was used to determine if the developmental effects were the result of gestational exposure, lactational exposure, or a combination of both. Postnatal survival was decreased in offspring exposed through gestation and lactation (5 mg/kg/day). Eye opening and body hair growth were delayed and body weight was reduced in offspring exposed during gestation (5 mg/kg/day), and gestation and lactation (3 and 5 mg/kg/day). No developmental delays were observed in offspring exposed via lactation only indicating that PFOA alters growth regulation in the developing fetus which persist as growth continues postnatally.

Abbott et al. (2007) examined activation of PPARα as a factor in the developmental toxicity of PFOA. Wild-type and PPARα null mice experienced full litter resorptions following gestational (GD1-17) PFOA exposure (≥5 mg/kg/day) indicating the mechanism of PFOA-induced resorptions was independent of PPARα expression. These resorptions may be due to insufficient trophoblast cell type differentiation and/or increased trophoblast cell necrosis (Suh et al., 2011). Postnatal survival was significantly decreased in wild-type offspring but not in PPARα null offspring indicating that PPARα expression was required for postnatal lethality (Abbott et al., 2007). Eye opening was significantly delayed in wild-type offspring, but not in PPARα null offspring although a trend was observed in those offspring for later eye opening. The results indicated that PPARα expression was important for eye opening but other PPARα independent factors may also play a role in its mechanism. Takacs and Abbott (2007) showed that PFOA can activate mouse PPARβ/δ which is expressed in developing tissue and suggested that inappropriate activation of PPARβ/δ could cause adverse effects. Further research needs to be conducted to fully elucidate the mechanism.

Mouse mammary gland development was another endpoint examined in prenatally PFOA-exposed offspring. White et al. (2007) found that dams dosed with PFOA GD1-17 and GD8-17 had significantly delayed mammary gland development (full of alveoli, visible adipose tissue, not well differentiated) at PND10 which is the peak of lactation in rodents. The delayed dam mammary gland development may play a role in the observed reduced offspring body weight gain if the quantity or quality of the milk is altered by PFOA (Lau et al., 2006; Abbott et al., 2007; Wolf et al., 2007; White et al., 2007). Restricted gestational exposure and cross-fostering studies showed that delayed offspring mammary gland development, observed PND1-63, occurred regardless of exposure duration or timing (gestation vs. lactation exposure). The developmental delays persisted even as the internal PFOA dose decreased (White et al., 2007, 2009, 2011; Macon et al., 2011). More studies need to be conducted to elucidate the MOA for dam and offspring mammary gland effects.

Mammary gland development was also affect by peripubertal exposure to PFOA (Yang et al., 2009, Zhao et al., 2010). Low doses (5 mg/kg/day) of PFOA from 3 to 7 weeks of age caused accelerated mammary gland development in C57BL/6 mice but delayed mammary gland development in BALB/c mice. Experiments examining the mechanism for accelerated mammary gland development showed that PFOA promotes steroid hormone production in the

ovaries and increases the levels of several mammary gland growth factors in C57BL/6 wild-type and PPARα-null mice. The mechanism for delayed mammary gland development following a peripubertal PFOA exposure needs to be examined.

Hines et al. (2009) found that low doses of PFOA given during gestation to CD-1 mice resulted in significant weight gain and increased serum insulin and leptin levels of the offspring in mid-life. The increased leptin levels, as well other hormone perturbations, may place PFOA into the environmental endocrine disruptor obesogen category similar to diethylstilbestrol (DES) (Newbold et al., 2007). In humans, increased leptin levels are associated with increased body fat and suggestive of a leptin-resistance mechanism of action for overweight (Considine et al., 1996). A similar relationship may occur in prenatally PFOA-exposed mice. Studies determining MOAs should be conducted to determine relevance to human health.

5.0 DOSE-RESPONSE ASSESSMENT

5.1 Dose-Response for Noncancer Effects

A Reference Dose (RfD) or Reference Concentration (RfC) is used as a benchmark for the prevention of long-term toxic effects other than carcinogenicity. RfD/RfC determination assumes that thresholds exist for toxic effects, such as cellular necrosis, significant body or organ weight changes, blood disorders, etc. The RfD is expressed in terms of milligrams per kilogram per day (mg/kg-day) and the RfC is expressed in milligrams per cubic meter (mg/m^3). The RfD and RfC are estimates (with uncertainties spanning perhaps an order of magnitude) of the daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

5.1.1 RfD Determination

The risk assessment for PFOA is fraught with a number of challenges including:

- Its toxicokinetic complexity
- The variability in half-lives between and within species
- The wealth of inconsistent data from epidemiological studies
- The robust but diverse database of animal studies
- Inconsistencies between some of the associations from the epidemiology studies and the effects from the animal studies
- The metabolic inertness of PFOA in living systems
- The number of animal studies that lack a NOAEL.

A subset of the challenges has an impact on dose-response, especially the toxicokinetic features that lead to differences in half-lives across species and in the case of rats, genders. Toxicokinetics also influence intraindividual and lifestage variability. Each of these toxicokinetic features highlights the importance of internal dose, and supports the utilization of a pharmakolinetic model as a component of the dose-response assessment.

Human Data. Studies have examined occupational and residential populations at or near large-scale PFOA production plants in the United States in an attempt to determine the relationship between serum PFOA concentration and various health outcomes suggested by the standard animal toxicological database. Endpoints monitored included standard clinical chemistry parameters, measures of cardiovascular risk, signs of organ damage, standard haematological endpoints, and diabetic or prediabetic conditions. Reporductive and developmental parameters were also evaluated in some epidemiology studies.

In reviewing the epidemiological monitoring data for PFOA it is important to remember that many individuals were coexposed to other fluorocarbons, and, although only serum PFOA measurements are often given, many individuals had other PFCs present in their serum. In no study was the actual exposure concentration or dose to the individuals known; thus, a quantitative exposure-response assessment based on the human data is not feasible.

In many C8 project studies, drinking water was identified as the primary contributor to exposure. However, indoor air and dietary exposures were possible and quantitative exposure

data are lacking for all routes. Similarly, occupational epidemiology studies cannot quantify exposures occurring away from the workplace. In all of the epidemiology studies the wide ranges of serum levels are likely a reflection, not only of intrahuman toxicokinetic variability, but also of diversity in external exposure sources. This is especially evident in the units used to express PFOA serum levels in occupational populations, μg/mL, versus in the general population, ng/mL. From the studies discussed in Section 4.1.1.1, mean serum levels in the occupational cohorts ranged from 1.02 – 4.63 μg/mL while those for the general population ranged from 1.05 – 354 ng/mL. The highest level seen in the general population is from residents serviced by a contaminated water district. For both the positive and negative findings summarized in the paragraphs below, mean or median serum PFOA levels fall within these ranges for worker and general populations.

The epidemiological studies have found a positive association between serum PFOA concentration and total cholesterol in the general population (Steenland et al., 2009; Frisbee et al., 2010; Nelson et al., 2010) and in worker populations (Olsen et al., 2001a,b, 2003; Sakr et al., 2007a,b; Costa et al., 2009) but no consistent trends in LDL, VLDL, or HDL levels. A positive association has been shown between serum PFOA concentration and increased liver enzymes and/or decreased bilirubin in both worker and general populations (Sakr et al., 2007,a,b; Olsen and Zobel, 2007; Costa et al., 2009; Lin et al., 2010; Gallo et al., 2010), chronic kidney disease in the general population (Shankar et al., 2011), and the odds of experiencing early menopause (Knox et al., 2011). Maternal or child plasma levels of PFOA were positively associated with decreased antibody titers in children after vaccination (Grandjean et al., 2012), obesogenic effects in female children at 20 years of age (Halldorsson et al., 2012), delayed puberty in girls (Lopez-Espinosa et al., 2011), and parent reported ADHD (Hoffman et al., 2010).

No association between serum PFOA and risk of dying from ischemic heart disease has been found in several worker populations (Sakr et al., 2009; Leonard et al., 2008; Lundin et al., 2009). No consistent associations have been found between hyperglycemia, type II diabetes (MacNeil et al., 2009; Lin et al., 2009; Nelson et al., 2010), thyroid homeostasis (Olsen et al., 2001a,2003; Sakr et al., 2007a; Olsen and Zobel, 2007; Costa et al., 2009; Emmett et al., 2006; Bloom et al., 2010), fertility, fecundity, and birth outcome (Fei et al., 2009; Monroy et al., 2008; Hamm et al., 2009; Washino et al., 2009; Nolan et al., 2010; Stein et al., 2009). Maternal or child plasma PFOA levels were not associated with developmental milestones in children (Fei et al., 2008b), child risk of hospitalization for infectious diseases (Fei et al., 2010), or attainment of puberty (Christensen et al., 2011).

Animal Data – Long Term Studies. Effects in animal studies are most commonly those associated with activation of the PPARα receptor leading to peroxisome proliferation. These include increased liver weight, decreases in triglycerides, cholesterol, and lipoprotein, plus increases in ALT and/or AST. However, the mechanisms for other effects, such as decreased body weight, immunological effects, and developmental delays, are unknown, yet may be relevant to human health risk assessment.

As an initial step in the dose-response assessment, U.S. EPA identified a suite of animal studies with low dose NOAELs and/or LOAELs as potential candidates for development of a chronic Reference Dose (RfD). These studies are listed in Table 5-1. The candidate studies were selected based on their low dose NOAEL and/or LOAEL, a duration of ≥7 weeks, use of a

control, and two or more doses. Table 5-1 does not include the data from human epidemiology studies because, although they include information on serum levels, they do not identify exposure sources or external doses.

TABLE 5-1. NOAEL/LOAEL data for Oral Subchronic and Chronic Studies of PFOA

Species	Study Duration	NOAEL mg/kg/day	LOAEL mg/kg/day	Critical Effects (s)	Reference
Monkey Male	90 days	none	3	Increased relative pituitary weight	Goldenthal, 1978b
Monkey Female	90 days	3	10	Decreased absolute and relative heart weight	Goldenthal, 1978b
Monkey Male	26 weeks	none	3	Increased absolute liver weight (hepatocellular hypertrophy) and mean liver-to-body weight percentages	Thomford, 2001b, Butenhoff et al., 2002
Rat Male	90 days	0.56	1.72	Hepatocyte necrosis, increased absolute liver weight	Goldenthal, 1978a
Rat Male	13 weeks	0.06	0.64	Increased absolute and relative liver weight with hepatocellular hypertrophy	Palazzolo, 1993; Perkins et al., 2004
Rat Male	2 years	1.3	14.2	Decreased body weight gain, increased kidney weight, and hepatic cystoid degeneration, megalocytosis, decreased red blood cell count, hemoglobin concentration, and hematocrit values, testicular vascular mineralization, pulmonary hemorrhage	Butenhoff et al., 2012
Rat Male F0 generation	84 days	none	1	Increased absolute liver and kidney weight	York, 2002; Butenhoff et al., 2004a; York et al., 2010
Rat Female F0 generation	127	30	none		York, 2002; Butenhoff et al., 2004a; York et al., 2010
Rat Male F1 generation	10 weeks	none	1	Decreased body weights and weight gains increased absolute and relative liver weights	York, 2002; Butenhoff et al., 2004a; York et al., 2010
Rat Female	90 days	22.4	76.5	Increased absolute and relative liver weight	Goldenthal, 1978a
Rat Male and Female	2 years	1.3 (m) 1.6 (f)	14.2 (m) 16.1 (f)	M: Decreased body weight gain; histopath. lesions in liver, testes, and lungs. F: Decreased body weight gain and decreased red blood cell count, hemoglobin, concentration, and hematocrit values	Butenhoff et al., 2012
Rat Female F1 Generation	10 weeks	10	30	Female: Delay in sexual maturity, decreased body weight and weight gain	York, 2002; Butenhoff et al., 2004a; York et al., 2010

When examining the effects associated with the low dose exposures summarized in Table 5-1, changes in relative and/or absolute liver weight appear to be the common denominator for

monkeys and rats (York, 2002; Butenhoff et al., 2004a; Goldenthal, 1978a; Palazzolo, 1993; Perkin et al., 2004; Thomford, 2001b, Butenhoff et al., 2002) with or without other hepatic indicators of adversity (Palazzolo, 1993; Perkin et al., 2004; Thomford, 2001b, Butenhoff et al., 2002). Serum PFOA levels, where available, associated with increased liver weight were 81 and 41 μg/mL for the male monkey and rat, respectively. Body weight effects were seen in several studies (York, 2002; Butenhoff et al., 2004a; Butenhoff et al., 2012). Testicular effects were observed by Butenhoff et al. (2012) and in the chronic one-dose study by Beigel et al. (2001). There were developmental delays for males and females in the two-generation study published by York (2002) and Butenhoff et al. (2004a).

Four of the studies in Table 5-1 lack a NOAEL and have LOAELs that range from 1 to 3 mg/kg/day. The NOAELs for the remaining 7 studies range from 0.06 to 22.4 mg/kg/day. Male monkeys and rats appear to respond at doses that are lower than their female counterparts. No long-term studies in mice were identified. Since NOAELs and LOAELs are to some extent the product of concentration or dose level selection, examination of the dose information in Table 5-1 suggests that several data sets have the potential to be co-critical in the dose-response evaluation.

Animal Data –Short Term Studies. A number of studies in mice have also identified adverse effects following low dose exposures. The studies fall into two clusters, those evaluating developmental or reproductive effects and those with a focus on immunological effects. The critical shorter-term studies in rats and mice are summarized in Table 5-2.

TABLE 5-2. Shorter term and Developmental Oral Exposure Studies					
Species	**Study Duration**	**NOAEL (mg/kg/day)**	**LOAEL (mg/kg/day)**	**Critical Effect(s)**	**Reference**
Rat					
Rat Male	29 days	1	10	Increased absolute and relative liver weight, focal liver necrosis	Loveless et al., 2008
Mouse					
Mouse Male	29 days	0.3	1	Increased absolute and relative liver weight, decreased relative spleen weight, moderate-severe liver hypertrophy with single cell and focal necrosis	Loveless et al., 2008
Mouse Male	29 days	1	0.3	Decreased spleen weight	Loveless et al., 2008
Mouse Female	15 days	none	0.94	Increased absolute and relative liver weight (1 and 15 days post dose)	DeWitt et al., 2008
Mouse Female	15 days	1.88	3.75	Decreased IgM (1 days post dose), increased IgG (15 days post dose), decreased absolute and relative spleen weight (1 day post dose)	DeWitt et al., 2008
Mouse Female	17 (pups) /18 (dams) days	none	1	Increased absolute maternal liver weight, reduced ossification (calvarin, enlarged fontanel), accelerated onset of puberty in male offspring	Lau et al., 2006
Mouse Female	17 days GD1-17	none	3	Increased absolute and relative maternal liver weight, delayed offspring eye opening and body hair growth, increased offspring relative liver weight, decreased offspring body weight, delayed mammary gland development (female offspring)	Wolf et al., 2007; White et al., 2009
Mouse Female	11 days GD7-17	none	5	Increased maternal and pup relative liver weight, delayed offspring eye opening and hair growth, decreased male offspring body weight, delayed mammary gland development (female offspring)	Wolf et al., 2007; White et al., 2009

As was the case with the longer-term studies, increased liver weight was a common effect among the shorter-term studies summarized in Table 5-2. Increases in absolute or relative liver weights were reported in six of the eight studies (Lau et al., 2006; Wolf et al., 2007; DeWitt et al., 2008; Loveless et al, 2008; Table 5-2). The co-occurring effects at the LOAEL were effects on spleen, thymus, liver, and/or developmental endpoints. Four of the eight studies lacked a NOAEL. For those studies with a NOAEL, the value ranged from 0.3 to 1.88 mg/kg/day while the LOAELs ranged from 0.3 to 10 mg/kg/day. Serum PFOA levels associated with effects in female mice ranged from 21.9 – 36.3 µg/mL. In both cases, the range of values across studies is narrow with overlap between the NOAELs and LOAELs. In all instances the durations of exposure in shorter term studies were less than 29 days, suggesting that physiological responses

to PFOA occur early in the exposure continuum and at doses comparable to those observed in the long term studies.

5.1.1.1 Benchmark Dose Approach

As a second step in the dose-response analysis, benchmark modeling of dose-response for the liver weight changes from the studies wherein this was a critical effect was conducted. The data were modeled using benchmark dose software (BMDS) v. 2.1.2 for a continuous dataset with modeled variance and restricted slope where applicable. A 10% increase in absolute liver weight was chosen as the benchmark response (BMR) for the initial analysis. Although it is a biomarker for systemic exposure in rodents rather than a biomarker of adversity, it serves as a lowest common denominator for loss of homeostasis in a sensitive species.

PFOA is not metabolically altered during absorption, distribution and excretion. Systemic homeostasis is lost when renal excretion is no longer balanced with absorption. There is clear evidence that PFOA has a high affinity for the proteins that compose the PPARα receptor of rodents resulting in hypertrophy and increased liver weight as an early signature of exposure. Thus, both liver responses serve as biomarkers for the loss of homeostasis and can be extrapolated to species other than PPARα sensitive-rodents. The studies of Nakamura et al. (2009) and Albrecht et al. (2013) of mice with the humanized PPAR-α receptor did not observe significant changes in liver weight. However, there were changes in humanized mice that occurred at doses that exhibited the liver weight effects in wild type mice and support its use as a biomarker for loss of homeostasis. Specifically, humanized mice exhibited changes in gene products that modulate lipid metabolism (Albrecht et al., 2013; Nanamura et al., 2009) at doses comparable to those associated with liver weight effects in wild-type mice.

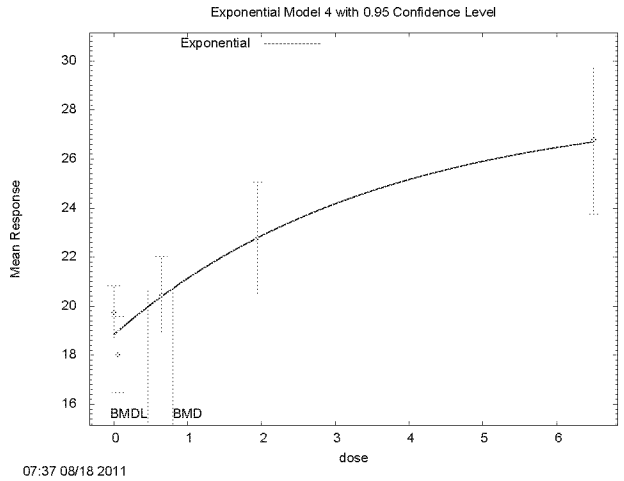
Results of the benchmark analyses are shown in Table 5-3 where goodness-of-fit information [p value and Akaike's Information Criterion (AIC)] was used to choose the best model for each data set. Liver weight data were available in the male rat (Butenhoff et al., 2004a; Palazzolo, 1993; Perkins et al., 2004; York, 2002; and York et al., 2010), male mouse (Loveless et al., 2008), and female mouse (Lau et al., 2006). For each data set, the BMD for a 10% increase in liver weight and the lower-bound confidence limit on the BMD (BMDL) are provided.

TABLE 5-3. Benchmark Dose Modeling for a 10% Increase in Liver Weight					
Species	**Best fit Model**	**BMD_{10} mg/kg/day**	**$BMDL_{10}$ mg/kg/day**	**Notes**	**Reference**
Rat					
Rat Male	Exponential: Model 4	0.798012	0.456224	Increased absolute and relative liver weight	Palazzolo, 1993; Perkins et al., 2004
Rat Male	Exponential Model 4	0.241376	0.151833	Increased absolute and relative liver weight	Loveless et al., 2008
Rat Male F0 generation	Exponential: Model 4	0.399087	0.274007	Increased absolute and relative liver weights	York, 2002; Butenhoff et al., 2004a; York et al., 2010
Rat Male F1 generation	Exponential: Model 4	0.599409	0.3655	Significant increases in absolute and relative liver weights	York, 2002; Butenhoff et al., 2004a; York et al., 2010
Mouse					
Mouse Male	Exponential: Model 4	0.099268	0.0680743	Increased absolute and relative liver weight	Loveless et al., 2008
Mouse Female	Exponential: Model 4	0.292244	0.246874	Increased maternal absolute liver weight	Lau et al., 2006

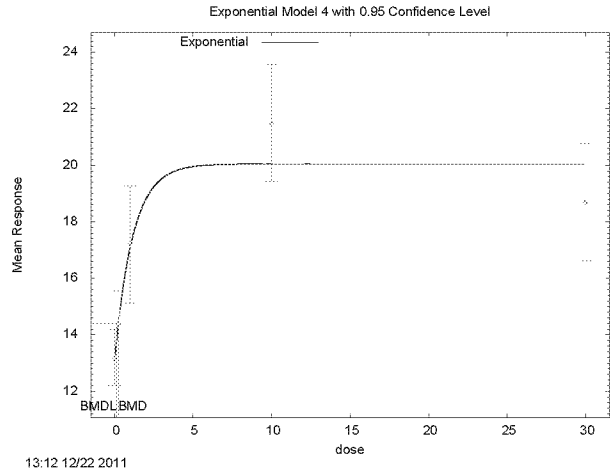
Data from Thomford (2001b) for monkeys and Goldenthal (1978) for the male rat were not presented because the low number of animals (n= 2 to 6) in each dose group resulted in highly variable lower bound estimates. An attempt was also made to use the BMDS to model liver-to-body weight ratio data from the studies where this information was available. However, all models failed with $p<0.1$ indicating that the fit was not reliable

Taken together, the analyses presented in Table 5-3 identify BMD values that vary by less than an order of magnitude (about 0.1 to 0.8 mg/kg/day) for a 10% increase in liver weight despite differences in species. Likewise, the values for the calculated lower-bound estimates did not vary greatly (about 0.1 to 0.5 mg/kg/day) indicating similarity in response between species. It is interesting to compare the empirical results presented in Table 5-1 with the BMD model results in Table 5-3. For the six data sets modeled, a NOAEL was not identified in four and was 0.06, 0.3, and 0.56 mg/kg/day in the remaining three studies. The LOAEL was 1 mg/kg/day in four studies and 0.64, 1.72, and 3.0 mg/kg/day in the others. The $BMDL_{10}$ values all fall below the experimental LOAELS.

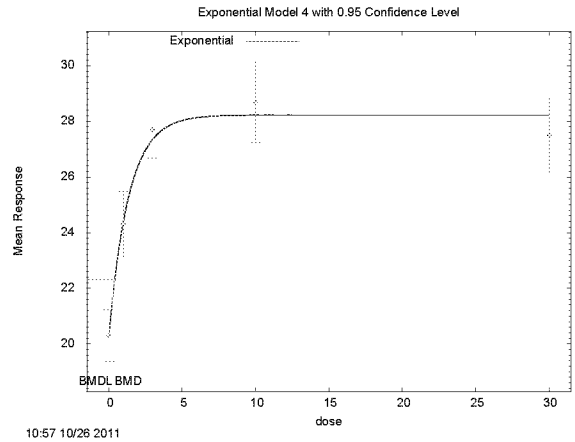
The model results shown in Table 5-3 were chosen based on goodness-of-fit criteria as described above. The modeling output for all studies is included in Appendix 1. A subset of the modeled data is used in the RfD derivation (section 3.1.1.3). Figure 5.1 provides the graphic results from those datasets which were selected as critical for the RfD derivation. Data from the F_1 generation male rats (York, 2002; Butenhoff et al., 2004a; York et al., 2010) were not used because of uncertainties about the timing for the developmental changes in the renal tubular transporters that differentiate male rats from female rats (see Section 3.4). The developmental toxicity study in female mice (Lau et al., 2006) supports the endpoint of liver weight effects and is included in Figure 5-1 despite the limitation posed by its 17-day, relatively short exposure duration.



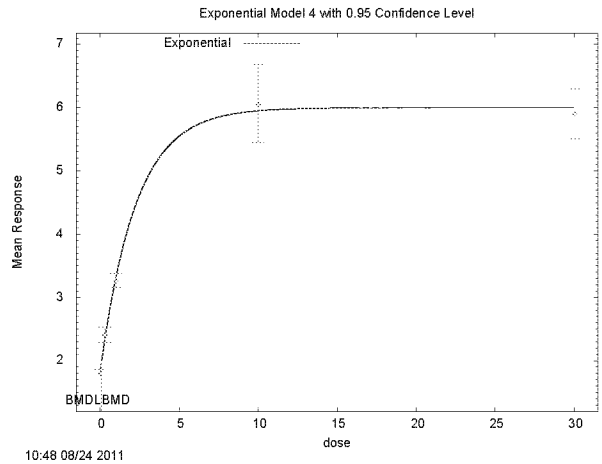
Male Rat (Palazzolo, 1993; Perkins et al., 2004)



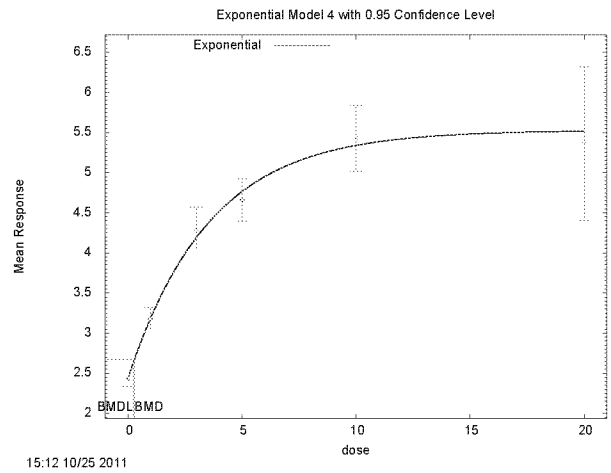
Male Rat (Loveless et al., 2008)



F_0 Male Rat (Butenhoff et al., 2004a; York, 2002; York et al., 2010)



Male Mouse (Loveless et al., 2008)



Female mouse (Lau et al., 2006)

FIGURE 5-1. BMDS graphic output from selected model runs.

5.1.1.2 Pharmacokinetic Model approach

In linking chemical exposure to toxic endpoints, careful consideration of the pharmacokinetics is crucial. This is especially true for PFOA, where inter-species and gender variation in clearance half-life can vary by several orders of magnitude. If the toxicological endpoints are assumed to be driven by internal concentrations, then it is the internal exposure that must be calculated and considered across species. Differences in pharmacokinetics (*e.g.*, male rats excrete PFOA less quickly than females) and differences across species produce differences in the external dose needed to achieve the same internal dose.

PFOA has been found to have dose-dependent kinetics. Although repeated doses rapidly result in quasi-equilibrium blood concentrations, a single dose results in a much longer half-life than would be consistent with a rapid approach to quasi-equilibrium (Andersen et al. 2006, Lou et al. 2009). Using a simple, linear pharmacokinetic model (e.g. a one- or two-compartment model) to predict internal dose resulted in estimated exposures due to repeated doses that were greater than actually seem to occur (Butenhoff et al. 2004, Lou et al. 2009).

The saturable renal resorption model (Andersen et al. 2006: Section 3.5.1) has been used to describe how PFOA can reach steady state faster than the elimination half-life would indicate. The mechanism for this phenomenon is suggested to be the result of saturable resorption from the kidney filtrate: Unbound compound in plasma is filtered at the kidney glomerulus and passes to the proximal tubules where it is reabsorbed by transporters and re-enters systemic circulation. If the amount of compound in the filtrate exceeds the ability of the transporters to reabsorb it, it is rapidly excreted so that additional doses do not affect the steady state serum levels.

In the Lou et al. (2009) report, the one and two compartment models were able to reasonably predict the serum levels for single 1 and 10 mg/kg doses, but not at the high dose of 60 mg/kg/day where excretion exceeded transporter resorption capability and serum values declined more rapidly than predicted based on the responses at 1 and 10 mg/kg/day (Section 3.5.1). Elimination from the body was much faster at the high dose than it was for the lower doses. This behavior is consistent with the saturable resorption model: at low doses excretion becomes balanced with uptake. Thus, plasma levels remain at steady state as long there is no increase in transporter density in the membrane of the renal tubule and dosing is constant. When a dose was large enough to exceed resorption capacity, the excretion rate increases but simple one- and two-compartment models do not allow for excretion rate changes. In Lou et al. (2009) the addition of a saturable resorption component to the model greatly improved the fit for the 60 mg/kg single dose and 20 mg/kg/day repeat doses, while still describing the 1 and 10 mg/kg doses.

The biological basis for the saturable resorption model parameters is still uncertain (Lou et al., 2009), particularly with respect to the idea that glomerular filtration alone is responsible for the movement of PFOA into the renal filtrate given that PFOA is highly protein bound (Kerstner-Wood et al., 2004) and export transporters are located along the apical and basal membranes of the renale tubular cells. Protein binding kinetics will impact the levels available for loss through glomerular filtration because the amount of free PFOA in serum will vary with the saturation of serum protein-binding sites. Some loss of PFOA to the renal filtrate will also

occur by way of tubular export transporters. However, the qualitative behavior of the saturable resorption model is excellent (Andersen et al., 2006; Lou et al., 2009).

The saturable resorption model is an empirical model similar to the commonly used one- and two-compartment models. Due to the empirical nature of the saturable resorption mechanism present in the Tan et al. (2008) and Loccisano et al. (2011, 2012a,b) models, no existing model for PFOA PK allows extrapolation between species. Resorption parameters cannot be directly linked to the properties of the relevant serum transport proteins or membrane transporters. Therefore the saturable resorption model parameters must be estimated using species-specific pharmacokinetic data.

Pharmacokinetic data (serial blood concentrations following treatment with known quantities of PFOA) were collected for three species: cynomolgus monkey (Butenhoff et al., 2004), Sprague Dawley rat (Kemper et al., 2003), and mouse. Data were available for two strains of mouse and were analyzed separately: CD1 (Lou et al., 2009) and C57BL6/N (Dewitt et al., unpublished). Due to the pronounced difference in the pharmacokinetics of male and female rats, the two genders were fit separately. For mice, only female data were used. For monkeys a limited amount of female data was used jointly with male data, assuming the only difference between the genders for monkey was bodyweight.

The available pharmacokinetic studies were not designed with the expectation of non-linear pharmacokinetics. Accordingly, many parameters associated with saturable resorption may be uncertain. For example, the Kemper et al. (2003) studies (Section 3.3) focused upon single doses, although the relatively large highest dose (25 mg/kg) may be sufficient to cause non-linearities such as that observed for a 60 mg/kg dose in CD1 mice (Lou et al., 2009) described above. In the absence of ideal data, the question of parameter values becomes one of what range of values is consistent with the data, rather than which single value is most consistent. For the monkey, some of the data came from an intravenous administration study in males and females (Butanoff et al., 2004b). Considering a range of possible values that might equally well explain the data can be addressed with Bayesian statistical analysis. In this case the Bayesian analysis was used to determine parameter distributions for all five data sets considered.

A non-hierarchical model for parameter values has been assumed; i.e. there is a single value shared by all individuals of the same species/strain and gender (except for rat, where there is a clear gender difference in renal excretion of PFOA). Body-weight and treatment (number and magnitude of doses) are the only parameters that may vary between individuals. However, with the exception of the Kemper et al. (2003) data, individual bodyweights were not available so that a default bodyweight was used for each species and gender. In model development it was assumed that animals were sacrificed at the end of the external exposure; i.e. there was no continued internal exposure after dosing had stopped.

Bayesian analysis allows formal inclusion of prior knowledge in the form of set distributions on the parameters being estimated (Gelman et al., 2004). Given that empirical pharmacokinetic parameters can have a wide range of values, vague, bounded prior distributions are appropriate (Wambaugh et al., 2008). For all estimated parameters the prior knowledge values were assumed to be distributed log-normally. This constrained the parameters to positive values. The mean and variances assumed for the CD1 mice, male and female Sprague-Dawley

rats, and Cynomolgus monkeys are given in Table 5-4. For the C57BL/6N mice the available data were insufficient to achieve a converged statistical analysis, so the posterior parameter distributions for the CD1 mouse were used, with the variances increased ten-fold.

TABLE 5-4. Description of prior distributions used to analyze all but the C57BL/6N mice

			Bounds	
Parameter	**Mean**	**Variance**	**Lower**	**Upper**
k_a	1	1000	1/6	10^5
V_{cc}	1	1000	10^{-10}	10^5
k_{12}	1	1000	10^{-10}	10^5
$R_{V2:V1}$	1	0.5	10^{-10}	100
T_{maxc}	1	1000	10^{-10}	10^5
k_T	1	1000	10^{-10}	10^5
free	0.01	0.5	10^{-10}	1
Q_{filc}	1	1000	0.01	10^5
V_{filc}	1	1000	10^{-10}	10

Parameters were log-normally distributed with the mean and variance listed. Bounds were used to reduce the time spent sampling in areas thought to have low probability, and were expanded when large posterior mass at the bounds indicated that the bounds were too narrow. For the C57BL/6N the posterior parameter distributions for the CD1 mouse was used, with the variances increased ten-fold.

The deep tissue compartment of the Andersen *et al.* (2006) model is characterized in terms of the rates to and from that compartment (k_{12} and k_{21}, respectively). This corresponds to a volume of distribution $V_2 = k_{12}*V_1/k_{21}$, so that the ratio of the volume of the second compartment to the first is equal to the ratio of k_{12} to k_{21} ($R_{v2:v1} = k_{12}/k_{21}$). In order to enforce the assumption that the primary (serum) compartment contains a significant portion of the PFOA, it was assumed that the volume of the deep tissue compartment was constrained to be no more than 100 times greater than the volume of distribution of the serum. For this reason the ratio of the two volumes is estimated, rather than the rate from the second compartment to the first compartment. The rate of flow from the deep tissue back to the serum was calculated as $k_{21} = k_{12}/R_{v2:v1}$.

Bayesian analysis was performed using Markov Chain Monte Carlo (Gelman *et al.* 2004). The crux of a Bayesian analysis is the assessment of whether the distributions of values in the Markov Chain reflect the posterior distribution of the statistical model given the priors and the data. The distribution of parameter values were considered to "burned in" (*i.e.*, true draws from the posterior combination of the prior assumptions and the available data which were independent of the assumed starting values) when they passed the Heidelberger and Welch Stationarity test (Heidelberger and Welch 1983) as implemented in the Coda Package (Best *et al.* 1995).

For all pharmacokinetic parameter values (Table 5-5) a mean and a 95% interval are reported, summarizing the distribution of parameter values found to be consistent with the available data. Parameters such as the flow to and volume of the filtrate compartment were found to be very uncertain as in Anderson et al. (2006) and Lou et al (2009). Between male and female rats parameters were largely similar; including the affinity of the putative resorption transporters but the maximum resorption transport rate was nearly 200 times greater for the males. The median fraction of blood flow to the filtrate (Q_{filc}) was physiologically reasonable (less than or equal to the fraction of blood flow to the kidney) in all cases. In contrast the Lou et al. (2009) model allowed an unreasonably high portion of cardiac output to pass through the kidney in

order to optimize the fit to the data. Although not totally based on biology, the form of the saturable resorption model seems to be an appropriate framework for the projection of internal PFOA serum dose.

TABLE 5-5. Estimated and assumed pharmacokinetic parameters used in model development

Parameter	Units	CD1 Mouse Female (Lau et al., 2009)	C56BL/6N Mouse Female (DeWitt et al. unpublished)	Sprague Dawley Rat Female (Kemper et al., 2003)	Sprague Dawley Rat Male (Kemper et al., 2003)	Cynomolgus Monkey male and female (Butenhoff et al., 2004)
Body Weight[a]	kg	0.025	0.025	0.20 (0.16 – 0.23)a	0.24 (0.21 – 0.28)a	7 (m) 4.5 (f)
Cardiac output[b]	$L/h/kg^{0.74}$	8.68	8.68	12.39	12.39	19.8
k_a	1/h	290 (0.6 – 73000)	340 (0.53 – 69000)	1.7 (1.1 – 3.1)	1.1 (0.83 – 1.3)	230 (0.27 – 73000)
V_{cc}	L/kg	0.18 (0.16 – 2.0)	0.17 (0.13 – 2.3)	0.14 (0.11 – 0.17)	0.15 (0.13- 0.16)	0.4 (0.29 – 0.55)
k_{12}	1/h	0.021 ($3.1x10^{-10}$ – $3.8x10^{4}$)	0.35 (0.058 – 52)	0.098 (0.039 – 0.27)	0.028 (0.0096 – 0.08)	0.0011 ($2.4x10^{-10}$ – $3.5x10^{4}$)
$R_{v2:v1}$	Unitless	1.07 (0.26 – 5.84)	53 (11 – 97)	9.2 (3.4 – 28)	8.4 (3.1 – 23)	0.98 (0.25 – 3.8)
T_{maxc}	M/h	4.91 (1.75 – 2.96)	2.7 (0.95 – 22)	1.1 (0.25 – 9.6)	190 (5.5 – 50000)	3.9 (0.65 – 9700)
k_T	M	0.037 (0.0057 – 0.17)	0.12 (0.033 – 0.24)	1.1 (0.27 – 4.5)	0.092 ($3.4x10^{-4}$ – 1.6)	0.043 ($4.3x10^{-5}$ – 0.29)
Free	Unitless	0.011 (0.0026 – 0.051)	0.034 (0.014 – 0.17)	0.086 (0.031 – 0.23)	0.08 (0.03 – 0.22)	0.01 (0.0026 – 0.038)
Q_{filc}	L/h	0.077 (0.015 – 0.58)	0.017 (0.010 – 0.081)	0.039 (0.014 – 0.13)	0.22 (0.011 – 58)	0.15 (0.02 – 24)
V_{filc}	L/kg	$9.7x10^{-4}$ ($3.34x10^{-9}$ – 7.21)	$7.6x10^{-5}$ ($2.7x10^{-10}$ – 6.4)	$2.6x10^{-5}$ ($2.9x10^{-10}$ – 28)	0.0082 ($1.3x10^{-8}$ – 7.6)	0.0021 ($3.3x10^{-9}$ – 6.9)

Estimated and assumed pharmacokinetic parameters for the Andersen et al. (2006) model. Means and 95% interval from Baysian analysis are reported. For some parameters the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values). As part of the Bayesian formalism, it is the posterior distribution of parameter values, as opposed to the mean values, that is used to make the predictions.
a Estimated average bodyweight for species used except with Kemper study where individual rat weights were available and assumed to be constant.
b Cardiac outputs obtained from Davies and Morris (1993)

If there is large uncertainty for parameters that greatly influence the predicted value, then the distribution of predicted values will be wide. If the quantity being predicted is not sensitive to the uncertain parameter, then the distribution of the predicted value will be unaffected (Wambaugh *et al.* 2008). Assessing parameter uncertainty allows appropriate confidence in model predictions.

For each study with a toxicological endpoint and LOAEL, the AUC and final serum concentrations were determined for the exposure duration investigated in that study. These values are summarized in 8-6 for rats, 8-7 for mice, and 8-8 for monkeys. In order to make a rough assessment of the validity of the model predictions, a final serum concentration was predicted for each treatment so that it could be compared to measured serum values. The

predicted final serum concentration is the estimate for serum concentration at the time of sacrifice. Generally these values were similar. They differed by a factor of four when strains were different and closer to a factor of two when predicting using parameters from the same strain. Because these predictions do not perfectly match the measured serum concentrations, there remains uncertainty about the exposure estimates that has not been fully characterized.

TABLE 5-6. Predicted Final Serum Concentration and Time-integrated Serum Concentration (AUC) for Studies in Rats

Study	Species/ Strain	Exposure Duration	Oral Doses mg/kg/day	Measured Final Serum value µg/ml	Species/ Strain Used for Prediction	Predicted Final Serum Value µg/ml	Predicted AUC mg/L*h
Palazzolo, 1993; Perkins et al., 2004	Rat (M) ChR-CD	13 weeks Diet	0.06 0.64 1.94 6.50	7.1 (1.2) 41 (13) 70 (16) 138 (34)	Rat (M) Sprague-Dawley	3.8 (0.0955) 34.8 (0.865) 79.5 (3.84) 139 (13.1)	7310 (188) 69900 (1640) 170000 (6770) 331000 (27100)
York, 2002; Butenhoff et al., 2004a; York et al., 2010	Rat (M) Sprague-Dawley	F0M: 10 wk pre mating-mating Oral gavage	1 3 10 30	NT NT 51.5 [s] 45.3	Rat (M) Sprague-Dawley	49.9 (1.53) 102 (6.5) 153 (17.3) 169 (27.7)	93500 (2460) 207000 (11000) 351000 (35100) 422000 (74600)

Numbers in parentheses indicate standard deviation
M= male; s=serum; NT=not tested
Since the Kemper (2003) data were not tied to toxicological endpoints and were only used in model development they are not included in this table.

TABLE 5-7. Predicted Final Serum Concentration and Time-integrated Serum Concentration (AUC) for Studies in Mice							
Study	**Species/ Strain**	**Exposure Duration**	**Oral Doses mg/kg/d**	**Measured Serum Value µg/ml**	**Species/ Strain Used for Prediction**	**Predicted Final Serum Value µg/ml**	**Predicted AUC mg/L*h**
Wolf et al., 2007; White et al., 2009	Mouse (F) CD-1	GD1-17[a] Oral gavage	3 5	NT NT	Mouse (F) CD-1	25 (2.22) 25.6 (2.26)	31700 (1740) 38600 (2050)
		GD7-17 Oral gavage	5	24.8	Mouse (F) CD-1	29 (2.55)	23300 (1160)
DeWitt et al., 2008	Mouse (F) C57BL/6N	15 days Drinking water	0.94 1.88 3.75 7.5 15 30	NT[b] NT[b] 35.3 42.8 50.0 162.6	Mouse (F) C57BL/6N	29.7 (1.58) 51.9 (1.89) 70.2 (2.57) 81.4 (3.91) 94.7 (11.8) 117 (29.3)	7320 (557) 13800 (952) 22400 (1330) 30500 (1520) 40100 (4470) 55900 (12000)
Lau et al., 2006	Mouse (F) CD-1	GD1-17 Oral gavage	1 3 5 10 20 40	21.9[c] 40.5[c] 71.9[c] 116[c] 181[c] 271[c]	Mouse (F) CD-1	57.6 (3.82) 87.2 (7.93) 95.2 (7.41) 106 (5.84) 121 (11) 148 (30.2)	16500 (1010) 33600 (2690) 40900 (2950) 51100 (2810) 65400 (6480) 90400 (17500)

Numbers in parentheses indicate standard deviation
NA = not applicable; could not be determined
F=female; GD = gestation day; s=serum; NT=not tested
[a]Sacrificed on PND 22.
[b]DeWitt (2008) had 0.94 and 1.88 mg/kg/day dose groups in a second experiment.
[c]The Lau et al. (2006) serum data were provided by the author for animals treated GDs 1-17.

TABLE 5-8. Predicted Final Serum Concentration and Time-integrated Serum (AUC) in Studies of Monkeys							
Study	**Species/ Strain**	**Exposure Duration**	**Oral Doses mg/kg/day**	**Measured Serum value µg/ml**	**Species/ Strain Used For Prediction**	**Predicted Final Serum Value µg/ml**	**Predicted Exposure (AUC) mg/L*h**
Thomford, 2001b, Butenhoff et al., 2002, 2004b	Monkey (M) Cyano-molgus	26 weeks Oral capsule	3 (N=3) 10 (N=4) 30/20 (N=3)	117.9 (87.6-141) 77.35 (55.4-96.5) 283.2 (61.7-489)	Monkey Cyno-molgus	89.1 (12.4) 121 (14) 149 (31)	387000 (50800) 565000 (61100) 710000 (144000)

Numbers in parentheses indicate standard deviation
M= male

The AUC for the LOAEL or NOAEL of each data set can be used to determine an average serum concentration by dividing it by the duration of the study in days with adjustment for the hours in a day. The following equation is used for the conversion:

Average Serum Concentration = AUC (mg/L*h) x 1 day/24 hours ÷ exposure duration (days)

For example, in a case where the AUC was 30,000 mg/L*h and the study duration was 90 days, the Average Serum Concentration would be calculated as follows:

Average Serum Concentration = 30,000 mg/L*h ÷ (90 days x 24 h/day) = 13.89 mg/L

This calculation has the advantage of normalizing the serum concentration across the exposure durations to generate a uniform metric for internal dose in situations where the dosing durations varied and serum measurements were taken immediately prior to sacrifice. The averaged serum concentration is a hybrid of the AUC and the maximum serum concentration. Compared across studies, PFOA average serum concentration appears to be a stable reflection of internal dosimetry.

Table 5-9 provides the AUC from the model, the dosing duration from each of the modeled studies, and the resultant average serum concentration. The internal doses associated with the effect levels (LOAELs) differ by less than an order of magnitude (20.3 mg/L to 88.6 mg/L) while the AUC values differ by over two orders of magnitude (7320 mg/L*h to 387000 mg/L*h). Given the differences in external doses, the projected serum levels are proportionally quite similar.

TABLE 5-9. Average Serum concentrations Derived from the AUC and the duration of Dosing					
Study	**Dosing duration days**	**NOAEL (AUC mg/L*h)**	**NOAEL (Av serum mg/L)**	**LOAEL (AUC mg/L*h)**	**LOAEL (Av serum mg/L)**
Dewitt, 2008 liver	15	None	None	0.94 (7320)	20.33
Dewitt, 2008 Immunotox	15	1.88 (13800)	38.33	3.75 (22400)	62.22
Lau, 2006 liver	17	None	None	1 (16500)	40.44
Palazzolo, 1993 Liver	91	0.06 (7310)	3.35	0.64 (69900)	32.00
Thomford, 2001b; Butenhoff et al., 2004 Liver	182	None	None	3 (387000)	88.60
Wolf, 2007 gd 7-17 Liver	11	None	None	5 (23300)	88.26
Wolf, 2007 gd 1-17 Liver	17	None	None	3 (31700)	77.70
York, 2002 liver	84	none	none	1 (93500)	46.38

Table 5-9 identifies 20.33 mg/L as the lowest concentration associated with an increase in liver weight in female mice (DeWitt et al, 2008). Average serum values for increased relative liver weight in male rats (Palazzolo et al., 1993) and female mice (Lau et al. 2006) are no more than 2-fold higher than the average serum value in DeWitt et al. (2008). Thus, it appears that the LOAELs are roughly consistent across gender, species, and treatment with respect average serum concentration. Assuming that mode of action and susceptibility to toxicity do not vary and that pharmacokinetics alone explains variation, it is reasonable to expect similar concentrations to cause similar effects in humans.

The predicted serum concentrations can be converted into an oral equivalent dose at steady state by recognizing that, at steady state, clearance from the body must equal dose to the body. Clearance can be calculated if the rate of elimination (derived from half-life) and the volume of distribution are both known.

Measures of half-life in humans have been determined for both workers and the general population (Section 3.5.2). Olsen et al. (2007) gives the human half-life as 3.8 years for PFOA in an occupationally exposed U.S. cohort. Bartell et al. (2010) determined a value of 2.3 years based on the decline in serum levels among members of the general population exposed via drinking water in the area near the Dupont Works plant in Washington, WV after the drinking water concentrations decreased. The Office of Water has chosen to use the Bartell et al. (2010) half-life value because it is appears to be the one most relevant to scenarios where exposures result from ingestion of contaminated drinking water by members of the general population.

Thompson et al. (2010) gives a volume of distribution of 0.17 L/kg bw (Section 3.5.3). The volume of distribution is defined as the total amount of PFOA in the body divided by the blood or serum concentration. The volume of distribution was calibrated using human serum concentrations and exposure data from NHANES and assumes that most PFOA intake came

from contaminated drinking water. The value for volume of distribution was calibrated so that the model prediction of elevated blood levels of PFOA was consistent with the values from NHANES. Lack of consideration of PFOA contributions from sources other than drinking water sources is a weakness of the volume of distribution value determined by Thompson et al. (2010), however this estimate is not radically different from the 0.198 L/kg bw determined for the monkey in the study by Buttanhoff et al (2004).

The half life ($t_{½}$) and volume of distribution (V_d) are utilized to calculate the clearance for PFOA according to the following equation assuming first order kinetics for clearance (CL) (Medinsky and Klaassen, 1996):

$$CL = V_d \times (\ln 2 \div t_{1/2}) = 0.17\ L/kg_{bw}/day \times (0.693 \div 839.5\ days) = 0.00014\ L/kg\ bw/day$$

Where:

V_d	=	0.17 L/kg
ln 2	=	0.693
$t_{1/2}$	=	839.5 days (2.3 years x 365 days/year = 839.5 days)

These values combined give a clearance of 1.4×10^{-4} L/kg BW/day.

Scaling the derived average concentrations (in mg/L) for the NOAELs and LOAELs in Table 5-10 gives predicted oral human equivalent doses (HEDs) in mg/kg/day for each corresponding serum measurement. The HED values are the predicted human oral exposures necessary to achieve serum concentrations equivalent to the LOAEL (and NOAEL where available) in the animal toxicity studies. Note that this scaling assumes linear human kinetics in contrast to the non-linear phenomena observed at high doses in animals.

Thus, HED = average serum concentration (in mg/L) x CL

Where:

Average serum is from model output in Table 5-9
CL = 0.00014 L/kg bw/day

TABLE 5-10. Human Equivalent Doses Derived from the Modeled Animal Average Serum Values							
Study	**Dosing duration days**	**NOAEL mg/kg/d**	**NOAEL Av serum mg/L**	**HED mg/kg/d**	**LOAEL mg/kg/d**	**LOAEL (Av serum) mg/L**	**HED mg/kg/d**
Dewitt, 2008 liver	15	None	-	-	0.94	20.33	0.0028
Dewitt, Immunotox	15	1.88	38.33	0.0054	3.75	62.22	0.0087
Lau, 2006 liver	17	None	-	-	1	40.44	0.0057
Palazzolo, 1993 Liver	91	0.06	3.35	0.00047	0.64	32.00	0.0045
Thomford, 2001b liver	182	None	-	-	3	88.60	0.0124
Wolf, 2007 GD 1-17 liver	17	None	-	-	3	77.70	0.0109
Wolf, 2007 gd 7-17 Liver	11	None	-	-	5	88.26	0.0124
York 2002 male F_0 liver	84	None	-	-	1	46.38	0.0065

5.1.1.3 RfD Quantification

There are several acceptable points of departure (PODs) that can be used in the process of identifying the POD for RfD development:

- NOAEL or LOAEL values
- Lower 95% confidence bounds on the BMD (BMDLs), and
- Human Equivalent Doses (HED).

Studies that have more than one POD for the same NOAEL or LAOEL are summarized in Table 5-11. There are very few NOAELs that have been identified for PFOA; LOAELs are the more common benchmark identified through classical toxicological studies. Some of the studies with NOAELs and/or LOAELs are chronic and others are short term. Modeling of dose response to identify a BMD and BMDL was successful for only a subset of studies. All benchmark models targeted a 10% increase in liver weight as a common metric for effect. This selection was not made based on toxicity but on the desire to find a common denominator against which to evaluate dose-response across studies and justified by the fact that other adverse effects accompanied the LOAEL for increased liver weight in some cases.

The subset of studies that were amenable for derivation of HED based on average serum measurements from the pharmacokinetic model was limited because of the need to have dose and species-specific serum values for model input. Most entries in Table 5-11 provide at least two potential PODs for consideration; only one provides five values (Palazzolo et al. 1993).

TABLE 5-11. RfD Point of Departure Options (mg/kg/day) from the PFOA Animal Studies						
Studies	**NOAEL**	**LOAEL**	**$BMDL_{10}$**	**HED NOAEL**	**HED LOAEL**	**Notes**
Dewitt, 2008 female mouse	none	0.94			0.0028	Increased absolute and relative liver weight;
Dewitt, 2008 female mouse	1.88	3.75		0.0054	0.0087	Decreased IgM, increased IgG, decreased absolute and relative spleen weights;
Lau, 2006, GD 1-17, mouse	none	1	0.247		0.0057	Increased maternal liver weight
Loveless et al., 2008 male rat	1	10	0.152			Increased liver weight, focal liver necrosis
Loveless et al., 2008 mouse male	0.3	1	0.0681			Increased liver weight, liver hypertrophy with single cell and focal necrosis
Palazzolo et al, 1993 male rat	0.06	0.64	0.456	0.00047	0.0045	Increased liver weight, hepatocellular hypertrophy
Wolf, 2007 gd 7-17 Liver	None	5			0.0109	Increased maternal relative liver weight 23 days after last dose, reduced male pup body weight at birth
Wolf, 2007 gd 1-17	none	3			0.0124	Increased maternal absolute and relative liver weight 23 days after last dose
York 2002 male F_0	none	1	0.274		0.0065	Increased liver and kidney weight

As explained previously, human data have identified significant relationships between serum levels and specific indicators of adverse health effects but lack the exposure information for dose-response modeling. For this reason none of the human studies provided an appropriate POD for RfD derivation. The pharmacokinetically-modeled average serum values from the animal studies are restricted to the animal species selected for their low dose response to oral PFOA intakes. Extrapolation to humans adds a layer of uncertainty that must be accommodated in deriving the RfD.

Each of the POD values represented in Table 5-11 requires a different quantification approach. Thus, EPA has systematically examined the impact of POD on outcome through three sets of calculations as follows:

- Derivation from 5 PODs: NOAEL, LOAEL $BMDL_{10}$, HED_{NOAEL}, and HED_{LOAEL} from the same study (Palazzolo et al., 1993)
- Derivation from the $BMDL_{10}$ values
- Derivation from HED values derived from modeled average serum values.

The Palazzolo et al. (1993) study is the only one among the studies selected for consideration as candidates for RfD determination that offers the opportunity to examine the impact of five PODs on outcome. It is the only study with a NOAEL, LOAEL, $BMDL_{10}$, and

HED values. Thus, it is ideally suited for use in examining the impact of quantification approach on the RfD outcome. The critical effect was a significant increase in liver weight accompanied by hepatic hypertrophy and increased palmitoyl Co A oxidase activity. As described earlier, the increase in liver weight was selected as a low dose common biomarker for systemic loss of homeostasis across studies. In other studies, adverse reproductive (Lau et al., 2006) or immunological effects in mice (Loveless et al., 2008) co-occurred in combination with the increased liver weights at the LOAEL justifying selection of the liver as a common metric.

Table 5-12 provides the potential RfDs derived from the five individual Palazzolo et al (1993) PODs.

TABLE 5-12. The Impact of Quantification Approach on the RfD outcome for the PODs from the Palazzolo et al (1993) Study

POD	POD dose mg/kg/day	UF_H	UF_A	UF_L	UF_S	UF_D	UF_{total}	Potential RfD mg/kg/day
NOAEL	0.06	10	219	-	10	-	21400	0.000003
LOAEL	0.64	10	219	10	10	-	214000	0.000003
$BMDL_{10}$	0.456	10	219	-	10	-	21400	0.00002
HED_{NOAEL}	0.00047	10	3	-	-	-	30	0.00002
HED_{LOAEL}	0.0045	10	3	10	-	-	300	0.00002

There is good agreement between the adjusted PODs from the HEDs from the modeled average serum levels and the $BMDL_{10}$ value. The adjusted PODs derived from the NOAEL and LOAEL are about an order of magnitude more conservative than those from the $BMDL_{10}$ and HEDs. The differences in outcome reflect the differences in the quantification approach rather than the experimental data utilized. The $BMDL_{10}$ value more closely approximates the study LOAEL rather than the study NOAEL.

Uncertainty Factor (UF) Application.

UF_H A ten-fold adjustment is assigned to account for intrahuman variability and applied for all PODs.

UF_A. Determination of the interspecies uncertainty factor for the NOAEL, LOAEL and BMDL10 requires application of the equation for first order kinetics in order to determine the pharmacokinetic adjustment associated with differences in half-life between humans and male rats. The equation that describes first order kinetics is as follows (Medinksy and Klaassen, 1996):

$$CL = V_d \times (\ln 2 \div t_{1/2})$$

Where:

$V_d = 0.17$ L/kg
Ln 2 = 0.693
Half-life = 11.5 days for rats and 839.5 days for humans

$CL_{rat} = 0.17$ L/kg x (0.693 ÷ 11.5 days) = 0.01024 L/kg/day

CL_{human} = 0.17 L/kg ÷ (0.693 ÷ 839.5 days) = 0.00014 L/kg/day

The ratio of CL_{rat} to CL_{human} (0.01024 L/kg/day ÷ 0.00014 L/kg/day = 73) is used as the pharmacokinetic adjustment for differences between species. The total UF_A requires an additional 3-fold factor for species differences in pharmacodynamics (73 x 3 = 219).

UF_L A ten-fold UF was applied for the LOAEL and HED_{LOAEL} to account for using an effect level as the POD. A UF of 1 was used for the $BMDL_{10}$ and the NOAEL-derived PODs following Agency policies.

UF_S A ten-fold factor was applied to the NOAEL, LOAEL, and $BMDL_{10}$ PODs to account for less than lifetime exposure. The HEDs reflect steady state serum values from the pharmacokinetic model and reduce the UF_S to 1 for the HED PODs.

UF_D In all cases the uncertainty factor for the strength of the database (UF_D) is 1. The data base for oral PFOA exposure studies is essentially complete although mechanistic questions relative to the MOA have not yet been fully elucidated.

$BMDL_{10}$ PODs. The lowest, species-specific benchmark dose values in Table 5-11 are those from the Loveless et al. (2008) study in the male mouse and male rat. As was the case with the Palazzolo et al. (1993) study, the effect modeled was a 10% change in liver weight. In this case, the LOAELs from the Loveless et al. (2008) publication for both species included co-critical focal hepatic cellular necrosis as well as decreased spleen weight for the mouse. The $BMDL_{10}$ for the mice was lower than that for the rats (Table 5-11).

The data for the York et al. (2002) and Palazzolo et al. (1993) are included in Table 5-13 for comparison with the Loveless et al. results in the rat. In York et al. (2002), the change in liver weight was accompanied by a change in kidney weight. A UF_L of 10 was applied to account for the intraindividual variability.

TABLE 5-13. The Impact of Quantification Approach on the RfD Outcome for PODs from the BMDLs

POD	Dose mg/kg/day	UF_H	UF_A	UF_L	UF_S	UF_D	UF_{total}	Potential RfD mg/kg/day
$BMDL_{10\ rat\ Loveless}$	0.152	10	219	-	10	-	21900	0.000007
$BMDL_{10\ mouse\ Loveless}$	0.0681	10	150	-	10	-	15000	0.000005
$BMDL_{10\ rat\ Palazzolo}$	0.456	10	219	-	10	-	21900	0.00002
$BMDL_{10\ rat\ York}$	0.274	10	219	-	10	-	21900	0.00001

The $BMDL_{10}$ values from the Loveless et al. (2008) 29-day studies are lower than those from the longer-term Palazzolo et al. (1993) and York et al. (2002) studies but differ by a maximum factor of 4 which is relatively small when considering the magnitude of the total uncertainty factors.

Uncertainty Value Application

The UF_H, UF_L, UD_S and UF_D values are assigned as described for the Palazzolo et al. (1993) data in Table 5-11.

The UF_A for the rat component of the Loveless et al (2008) study is the same as that described above for the Palazzolo et al (1993) study. It is necessary to derive the pharmacokinetic component of UF_A for the mouse and monkey using half-life and volume of distribution to calculate the clearance ratio as was done for the rat:

Mouse
CL_{mouse} = 0.17 L/kg x (0.693 ÷ 15.6 days) = 0.00697 L/kg/day
CL_{human} = 0.17 L/kg x (0.693 ÷ 839.5 days) = 0.00014 L/kg/day

The ratio of CL_{mouse} to CL_{human} (0.00697 L/kg/day ÷ 0.00014 L/kg/day = 50) is used as the pharmacokinetic adjustment for differences between species. Thus, the total UF_A adds a 3-fold factor for species differences in pharmacodynamics between mice and humans (50 x 3 = 150).

HED PODs. The HEDs derived from Palazzolo et al. (1993), Dewitt et al. (2008), Lau et al. (2006), Thomford (2001), and York et al. (2002) were each examined as the potential basis for the RfD. Only two of these studies, Palazzolo et al. (1993) and Dewitt et al., (2008), contained a NOAEL from which the HED could be derived. Because the immunological effects which were the basis of the NOAEL in the Dewitt et al. study occurred at a dose about four-fold higher than the effects on liver weight, this value is not included in Table 5-14. The Dewitt et al. (2008) LOAEL HED based on increased liver weight has the lowest quantitative values from among these studies. LOAEL HEDs from the other studies are about twice the Dewitt et al. (2008) value. The Lau et al. (2006) LOAEL HED included reduced fetal ossification; accelerated puberty in male offspring as co-critical effects. The York et al. (2001) study included increased kidney weight as co-critical at the LOAEL and the Palazzolo et al. (1993) study reported hepatic hypertrophy and increased palmitoyl Co A oxidase activity.

TABLE 5-14. The Impact of Quantification Approach on the RfD Outcomes for the HEDs from the Pharmacokinetic Model Average Serum Values

POD	Value mg/kg/day	UF_H	UF_A	UF_L	UF_S	UF_D	UF_{total}	Potential RfD mg/kg/day
$PK\text{-}HED_{NOAEL\ Palazzolo}$	0.00047	10	3	-	-	-	30	0.00002
$PK\text{-}HED_{LOAEL\ Palazzolo}$	0.0045	10	3	10	-	-	300	0.00002
$PK\text{-}HED_{LOAEL\ Dewitt}$	0.0028	10	3	10	-	-	300	0.000009
$PK\text{-}HED_{LOAEL\ Lau}$	0.0057	10	3	10	-	-	300	0.00002
$PK\text{-}HED_{LOAEL\ York}$	0.0065	10	3	10	-	-	300	0.00002
$PK\text{-}HED_{LOAEL\ Thomford}$	0.0124	10	3	10	-		300	0.00004

The outcomes for potential RfD values as determined in Tables 5-11, 5-12, and 5-13 differ by about an order of magnitude (0.000003 mg/kg/day to 0.00004 mg/kg/day). Of the values derived from the HED values one is 0.000009 mg/kg/day (Dewitt et al., 2008) and 4 are 0.00002 (Palazzolo et al., 1993; Lau et al., 2006; York et al., 2002). These results demonstrate the ability of the model to normalize the animal data across species, strain, gender, and exposure duration.

The Palazzolo et al. (1993) and York et al. (2002) studies were conducted in male Sprague Dawley rats with durations of 91 days via diet and 84 days via gavage, respectively. The Lau et al. (2006) study was conducted in pregnant female rats with an 18-day average exposure via gavage leading to increased liver weights in the dams and developmental effects in their male offspring. The Dewitt et al. (2008) drinking water study was conducted in female C57BL/6 mice with a 15-day exposure. The Thumford (2001b) study was conducted in male cynomolgus monkeys for 182 days; the PFOA was administered in capsules.

Uncertainty Value Application

The UF_H, UF_L, UF_S and UF_D values are assigned as described for the HED LOAEL entry for the Palazzolo et al. (1993) data in Table 5-12.

The UF_A is 3 for each study because the HED was derived using the steady state serum values from the pharmacokinetic model. The 3-fold factor is applied to account for toxicodynamic differences between the animals and humans.

RfD Selection Based on the consistency of the response and with recognition of the use of liver weight having been chosen as a common denominator in the RfD analysis for loss of homeostatisis and protection against co-occurring adverse effects, the 0.00002 mg/kg/day outcome is selected as the RfD for PFOA. This value is the outcome for all modeled rat and mouse serum values except for the Dewitt et al. (2008) 15-day study with an impact on liver weight but not the co-monitored immunological effects. The liver endpoint in the Lau et al. (2006) and York et al. (2002) studies were accompanied by developmental effects and effects on kidney weights, respectively. The modeled serum value from Thumford (2001) based on liver effects in the monkey, also strongly supports the chosen RfD.

The results from the modeled serum values are supported by the benchmark dose results wherein the Palazzolo et al. (1993) POD supports a potential RfD of 0.00002 mg/kg/day for change in liver weight. Other than Lau et al. (2006) all of the studies supporting an RfD of 0.00002 mg/kg/day are studies with exposures to PFOA for $\geq$ 84 days meeting the duration requirements for determination of a lifetime exposure value. Confidence in the RfD is medium because of the uncertainties surrounding mode of action for some of the observed effects and the role of saturable renal resorption in determining the body burden from chronic exposures.

5.1.2 RfC Determination

Derivation of the RfC requires evaluation of toxicity data for concentration-response and identification of target organs and noncancer effects. Limited data from human epidemiology and animal toxicity studies were available with which to evaluate the potential health effects resulting from continuous inhalation exposure to PFOA. The available data base, summarized below for human and animal data, does not meet the minimal requirements for derivation of the RfC as defined by US EPA (1994). Thus, the RfC for PFOA is not recommended or derived.

Human Data. Studies have examined occupational and residential populations at or near large-scale PFOA production plants in the United States in an attempt to determine the relationship between serum PFOA concentration and various health outcomes suggested by the standard

animal toxicological database. While inhalation is an important route of exposure to workers, drinking water was identified as the primary contributor to exposure in the general population. In all of the epidemiology studies, wide ranges of serum levels were reported which are likely a reflection, not only of intra-human toxicokinetic variability, but also of diversity in external exposure sources and routes of exposure. Thus the data cannot be clearly applied to quantification of dose-response via inhalation

Animal Data. Inhalation toxicity data in laboratory animals were limited to acute exposure, single and repeated exposures for pharmacokinetic studies, and a developmental toxicity study in rats. No subchronic or chronic inhalation toxicity studies in animals were available for assessment. Generally, adverse effects observed following inhalation exposure to PFOA were similar to effects following exposure to an irritating dust. For male rats exposed to PFOA as a dust in air, the 4-hour LC_{50} was 980 mg/m^3 with adverse clinical signs of body weight loss, irregular breathing, red discharge around the nose and eyes, and corneal opacity and corrosion (Kennedy et al., 1986; 2004).

Distinct toxicokinetic differences between male and female rats were found following single and repeated inhalation exposures. Sprague-Dawley rats were exposed nose-only to PFOA aerosols of 0, 1, 10, or 25 mg/m^3 for 6 hours or for 6 hours/day, 5 days/week, for three weeks (Hinderliter, 2003). Absorption was indicated in both males and females after single and repeated exposures with plasma PFOA concentrations proportional to exposure concentration. The Cmax values were approximately 2-3 times higher in males than in females and persisted for up to six hours in males compared to just one hour in females. Similarly, the elimination of PFOA was rapid by females at all exposure levels, and by 12 hours after exposure the plasma levels had dropped below the analytical limit of quantitation (0.1 μg/ml). In males, the plasma concentration remained approximately 90% of the peak concentration at all exposure levels at 24 hours after exposure, and steady state was reached following repeated exposures. While these results clearly show toxicokinetic differences between male and female rats, toxicity data were not included for use in quantitative risk assessment.

In a developmental toxicity study, pregnant Sprague-Dawley rats were exposed whole-body to PFOA dust concentrations of 0, 0.1, 1, 10, or 25 mg/m^3 for 6 hours/day on GDs 6-15 (Staples et al., 1984). Dams were either sacrificed on GD 21 or allowed to litter and rear their offspring until PND 35. Maternal toxicity at 10 and 25 mg/m^3 consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, lethargy (high-concentration group only), and decreased body weight and food consumption. Five out of 24 dams died during treatment at 25 mg/m^3. Significantly increased mean liver weight ($p < 0.05$) was seen at 25 mg/m^3. No effects were observed on the maintenance of pregnancy or fetal and pup survival. At 25 mg/m^3, mean offspring body weight was lower than that of controls on GD 21 and throughout lactation.

5.2 Dose-Response for Cancer Effects

As discussed in section 4.4.4, there is some evidence that PFOA exposure may be associated with an increased risk for cancer from the human epidemiology database and animal studies. The Dupont (2003b) report on the West Virginia Washington Works Plant found statistically significant elevations in cancer of the bladder, kidney, and urinary tract organs and a

nonstatistically significant elevation in leukemia incidence. The findings for an increased incidence of leukemia from two earlier worker studies were statistically significant, but both had methodological limitations (Walrath and Burke, 1989; Karns and Fayerweather, 1991). The mortality data for liver cancer, pancreatic cancer, prostate cancer, and testicular cancer from human epidemiological studies all showed no association to exposure.

The only chronic bioassays of PFOA were conducted in rats (Butenhoff et al., 2012; Biegel et al, 2001). The two studies support a positive finding for the ability of PFOA to be weakly tumorigenic in one or more organs of male but not female rats. There are no carcinogenicity data from a second animal species. The study by Butenhoff et al. (2012) examined males and females; the Biegel et al. study only evaluated males. The tumor types observed were:

- Liver (Butenhoff et al., 2012)
- Leydig Cell (Butenhoff et al., 2012; Biegel et al., 2001)
- Pancreatic Ascinar Cell (Biegel et al., 2001)

The dose response information and tumors incidence data from the Butenhoff et al. (2012) and Biegel et al. (2001) study are summarized in Table 5-15, below. The data are limited in that only Butenhoff et al. tested more than one dose and only one tumor-type (Leydig Cell adenoma) demonstrated a dose-response relationship.

TABLE 5-15. Summary of Tumor Data from Animal Studies

Tissue	Concentration in Diet (ppm)			Tumor Type	Reference
	0[a]	30	300		
Liver Male	7/50	2/50	10/50	Hepatocellular carcinoma	Butenhoff et al., 2012
Liver Male	0/80	NT	0/76	Hepatocellular carcinoma	Biegel et al., 2001
Liver Male	2/80	NT	10/76	Hepatocellular adenoma	Biegel et al., 2001
Liver Female	0/50	0/50	2/50	Hepatocellular carcinoma	Butenhoff et al., 2012
Testes Male	0/50	2/50	7/50	Leydig Cell adenomas	Butenhoff et al., 2012
Testes Male	0/80	NT	8/76	Leydig Cell adenomas	Biegel et al., 2001
Pancreas Male	1/80	NT	0/76	Acinar Cell carcinoma	Biegel et al., 2001
Pancreas Male	0/80	NT	7/76	Acinar Cell adenoma	Biegel et al., 2001

[a] The value reported is for the ad libitum control
NT = Not tested

There are some data that provide support for the hypothesis that the PPARα agonism MOA is wholly or partially linked to each of the observed tumor types. The data support a PPARα MOA for the liver tumors, and thus, mitigate concern for their relevance to humans. PPARα is found in human livers and, when activated, is linked through activation to a number of metabolic responses but not to the large scale peroxisome proliferation associated with tumors in rats and other rodent species.

PPARα activation may also play a role in the other tumor types observed, however for the Leydig tumors the PPARα involvement is indirect. The favored hypothesis for the DNA replication errors responsible for induction of Leydig tumors are postulated be a consequence of the following sequence of events:

- Decreased testosterone synthesis
- Increased GnRH and increased levels of leutinizing hormone leading to chronic stimulation of Leydig cells by growth-stimulating mediators including IGF-1, TGF-α, leukotrienes and various free radicals (Clegg et al., 1997; Li et al, 2011).

There are some experimental data that demonstrate systemic effects of PFOA leading to decreased testosterone and increased estradiol as a result of increased activity of aromatase, the cellular enzyme responsible for the metabolic conversion of testosterone to estradiol (Biegel et al., 1995). However, more data to support the relationship of PFOA to intermediate steps in the proposed MOAs are needed.

Current MOA theories for the PACT tumors are linked to the impact of either the mitogenic effects elevated testosterone levels or intestinal tissue hormones (cholecystokinin and/or gastrin) in promoting proliferation of acinar cell preneoplastic foci (Osboourn et al., 1997; Klaunig et al., 2003). PACT tumors are most commonly found in rats but also occur in humans. Because PFOA is associated with decreased rather than increased levels of testosterone the mechanistic link between PFOA exposure and PACT is more likely associated with gastric hormone changes possibly associated with alterations in bile composition. Some of the membrane transporters that are impacted by PFOA function in transport of bile components from the liver to the gallbladder and thereby to the intestines. Cholecystokinen and gastrin stimulate contraction of the gallbladder and release of bile into the intestines. Data to support this hypothesis are not available for PFOA. Minimal data for an effect on bile composition are available for the PPARα activating agent Wyeth-14,643 (Osborn et al., 1997).

Under the EPA 2005 cancer guidelines the evidence for the carcinogenicity of PFOA is considered *suggestive* because only one species has been evaluated and the tumor responses occurred primarily in males. Dose-response data are only available for the Leydig Tumors in one study. In cases where the evidence is suggestive, the Agency generally does not attempt dose-response quantification. However, if the evidence includes a well-conducted study with adequate data to support modeling of dose-response, quantitative analyses may be useful in providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.

The increase in hepatocellular tumors did not show a direct relationship to dose in male rats and was not significantly elevated in either males or females at the high dose when compared to controls. There was a dose-related significant increase in Leydig cell tumors in male rats in the Butenhoff et al. (2012) study which was confirmed by the high dose in the single dose mechanistic study by Biegel et al. (2011). At the high dose (300 ppm in the diet; 14.2 mg/kg/day) tumors were found in 14% of the male rats at the end of 2 years in the Butenhoff et al. (2012) study and 4% at the low dose (1.3 mg/kg/day). In the Biegel et al (2001) study 11% were affected at a dose of 300 ppm in the diet (13.6 mg/kg/day). In each case there were no Leydig cell tumors in the controls. The PACT tumors, only detected in the single dose Biegel et

al (2001) study, do not support quantification.

EPA modeled the dose-response for the Leydig cell tumors from Butenhoff et al. (2012) using the Agency benchmark dose software (BMDS) v 2.3.1. The multistage cancer model was used for a dichotomous dataset to predict the dose at which a 4% increase in tumor incidence would occur. The 4% was chosen as the low-end of the observed response range within the study results. Both the first and second degree polynomials gave identical goodness-of-fit criteria (p value and AIC). Results are shown in Table 5-16.

TABLE 5-16. Model Results for Leydig Cell Tumors (Buttenhoff et al. (2012)		
	BMD (mg/kg/day)	**BMDL (mg/kg/day)**
4% increase	3.51	1.99
4% increase	3.51	1.99
AIC = 62.6936	P = 0.2245	

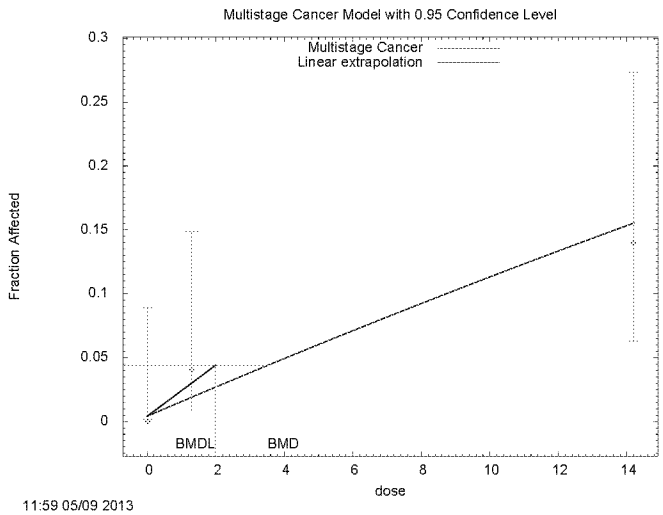


FIGURE 5-2. BMD Model Results for Leydig Cell Tumors (Buttenhoff et al., 2012)

The Cancer Slope Factor (CSF) for PFOS is is derived from the $BMDL_{04}$ of 1.99 mg/kg/day after converting the animal BMDL to a Human Equivalent Dose (HED) using body weights the ¾ power. The HED is calculated as follows:

$$HED = \text{Animal BMDL} \times (\text{animal body weight})^{1/4} \div (\text{human body weight})^{1/4}$$

$$HED = 1.99 \text{ mg/kg/day} \times [(0.523 \text{ kg}^{1})^{1/4} \div (70 \text{ kg})^{1/4}] = 1.99 \text{ mg/kg/day} \times 0.29 = 0.58 \text{ mg/kg/day}$$

[1] Body weight for male Sprague Dawley rats (chronic Exposures) U.S. EPA, 1988

Where:

1.99 mg/kg/day	=	$BMDL_{04}$ for Leydig Cell tumors
0.29	=	The dosimetric adjustment factor

The CSF is calculated from the $BMDL_{04}$ HED as follows

CSF = response ÷ $BMDL_{04}$ HED

CSF = 0.04 ÷ 0.58 mg/kg/day = 0.07 $(mg/kg/day)^{-1}$

The CSF should not be used at doses greater than 0.58 mg/kg/day which is the human equivalent dose corresponding to the point of departure for the 4% incidence of Leydig Cell tumors follow lifetime exposure to PFOA. The observed dose-response relationships do not continue linearly above this level and the fitted dose-response models better characterizes the dose-response for the higher exposures. The HED of 0.58 mg/kg/day is nearly 30,000-fold greater than the RfD (0.58 mg/kg/day ÷ 0.00002 mg/kg/day = 29,000). Thus, the proposed RfD, based on increased liver weight as a common denominator for loss of homeostasis and protection of co-occurring effects, will also be protective of Leydig cell tumors.

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APPENDIX A

Contents: Benchmark Dose Modeling Output Files 10% increased liver weight for:

1) Palazzolo, 1993; Perkins et al., 2004: male rat;
2) Loveless et al., 2008: male rat;
3) York, 2002; Butenhoff et al., 2004a; York et al., 2010: male rat;
4) Loveless et al., 2008: male mouse;
5) Lau et al., 2006: female mouse

1) Palazzolo, 1993; Perkins et al., 2004: male rat

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        Exponential Model. (Version: 1.7;  Date: 12/10/2009)

                                                  Thu Aug 18 07:37:14 2011
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 BMDS Model Run
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact
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Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
Lnalpha	-11.0843	-11.0843	-11.0843	-11.0843
rho	4.39551	4.39551	4.39551	4.39551
a	19.3889	19.3889	17.1285	17.1285
b	0.0525111	0.0525111	0.329637	0.329637
c	--	--	1.64165	1.64165
d	--	1	--	1

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Lnalpha	-10.3398	-10.3398	-11.3983	-11.4672
rho	4.17399	4.17399	4.50168	4.523
a	19.3333	19.3333	18.8392	18.9098
b	0.055133	0.055133	0.281713	0.393804
c	--	--	1.49669	1.42426
d	--	1	--	1.2698

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	15	19.73	2.01
0.06	15	18.03	2.81
0.64	15	20.44	2.87
1.94	15	22.74	4.21
6.5	15	26.78	5.47

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	19.33	2.75	0.5588
	0.06	19.4	2.769	-1.913
	0.64	20.03	2.96	0.5396
	1.94	21.52	3.437	1.379
	6.5	27.67	5.809	-0.5906
3	0	19.33	2.75	0.5588
	0.06	19.4	2.769	-1.913
	0.64	20.03	2.96	0.5396
	1.94	21.52	3.437	1.379
	6.5	27.67	5.809	-0.5906
4	0	18.84	2.482	1.39
	0.06	19	2.529	-1.479
	0.64	20.38	2.964	0.07453
	1.94	22.78	3.806	-0.03971
	6.5	26.7	5.441	0.05896
5	0	18.91	2.496	1.273
	0.06	18.98	2.516	-1.46
	0.64	20.19	2.894	0.3352
	1.94	22.99	3.882	-0.2494
	6.5	26.64	5.416	0.1028

Other models for which likelihoods are calculated:

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-132.6899	6	277.3799
A2	-123.7484	10	267.4967
A3	-125.6728	7	265.3456
R	-153.0718	2	310.1437
2	-129.1329	4	266.2658
3	-129.1329	4	266.2658

4	-126.8109	5	263.6218
5	-126.6814	6	265.3627

Additive constant for all log-likelihoods = -68.92. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	58.65	8	< 0.0001
Test 2	17.88	4	0.001301
Test 3	3.849	3	0.2783
Test 4	6.92	3	0.07449
Test 5a	6.92	3	0.07449
Test 5b	0	0	N/A
Test 6a	2.276	2	0.3204
Test 6b	4.644	1	0.03116
Test 7a	2.017	1	0.1555
Test 7b	4.903	2	0.08616
Test 7c	0.2591	1	0.6108

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

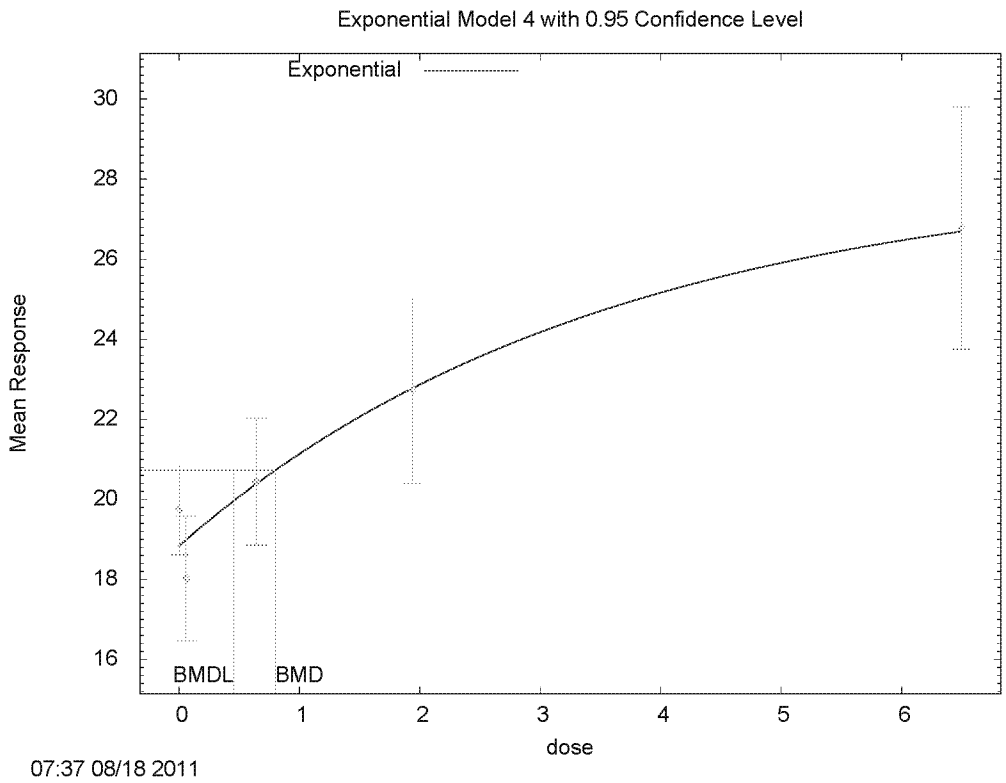
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	1.72873	1.34495
3	1.72873	1.34495
4	0.798012	0.456224
5	0.902365	0.468724



2) Loveless et al., 2008: male rat

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        Exponential Model. (Version: 1.7;  Date: 12/10/2009)

                                                  Thu Dec 22 12:43:43 2011
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 BMDS Model Run
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.

   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
Lnalpha	-7.96162	-7.96162	-7.96162	-7.96162
rho	3.3934	3.3934	3.3934	3.3934
a	15.5663	15.5663	12.54	12.54
b	0.00879874	0.00879874	0.0515133	0.0515133
c	--	--	1.80024	1.80024
d	--	1	--	1

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Lnalpha	-4.07115	-4.07115	-10.0648	-10.3628
rho	2.30379	2.30379	4.14318	4.24407
a	15.7112	15.7112	13.0813	13.1947
b	0.00896759	0.00896759	0.861545	0.977136
c	--	--	1.5326	1.51565
d	--	1	--	1.36232

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	13.2	1.4
0.3	10	14.4	1.6
1	10	17.2	2.9
10	10	21.5	2.9
30	10	18.7	2.9

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	15.71	3.118	-2.547
	0.3	15.75	3.128	-1.368
	1	15.85	3.15	1.352
	10	17.19	3.457	3.947
	30	20.56	4.251	-1.385
3	0	15.71	3.118	-2.547
	0.3	15.75	3.128	-1.368
	1	15.85	3.15	1.352
	10	17.19	3.457	3.947
	30	20.56	4.251	-1.385
4	0	13.08	1.342	0.2798
	0.3	14.67	1.701	-0.4985
	1	17.1	2.339	0.1288
	10	20.05	3.249	1.414
	30	20.05	3.25	-1.312
5	0	13.19	1.34	0.01248
	0.3	14.36	1.604	0.07811
	1	17.42	2.416	-0.2836
	10	20	3.24	1.466
	30	20	3.24	-1.268

Other models for which likelihoods are calculated:

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
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Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-66.95077	6	145.9015
A2	-62.37207	10	144.7441
A3	-63.18657	7	140.3731
R	-91.38557	2	186.7711
2	-86.12482	4	180.2496
3	-86.12482	4	180.2496
4	-65.31811	5	140.6362

5	-64.98792	6	141.9758

Additive constant for all log-likelihoods = -45.95. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	58.03	8	< 0.0001
Test 2	9.157	4	0.05728
Test 3	1.629	3	0.6528
Test 4	45.88	3	< 0.0001
Test 5a	45.88	3	< 0.0001
Test 5b	-1.563e-012	0	N/A
Test 6a	4.263	2	0.1187
Test 6b	41.61	1	< 0.0001
Test 7a	3.603	1	0.05769
Test 7b	42.27	2	< 0.0001
Test 7c	0.6604	1	0.4164

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

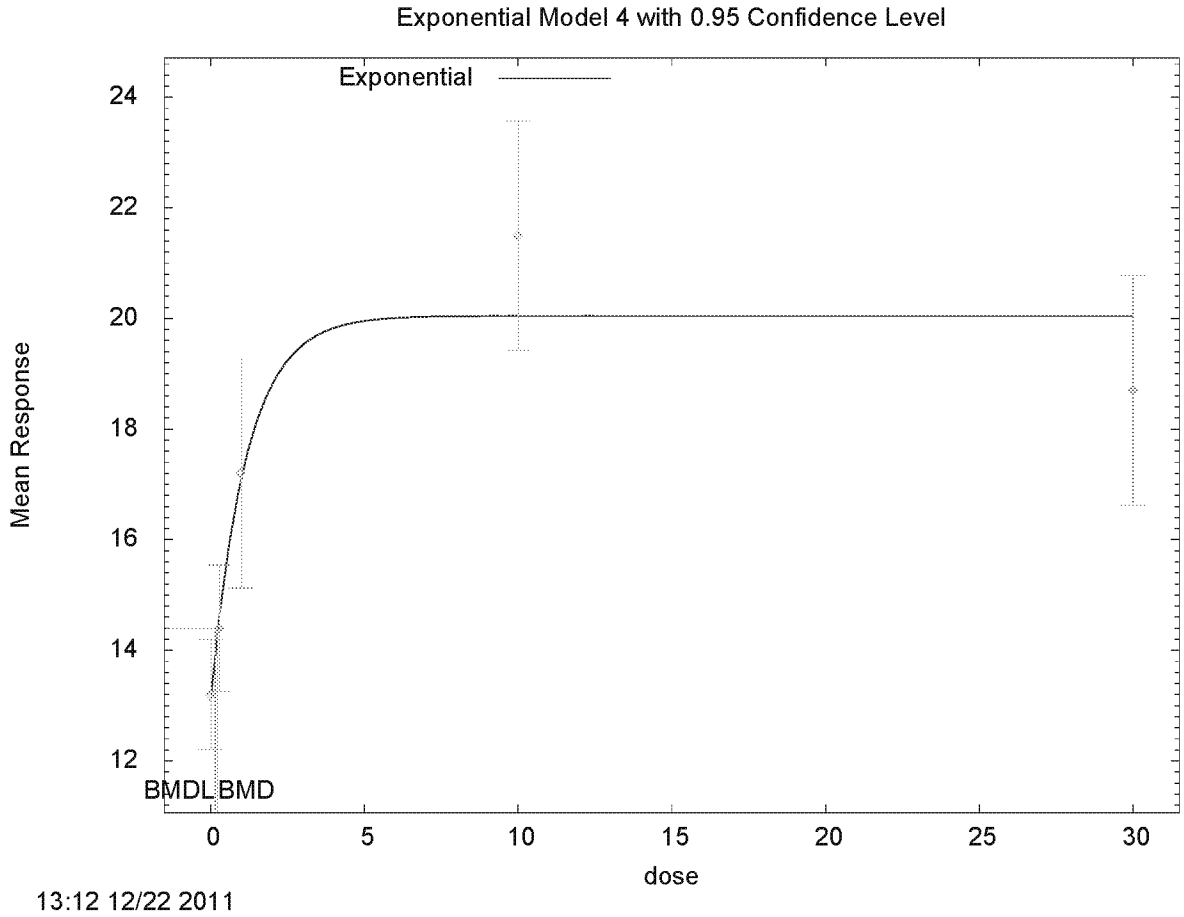
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	10.6283	6.02231
3	10.6283	6.02231
4	0.241376	0.151833
5	0.331813	0.161379


Exponential Model 4 with 0.95 Confidence Level
Exponential
Mean Response
24
22
20
18
16
14
12
BMDL
BMD
0
5
10
15
20
25
30
dose
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3) York, 2002; Butenhoff et al., 2004a; York et al., 2010

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        Exponential Model. (Version: 1.7;  Date: 12/10/2009)

                                                   Wed Oct 26 10:57:26 2011
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 BMDS Model Run
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.

   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-3.83873	-3.83873	-3.83873	-3.83873
rho	1.89443	1.89443	1.89443	1.89443
a	24.2168	24.2168	19.285	19.285
b	0.00591038	0.00591038	0.0671202	0.0671202
c	--	--	1.56261	1.56261
d	--	1	--	1

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	5.96811	5.96811	-4.75762	-4.61574
rho	-0.961701	-0.961702	2.17955	2.13551
a	24.5758	24.5758	20.2791	20.2956
b	0.0049661	0.0049661	0.737783	0.745578
c	--	--	1.39208	1.38868
d	--	1	--	1.12767

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	30	20.3	2.5
1	30	24.3	3.2
3	30	27.7	2.7
10	30	28.7	3.9
30	29	27.5	3.7

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	24.58	4.24	-5.524
	1	24.7	4.23	-0.5157
	3	24.94	4.209	3.585
	10	25.83	4.14	3.801
	30	28.52	3.947	-1.397
3	0	24.58	4.24	-5.524
	1	24.7	4.23	-0.5157
	3	24.94	4.209	3.585
	10	25.83	4.14	3.801
	30	28.52	3.947	-1.397
4	0	20.28	2.462	0.04647
	1	24.43	3.016	-0.2328
	3	27.36	3.412	0.5445
	10	28.23	3.53	0.7368
	30	28.23	3.531	-1.114
5	0	20.3	2.476	0.009804
	1	24.34	3.005	-0.06777
	3	27.52	3.427	0.2834
	10	28.18	3.515	0.8048
	30	28.18	3.515	-1.048

Other models for which likelihoods are calculated:

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-247.2345	6	506.469
A2	-242.9088	10	505.8177
A3	-244.9146	7	503.8291
R	-296.4613	2	596.9226
2	-286.6477	4	581.2954
3	-286.6477	4	581.2954
4	-245.8857	5	501.7713
5	-245.8276	6	503.6552

Additive constant for all log-likelihoods = -136.9. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	107.1	8	< 0.0001
Test 2	8.651	4	0.07043
Test 3	4.011	3	0.2602
Test 4	83.47	3	< 0.0001
Test 5a	83.47	3	< 0.0001
Test 5b	-2.274e-013	0	N/A
Test 6a	1.942	2	0.3787
Test 6b	81.52	1	< 0.0001
Test 7a	1.826	1	0.1766
Test 7b	81.64	2	< 0.0001
Test 7c	0.1162	1	0.7332

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

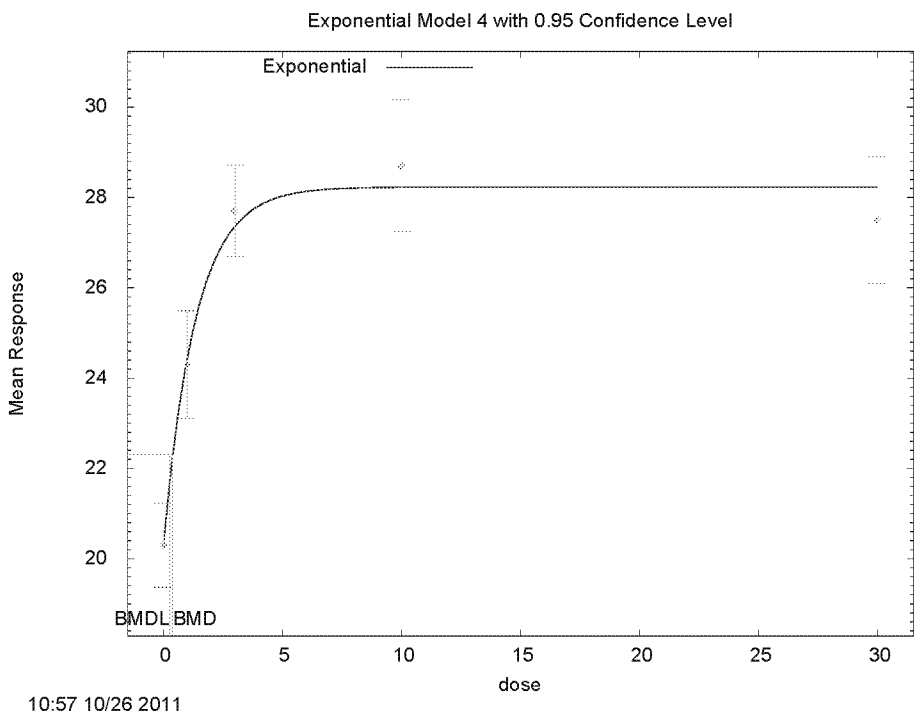
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	19.1922	13.6199
3	19.1922	13.6199
4	0.399087	0.274007
5	0.45764	0.276304



4) Loveless et al., 2008: male mouse

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        Exponential Model. (Version: 1.7;  Date: 12/10/2009)

                                                   Wed Aug 24 10:48:22 2011
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 BMDS Model Run
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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   rho is set to 0.
   A constant variance model is fit.

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                                  Initial Parameter Values
Variable       Model 2        Model 3          Model 4        Model 5
--------       -------        -------          -------        -------
  lnalpha      -0.698397      -0.698397        -0.698397      -0.698397
  rho(S)       0              0                0              0
  a            2.65446        2.65446          1.691          1.691
  b            0.0323602      0.0323602        0.0970613      0.0970613
  c            --             --               3.76286        3.76286
  d            --             1                --             1

      (S) = Specified
```

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.636609	0.636609	-0.686179	-0.686255
rho	0	0	0	0
a	3.06456	3.06456	1.8131	1.8194
b	0.0242388	0.0242388	0.446049	0.450771
c	--	--	3.30881	3.29563
d	--	1	--	1.01732

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	20	1.78	0.18
1	20	3.27	0.23
0.3	20	2.41	0.26
30	20	5.9	0.85
10	20	6.06	1.32

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	3.065	1.375	-4.179
	1	3.14	1.375	0.4237
	0.3	3.087	1.375	-2.202
	30	6.341	1.375	-1.435
	10	3.905	1.375	7.01
3	0	3.065	1.375	-4.179
	1	3.14	1.375	0.4237
	0.3	3.087	1.375	-2.202
	30	6.341	1.375	-1.435
	10	3.905	1.375	7.01
4	0	1.813	0.7096	-0.2086
	1	3.319	0.7096	-0.3117
	0.3	2.337	0.7096	0.4575
	30	5.999	0.7096	-0.6252
	10	5.951	0.7096	0.688
5	0	1.819	0.7095	-0.2483
	1	3.318	0.7095	-0.3055
	0.3	2.331	0.7095	0.4988
	30	5.996	0.7095	-0.6055
	10	5.955	0.7095	0.6605

Other models for which likelihoods are calculated:

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-15.08015	6	42.16031
A2	40.89337	10	-61.78674
A3	-15.08015	6	42.16031
R	-114.7709	2	233.5419
2	-81.83045	3	169.6609
3	-81.83045	3	169.6609
4	-15.69106	4	39.38211
5	-15.68723	5	41.37446

Additive constant for all log-likelihoods = -91.89. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	311.3	8	< 0.0001
Test 2	111.9	4	< 0.0001
Test 3	111.9	4	< 0.0001
Test 4	133.5	3	< 0.0001
Test 5a	133.5	3	< 0.0001
Test 5b	-7.788e-012	0	N/A
Test 6a	1.222	2	0.5429
Test 6b	132.3	1	< 0.0001
Test 7a	1.214	1	0.2705
Test 7b	132.3	2	< 0.0001
Test 7c	0.007649	1	0.9303

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. Consider running a non-homogeneous variance model.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

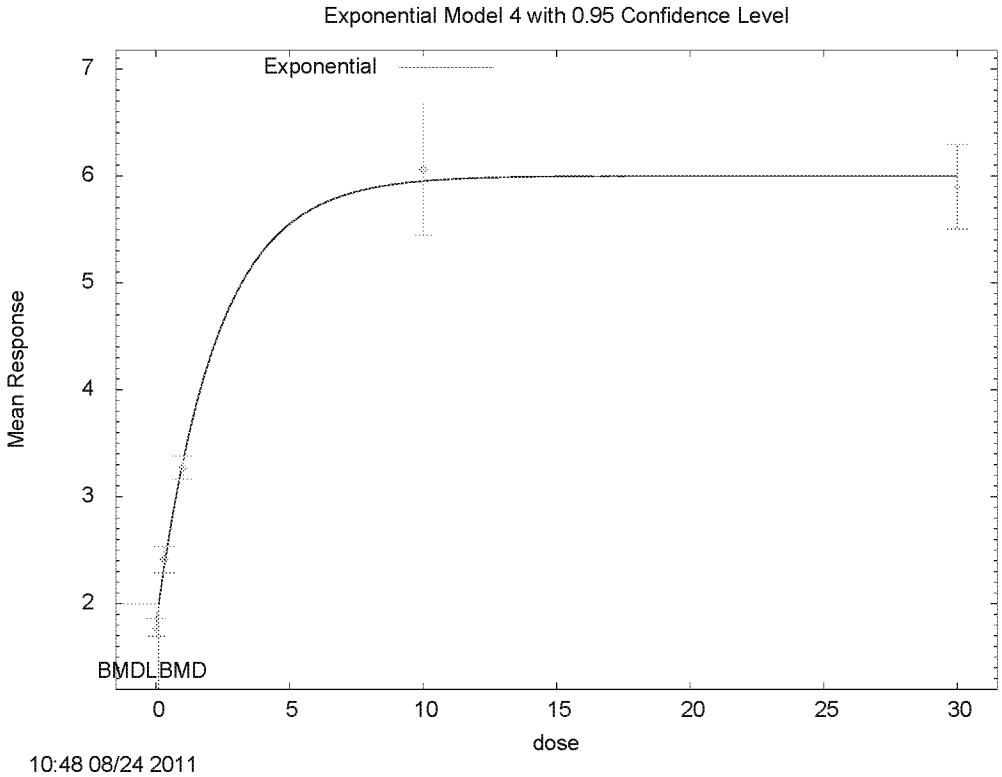
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	3.93213	3.38551
3	3.93213	3.38551
4	0.099268	0.0680743
5	0.104178	0.0681103


Exponential Model 4 with 0.95 Confidence Level
Exponential
Mean Response
7
6
5
4
3
2
BMDL BMD
0
5
10
15
20
25
30
dose
10:48 08/24 2011

5) Lau et al., 2006: female mouse

```
=====================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
                                                  Tue Oct 25 15:12:47 2011
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.

   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-5.21753	-5.21753	-5.21753	-5.21753
rho	2.68479	2.68479	2.68479	2.68479
a	2.91529	2.91529	2.3085	2.3085
b	0.0531403	0.0531403	0.149585	0.149585
c	--	--	2.46979	2.46979
d	--	1	--	1

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-5.42859	-5.42858	-4.62127	-4.62765
rho	3.52242	3.52242	2.25579	2.26038
a	2.63415	2.63415	2.4311	2.43306
b	0.0990961	0.0990961	0.279706	0.286892
c	--	--	2.27404	2.25849
d	--	1	--	1.03267

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev

0	40	2.43	0.29
1	15	3.17	0.27
3	16	4.3	0.51
5	20	4.66	0.56
10	15	5.43	0.74
20	5	5.36	0.77

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	2.634	0.3648	-3.54
	1	2.909	0.4343	2.331
	3	3.546	0.6158	4.897
	5	4.323	0.873	1.724
	10	7.096	2.089	-3.088
	20	19.12	11.97	-2.57
3	0	2.634	0.3648	-3.54
	1	2.909	0.4343	2.331
	3	3.546	0.6158	4.897
	5	4.323	0.873	1.724
	10	7.096	2.089	-3.088
	20	19.12	11.97	-2.57
4	0	2.431	0.2702	-0.02564
	1	3.187	0.3666	-0.1777
	3	4.19	0.4992	0.8807
	5	4.763	0.5769	-0.8022
	10	5.34	0.6562	0.5341
	20	5.517	0.6809	-0.5152
5	0	2.433	0.2701	-0.07155
	1	3.17	0.3643	-0.002448
	3	4.195	0.4999	0.8422
	5	4.778	0.5791	-0.91
	10	5.338	0.6564	0.5436
	20	5.488	0.6773	-0.4226

Other models for which likelihoods are calculated:

```
Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	29.04002	7	-44.08005
A2	44.97661	12	-65.95321
A3	43.40484	8	-70.80967
R	-80.9645	2	165.929
2	-15.64228	4	39.28456
3	-15.64228	4	39.28456

4	42.25345	5	-74.50691
5	42.2837	6	-72.56741

Additive constant for all log-likelihoods = -102. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	251.9	10	< 0.0001
Test 2	31.87	5	< 0.0001
Test 3	3.144	4	0.5341
Test 4	118.1	4	< 0.0001
Test 5a	118.1	4	< 0.0001
Test 5b	-9.564e-012	0	N/A
Test 6a	2.303	3	0.512
Test 6b	115.8	1	< 0.0001
Test 7a	2.242	2	0.3259
Test 7b	115.9	2	< 0.0001
Test 7c	0.0605	1	0.8057

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

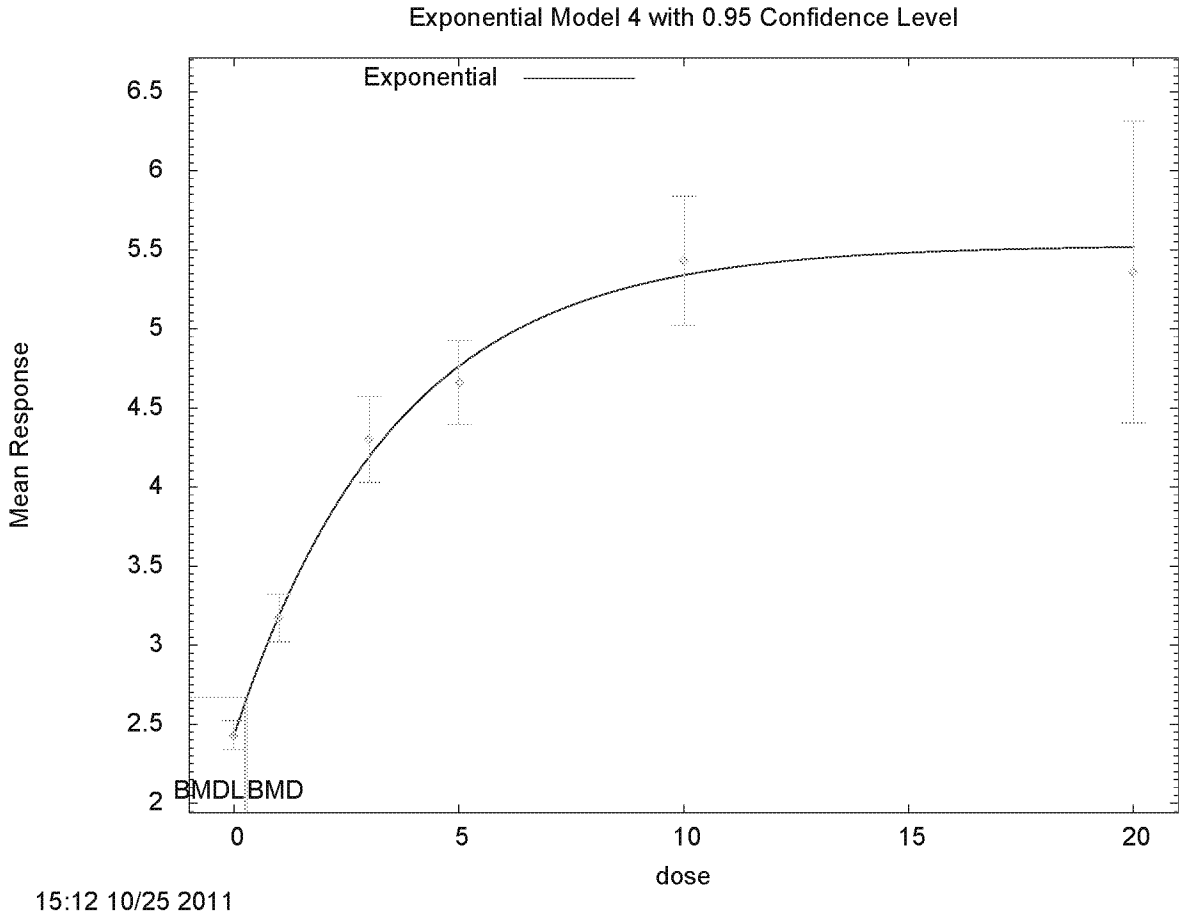
Specified Effect = 0.100000

Risk Type = Relative deviation

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	0.961795	0.832065
3	0.961796	0.832065
4	0.292244	0.246387
5	0.312258	0.246874


Exponential Model 4 with 0.95 Confidence Level
Exponential
Mean Response
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
BMDL
BMD
0
5
10
15
20
dose
15:12 10/25 2011

APPENDIX B

Multistage Model for Leydig Cell Tumors

```
 ======================================================================
         Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
         Input Data File: C:/1Data/MyFiles/PFOA-PFOS/PFOA
Docs/msc_Leydig_Opt.(d)
         Gnuplot Plotting File:  C:/1Data/MyFiles/PFOA-PFOS/PFOA
Docs/msc_Leydig_Opt.plt
                                                  Thu May 09 11:59:27 2013
 ======================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Col2
   Independent variable = Col1

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                   Default Initial Parameter Values
                      Background =    0.0132945
                         Beta(1) =    0.0097738
                         Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)
                 have been estimated at a boundary point, or have been
specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1        -0.64

   Beta(1)        -0.64            1



                                  Parameter Estimates
```

95.0% Wald Confidence Interval

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.00409839	*	*	*
Beta(1)	0.0116288	*	*	*
Beta(2)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.3468	2	1.40286	1	0.2362
Reduced model	-34.0451	1	10.7995	2	0.004518

AIC: 62.6936

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0041	0.205	0.000	50	-0.454
1.3000	0.0190	0.952	2.000	50	1.084
14.2000	0.1557	7.784	7.000	50	-0.306

Chi^2 = 1.48 d.f. = 1 P-value = 0.2245

Benchmark Dose Computation

Specified effect = 0.04

Risk Type = Extra risk

Confidence level = 0.95

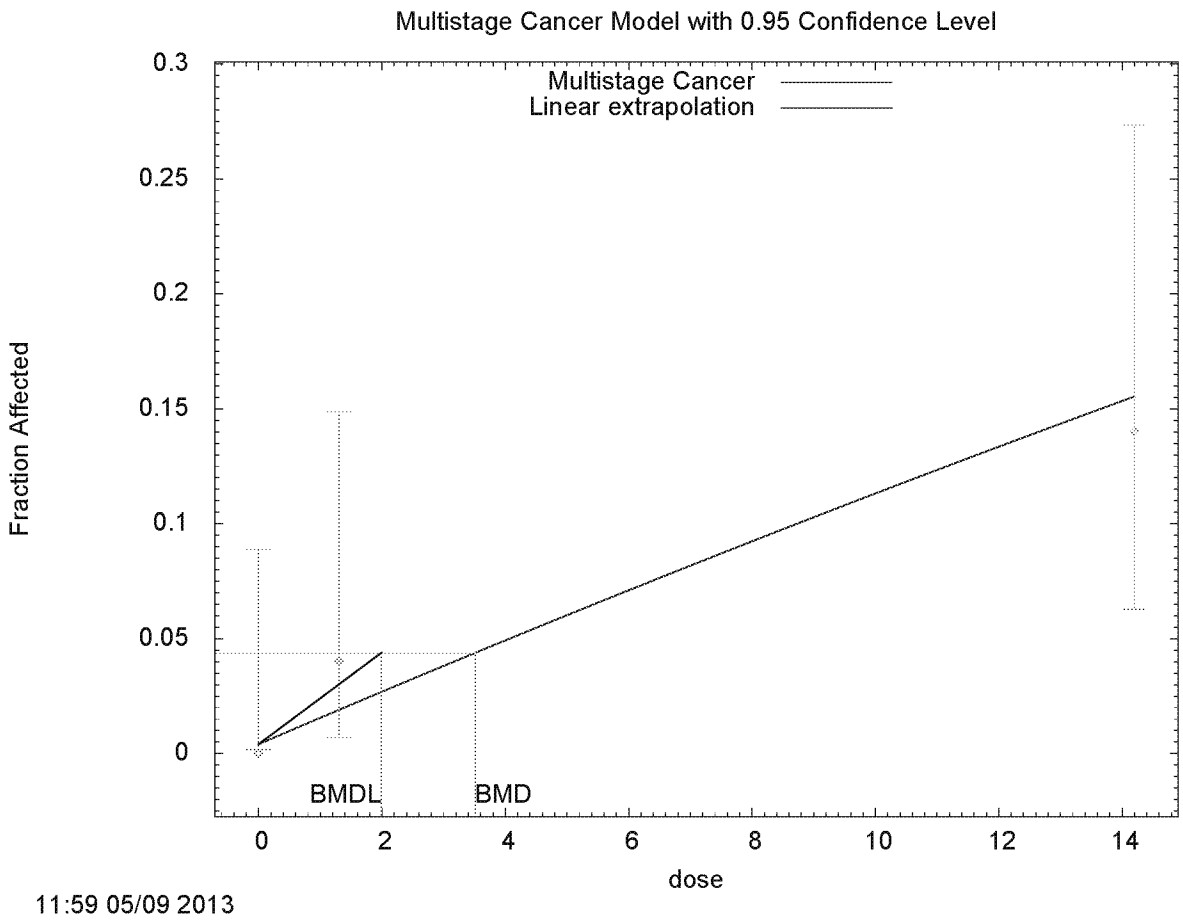
BMD = 3.51044

BMDL = 1.99346

BMDU = 10.7788

Taken together, (1.99346, 10.7788) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0200656




```
 ======================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/1Data/MyFiles/PFOA-PFOS/PFOA
Docs/msc_Leydig_Opt.(d)
        Gnuplot Plotting File:  C:/1Data/MyFiles/PFOA-PFOS/PFOA
Docs/msc_Leydig_Opt.plt
                                                Thu May 09 12:05:42 2013
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Col2
   Independent variable = Col1

 Total number of observations = 3
 Total number of records with missing values = 0
```

Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0132945
Beta(1) = 0.0097738

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.64
Beta(1)	-0.64	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.00409839	*	*	*
Beta(1)	0.0116288	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.3468	2	1.40286	1	0.2362
Reduced model	-34.0451	1	10.7995	2	0.004518

AIC: 62.6936

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0041	0.205	0.000	50	-0.454
1.3000	0.0190	0.952	2.000	50	1.084
14.2000	0.1557	7.784	7.000	50	-0.306

Chi^2 = 1.48 d.f. = 1 P-value = 0.2245

Benchmark Dose Computation

```
Specified effect =           0.04

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3.51044

            BMDL =        1.99346

            BMDU =         8.7003

Taken together, (1.99346, 8.7003 ) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0200657
```