SIDS Initial Assessment Report

After

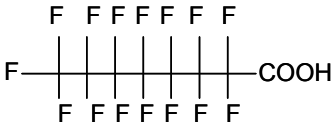
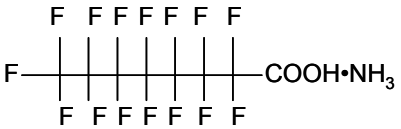
SIAM 22

Paris, France, 18-21April 2006

1.	**Chemical Name:**	**Ammonium Perfluorooctanoate & Perfluorooctanoic Acid**
2.	**CAS Number:**	**3825-26-1 & 335-67-1**
3.	**Sponsor Country**	
	Environment and Exposure	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Alexander Nies Postfach 12 06 29 D- 53048 Bonn
	Human Health part	US-EPA
4.	**Shared Partnership with:**	Environmental and Exposure part: UBA and BUA, Germany DuPont de Nemours (Belgium) BVBA
5.	**Roles/Responsibilities of the Partners:**	
•	Name of industry sponsor /consortium	DuPont de Nemours (Belgium) BVBA A Spinoystraat 6 B-2800 Mechelen Belgium Dr. Watze de Wolf Eric van Wely
•	Process used	Sharing process: US-EPA: Human Health part Germany: Environmental and Exposure part
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Programme?	

7. Review Process Prior to the SIAM:	1.) last literature search (update): June 2006 (Toxicology) June 2005 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms. - March 2006 (Exposure): company information, databases as named above. 2.) Other review processes: Mammalian Toxicology: 1) External Review of US EPA report-written procedure, April 2003; 2) U. S. EPA Science Advisory Board , February 2005 Ecotoxicology: OECD ICCA review process including addition of few relevant current information and publications
8. Quality check process:	Internal crosscheck
9. Date of Submission:	1.) January 20th 2006 for SIAM 22 2.) written procedure before SIAM 24
10. Comments:	For the assessment of environmental hazards, with some minor exceptions, only data published before 2006, March1st is considered.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	335-67-1	3825-26-1
Chemical Name	Perfluorooctanoic Acid (PFOA)	Ammonium Perfluorooctanoate (APFO)
Structural formula		

SUMMARY CONCLUSIONS OF THE SIAR

Chemical Identity

For the purposes of this document, the anion of PFO (perfluorooctanoate) is frequently referenced as PFOA or APFO. APFO and PFOA are sometimes used interchangeably. Perfluorooctanoic Acid (PFOA) is a fully fluorinated carboxylic acid. APFO is the ammonium salt of PFOA.

Human Health

APFO is the ammonium salt of PFOA and the two substances are metabolically equivalent. PFOA is a strong acid and it is expected to dissociate in biological media.

Several epidemiology and medical surveillance studies have been conducted on workers employed at various APFO manufacturing sites in the U.S. Most of the studies were cross-sectional and focused primarily on males. A retrospective cohort mortality study demonstrated a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures PFOA. However, in an update to this study in which more specific exposure measures were used, a significant association for prostate cancer was not observed. Other mortality studies lacked adequate exposure data which could be linked to health outcomes. A study which examined hormone levels in workers reported an increase in estradiol levels in workers with the highest PFOA serum levels; however, these results may have been confounded by body mass index. Cholesterol and triglyceride levels in workers were positively associated with PFOA exposures, which is inconsistent with the hypolipidemic effects observed in rat studies. A statistically significant positive association was reported for PFOA and T3 levels in workers but not for any other thyroid hormones.

Little information is available concerning the pharmacokinetics of PFOA and its salts in humans. Preliminary results of a 5-year half-life study in 26 retired workers indicate that the mean serum elimination half-life of PFOA in these workers was 3.8 years (1378 days, 95% CI, 1131-1624 days) and the range was 1.5-9.1 years.

The pharmacokinetics of PFOA in non-human primates has been studied both in classical intravenous elimination studies using three male and three female cynomolgus monkeys and in a six-month, repeat-oral-dose toxicology study in male cynomolgus monkeys with recovery. These studies confirmed urinary elimination as the primary excretion mode. From the intravenous study, in which males and females were given a 10 mg/kg dose, mean elimination half-lives were 20.9 days for males and 32.6 days for females. In the six-month study, monkeys were dosed with 3, 10, or 30/20 mg/kg-d ammonium PFOA. Steady-state serum concentration was reached within

four to six weeks, with steady state levels lower than those that would be predicted based on the elimination rates and not in linear proportion to dose. Males in the six-month toxicology study had elimination half-life rates approximating 20 days.

Studies in adult rats have shown that the ammonium salt of PFOA (APFO) is absorbed following oral and inhalation exposure; less absorption occurs following dermal exposure. Serum pharmacokinetic parameters and the distribution of PFOA has been examined in the tissues of adult rats following administration by gavage and by intravenous (i.v.) and intraperitoneal (i.p.) injection. PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. The distribution of PFOA is predominantly extracellular. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats.

There are gender differences in the elimination of PFOA in adult rats following administration by gavage and by i.v. and i.p.injection. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8-16 hours, while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138-202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic anion transporters); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

Several recent studies have been conducted to examine the kinetics of PFOA in the developing rat. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. The gender difference in elimination is developmentally regulated; between 4-5 weeks of age, elimination assumes the adult pattern and the gender difference becomes readily apparent. Distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

In acute toxicity studies in animals using APFO, the oral LD_{50} values for CD rats were >500 mg/kg for males and 250-500 mg/kg for females, and <1000 mg/kg for male and female Wistar rats. There was no mortality following inhalation exposure of 18.6 mg/l APFO for one hour in rats. The dermal LD_{50} in rabbits was determined to be greater than 2000 mg/kg. APFO is a primary ocular irritant in rabbits, while the data regarding potential skin irritancy are conflicting.

APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation. APFO 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. APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO 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 metabolic activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in mouse embryo fibroblasts and in the mouse micronucleus assay.

Repeat-dose studies have been conducted in non-human primates. In a 13-week study with Rhesus monkeys, exposure to doses of 30 mg/kg-day or higher resulted in death. Clinical signs of toxicity were noted at doses as low as 3 mg/kg-day. Unlike rodent studies, analyses of the serum and liver levels did not reveal a gender difference in monkeys, but the sample size was very

small. In a 6-month study of male cynomolgus monkeys, there was a steep dose response curve for mortality. Increases in liver weight were noted at doses as low as 3 mg/kg-day, but there was no evidence of peroxisome proliferator-activated receptor alpha activity (PPARα). The LOAEL for the study was 3 mg/kg-day, and a NOAEL was not identified.

Repeat-dose studies in rats and mice demonstrated that the liver is the primary target organ. Due to gender differences in elimination, adult male rats exhibit effects at lower administered doses than adult female rats. Dietary exposure to APFO for 90 days resulted in significant increases in liver weight and hepatocellular hypertrophy in female rats at 1000 ppm (76.5 mg/kg-day) and in male rats at doses as low as 100 ppm (5 mg/kg-day). Chronic dietary exposure of rats to 300 ppm (males, 14.2 mg/kg-day; females, 16.1 mg/kg-day) APFO for 2 years resulted in increased liver weight, hepatocellular hypertrophy, hematological effects, and testicular masses in males; and reductions in body weight and hematological effects in females.

The carcinogenic potential of PFOA has been investigated in two dietary carcinogenicity studies in rats. Under the conditions of these studies, there is some evidence that PFOA is carcinogenic, inducing liver tumors, Leydig cell tumors (LCT), and pancreatic acinar cell tumors (PACT) in male rats. The evidence for mammary fibroadenomas in the female rats is equivocal since the incidences were comparable to some historical background incidences. There is sufficient evidence to indicate that PFOA is a PPARα-agonist and that the liver carcinogenicity (and toxicity) of PFOA is mediated by PPARα in the liver.

PFOA appears to be immunotoxic in mice. Feeding mice a diet containing 0.02% PFOA resulted in adverse effects to both the thymus and spleen. In addition, this feeding regimen resulted in suppression of the specific humoral immune response to horse red blood cells, and suppression of splenic lymphocyte proliferation. The suppressed mice recovered their ability to generate a humoral immune response when they were fed a diet devoid of PFOA. Studies using transgenic mice showed that the PPARα was involved in causing the adverse effects to the immune system.

In an oral prenatal developmental toxicity study in rats, the LOAEL and NOAEL for maternal toxicity were 150 mg/kg-day and 5 mg/kg-day, respectively. There was no evidence of developmental toxicity after exposure to doses as high as 150 mg/kg-day. In a rat inhalation developmental toxicity study, the NOAEL and LOAEL for maternal toxicity were 1 and 10 mg/m3, respectively. The NOAEL and LOAEL for developmental toxicity were 10 and 25 mg/m3, respectively. In a rabbit oral prenatal developmental toxicity study there was a significant increase in skeletal variations after exposure to 5 mg/kg-day APFO, and the NOAEL was 1.5 mg/kg-day. There was no evidence of maternal toxicity at 50 mg/kg-day, the highest dose tested. In a mouse oral developmental toxicity study, there was evidence of maternal toxicity and developmental toxicity and the authors calculated benchmark doses for a variety of endpoints. Decreased weight gain and increased liver weight was observed in adult females; the BMD5 and BMDL5 estimates for decreases in maternal weight gain were 6.76 and 3.58 mg/kg, respectively, and the BMD5 and BMDL5 estimates for increases in maternal liver weight were 0.20 mg/kg and 0.17 mg/kg, respectively. The BMD5 and BMDL5 estimates for the incidence of full-litter resorptions and neonatal mortality (determined by survival to weaning) observed at the 5 mg/kg/day dose group 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 BMD5 and BMDL5 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 BMD5 and BMDL5 estimates for reduced phalangeal ossification were <1 mg/kg. BMD5 and BMDL5 estimates for reduced fetal weight at term were estimated to be 10.3 and 4.3 mg/kg, respectively.

A variety of endpoints were evaluated throughout different lifestages in a two-generation reproductive toxicity study in rats exposed to 0, 1, 3, 10, or 30 mg/kg/day APFO. In that study, a re-

duction in F1 pup mean body weight on a litter basis was observed during lactation (sexes combined) in the 30 mg/kg-day group. F1 male pups in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. F1 female pups in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. Reproductive indices were not affected in the F1 animals. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 male and female pups in the 30 mg/kg-day group. No effects were observed in the F2 pups. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation. Adult systemic toxicity consisted of reductions in body weight in both the F0 and F1 animals.

Environment

Both PFOA and APFO are solid at environmental relevant temperatures. The melting point for PFOA is 54.3°C and the boiling point is 190°C at 1013 hPa. APFO starts to decompose above 105°C. At 20°C, the water solubility of PFOA is 9.5 g/l and of APFO >500 g/l. When dissolved in water, the strong acid PFOA (pKa 2.5) dissociates.

Pure PFOA at room temperature has moderate vapor pressure (2.3 Pa). The vapor pressure of APFO is much lower with 0.008 Pa. APFO or PFOA dissolved in water dissociate to ions. Although the dissociated fraction is not subject to volatilization, depending on the pH, pure PFOA might volatilize from water to a certain degree.

Due to emissions for more than 50 years, PFOA is distributed worldwide in the marine environment, and hence may be transported to remote areas via the aqueous phase and the atmospheric phase. However, the significance of these sources are not currently known. Both atmospheric and aquatic transport mechanisms are actively being investigated.

Possible substances subject to atmospheric long-range transport are PFOA precursors rather than PFOA itself. Potential precursors are fluorotelomer alcohols, -olefins and perfluoroalkyl sulfonyl derivates. These substances are degraded by OH radicals via gas-phase reactions to result partially in PFOA. The relative environmental significance of these sources is not known at this time.

Due to the stability of the C-F bond, PFOA is persistent in the environment. No degradation could be observed in the studies on abiotic or biological degradability in water. Also the examinations on photolytic and photochemical degradation in air indicate high stability under environmentally relevant conditions. The half-life of the reaction with OH-radicals in the atmosphere is 130 days.

According to the low adsorption potential and the water-solubility, PFOA is mobile in soil.

The available ecotoxicological studies using APFO indicate a low acute toxicity for aquatic organisms. In the short term tests using fish, invertebrates, and algae, effective concentrations were as follows:

Oncorhynchus mykiss	LC_{50} (96 h)	= 707 mg/l (nominal)
Daphnia magna	EC_{50} (48 h)	= 480 mg/l (measured)
Pseudokirchneriella subcapitata	$EC_{50growth\ rate/biomass}$ (72 h)	> 400 mg/l (nominal)

The NOEC values determined in the chronic tests using fish and daphniae, were 40 mg/l (*O. mykiss,* NOEC, 85 d, measured) and 20 mg/l (*D. magna*, NOEC, 21 d, measured). In a 10 day study using *Chironomus tentans* no effects were observed up to a nominal concentration of 100 mg/l. In addition to that, the following information about effects on community level (indoor and outdoor microcosm studies) is available:

Zooplankton community	35 d-$LOEC_{species\ richness}$ = 10 mg/l	(nominal)
Myriophyllum spicatum	35 d-EC_{10} = 5.7 mg/l	(measured)
Myriophyllum spp.	35 d-NOEC = 23.9 mg/l	(measured)

In several tests on effects using activated sludge, no inhibition of microbial activity was measured up to a nominal concentration of 1000 mg/l.

Concerning the effects on terrestrial organisms, in a test using *Caenorhabditis elegans* without analytical verification, an EC_{50} (48 h) of 973 mg/l (nominal) was calculated. In a chronic study using the same species, a reduction of abundance and egg production was observed at the 4th generation at a concentration of $4.1x10^{-3}$ mg/l (nominal). The NOEC for this endpoints was $4.1x10^{-4}$ mg/l.

In tests with the rainbow trout *Oncorhynchus mykiss* a bioconcentration factor (BCF) of 0.038 and bioaccumulation factors (BAF) for organs of 27 (blood), 8.0 (liver) and 4.0 (carcass) were obtained. These laboratory studies indicate a low bioaccumulation potential in fish. Some monitoring data suggest a low biomagnification potential in aquatic food webs, while in some marine and Canadian Arctic mammalian food web studies a potential for biomagnification has been suggested. Further elucidation of the mechanisms leading to uptake and accumulation in biota is required.

Exposure

APFO is used as a processing aid in the production of fluoropolymers. In 2002, its world-wide production was about 200-300 metric tons. Entry into the environment occurs during production and use of PFOA / APFO. Other sources for releases to the environment are residual contents of PFOA in fluoropolymer and fluoroelastomer products, PFOA as a byproduct in end products and fire-fighting foams containing perfluorocarboxylates, PFOA contaminations in perfluorooctyl sulfonyl (PFOS) based products, and PFOA contamination in fluorotelomer products. An indirect source for PFOA in the environment is the degradation (biotic and abiotic) of some fluorotelomer-based products.

The global distribution of PFOA was demonstrated by several monitoring studies. Elevated PFOA concentrations were measured near industrialized and urbanized regions. PFOA could be detected in air in concentrations in the range of pg/m³, ng/g dw in soil, in sediment, suspended matter, and sewage sludge.

PFOA concentrations up to 67,000 ng/l and 3,200,000 ng/l were analysed in sewage effluent and landfill effluent. Sporadically, PFOA was determined in ground water samples (up to 3,400,000 ng/l). In fresh water samples (rivers, lakes, rain water) PFOA was regularily measured. The maximum concentration determined was 11,300 ng/l. Elevated concentrations of PFOA were also detected in coastal waters near industrialized and urbanized areas; the maximum concentration was 15,300 ng/l.

In freshwater and salt water fish PFOA was detected occasionally. The maximum concentration (91 ng/g ww) was found in common shiner (liver samples) after a spill of fire retardant foam.

The highest PFOA concentration in birds was determined in liver samples of cormorants (450 ng/g ww). However, it should be noted that for this colony of cormorants the highest value (450 $\mu g.kg^{-1}$ ww) appeared to be an outlier as the concentration was 4.5 times greater than the standard deviation of the mean. The occurrence of PFOA even in remote areas, was demonstrated by analysis of polar bear liver samples (highest concentration: 55.8 ng/g ww). Liver samples of other mammals (e.g. seals, whales, walrus, dolphin) contained PFOA; concentrations up to 62 ng/g ww.

In addition to the environmental measurements, PFOA was regularly analysed in human blood samples. While the pathways of human exposure to PFOA and its salts are unknown, there are limited data on PFOA blood serum levels in both occupationally- and non-occupationally-exposed populations. It has been detected in samples of human blood (plasma, serum and whole blood), liver, seminal plasma, and breast milk from several countries throughout the world, including the US, Canada, Columbia, Poland, Belgium, India, Korea, Sri Lanka, Japan, Sweden and Germany. Preliminary US reports indicate that individuals living near a facility that uses PFOA have much higher PFOA serum concentrations than the levels previously reported for US populations.

The routine finding of PFOA in human blood initiated research on the sources of human exposure. Fluoropolymers and fluoroelastomers are typically manufactured through telomerization. As APFO is an essential processing aid in this process, trace amounts of PFOA may be generated as an unintended by-product.

Fluorotelomer-based products have numerous uses in many industrial and consumer products, including soil-, stain-, grease-, and water-resistant coatings on textiles and carpet, personal care products, and nonstick coatings on cookware, and uses in the automotive, mechanical, aerospace, chemical, electrical, medical, and building and construction industries. Consumer exposure to PFOA due to impurities in the finished products can not be excluded. However, based on analysis of consumer articles and using a simple compartmental model estimation, an explanation for the PFOA concentrations found in humans can not be given.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:

The chemical is a candidate for further work. The chemicals possess properties indicating a hazard for human health (eye irritation; subchronic toxicity; potential carcinogenicity; developmental toxicity). In the US, data collected in the National Health and Nutrition Examination Survey (NHANES) will provide data on exposure profiles of individuals across the U.S. Epidemiologic studies have not shown conclusively an association of PFOA exposure and health outcomes but most of the studies were cross-sectional; further work is needed to understand any potential associations. Further work on the species differences in toxicokinetics and mode of action to enhance our ability to predict risk in humans is currently underway. Member countries are invited to perform an exposure assessment and then if indicated, a risk assessment.

Environment:

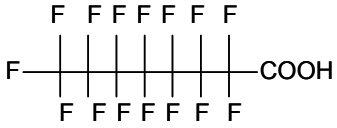
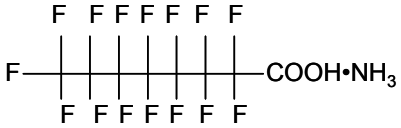
The chemical is a candidate for further work. PFOA is persistent in the environment. The primary environmental sink is the aqueous phase. PFOA tends to be dissipated into organisms and is eliminated from the body very slowly. Laboratory studies and monitoring data in some aquatic food webs indicate a low bioaccumulation potential in fish, but other data suggest a potential for biomagnification, e.g. in marine mammals and Canadian Arctic food webs. Hence, for substances like PFOA, bioconcentration values in fish may not be the most relevant endpoint to consider. For PFOA, biomagnification may occur in air-breathing species (e.g., terrestrial mammals, birds and marine mammals). Further elucidation of the mechanisms leading to uptake and accumulation in biota is required.

The main industrial sources of environmental emissions appear to have been identified. Further research is needed to quantify the sources leading to the ubiquitous environmental distribution and human exposure. In conclusion, member countries are invited to perform an exposure assessment (consideration should be given to precursors to PFOA) and, if indicated, a risk assessment. Member countries are invited to consider risk management measures, e.g. environmental emission reductions.

SIDS Initial Assessment Report

1.0 IDENTITY

1.1 Identification of the Substance

CAS Number:	335-67-1	3825-26-1
IUPAC Name:	Pentadecafluorooctanoic Acid (PFOA)	Ammonium Pentadecafluorooctanoate (APFO)
Molecular Formula:	$C_7F_{15}COOH$	$C_7F_{15}COO^-NH_4^+$
Structural Formula:		
Molecular Weight:	414.07 g/mol	431.10 g/mol
Synonyms:	Perfluorooctanoic Acid; PFOA; Pentadecafluoro-1-octanoic acid; Perfluorocaprylic acid; Perfluoroheptanecarboxylic acid; Perfluoro-n-octanoic acid; Pentadecafluoro-n-octanoic acid; Pentadecafluorooctanoic acid; n-Perfluorooctanoic acid 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6, 7,7,8,8,8-pentadecafluoro	Ammonium Perfluorooctanoate; APFO; C-8; Ammonium pentadecafluorooctanoate; Ammonium perfluorocaprylate; DS 101; FC 1015; FC 143; FX 1006; Fluorad® FC 143; Perfluorooctanoic acid ammonium salt; Unidyne® DS 101-20

There are a number of perflurorooctanoate salts in commercial use but only in very small quantities (est. < 1 metric ton•yr-1). Examples are:

- Potassium perfluorooctanoate CAS # 2395-00-08
- Sodium perfluorooctanoate CAS # 335-95-5
- Silver perfluorooctanoate CAS # 335-93-3

These salts are considered in chapter “

2.2.1.3 Other Direct PFOA/APFO Sources”. Other aspects of the salts different from APFO are not subject of this assessment report.

For the purposes of this document, the anion of PFO (perfluorooctanoate) is frequently referenced as PFOA or APFO. APFO and PFOA are sometimes used interchangeably.

1.2 Purity/Impurities/Additives

PFOA itself is mainly produced and used as ammonium perfluorooctanoate (APFO). The purity and impurity of APFO depends on the manufacturing process perfluorooctyl iodide oxidation and electro-chemical fluorination.

Industrial processes for the synthesis of PFOA

Electrochemical Fluorination (ECF)

$H(CH_2)_7COF + HF + e^-$ → $F(CF_2)_7COF$ → $F(CF_2)_7CO_2NH_4$

Octanoic acidfluoride **APFO**

Perfluorooctyl Iodide Oxidation

$F(CF_2)_8I + [O]$ → $F(CF_2)_7COOH$ → $F(CF_2)_7CO_2NH_4$

Perfluorooctyl Iodide **APFO**

Process	Perfluorooctyl Iodide Oxidation	Electrochemical Fluorination
Purity of the commercial product:	> 99%[1]	99%[1]
Impurities:	• ≤ limit of quantification (≤0.01%) of each of the following acids PFNA, PFDA, PFUA, PFDDA, PFTDA	• C-5 through C-7 isomers • 0.2 % PFNA • ≤ limit of quantification (≤0.01%) of each of the following acids PFDA, PFUA, PFDDA, PFTDA
Linear / Branched	100% linear[2]	< 30% branched (Simons, 1949; Kissa, 2001)

PFNA: perfluorononanoic acid, PFDA: perfluorodecanoic acid, PFUA: perfluoroundecanoic acid, PFDDA: perfluorododecanooic acid, PFTDA: perfluorotridecanoic acid

1 Puritiy after purification.

2 With regard to APFO used as a processing aid to manufacture certain fluoropolymers, the telomerisation process produces a range with different alkyl chain lengths. However the starting product perfluorooctyl iodide is refined and distilled to a high purity prior to production of APFO. High purity APFO is necessary to produce PTFE.

1.3 Physico-Chemical properties

Table 1 Summary: Perfluorooctanoic Acid (PFOA) Physico-Chemical Properties

Property	Value	Reference	IUCLID
Substance type	Organic compound		1.1.1
Physical state	Solid		1.1.1
UV absorption in water	no absorption < 290 nm	Hori et al., 2005	
Melting point (°C)	54.3 44 - 56.5 (6 references)	Lide, 2003 Beilstein, 2005	2.1
Boiling point (°C)	188 (1013.25 hPa) 189 (981 hPa)	Lide, 2003 Kauck and Diesslin, 1951	2.2
Density	1.792 g/cm^3 (20° C)	HSDB, 2005	2.3
Vapour pressure (Pa)	4.2 (25° C) extrapolated from measured data 2.3 (20° C) extrapolated from measured data 128 (59.3° C) measured	Kaiser et al., 2005; Washburn et al., 2005 Washburn et al., 2005 Washburn et al., 2005	2.4
Partition coefficient n-octanol/water (log value)	*		
Henry's law constant	**		
Water solubility (g·L^{-1})	9.5 (25° C)	Kauck and Diesslin, 1951	2.6.1
Solubility in organic solvents	-		
pH value	2.6, 1 g/l (20° C)	Merck, 2005 (reliability not assignable)	2.6.1
pKa	2.5 2.8 1.5-2.8	Gilliland, 1992, Ylinen et al., 1990 (reliability not assignable) Brace, 1962 Kissa, 2001	2.12
Critical Micelle Concentration (g·L^{-1})	3.6 - 3.7	Kissa, 1994	
Conversion factor for the vapour phase	1 ppm = 17.21 mg/m^3		

* For the surface active PFOA Kow is not measurable (USEPA, 2005).

** Henry's law constant cannot be calculated from vapor pressure and solubility (dissociating substance).

Table 2 Summary: Ammonium Perfluorooctanoate (APFO) Physico-Chemical Properties

Property	Value	Reference	IUCLID
Substance type	Organic compound		1.1.1
Physical state	Solid		1.1.1
UV absorption in water	no absorption < 290 nm (acid)	Hori et al., 2005	1.1.2
Melting point (°C)	130 (decomposition) 157 - 165 (decomposition starts above 105° C)	3M, 1987 (reliability not assignable) Lines and Sutcliffe, 1984	2.1
Boiling point (°C)	Decomposition	Lines and Sutcliffe, 1984	2.2
Density	0.6-0.7 g/cm^3 (20° C)	Grifith and Long, 1980	2.3
Vapour pressure (Pa)	0.0081 (20° C) calculated from measured data 3.7 (90.1° C) measured	Washburn et al., 2005 Washburn et al., 2005	2.4
Partition coefficient n-octanol/water (log value)	*		2.5
Henry's law constant	**		3.3.2
Water solubility (g·L^{-1}) at 20 °C	> 500	Shinoda, Hato, and Hayashi´, 1972 3M, 1987 (reliability not assignable)	2.6.1
Solubility in organic solvents (g·L^{-1})	Heptane, Toluene : 0 Methanol, Acetone : >500	3M, 1987 (reliability not assignable)	2.6.1
pH value in water at 23 °C	approx. 5	3M, 1987 (reliability not assignable)	
Begin of thermal decomposition (°C)	130 above 105	3M, 1987 (reliability not assignable) Lines and Sutcliffe, 1984	
Critical Micelle Concentration ((g·L^{-1})	(see table 1)		
Conversion factor for the vapour phase	1 ppm = 17.92 mg/m^3		

* Kow is not measurable for the surface active PFOA (USEPA, 2005).

** Henry's law constant cannot be calculated from vapor pressure and solubility (dissociating substance).

2.0 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

As stated before, PFOA is mainly produced as ammonium perfluorooctanoate – APFO.

The most important industrial processes for the synthesis of PFOA (as APFO) are Electrochemical Fluorination (ECF), and Perfluorooctyl Iodide Oxidation. ECF starts with octanoic acidfluoride and hydrogen fluoride which are transformed to APFO in multiple steps.

In the Perfluorooctyl Iodide Oxidation process, the perfluorooctyl iodide is oxidised to PFOA. PFOA is further transformed to its ammonium salt APFO which is the marketed form.

Industrial processes for the synthesis of PFOA

Electrochemical Fluorination (ECF)

$H(CH_2)_7COF + HF + e^- \rightarrow F(CF_2)_7COF \rightarrow F(CF_2)_7CO_2NH_4$

Octanoic acidfluoride **APFO**

Perfluorooctyl Iodide Oxidation

$F(CF_2)_8I + [O] \rightarrow F(CF_2)_7COOH \rightarrow F(CF_2)_7CO_2NH_4$

Perfluorooctyl Iodide **APFO**

2.1.1 Historical and Current Production

From 1947, the electrochemical fluorination (ECF) process was used worldwide to manufacture the majority of APFO (Simons (1949)), and was 80-90% in 2000 (Prevedouros et al. (2006)). The largest production sites were in the U.S. (ECF process ceased in 2002) and Belgium (production ceased in 2002), the next largest in Italy and small scale producers in Japan.

From about 1975 to 2002, the remaining 10-20% of APFO was made by direct oxidation of perfluorooctyl iodide at one site in Germany (production ceased in 2004) and at least one site in Japan, and DuPont in the U.S (production started in 2002). APFO has been mostly used in making fluoropolymers (see below). In 2002, DuPont and Daikin committed to selling APFO only for use in the fluoropolymer industry (SPI, 2002). Additional limited research quantity production, use and disposal have taken place in numerous academic and industrial locations worldwide over the past fifty years as indicated by patents and papers in the scientific literature (Prevedouros et al., 2006; USEPA Dockets EPA-HQ-OPPT-2003-0012, EPA-HQ-OPPT-2004-0112 and EPA-HQ-OPPT-2004-0113).

By 2002, the principal worldwide APFO manufacturer by the ECF process, 3M, discontinued external sales and ceased production leaving only a number of relatively small producers in Europe and in Asia (OECD, 2004) compared to potential Russian producers. New APFO production capacity based on perfluorooctyl iodide commenced in the U.S. by DuPont in late 2002 (DuPont, 2005a). At the time of shut down, 3M listed their APFO capacity at 227 metric tonnes per year. Between 1992 and 2002, 3M's total PFOA production averaged 113 metric tonnes (250,000 pounds) annually (Wendling, 2003). Approximately 97% of the PFOA produced by 3M was used by its industrial customers and its own processes as a fluoropolymer processing aid, and the remainder was used in medical film coatings and electronics applications. DuPont's capacity was given as 136 metric ton-

nes per year at the time of start up (USEPA docket AR-226-1094). Current producers are listed in Table 3. Estimated historical production is provided in Table 4.

Table 3 Current Manufacturers of APFO (DuPont 2005)

Region	Producer(s) in 2005
Europe	Miteni (Italy) some production in Russia
USA	DuPont
South America	None
Asia	Daikin (Japan), several Chinese Producers

Table 4 Estimated Industry-Wide Global Historic APFO Manufacture

Time Period	Number of Years	Estimated Global Average Annual APFO Production (tonnes/year)	Estim. Global Average APFO Prod. (cumulative tonnage over the period)
From 2003	3	Data not available	Data not available
1995-2002	8	200 – 300	1600 – 2400
1980-1994	15	100 – 150	1500 – 2250
1965-1979	15	30 – 50	450 – 750
1951-1964	14	5 – 25	70 – 350
			3600 – 5800*

* Rounded to the nearest 100 t = metric tonnes data according to Prevedouros et al. (2006)

For information about emission reduction programes see Section "2.2.1.6 Measures and programes for the reduction of APFO/PFOA emissions".

2.1.1.1 Production in the European Union, Italy

The only known producer of APFO in the European Union in 2005 is Miteni S.p.A. (owned by Mitsubishi Corp.). Miteni produces APFO at Trissino (Vincenza) using the electrochemical fluorination process (ECF). The actual production volume per year is in the range <100 tonnes. All of the volume produced is sold to customers outside the production site. An overview of Miteni's historic PFOA production is listed in **Error! Reference source not found.** (Miteni, 2005).

Table 5 Estimated Historic APFO Manufacture at Trisono, Italy

Time Period	Number of Years	Estimated Average Annual* APFO Production [t/yr]
2003-2004	2	40
1995-2002	8	30
1980-1994	15	20
1965-1979	15	10
1951-1964	14	0

* Rounded to the nearest 10 t metric tonnes (Miteni, 2005)

2.1.1.2 Production in the European Union, Germany

Prior to 2004 virgin PFOA (as APFO) was produced in the Industrial Park Gendorf in Germany.

The virgin APFO-production at the Gendorf site was ceased in 2004. The former production capacity was 5 to 40 metric tonnes/year. APFO was produced by oxidation of $C_8F_{17}I$. Virgin APFO production in former years is listed in Table 6.

Table 6 Historic APFO production at Gendorf, Germany (Dyneon, 2005)

	2003	2002	2001	2000-1995	1994-1990
Production [tonnes/year]	5	10	40	20-25	5 – 15

Additional volumes of APFO were supplied by recovery and recycling from waste streams resulting in the reduced demand for virgin material in recent years (Dyneon, 2005).

2.1.2 Current and Historical Use

The largest direct use of PFOA is as the ammonium salt as a processing aid in the manufacture of certain fluoropolymers. In this process APFO is used as an emulsifier in aqueous solution during the emulsion polymerisation of tetrafluoroethylene. APFO is not consumed during the polymerization process. The remaining APFO may be released into air or water, remain in the fluorpolymer product, be recycled, or be destroyed during preparation of the fluorpolymer (SPI, 2005).

In 2002, there were thirty- two fluoropolymer manufacturing sites worldwide located in North America (eight), Japan (seven), China (seven), Europe (seven), Russia (two) and India (one) with a total fluoropolymer manufacturing capacity of 144,000 metric tonnes (Prevedouros et al., 2006).

The ammonium salt of PFOA, ammonium perfluorooctanoate (APFO) is used in the production of most, but not all fluoropolymers. It is also possible to use other perfluorocarboxylates as polymerization aids. For example for the production of Polyvinylidene fluoride (PVDF) some producers use ammonium perfluorononanoate (APFN) instead of APFO. This depends on process and producer. The production and consumption of all fluoropolymers has grown steadily and, along with it, the usage of APFO. Table 7 shows the worldwide consumption of fluoropolymers for 2001 (Ring, Kalin, and Kishi, 2002). An estimated 94,300 metric tonnes of fluoropolymers were consumed worldwide in 2001.

Table 7 Global Fluoropolymer Production by Type for 2001, Excludes Chinese and Russian Production [thousand metric tonnes]

Fluoropolymer Grade	US	Western Europe	Japan	Totals
PTFE (polytetrafluoroethylene)	19	16	12	47
FEP (fluorinated ethylene propylene)	10	1.5	3.7	15
PVDF (polyvinylidene fluoride)	11	6.1	1.2	19
Other	6.5	1.4	5.9	14
Total	47	25	23	95

Figures derived from Ring, Kalin, and Kishi, 2002.

APFO consumption can be estimated for the various fluoropolymers that use it. The usage rate generally depends on the polymer type and the specifics of a given producer's manufacturing process. Excluding PVDF, the consumption of APFO for other fluoropolymer types is estimated to be 200-300 metric tonnes (Prevedouros et al., 2006). This range of estimated consumption divided by the worldwide fluoropolymer production of 76,000 metric tonnes (94,300 metric tonnes less 18,300 metric tonnes PVDF consumption from Table 7) would yield a rough range of APFO usage factors of 0.27% to 0.40% on a dry polymer basis.

Applying these factors to the estimated production volumes in Table 7, a very rough usage pattern by main regions results. This is shown in Table 8 below for fluoropolymers excluding PVDF.

Table 8 Range of Estimated Global APFO Consumption for Fluoropolymers Production (excl. PVDF) for 2001 at Usage Factors Ranging from 0.27% to 0.40% (metric tonnes of APFO rounded to the nearest 5 tonnes)

Fluoropolymer Grade	US	Western Europe	Japan	Totals
PTFE (Polytetrafluoroethylene)	50 - 75	40 - 65	30 - 50	120 - 190
FEP (fluorinated ethylene propylene)	25 - 40	5 - 10	10 - 15	40 - 65
PVDF (Polyvinylidene fluoride)	N/A	N/A	N/A	N/A
Other	15 - 25	5	15 - 25	35 – 55
Total	90 - 140	50 - 80	55 - 90	195 - 310

Note: Excludes Chinese and Russia Consumption

Similarly, using historical production estimates and the usage factors ranging from 0.27% to 0.40%, the estimated historic usage for the manufacture of fluoropolymers by major region, as shown in Table 9 below.

Table 9 Estimated Global Historical APFO Usage for Fluoropolymers Production since 1980 (excl. PVDF) at Usage Factors ranging from 0.27% to 0.40% (metric tonnes of APFO rounded to the nearest 50 tonnes)

Period	US	Western Europe	Japan	Totals
1980-1984	150 – 250	100 – 150	100	350 – 500
1985-1989	200 – 300	150 – 250	200 – 300	550 – 850
1990-1994	250 – 400	200 – 300	200 – 300	650 – 1000
1995-1999	300 – 500	300 – 400	300 – 400	900 – 1300
2000-2001	150 – 200	100 – 150	150 – 200	400 – 550
totals	1050 – 1650	850 – 1250	950 – 1300	2750 – 4200

Underlying fluoropolymer production volumes derived from Ring, Kalin, and Kishi (2002) (CEH Marketing Research Report).

Considering that these data exclude Russia and China APFO consumption, the total agrees well with the estimated range of APFO produced for the same period in Table 4.

The largest global manufacturers of fluoropolymers and fluoroelastomers and their respective market share are listed in Table 10.

Table 10 Current manufacturers of fluoropolymers and -elastomers, estimation of market share. (Not all manufacturers use APFO as a polymerisation processing aid.)

Company	Rough estimation of market share in 2004 [%]
DuPont	27
Dyneon	16
Daikin	15
AsahiGlass	11
Solvay Solexis	10
DDE (JV Dow/DuPont)*	9
Atochem**	4
Others	8

* DDE joint venture disbanded July 1st 2005 with fluoroelastomers remaining in DuPont at Dordrecht site (NL)

** Arkema: new name since 2005

2.1.2.1 Use as processing aid in the manufacture of fluoropolymers and fluoroelastomers in the EU

Corresponding to the companies, as mentioned in Table 10, the manufacturing facilities for fluoropolymers and fluoroelastomers in the European Union are listed in Table 11. Downstream users of fluoropolymers (for example in the form of APFO containing dispersions) are not included.

Table 11 Fluoropolymers and -elastomers manufacturing sites in the EU (Plastics Europe 2005)

Company	Manufacturing site, located in the EU
DuPont	Dordrecht, Netherlands
Dyneon	Gendorf, Germany
Daikin	Pierre-Benite (Lyon), France Oss, Netherlands
Solvay Solexis	Spinetta Marengeo, Italy Tavaux, France
Asahi Glass	Thornton Cleveleys, Lancashire, United Kingdom
Arkema*	Tavaux, France

* name before 2005: Atochem

2.1.2.2 PFOA for Non-Fluoropolymer Uses

3M had produced limited quantities of PFOA (approximately 3.6 metric tonnes (8,000 pounds) annually) for non-fluoropolymer use (Wendling 2003). The primary use of PFOA unrelated to the production of fluoropolymers occurred in medical film applications where PFOA served as an antistatic additive in coatings sandwiched between multi-layer medical films. In addition, a small amount (approximately 0.23 metric tonnes (500 pound per year), was derivatized as an ester to form a methacrylate co-polymer employed in the electronics industry as a humidity barrier in printed circuit boards and to secure silicon oil on precision bearings (Wendling 2003). PFOA was also used in a number of products as a surfactant i.e. cleaning formulas, and dispersions were used in other minor uses with direct environmental releases i.e. lubricants.

2.2 Environmental Exposure and Fate

Direct and indirect sources of perfluorocarboxylates have been recently reviewed (Prevedouros et al., 2006). A number of other studies have considered the environmental fate of fluorotelomer alcohols as an indirect source of PFOA in the environment (Dinglasan 2004, Ellis et al. 2004, Hurley et al. 2004, Lange et al. 2002, Martin et al. 2004c, Wallington et al. 2006, Wang et al 2005a, Wang et. al 2005b). Current understanding of the environmental behavior of PFOA and PFOA precursors is evolving and may change as new data are generated. These new data are also discussed in the following sections.

2.2.1 Sources of Environmental Exposure

2.2.1.1 Production of PFOA/APFO

Current producers of the ammonium salt of PFOA (called APFO) are listed in Table 3. There is minimal information available about emissions for producers in Europe and USA. No information is available for production outside the EU and USA.

In 2005 the only production facility in the EU is the Miteni plant, located at Trissino, Italy. The former Hoechst and later Clariant production at Gendorf, Germany, ceased in 2004.

USA, Alabama, Decatur (3M)

The 3M Corporation produced PFOA at various facilities in the US from 1969 to 2002 using electrochemical fluorination (ECF) technology. Currently the 3M subsidiary Dyneon manufactures fluoropolymers at its Decatur, Alabama plant and another company, Daikin America also manufactures fluoropolymers nearby. While not currently manufactured in Decatur, PFOA used in the manufacturing process of fluoropolymers is emitted to the surrounding air. It was estimated that approximately 5 kg (11 pounds) of PFOA per year are being emitted from the 3M Dyneon facility; the adjacent Daikin facility has air emissions of approximately 204 kg(450 pounds) per year (Wendling 2004 EPA-HQ-OPPT-2004-0112-0002). In addition, from 1978 to 1998, 3M incorporated sludge from the on-site wastewater treatment plant via subsurface injection in an on-site area designated as the Sludge Incorporation Area. It is not known how much total PFOA contaminated sludge was buried at this site; however, a USEPA hazard assessment for PFOA and its salts reported that less than 227 kg (500 lbs.) were buried in 1997 (USEPA 2002). Treated process wastewater containing PFOA was also discharged from the facility to Baker's Creek near its confluence with the Tennessee River. The average PFOA concentration in wastewater samples collected from the Decatur facility was 602, 766, 1,028, and 310 ppb in sampling data from 1998, 1999, 2000, and 2001, respectively (Santoro 2003). The average PFOA concentration in wastewater samples at this location in January and May 2003 were 58 and 88.3 ppb, respectively. For 1999, 3M estimated total PFOA-compound (PFOA and related salts) water releases of < 13.6 metric tonnes (30,000 lbs.) at its Decatur AL location (USEPA 2002). A data collection and monitoring program is underway and a screening level exposure assessment will characterize releases and exposures associated with this site. More data are being generated and will be made available to the public through the US EPA Docket in 2006 and 2007.

USA, Minnesota, Cottage Grove (3M)

Analysis of PFOA in the wastewater effluent from the 3M Cottage Grove, MN facility indicated wastewater emissions of roughly 20-2,000 ppb (Santoro 2003). The average concentration of PFOA in wastewater sample emissions from January-March 2000, September-October 2000, and December 2002 were 1,991, 216, and 180 ppb, respectively. The average PFOA concentration in wastewater samples from this facility for January-June 2003 ranged from 17.7 to 110.5 ppb (Santoro 2003). For 1999, 3M estimated total PFOA-compound wastewater releases of < 6.8 metric tonnes (15,000 lbs.) at its Cottage Grove MN location (USEPA 2002). For 1997, 3M estimated 0.885 metric tonnes (1,950 lbs.) of PFOA-compound stack releases at its Cottage Grove, MN location and another 2.0 metric tonnes (4,500 lbs.) from Cottage Grove incinerated offsite (USEPA 2002).

USA, North Carolina, Fayetteville (DuPont)

Information about emission according to Table 12 is given.

Table 12 Emissions from DuPont APFO production sites (DuPont, 2005a) [metric tonnes /year]

APFO Emissions		2002	2003	2004
APFO Manufacture	US	<0.1	<0.1	<0.1

* Prior to 2002 DuPont did not manufacture APFO, it was manufactured by 3M (for 3M emissions data see above paragraph).

EU, Italy Trissino (Miteni)

According to Miteni, information about production related emission of PFOA at Miteni's production site Trissino to air or water is not available (Miteni, 2005).

EU, Germany, Gendorf (Dyneon)

Emission to Air / Water / Terrestrial Compartment:
At the Gendorf site PFOA/APFO was produced in a closed process with marginal losses only. E.g. Emission to air before the shut down in 2004 was < 1kg/year. All emission quantities are included in the data provided in the use section for the Gendorf site (Dyneon, 2005).

Other sites

No information is available for the other production sites, for example in the UK, Poland, Russia, China, India or Japan). See Table 13 for the full list of fluoropolymer sites using PFOA as a polymerization aid.

2.2.1.2 Use of PFOA/APFO

During the manufacture of fluoropolymers, APFO is used to suspend and emulsify some fluoropolymers. APFO is not consumed but may be recycled or destroyed during preparation of the product, released into air or water or may remain in the product. One product containing a small amount of APFO is the aqueous fluoropolymer dispersion (AFD). AFD is used in the manufacture of products with the potential release of APFO during the manufacturing process (SPI, 2005). Based on 2003 survey data, approximately 15% of the APFO used to make fluoropolymers world-wide remained in products sold to customers in the form of AFD (SPI, 2005). The APFO content of commercially available AFD was reported to be typically <0.5% by weight (range of 0.7 to 0.2% by weight). In a survey undertaken by the Society of the Plastics Industry, Inc. and the Fluoropolymer Manufacturers Group in the U.S.A., general information on the manufacturing process and on-site sampling of representative processing facilities was collected to study APFO emissions from these sources. Several processes were studied including the manufacture of glass cloth coating and the manufacture/formulation of coating products for metal, glass, and specific polymeric substances. Common to these processes is a final step where the polymer is heated in order to melt it onto the substrate, with the temperature dependent on the type of fluoropolymer. However, steps until the final heating can release APFO through various waste streams. AFD is also used as an additive to dry cell batteries, in the manufacture of valve and pump packing materials, in the manufacture and coating of film, manufacture of impregnated felt cloth for the manufacture of air pollution control

"bag houses", and in the manufacture of fluoropolymer fibers to make a yarn, These manufacturing processes do not utilize a final high heat melting step. Results from this survey indicate that approximately 26% of the APFO present initially in the AFD is released to air, water, and solid waste streams and 62% decomposes (during the heating steps). The remaining 12% APFO is used in low temperature processes and its fate was described as "undetermined" by the project (SPI, 2005).

Main PTFE products sold in dry, bulk form (dry powders, granular resins) contain APFO in the order of 1-5 ppm (Cope, 1981).

Information about emission of APFO to the environment is only available for some sites. It is assumed that emissions of APFO take place at all fluoropolymer production sites. Some sites are named in Table 13. (In this table only fluoropolymer production sites are listed not sites of fluoropolymer downstream users. At sites of fluoropolymer downstream user's relevant APFO emissions also occur.)

The amounts of APFO used in 1991-1992 are illustrated in the following Table 13 (USEPA, 1992- AR226-2423; USEPA, 1993 - AR226-2070).

Table 13 Global PFOA/APFO Users in 1991 and 1992 - historical

Site	**1991 Amount** [metric tonnes (lbs)] **(USEPA, 1992- AR226-2423)**	**1992 Amount** [metric tonnes (lbs)] **(USEPA, 1993 - AR226-2070)**
DuPont, Washington Works, WV (USA)	32.3 (71,100)	30.5 (67,200)
Dordrecht Works (NL)	14.2 (31,400)	17.2 (37,950)
Shimizu	7.23 (15,950	6.67 (14,700)
DuPont, Chambers Works, NJ (USA)	0.90 (2,000)	0.48 (1,050)
DuPont, Spruance, VA (USA)	0.68 (1,500)	0.64 (1,400)
DuPont, Parlin NJ (USA)	1.13 (2,500)	
U.S. Major Customers	2.79 (6,150)	
Non-U.S. Major Customers	1.84 (4,060)	

Table 14 Fluoropolymer manufacturing site using PFOA as a polymerisation aid in the manufacture of PTFE or FEP including sites mentioned in the previous table (Will et al. 2005)

Country	**Name**	**City**
Americas		
USA	AGC Chemicals Americas Inc. (Asahi Glass)	Bayonne, NJ
USA	Solvay Solexis, Inc.	Orange, TX
USA	Daikin America Inc.	Decatur, AL
USA	DuPont	Parkersburg, WV
Europe		
EU		

Country	Name	City
Germany	Dyneon 3M	Gendorf
Italy	Ausimont S.p.A (now Solvay Solexis)	Spinetta-Marengo
Netherlands	DuPont	Dordrecht
UK	Asahi Glass UK (formerly ICI)	Thornton Cleveleys
Poland	Zakłady Azotowe w Tarnowie-Mościcach S.A.	Tarnow
Non-EU		
Russia	KCKK	Kirovo-Chepetsk
Russia	JSC "Halogen"	Perm
Asia		
Japan	Asahi Glass	Ichihara
Japan	Daikin Industries	Kashima
Japan	Daikin Industries	Settsu, Osaka
Japan	DuPont-Mitsui	Shimizu
China	Chenguang Res Institute	Zigong
China	Fuxin Hengtong Fluorchemical Co., Ltd.	Fuxin
China	Jiangsu Meilan Chemical Group Corp.	Taizhou
China	Jinan 3F Fluorochemical	Jinan
China	Shanghai 3F New Materials	Shanghai
China	Daikin Fluorochemicals (China) Co., Ltd.	Changshu, Jiangsu
China	Shangdong Dongyue Chemical Co., Ltd.	Zibo, Shangdong
India	Hindustan Fluorocarbons Ltd.	Bashir Bagh ,Hyderabad

DuPont

For 1999, DuPont estimated stack releases of 10.8 metric tonnes (24,000 lbs.) APFO at its Washington Works, WV location, plus another 7.26 metric tonnes (16,000 lbs.) from Washington Works that were incinerated offsite (USEPA 2002). For 1999, DuPont estimated the following APFO wastewater releases per location: Washington Works WV, 24.9 metric tonnes (55,000 lbs); Parlin NJ, 0.136 metric tonnes (300 lbs.); Spruance VA, 0.068 metric tonnes (150 lbs.); Chambers Works NJ, 4.3 metric tonnes (9,500 lbs.) (USEPA 2002). DuPont also estimated that 1.8 metric tonnes (3,900 lbs.) of APFO in waste sludge was landfilled on site in 1999 at their Chambers Works, NJ facility and 1.2 metric tonnes (2,600 lbs.) APFO was transferred offsite to a hazardous waste landfill from their Washington Works, WV plant.

Aggregated emissions from DuPont production sites from 1999-2004 are listed in Table 15.

Table 15 PFOA/APFO-emissions from DuPont sites (DuPont, 2005a) [metric tonnes /year]

APFO Emissions in metric tonnes		1999	2000	2001	2002	2003	2004
Fluoropolymer / Fluorotelomer Manufacture	US	51	47.6	26.3	15.9	11.7	2
	Asia	9.3	10.2	4.8	4.9	5.0	1.4
	Europe (2 sites)	4.5	6.2	4.8	2.1	2.5	3.6
Total		64.8	64	35.9	22.9	19.2	7

Emissions from DuPont's Dordrecht site located in The Netherlands, Europe are listed in Table 16.

Table 16 APFO-emissions from DuPont Dordrecht site (DuPont, 2005a)

APFO [metric tonnes / year]	**1999-2004**
Air	0 – 2
Water	1 – 4
Product	< 3
Emission to terrestrial compartment (land-fill)	< 0.5

USA, West Virginia, Washington Works (DuPont) - historical

A PFOA mass balance carried out for 1991 reported that 32.25 metric tonnes (71,100 lbs) of PFOA were purchased with an estimated disposal of 9.26 metric tonnes (20,410 lbs) and 5.10 metric tonnes (11,250 lbs) to the Ohio River and the Delaware River, respectively, 2.08 metric tonnes (4,590 lbs) were emitted to air in the Ohio River valley, 1.7 metric tonnes (3,700 lbs) were released to land and 5.0 metric tonnes (11,000 lbs) and 0.02 metric tonnes (50 lbs) were disposed of in dispersions and fine powder, respectively. The release of PFOA to the Ohio River can be separated into granular, fine powder/dispersion, and FEP sources at 0.072 (160), 1.3 (2,850), 7.9 metric tonnes/year (17,400 lbs/year), respectively. PFOA release to the Delaware was entirely as a dispersion supernate. PFOA that was either recovered or destroyed in FP driers or in FEP ovens represented 3.7 metric tonnes (8,100 lbs) total (3.4 (7,500) and 0.32 metric tonnes (600 lbs), respectively) (USEPA, 1992 AR226-2423). Emissions of PFOA from the Washington Works facility in 1983 were 9.84 (21,700), 5.76 (12,700), 3.5 (7,700) and 3.6 metric tonnes (8,000 lbs) to water, air, off-plant disposal, and to product, respectively. In 1986, these values were 8.66 (19,100), 7.35 (16,200), 3.8 (8,300), and 3.8 metric tonnes (8,300 lbs), respectively (Zipfel, 1987).

EU, The Netherlands, Dordrecht Work (DuPont) – historical

An APFO mass balance carried out for 1990 reported that 14.2 metric tonnes (31,400 lbs) of PFOA were purchased with 9.62 (21,200), 3.22 (7,100), 0.18 (400), and 1.0 metric tonnes (2,300 lbs) disposed of to the Merwede River, to air, to the Dordrecht municipal disposal area, and to product dispersions, respectively. 0.18 metric tonnes (400 lbs) (or 1.2% of the feed) were either destroyed or recovered from FEP ovens (USEPA, 1992 AR226-2423).

Japan, Shimizu Works (DuPont) – historical

A PFOA mass balance carried out for 1990 reported that of the initial purchase of 7.23 metric tonnes (15,950 lbs), 5.81 (12,800), 0.23 (500), 0.05 (10), and 0.77 metric tonnes (1,700 lbs) were released to Sugura bay, to air, to land, and to product with 0.43 metric tonnes (950 lbs) destroyed by incinerator (USEPA, 1992 AR226-2423).

USA, other historical emissions

The DuPont Chambers Works facility reported that 0.71 (1,560), 0.16 (350), and 0.04 metric tonnes (90 lbs) of PFOA were released to the Delaware River, to air, and to product, respectively, in 1990. The Spruance facility reported the release of 0.68 metric tonnes (1,500 lbs) of PFOA to the James River in 1990 (USEPA, 1992 AR226-2423). A review of the PFOA emission reduction to the environment reported that of 52.0 (114,600), 54.7 (120,650), and 54.9 metric tonnes (121,100 lbs) used in 1990, 1991, and 1992, 87.4, 82.7, and 79.8% were released to the environment, respectively (USEPA 1993 AA226-2070). 3M estimated that 0.885 metric tonnes (1950 lbs) PFOA compounds were released to the air from vent stacks from the Cottage Grove facility in 1997 with releases occurring from 100-200 days/year (ATSDR, 2005).

EU, Germany, Gendorf works (Dyneon)

About 15 % of the 2004 fluoropolymer industry world market is produced by Dyneon. At the Gendorf site Dyneon's throughput of APFO at full production capacity utilization is in the range of 10-100 tonnes/year. A full production scale, advanced recovery and recycling system is in operation at this facility. This has reduced emissions of APFO to the 1 – 10 tonnes/year range. A similar AFPO recovery system is in place for fluoropolymer dispersion products. This ensures that they contain greatly reduced levels of APFO, thereby minimizing the releases of APFO at downstream processing locations. Manufacturers other than Dyneon also supply conventional fluoropolymer dispersions to downstream processors of these products (Dyneon, 2005).

Emission to air

Total releases of PFOA to air never exceeded 1 ton per year. Improvements to abatement and recovery technology have been routinely implemented to maintain a low air emission level even with increased production output. Emissions in 2005 were less than 0.5 tonnes per year.

- Before 1995 < 1 tonne/year
- 2005 <0.5 tonnes/year

Emission to Water

Abatement and recovery technology has been continuously improved and implemented over the past few years.

- Before 1995 1-10 tonnes/year
- 2005 1-5 tonnes/year

Emission with products (information for dispersions only)

Conventional dispersion products contain some residual APFO, typically about 1000 – 2000 ppm. Recovery technology for removal of APFO to below 10 ppm was developed and installed by Dyneon in 2004. Many fluoropolymer dispersions are now being produced with this new technology. 2005 is a transition year. Therefore an estimate of 2006 is provided.

- before 1995 1-10 tonnes/year
- 2005 <0.5 tonnes/year

Table 17 APFO emissions from Dyneon, Gendorf (Germany) production facility (Dyneon 2005)

APFO emissions in metric tonnes per year	**Before 1995**	**2005**
Air	< 1	< 0.5
Water	1-10	1-5
Product	1-10	< 0.5

2.2.1.3 Other Direct PFOA/APFO Sources

- Consumer and industrial products and fire-fighting foams containing perfluorocarboxylates including perfluorooctanoate (PFO): PFCAs and their derivatives have been used and are still present as additives and impurities in industrial and consumer products as well as in fire-fighting foams (Kissa (2001), Moody et al. (2002) and Prevedouros (2006)). As a consequence of residues and impurities of PFOA in some products and articles, use results in widespread release. Disposal of these products and articles at landfills is likely to lead to the release of PFOA to landfill effluents. Wastewater treatment plant discharge can also be a source (Boulonger(2005).

- APFN: Ammonium Perfluorononanoate (APFN) is manufactured primarily in Japan by ozonolysis of a mixture of linear fluorotelomer olefins (Asahi Glass Co., 1975). The commerical product is a mixture comprised of mainly odd acids 7-13 carbons in length. APFN is primarily used as a processing aid in fluoropolymer manufacture, most notably polyvinylidene fluoride (PVDF).

- Silver (Ag) and potassium (K) salts of PFOA have also been items of commerce produced and used in very small quantities (approx. < 1 metric ton per year).

2.2.1.4 Indirect Sources (Chemical substances which contain PFOA as an impurity or may degrade to PFOA)

Chemical substances that include the C_8F_{17} moiety may be a source for PFOA. Examples are the telomer alcohols, sulfonates, sulfonamides, carbonates, phosphates, phosphinic and phosphonic acid (Dookhith, 2001; Kissa, 2001; Martin et al., 2004a; Begley et al., 2005; Prevedouros et al., 2006). Manufacturing, use and degradation of these derivates may be PFOA sources.

Perfluoroalkyl precursor monomers

Fluorotelomer-based products have been manufactured since the early-1970's and used in many of the same industrial and consumer product applications as POSF-based products. Perfluoroalkyl iodide, Telomer A, is the basic raw material manufactured at one site in the U.S., one site in Germany and two sites in Japan. Residual fluorotelomer raw materials, such as 8:2 telomer alcohol, per-

fluoroalkyl iodide and perfluorosulfonamides are indirect sources of PFCAs. They may react during chemical processing or release to the environment and form PFCAs (including PFOA). PFOA, known and suspected PFOA precursors are present in many fluorotelomer-based products. Environmental degradation of fluorotelomer raw materials present in fluorotelomer-based products is a potential indirect source of PFCAs. Microbial degradation, in vivo metabolism in higher organisms and abiotic degradation have been shown to produce low levels of PFOA (Wang, 2005a and 2005b; Fasano et al. 2006, Prevedouros et al., 2006).

Recently, Dinglasan-Panlilio and Mabury (2006) identified the presence of residual unbound fluorotelomer alcohols in varying chain lengths (C6-C14) in seven commercially available and industrially applied polymeric and surfactant materials. It was determined that the fluorinated materials examined consist about of 0–1.6 % residual 8:2 Fluorotelomer alcohols of dry weight. Similarly, perfluoroalkyl sulfonamido based products, principally perfluorooctyl sulfonyl derivatives have been reported to contain residual raw material concentrations (e.g. sulfonamide alcohols, amides) at similar levels. (3M 1999c).

Fluorotelomer based products

Fluorotelomer-based products, monomers and polymers, may contain trace levels of PFCAs, may contain PFOA (monomeric products up to % level), may degrade to PFOA and may contain other impurities that may degrade to PFOA (e. g. Begley et al. 2005, TRP, 2003, Washburn et al. 2005, Prevedouros et al., 2006).

Impurities and unreacted monomers are present in telomer based polymer products. The 8:2 telomer alcohol is the most extensively studied of the unreacted raw materials present in fluorotelomer based polymers. It can be transformed by both abiotic and biotic processes to form PFOA.

Research suggests the existence of at least two biodegradation pathways, (alpha and beta oxidation) by which, for example, the 8:2 telomer alcohol would biodegrade to either an even carbon numbered perfluorocarboxylic acid, i.e., perfluorooctanoic acid, or an odd carbon numbered perfluorocarboxylic acid, i.e., perfluorononanoic acid (Wang et al 2005a, Wang, et. al 2005b, Dinglasan 2004, Lange, et al. 2002).

Wang et al (2005a) investigated the biodegradation of a 3-^{14}C radiolabelled 8:2 telomer alcohol in a dilute domestic activated sludge test system for 28 days. Biodegradation products were separated and quantified by LC/ARC (on-line liquid chromatography/accurate radioisotope counting). Transformation products were identified by quadrupole time of flight mass spectrometry. The alcohol strongly sorbed to the septa of the incubation containers with 41% of the mass of the added chemical found in septa extracts at the end of the study. However, the portion of the alcohol not sorbed to the septa was biodegraded to form PFOA and several intermediates. PFOA accounted for 2.1 percent of the total mass added. Other products included the 8:2 saturated acid at 27 percent of the total mass added and the 8:2 unsaturated acid 6 percent of the total mass added. Wang et al (2005a) suggest that this study indicates that in addition to the formation of PFOA, alternative pathways of degradation exist for telomer alcohols resulting in the mineralization of the perfluorinated carbon bonds. This was confirmed in Wang et al. (2005b).

In subsequent studies of the 3-^{14}C radiolabelled 8:2 telomer alcohol, Wang et al (2005b), analyzed the headspace of sealed vessels containing mixed bacterial cultures and vessels containing activated sludge from a domestic sewage treatment plant under continuous air flow for up to 4 months. The mixed bacterial culture from an industrial wastewater treatment sludge was enriched using 8:2 telomer alcohol. Using analytical methods previously described, a total of eight biodegradation products were identified, including three novel metabolites. No perfluorononanoic acid was observed in the test systems, suggesting that alpha-oxidation did not occur under the conditions of the test.

A similar observation was made by Wang et al. (2005a) and Dinglasan et al. (2004). In the mixed bacterial culture study, PFOA concentrations increased to day 56 and ultimately accounted for 6% of the total mass of the 8:2 telomer alcohol after 90 days. 1-^{14}C PFOA accounted for about 6% of the initial telomer alcohol after 56 days. In the continuous air flow and closed vessel activated sludge systems, $^{14}CO_2$ was detected in the headspace, and increased up to 12 percent of the available ^{14}C over 135 days with periodic addition of ethanol. A net increase of fluoride ion in the mixed bacterial culture equivalent to 12 percent of total mineralization of the alcohol was also observed. Without additional ethanol, however, only 0.4% $^{14}CO_2$ was reported during the study period using an activated sludge from a different source. The authors concluded that with the regular addition of an organic carbon source, mineralization of multiple -CF_2- groups was enhanced, and under conditions that may be present in a wastewater treatment plant, defluorination and mineralization of these groups to form shorter fluorinated carbon metabolites may occur.

When ^{14}C-PFOA was used as a starting material in a similar experiment, <0.6% of the ^{14}C mass balance was reported as $^{14}CO_2$ after 28 days of incubation. This suggests that the significant quantities of $^{14}CO_2$ reported in the 8:2 telomer alcohol experiment above using supplemental ethanol did not originate from the decarboxylation of ^{14}C-PFOA but, unlike from the decarboxylation of telomer alcohol metabolites such as the unsaturated acid metabolites (Wang et al. 2005b). The presence of perfluorohexanoic acid in the mixed bacterial system, accounting for approximately 1 percent of the concentration of 3-^{14}C radiolabelled 8:2 telomer alcohol from day 28 to day 90 of the test, also indicates that 8:2 telomer alcohol metabolites are undergoing defluorination and mineralization processes.

Dinglasan et al. (2004) studied the transformation of 8:2 telomer alcohol in aerobic mixed microbial systems over an 81 day test period. The mixed culture was collected from a contaminated groundwater site and was initially enriched on 1,2-dichloroethane. Degradation of the test substance was followed using gas chromatography with electron capture GC/ECD. Transformation products were identified using either gas chromatography/mass spectroscopy GC/MS or liquid chromatography/tandem mass spectroscopy LC/MS/MS. By day 7, 85% of the 8:2 telomer alcohol (initially present at 50 µg/l) was degraded and by day 16, concentrations of the telomer alcohol were below the detection limit of 2 µg/l. By day 52, 6% of the total mass of 8:2 telomer alcohol was converted to PFOA; however by day 81 this value was reduced to approximately 3 percent of the total mass. Unlike the findings of Wang, et al. (2005b), the 8:2 unsaturated acid was the most abundant metabolite comprising approximately 50 percent of the total mass of 8:2 telomer alcohol, with the 8:2 saturated acid accounting for less than 10 percent of the total mass of 8:2 telomer alcohol.

In a study of Lange (2002) using a commercial mixture of fluorotelomer alcohols containing primarily the 6:2, 8:2, or 10:2 forms, activated sludge from municipal wastewater treatment plants was shown to nearly completely remove the 4:2, 6:2, 8:2, 10:2, and 12:2 Fluorotelomer alcohols within 16 days, with moderate and little degradation for the 14:2 and 16:2 alcohols, respectively. Disappearance was measured through analysis of the extract using HPLC/MS. No biodegradation occurred in the abiotic controls. The C5–C12 perfluorocarboxylic acid degradation products were detected in the test cultures following the 16-day test period. Only levels of perfluorooctanoic acid were quantified and were found at 6–7% of the total FTOH starting material or 23% of the 8:2 telomer.

POSF-based products

Perfluorooctyl sulfonyl-based (POSF) products made by the ECF process contained PFCA impurities (3M, 2003a). POSF-based products have been manufactured at several sites in the U.S. as well at sites in Belgium, Italy, Germany and Japan. Production began in the 1950's. The principal (estimated 80-90% market share) manufacturer, 3M, phased out production starting in 2000 and completing its production phaseout in 2002. Global production by the major manufacturer of POSF as a

raw material in 2000 was approximately 3,700 tonnes. The global use, distribution and release of POSF-based products have been reported. Uses included industrial applications and consumer products (i.e. food-contact paper, spray cans for do-it-yourself application) worldwide. POSF-based products contained between 200-1,600 ppm of PFOA (solids basis). Although principally composed of fluorinated eight-carbon chains, the PFCA impurities in POSF-based product were a mixture of linear and branched (up to 30 wt%) chain isomers from four to nine carbons in length. Degradation of POSF-based residual raw materials and products is also a potential indirect source of PFCAs in the environment. (3M 1977, 1978, 2003a, and Boulanger et al., 2005; Prevedouros et al. 2006).

D'Eon et al. (2006) and Martin et al. (2006) estimated that in the atmosphere perfluoroalkane sulfonamides may degrade to the respective perfluorinated carboxylic acids in yields up to 45%.

Recent studies simulating the atmospheric degradation of N-ethyl perfluorobutanesulfonamide (N-EtFBSA), suggest that by analogy, N-ethyl perfluorooctanesulfonamide (N-EtFOSA) may also be a possible indirect source of perfluorocarboxylic acids, including PFOA in the environment (Martin et al. 2006). In smog chamber experiments using N-EtFBSA, as an environmental analog for N-EtFOSA, the atmospheric lifetime of N-EtFOSA was estimated as 20-50 days via reaction with photochemically generated hydroxyl radicals (Martin et al. 2006). N-EtFBSA was employed in the experimental design of the study rather than N-EtFOSA, because it is much more volatile, thus facilitating its vapor-phase introduction into the smog chamber. The results of these experiments suggest that the degradation of perfluorobutanesulfonamides (and by analogy, N-EtFOSA) may serve as atmospheric sources of some perfluorocarboxylates, including PFOA.

D'eon et al. (2006) conducted smog chamber experiments in order to deduce the atmospheric chemistry of N-methyl perfluorobutanesulfonamidoethanol (N-MeFBSE). The results of this study indicated that the atmospheric degradation of N-MeFBSE is rapid and results in the production of short chain perfluorocarboxylates among its degradation products. By analogy, it was concluded that similar degradation mechanisms would occur for the perfluorooctane compounds N-methylperfluorooctane sulfonamidoethanol (N-MeFOSE), which are widely distributed in the environment due to their previous use in oil and water repellent coatings for paper and packaging material. The authors of this study suggest that N-MeFOSE is capable of contributing to PFOS and perfluorocarboxylic acid deposition that is observed in remote global locations.

Biodegradation studies on N-EtFOSE are consistent with the suggestion that it is an indirect source of PFOA in the environment. In a screening test using activated sludge, N-EtFOSE was degraded to 34% of its initial concentration over 28 days (Rhoads et al., 2005). The major degradation products were 2-(N-ethylperfluorooctanesulfonamido) acetic acid (N-EtFOSAA) and perfluorooctanesulfinate (PFOSI), which account for 60% and 15% of the metabolites, respectively. PFOS accounted for 7% of the metabolites at 28 days, N-EtFOSA accounted for 4% of the metabolites and PFOA accounted for 2% of the metabolites. This work is in harmony with the results of Lange (2000). The biodegradability of N-EtFOSE (purity 99.9%) was measured in a 5-week screening study using wastewater treatment sludge as the inoculum (Lange, 2000). At an initial concentration of 0.047 µg/ml, complete biodegradation was reported in 14 days while at an initial concentration of 2.38 µg/ml, 90% biodegradation was reported in 35 days. The formations of metabolites over the study period were monitored using HPLC/MS. Major metabolites included N-EtFOSAA and 2-(perfluorooctanesulfonamido) acetic acid accounting for 85% of the total metabolites by day 35. Minor metabolites included N-EtFOSA (0.1% of the products at day 35), perfluorooctanesulfonamide (FOSA, at 5.0%), perfluorosulfinate (3.5%), PFOS (7%), and PFOA (0.6%).

Biodegradation of fluorotelomer-based polymers

The potential of telomer based polymer products to form PFOA and other perfluorochemicals from degradation of the polymer is currently being evaluated. A number of studies by industry, USEPA, and academics are underway examining the biodegradation of fluorotelomer-based polymers with various Rf linkages in soil and sludge systems. These studies will address questions about rates and mechanisms of degradation of fluorotelomer-based polymeric products.

The potential for the degradation of the polymer linkage is currently being examined. Biodegradation of a fluorinated monomeric acrylate (3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-heptadecafluorodecyl methacrylate) has been reported by Dinglasan and Mabury (2005). This methacrylate monomer was incubated with a sewage inoculum and fluorotelomer alcohol metabolites measured using GC/MS and electrospray LC MS/MS. By day 48, the total measured telomer acids represented 3% of the initial mass of methacrylate monomer. Microbial- and fungal-mediated biodegradation of polyurethanes has been reported (Howard, 2002; Nakajima-Kambe et al., 1999). This process occurs quite slowly for unsubstituted polymers and the rate is expected to decrease further with the presence of added substituents (Darby and Kaplan, 1968). Although data from long-term studies are not currently available for the fluorinated polymers, it is likely that the presence of perfluorinated substituents will increase the persistence of the polymer in the environment in comparison to an unsubstituted polymer by either physically blocking potential sites on the polymer backbone that might be available for microbial/fungal enzyme attack or the perfluoro substituent will prevent polar enzymes from reaching the active enzyme site (Boese et al., 2005).

In addition, the structure of the polymer backbone and the degree of substitution will also determine its biodegradability. Polyester backbones susceptible to esterase attack are expected to be considerably more degradable than polyethylene structures (Nakajima-Kambe et al., 1999). An inherent biodegradability screening test (OECD 302B) was conducted on a fluorotelomer-based acrylate polymer made predominantly from the fluorotelomer alcohol 2-perfluorooctylethanol (Boese et al., 2005). More than 99.9% of the 2-perfluorooctylethanol was bound as ester in the polymer with the remaining material reported as low molecular weight residuals. Analysis of metabolites was conducted using GC/MS and LC MS/MS. Ester cleavage of the polymer was not reported after 28 days (Boese et al., 2005).

The study was conducted using a municipal wastewater treatment sludge inoculum under aerobic conditions over 28 days. Samples were taken from the system on days 0, 4, 14, and 28 and prepared using solid phase extraction followed by analysis using High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) for predicted biodegradation products based on earlier (proprietary) biodegradation studies. Due to the polymeric nature of the starting material the appearance of degradation products was measured rather than the direct measurement of the loss of starting material. By day 4 of the study 114 % degradation of the initial concentration of the starting material to a major biodegradation product was observed and mass balance appeared to have been achieved. Four minor metabolites were also observed. An increase in degradation products over 28 days in the abiotic controls suggests hydrolytic degradation also occurred.

Biodegradation of the 8:2 fluorotelomer alcohol in soil to the 8:2 saturated and unsaturated acids after 7 days has also been reported (Liu et al. 2005).

Biodegradation of other polymeric structures

3M (2003d) has reported on the inherent aerobic aquatic biodegradation of a fluoroaliphatic polymeric ester. The specific chemical identities of the test substance and metabolites were proprietary and not reported. In this study “fluoroaliphatic polymeric esters” were seen to be 100% degraded in a modified SCAS test within 4 days.

Studies of other polymer structures are being conducted by US-EPA, Industry and academic researchers but to date but very limited preliminary data have been reported

Recent papers by Sinclair and Kannan (2006) and others have reported that PFOA and other PFCAs are formed during wastewater treatment. The source of PFOA and other PFCAs is unknown, but their formation would be consistent with the degradation of polymer, 8:2 telomer alcohol, or other PFCA precursors.

Indirect photolysis of precursors

Fluorotelomer alcohols and perfluoroalkyl sulfonamido compounds such as perfluorooctyl sulfonamido ethanol, have been detected in the atmosphere (Stock et al. 2004, Shoeib et al. 2005). The ambient concentration of 8:2 telomer alcohol was reported to range approximately 9-123 pg/m^3 in North American air (Stock et al. 2004). Perfluoroalkyl sulfonamide concentrations were similar to telomere alcohols in North America. These substances have sufficient vapor pressure to be present in air at ambient temperatures (Kaiser et al. 2004, Krusic et al. 2005).

The work of Ellis et al. (2004) and others (Martin et al. 2002, Hurley et al. 2004, Wallington et al. 2006) suggests that an additional indirect source of PFOA may be the atmospheric oxidation of fluorotelomer alcohols.

Ellis et al. (2004) conducted smog chamber experiments with mixtures of 4:2, 6:2, and 8:2 telomer alcohols using Cl atoms in the absence of NOx to initiate the oxidation of fluorotelomer alcohols. Cl atoms are believed to share a common mechanism with OH radicals for attack on fluorotelomer alcohols (the hydrogen atom abstraction from the -CH2- group bearing the alcohol functionality) and are believed by the authors to be a reasonable surrogate for OH radicals in atmospheric oxidation studies of Fluorotelomer alcohols. Cl atoms were employed in the smog chamber experiments because reaction of telomer alcohols with Cl proceeds at a much higher rate as compared to hydroxyl radicals thereby facilitating the ability to analyze the degradation products more readily. The results of the experiments demonstrated that the fluorotelomer alcohols were oxidized to form perfluorocarboxylic acids. The overall yield of perfluorocarboxylic acids at an intermediate time point in the degradation was approximately 5 percent with PFOA produced at carbon yields of approximately 1.5 percent. The authors noted that in the presence of NOx, as in polluted urban air, fluorotelomer alcohols will undergo a different series of reactions that do not directly lead to perfluorocarboxylic acid formation. However, in remote areas where the concentrations of HO_2 and other peroxy radicals are comparable to those of NO and NO_2 it is likely that reactions leading to perfluorocarboxylic acid formation will play an important role.

The atmospheric lifetimes calculated by Ellis et al. (2004), Martin et al. (2002), and Hurley et al. (2004b) using smog-chamber studies on the gas-phase atmospheric fate indicate that atmospheric long-range transport of the fluorotelomer alcohols is possible. In addition, the studies demonstrate that these precursor substances can form PFCAs as a result of atmospheric chemical degradation processes. For instance, gas-phase hydroxyl radical degradation of fluorotelomer alcohols yields 1-10% PFCA (Ellis et al. 2004) while the perfluoroalkyl sulfonamido substances gave PFCA yields up to 45%[3] (Martin et al. 2006, D'eon et al. 2006). The authors proposed that gas-phase atmospheric degradation of precursor substances in remote areas may be a source of PFOA and other PFCAs in polar and remote regions far from direct PFOA sources. (Ellis et al. 2004). 3-D global atmospheric transport modeling computing showed an estimated annual PFOA flux to the arctic of 0.4 tonnes per year (Wallington et al. 2006). In an unpublished presentation on global steady-state

[3] The authors of Martin et al. (2006) state regarding Ellis et al. (2004): "…for fluorotelomer alcohols … 14% of the perfluorocarbons are ultimately expected to be incorporated into PFCAs …"

multi-media fate modeling work, other researchers have suggested that 8:2 fluorotelomer alcohol preferentially partitions to air and has the potential for long range transport to Arctic regions (Klasmeier et al. 2004).

An atmospheric degradation mechanism for 8:2 telomer alcohol has been proposed and is summarized in Figure 1 (Wallington et al. 2006).

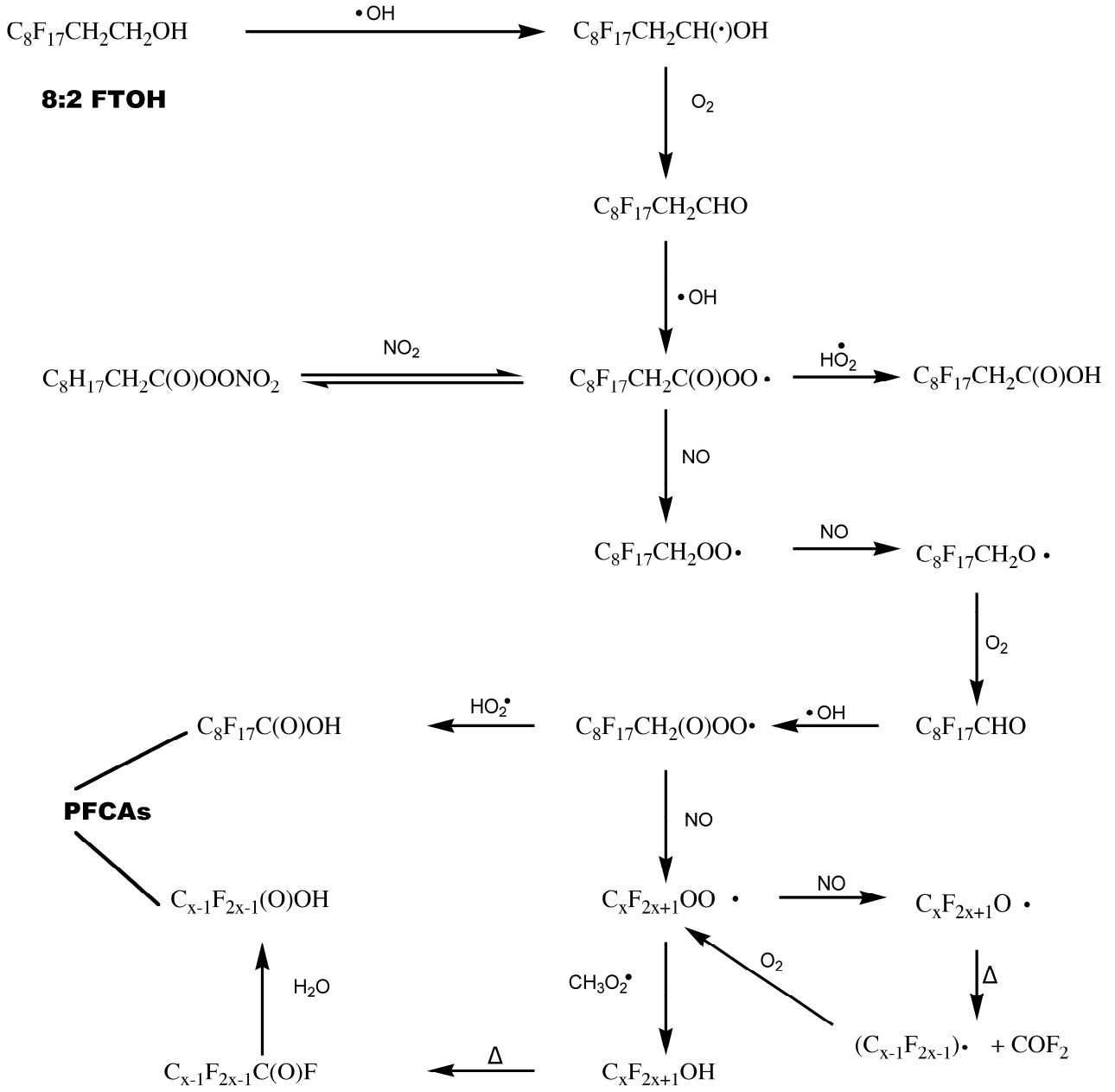


Figure 1 Proposed simplified atmospheric degradation pathway for 8:2 telomer alcohol (Wallington et al. 2006, modified).

Oxidation of 8:2 telomer alcohol proceeds by reaction with hydroxyl radicals and leads to the formation of perfluoroaldehyde in 100% yield, which also reacts with hydroxyl radicals and atmospheric oxygen producing acyl peroxy radicals ($C_8F_{17}CH_2C(O)O_2$). It is assumed that these radicals react with NO, NO_2 and hydroperoxy radicals leading to the formation of various end products. The authors reported these modeling results suggest the degradation of fluorotelomer alcohols contribute to PFCAs observed in remote locations. They concluded that it is likely that PFNA, PFOA, and other shorter PFCAs are significant products from 8:2 fluorotelomer alcohol oxidation and that

this can be a significant global source of perfluorocarboxylic acids. As a result of this oxidation, PFOA is likely to be ubiquitous in the Northern Hemisphere. The yield of PFOA and other PFCAs are of the correct order of magnitude to explain observed PFCAs levels in Arctic fauna (Wallington et al. 2006).

The gas-phase chemical degradation pathway for fluorotelomer alcohol has been well defined and investigated (Figure 1). However, additional research indicates that additional atmospheric processes may play an important role in the overall atmospheric fate of fluorotelomer alcohols. Fluorotelomer alcohols, while indeed volatile, may be removed to some extent from the gas-phase due to their strong adsorption properties. In partitioning studies, poly parameter linear free energy relationships were derived for fluorotelomer alcohols and olefins. The study concluded that environmental partitioning properties of fluorotelomer alcohols and olefins cannot be accurately predicted from correlations with existing values of Kow or vapor pressure. Interface sorption should be taken into account and modeling partitioning to bulk compartments does not accurately predict behavior. Partitioning to air may be 1-2 orders of magnitude higher then currently measured data suggest. (Goss et al. 2006). Heterogeneous uptake studies found that gas-phase 8:2 fluorotelomer alcohol was rapidly taken up by octanol droplets suggesting that partitioning to organic-containing cloud/fog droplets and aerosol particles may be an atmospheric loss mechanism (Li et al. 2006). Recent smog-chamber studies on perfluoroaldehydes, a key transformation product in the atmospheric pathway shown in Figure 1, show that perfluoroaldehyde photolysis may be an atmospheric degradation pathway (Chiappero et al. 2006).

Telomer alcohols have been detected in air in North America (Shoeib et al., 2006) and in precipitation (Scott et al. 2006b, DuPont, 2005). Existing data is insufficient to understand the transport and removal processes which control the behavior of these chemicals in the atmosphere.

In a different study, unreacted 8:2 telomer alcohol has also been shown to undergo indirect photolysis in natural waters yielding PFOA (Gauthier and Mabury 2005). Similar to the degradation pathway in the atmosphere, the degradation of 8:2 telomer alcohol and the subsequent production of PFOA is facilitated by hydroxyl radicals present in the water. In distilled water containing 10 mM hydrogen peroxide exposed to artificial sunlight, the photolysis half-life of 8:2 telomer alcohol was 0.83 ± 0.20 hours, and PFOA was formed at approximately 35% of the initial amount of 8:2 telomer alcohol (Gauthier and Mabury 2005). Using a lower initial starting concentration of 0.1 mM hydrogen peroxide resulted in a longer half-life (38.2 ± 6 hours), and the PFOA yield was about 12%. In similar experiments using natural water obtained from Lake Ontario, the half-life of 8:2 telomer alcohol was 93.2 ± 10.0 hours and the PFOA yield was approximately 7%.

In recently published studies, the gas-phase atmospheric chemistry of perfluoroalkyl sulfonamido substances was investigated and PFCAs were detected as products of the atmospheric chemistry. Perfluoroalkyl sulfonamido substances are thus a plausible source of PFCAs (Martin et al. 2006, D'eon et al. 2006). The quantitative contribution to PFOA presence in the environment and in remote regions has not been determined.

Thermal degradation of fluorinated compounds

Thermal degradation of fluoropolymers may result in formation of monomers and other PFOA precursors (Ellis et al. 2001). Perfluorinated carboxylic acids were identified as minor combustion products following the thermal degradation of polytetrafluoroethylene (Ellis et al. 2001). Some fluoropolymers lose greater then 1% mass/hour in air at maximum processing temperatures. The principal evolved gas in one study was carbonyl fluoride (COF_2) (Baker and Kasprzak, 1993).

In a study of Leung et al. (2002) thermal degradation of FEP (fluorinated ethylene propylene) showed to produce 50 ppm PFOA and the C6 perfluorocarboxylic acid each. For PTFE there were no findings (LOD not noted).

Other studies indicate that no PFOA was formed under simulated typical municipal incineration conditions of fabrics treated with fluorotelomer-based polymers (Yamada et al. 2005). Additional research is ongoing to help clarify the behavior in incineration (USEPA 2006). On April 16, 2003 an Enforceable Consent Agreement (ECA) was entered into by USEPA and several fluoropolymer manufacturers (AGC Chemicals Americas, Inc., Daikin America, Inc., Dyneon, LLC, and E.I. du Pont de Nemours and Company). The purpose of this ECA was to develop a laboratory-scale incineration testing program of fluoropolymers (USEPA 2003b USEPA Docket OPPT-2003-0071). The fluoropolymers to be tested include: Dry non-melt fluoropolymer resin (CAS 9002-84-0); Dry non-melt fluoroelastomer gum (CAS 27029-05-6); Aqueous dispersion (CAS 9002-84-0).

Begley et al. (2005) determined the PFOA content of PTFE film of non-stick cookware before and after heating empty pans to 320 °C. The PFOA content decreased from 4-75 µg/kg to below LOD. The authors conclude that significant amounts of PFOA are not generated and remain in the cookware after an extreme heating event.

2.2.1.5 Historical global estimation of sources

One estimation of the historical global environmental releases of PFCAs from indirect sources and direct sources has recently been compiled. These data and the corresponding sources are presented in Table 18 (based on Prevedouros et al., (2006). The snapshots of PFCA environmental releases in 2000 by carbon chain length shown in Figure 2 and Figure 3 were taken from this paper.

Table 18 Estimated Historical Global Releases of Perfluorocarboxylic Acids (PFCAs) based on (Prevedouros et al. 2006)

Environmental Input Source	Historical Time Period (years)	Estimated Total Global Historical PFCA Emissions (tonnes)
Other Direct PFCA Sources		
Fluoropolymer Manufacture (APFN)	1975-2004	400 - 1,400
Fluoropolymer Processing (APFN)	1975 - 2004	10 – 20
Aqueous Fire Fighting Foams (AFFF)	1965-1974	50 – 100
Consumer and Industrial Uses	1960-2000	40 – 200
Indirect PFCA Sources		
POSF-Based Products	1960-2002	
PFCA Residual Impurities	1960-2002	20 - 130
POSF-Based Precursor Degradation	1960-2002	1 - 30
POSF-Based AFFF	1970-2002	3 - 30
Fluorotelomer-Based Products	1974-2004	
PFCA Residual Impurities	1974-2004	0.3 - 30
Fluorotelomer-Based Precursor Degradation	1974-2004	6 - 130
Fluorotelomer-Based AFFF	1975-2004	< 1

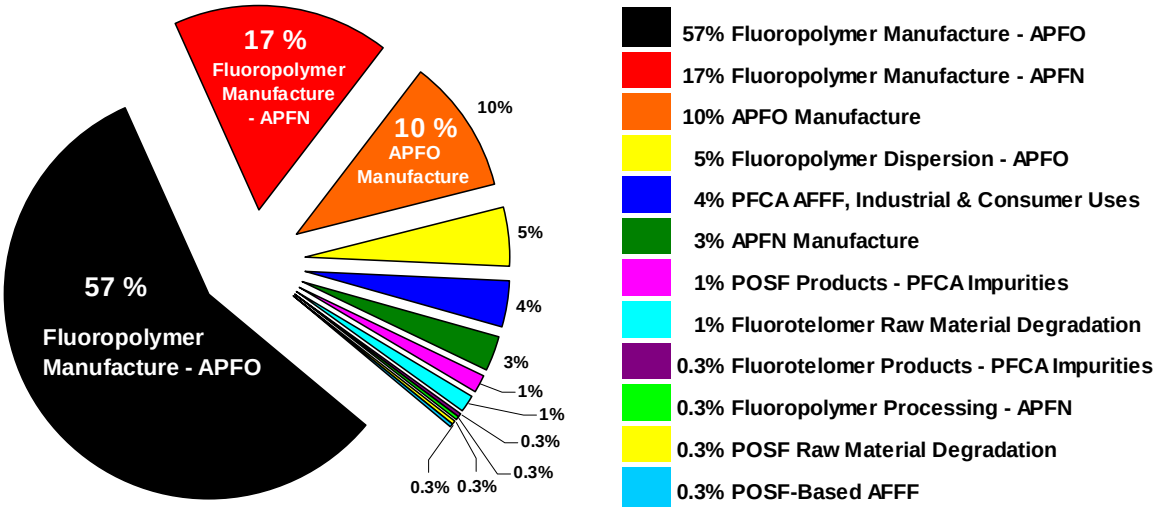


Figure 2 Global Historic PFCA Emissions by Source (Prevedouros et al., 2006)

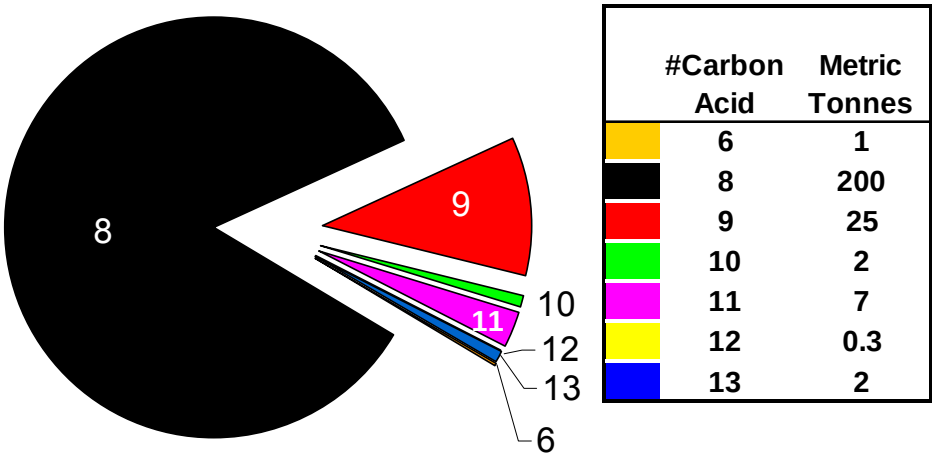


Figure 3 Estimated 2000 Global PFCA Emissions by Chain Length (Prevedouros et al., 2006)

2.2.1.6 Measures and programes for the reduction of APFO/PFOA emissions

In March of 2006, the US Environmental Protection Agency announced the EPA 2010/2015 PFOA stewardship program. The participation requires voluntary global corporate commitment to two goals:

1) A commitment to achieve, no later than 2010, a 95% reduction - measured from a year 2000 baseline - in both facility emissions to all environmental media of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals. In parallel, it requires an

equivalent reduction in product content levels of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals.

2) A commitment to working toward the elimination of PFOA, PFOA precursors, and related higher homologue chemicals from emissions and products by five years thereafter, or no later than 2015.

A number of projects are proposed or under way worldwide to achieve emissions reductions from fluoropolymer and fluorotelomer manufacturing.

Fluoropolymer manufacturers have recently installed additional capability to capture and recycle APFO. The global APFO manufacturing emissions have decreased from about 45 tonnes in 1999 to about 15 tonnes in 2004 and are expected to be 7 tonnes in 2006 (FMG, 2002). Aqueous APFO solutions have been processed for reuse in all regions. Dramatic (>90%) reductions in overall APFO/PFOA environmental releases over the past few years have been reported (DuPont, 2005a; Dyneon, 2004). The recent historical trend in APFO emissions from fluoropolymer manufacture shows a decrease from hundreds of tonnes in 1999 to less than 50 tonnes projected in 2006 (Prevedouros et al., 2006). For example, DuPont's Washington Works PFOA recovery program has resulted in a recovery of the 20% solution of C-8 from 5.0 metric tonnes (11,000 lbs) in 1992 to 58.0 metric tonnes (130,000 lbs) in 1997 (DuPont, 1998 – AR226-2084).

2.2.2 Photodegradation

Direct photolysis of APFO was examined in two separate studies (3M, 1979; 3M, 2001b) and photodegradation was not observed in either study. In the 3M (1979) study, a solution of 50 mg/l APFO in 2.8 liters of distilled water was exposed to simulated sunlight at 22±2 ºC. Spectral energy was characterized from 290-600 nm with a max output at ~360 nm. Direct photolysis of the test substance was not detected. However, the author noted that sample purity was not properly characterized which may have contributed to experimental error. This was also done with older, much less sensitive analytical methods. Current methods may be able to detect degradation products.

In the 3M (2001b) study, both direct and indirect photolysis were examined utilizing techniques based on USEPA and OECD guidance documents. To determine the potential for direct photolysis, APFO was dissolved in pH 7 buffered water and exposed to simulated sunlight (Scrano et al., 1999; Nubbe et al., 1995). For indirect photolysis, APFO was dissolved in 3 separate matrices and exposed to simulated sunlight for periods of time from 69.5 to 164 hours. These exposures tested how each matrix would affect the photodegradation of APFO. One matrix was a pH 7 buffered aqueous solution containing H_2O_2 as a well-characterized source of OH radicals (Ogata et al., 1983; Lunak and Sediak, 1992). This tested the propensity of APFO to undergo indirect photolysis. The second matrix contained Fe_2O_3 in water that has been shown to generate hydroxyl radicals via a Fenton-type reaction in the presence of natural and artificial sunlight (Kachanova and Koslov, 1973; Behar et al., 1966). The third matrix contained a standard solution of humic material. Neither direct nor indirect photolysis of APFO was observed based on loss of starting material. Predicted degradation products were not detected above their limits of quantitation. There was no conclusive evidence of direct or indirect photolysis whose rates of degradation are highly dependent on the experimental conditions. Using the iron oxide (Fe_2O_3) photoinitiator matrix model, the APFO half-life was estimated to be greater than 349 days.

According to Hori et al. (2004a, 2005) aqueous solutions of PFOA absorb light strongly from the deep UV-region to 220 nm. A weak, broad absorption band reaches from 220 to 270 nm (no absorption coefficient stated). The irradiation of a 1.35 mM PFOA solution (29.6 µmol) in water with light from a xenon-mercury lamp (no radiant flux stated) for 24 hours resulted in a ca. 40 % reduction (12.8 µmol) of PFOA concentration. Concentrations of CO_2 and fluoride increased simultaneously.

Small amounts (0.1-5 µmol) of short chain perfluorinated hydrocarbon acids (C_2-C_7) were detected. After irradiation about 12 % of total carbon and fluoride couldn't be quantified. Addition of high-pressure oxygen (4,800 hPa) and hydrogen peroxide increased the amount of fluoride and carbon dioxide detected. The addition of the photocatalyst tungsten heteropolyacid ($[PW_{12}O_{40}]^-$) or persulfate ($S_2O_8^{2-}$) (Hori et al., 2005) accelerates the reaction rate. Due to the short wave length used for irradiation (< 300 nm) the photodegradation described may be of limited relevance for an aqueous environment but may be used as a technical process.

Hori et al. (2004a) suggest using the degradation for the treatment of PFOA contaminated industrial wastewater. The photodegradation is not considered a pathway for environmental degradation of PFOA (absorption of light with wavelength below 300 nm).

Hurley et al. (2004) determined the rate constants of the reactions of OH radicals with a homologous series of perfluorinated acids (from trifluoroacetic acid to nonafluoropentanoic acid) in 700 Torr of air at 296 K. For C_3 to C_5 chain length had no discernible impact on the reactivity of the molecule. The rate constant $k(OH + F(CF_2)_nCOOH) = (1.69\pm0.22)\times10^{-13}$ cm^3 $molecule^{-1}$ s^{-1} for n = 2, 3, 4, respectively. Atmospheric lifetimes of $F(CF_2)_nCOOH$ with respect to reaction with OH radicals are estimated to be approximately 230 days for n = 1 and 130 days for n > 1. (Calculation of lifetime by comparison with CH_3CCl3 (half-life 5.99 years, $k = 1.0\times10^{-14}$ cm^3 $molecule^{-1}$ s^{-1}).) The authors conclude, that the major atmospheric loss mechanism of perfluorinated carboxylic acids is dry and wet (particle mediated) deposition which occur on a time scale which is probably of the order of 10 days. Reaction with OH is a minor atmospheric loss mechanism for perfluorinated carboxylic acids.

Conclusion

PFOA has not been shown to undergo direct photodegradation at environmentally relevant conditions (wavelength of light > 300 nm). There is an indication (conclusion by analogy) of slow indirect photodegradation in air (half-life 130 days).

2.2.3 Stability in Water

The 3M Environmental Laboratory (2001a) performed a study of the hydrolysis of PFOA. The study procedures were based on USEPA's OPPTS Guideline Document 835.2110 (USEPA, 1998); although the procedures do not fulfill all the requirements of the guideline, they were more than adequate for these studies. Results were based on the observed concentrations of PFOA in buffered aqueous solutions as a function of time. The chosen analytical technique was high performance liquid chromatography with mass spectrometry detection (HPLC/MS).

During the study, samples were prepared and examined at six different pH levels from 1.5 to 11.0 over a period of 109 days. Experiments were performed at 50 °C and the results extrapolated to 25 °C. Data from two of the pH levels (3.0 and 11) failed to meet the data quality objective and were rejected. Also rejected were the data obtained for pH 1.5 because ion pairing led to artificially low concentrations for all the incubation periods. The results for the remaining pH levels (5.0, 7.0, and 9.0) indicated no clear dependence of the degradation rate of PFOA on pH. From the data pooled over the three pH levels, it was estimated that the hydrolytic half-life of PFOA at 25°C is greater than 92 years, with the most likely value of 235 years. From the mean value and precision of PFOA concentrations, it was estimated the hydrolytic half-life of PFOA to be greater than 97 years.

Conclusion

PFOA is hydrolytically stable in water.

2.2.4 Transport between Environmental Compartments

2.2.4.1 Model calculations

An octanol-water partition coefficient (K_{ow}) cannot be determined for the surface active PFOA. Therefore calculating the distribution between environmental compartments with models using Kow (for example the Mackay model) is not applicable.

2.2.4.2 Volatility

Different studies have reported different values for PFOA vapor pressure. The vapor pressure of PFOA was determined experimentally from 60° to 190° C, using a dynamic procedure where the equilibrium temperature is measured at controlled pressure (Kaiser et al. 2005). The extrapolation of the measured pressure to lower temperature was based on a fitted Antoine equation. For 20° C a vapor pressure of 2.3 Pa was extrapolated.The vapor pressure of APFO was determined based on a standard procedure described by the USE PA in which the quantity of APFO transported by a known volume of a carrier gas is measured. Measurements reached from 90° to 165° C. A vapor pressure of 0.008 Pa was extrapolated for 20°C (Washburn et al. 2006) with a fitted Antoine equation.

Studies with wash bottles (impinger studies) were performed to examine the volatility of aqueous APFO solutions (3M, 1993). Solutions of APFO containing ammonium acetate in water/1-propanol (50:50) or phase transfer agents, e.g., n-alkyldimethylbenzylammonium chloride were blown with 280 liters of air at a flow rate of 1 L/min. (3M, 1993). The results indicate there is some loss of APFO, but most of the solutions retained over 80% or more of the APFO. The average retention was 92% for APFO, indicating that some APFO disappeared out of the solutions. However, some of the solutions, particularly the n-alkyldimethylbenzylammonium chloride solution, appear to retain all the fluorochemicals. (3M, 1993). The authors concluded that it is unlikely that this fluorochemical was removed by bubbling air through water due to its very low vapor pressure. Authors suggested that a more plausible mechanism for loss from the solution phase is concentration of the surfactants in foam and loss from the bubbled solutions as foam or micro-droplets.

The physical-chemical properties of PFOA and its anion PFO are distinctly different and govern the expected environmental fate and transport. PFOA is an acid with a pKa of about 3 (see chapter “1.3 Physico-Chemical properties”). At pH values of 4.5, approximately 97% of this chemical is present as the perfluorocarboxylate anion. The remaining 3% are protonated. In the aqueous phase, at pH values between 6 and 8, lower amounts of PFOA (< 3%) are in equilibrium with PFO. However, the protonated form has sufficient volatility to leave surface and atmospheric water and/or soil, and generating a slow release of PFOA into the atmosphere. The environmental relevance of this release is unknown.

Conclusion

While perfluorooctanoate, the anionic form, is not volatile, pure PFOA (protonated) is moderately volatile. When dissolved in water the strong acid PFOA dissociates. The degree is dependent on the pH. Environmental conditions control partitioning between environmental media

2.2.4.3 Long-Range Transport

PFOA, as the anion perfluorooctanoate, PFO, has been detected in remote areas of the world in monitoring programs involving various abiotic and biotic samples. For example, PFOA has been

measured in air (Shoeib et al. 2006) and biota such as polar bears and seals in the Canadian Arctic (see section 2.2.8b Environmental Monitoring, DeSilva and Mabury 2004, Martin et al. 2004c). No information is available about current or historical use of PFOA or related substances in the Arctic. A possible explanation for this finding is the long-range transport of either PFOA or potential precursors. Two possible transportation pathways include atmospheric and aquatic transport.

Atmospheric Transport

Due to the relative vapor pressures of APFO, PFOA, and PFO, the chemical form potentially most subject to gas-phase atmospheric transport is PFOA. Franklin (2002) suggested that in the presence of water in air (humidity), gaseous PFOA condenses to aerosol particles and dissociates to the corresponding perfluorooctanoate, resulting in a low vapour pressure. According to Franklin (2002), the atmospheric lifetime of PFOA (respectively its salts) was calculated in the order of days when emitted from a ground source.

Additional sources of PFOA to the atmosphere are the degradation or transformation of precursors, which could lead to indirect environmental releases. Potential precursors include related fluorinated chemicals which are detectable in the atmosphere (e.g., fluorotelomer alcohols, olefins, and perfluoroalkyl sulfonamido substances) which can degrade in the atmosphere or after deposition to the surface to PFOA (precursor substances and degradation mechanisms see section "2.2.1.4 Indirect sources"). Calculations using a three-dimensional global atmospheric chemistry model (IMPACT) indicate that 8:2 fluorotelomer alcohol (widely used in industrial and consumer products) degrades in the atmosphere to give PFOA (Wallington et al., 2006).. FTOHs have sufficient vapor pressure to be present in air (Prevedouros et al., 2006). Smog chamber studies prove the potential for FTOHs to react in the atmosphere with ubiquitous OH radicals to yield PFOA (Ellis et al., 2004). Telomer A ($\geq$ C8) is the initial product for the manufacturing of C8 telomer alcohols. Ellis et al. (2004) showed that the atmospheric lifetime of short chain FTOHs, as determined by their reaction with OH radicals, was approximately 20 days.

However, there is not enough data available to estimate how much the different sources contribute to the PFOA detected in the Arctic and in biota of remote areas. While there is evidence for the possible role of precursors for the long-range atmospheric transport of PFOA, the extent to which these precursors and their transformation may explain the concentrations of PFOA found in remote areas such as the Canadian Arctic is uncertain.

Aquatic Transport and Marine Aerosols

Another possible mechanism for the transport of PFOA to the Canadian Arctic is aquatic transport (Prevedouros et al., 2006). Given PFOA's environmental persistence, high water-solubility and the fact that PFOA and related substances have been emitted to air and water for approximately 50 years and may have accumulated in the oceans, a hypothesis has been presented to suggest ocean water transport as a possible pathway explaining the presence of PFOA in the Canadian Arctic. Currently there is insufficient data to evaluate the significance of this potential pathway .

Several researchers have indicated that the timelines involved with transport via ocean currents could not account for what appears to be rapidly increasing levels of perfluorinated substances in certain Arctic biota (Smithwick et al. 2006). While PFOA has been detected in coastal water and seawater even in remote areas (see section "2.2.8 Environmental Monitoring", Caliebe et al. 2004, Yamashita et al. 2005), the extent to which this may be due to ocean or atmospheric transport is uncertain. Ocean water transport of perfluorocarboxycyclic compounds is a combination of :a) discharges of PFCAs to surface waters and transport to oceans; b) atmospheric loadings of PFCAs to surface waters and transport to oceans; and c) discharge of precursors to surface waters, transformation to PFCAs and transport to oceans (Prevedouros et al. 2006).

In addition to the possible role of aquatic transport via oceans to the Arctic, the possibility of atmospheric transport of PFOA on marine aerosols has been proposed (Prevedouros et al., 2006). Due to its nature as surfactant, PFOA is expected to be enriched on the water surface. As hypothesized, marine aerosols may be generated from this PFOA enriched surface through gas-bubble production and collapse through breaking waves and rough sea conditions. The sea surface micro-layer may thus, supply the atmosphere with PFOA-rich particles which undergo atmospheric transport over, at least, short distances. Studies are needed to determine whether and to what extent marine aerosols contain PFOA and contribute to their global transport. The determination of whether perfluorocarboxylic acids are present, and to what extent, in marine aerosols, and whether this contributes to their global transport, is the subject of ongoing scientific investigations (Prevedouros et al. 2006).

Conclusion

Pure PFOA at room temperature has moderate vapor pressure (2.3 Pa). The vapor pressure of APFO is much lower with 0.008 Pa. APFO or PFOA dissolved in water dissociate to ions. Although the dissociated fraction is not subject to volatilization, depending on the pH, pure PFOA is expected to volatize from water to a certain degree.

Due to emissions for more than 50 years, PFOA is distributed worldwide in the marine environment, and hence may be transported to remote areas via the aqueous phase and the atmospheric phase. However, the significance of these sources are not currently known. Both atmospheric and aquatic transport mechanisms are actively being investigated.

PFOA and PFOA precursors including fluorotelomer alcohols, olefins and perfluoroalkyl sulfonyl derivates are subject to long range transport. The relative environmental significance of these sources are not known currently.

2.2.5 Biodegradation

A number of studies have examined the biodegradation potential of PFOA in aerobic and anaerobic conditions. Data have shown that PFOA is a highly persistent substance unlikely to undergo rapid biodegradation in the environment. Slow degradation may be possible under certain conditions.

Using an acclimated sludge inoculum, the biodegradation of APFO was investigated using a shake culture study modeled after the Soap and Detergent Association's presumptive test for degradation (3M, 1978). Both thin-layer and liquid chromatography did not detect the presence of any metabolic products over the course of 2 1/2 months indicating that PFOA does not readily undergo biodegradation. In a related study, 2.645 mg/l APFO was not measurably degraded in activated sludge inoculum (Pace Analytical, 2001). Test flasks were prepared using a mineral salts medium, 1 ml methanol, and 50 ml settled sludge. Analysis was conducted with a HPLC/MSD system. Although the results were deemed unreliable due to a lack of description of experimental protocols or indications of a high degree of experimental error, several other studies conducted between 1977-1987 also did not observe APFO biodegradation (3M, 1979; 3M, 1980b; 3M, 1985; Pace Analytical, 1987). In addition, a study conducted by Oakes et al. (2004) indicated little biotic or abiotic degradation of PFOA on a time scale of 35 days, i.e., the PFOA exposure concentrations were stable over time and ranged from 84.5 % to 114.5 % of the initial concentrations.

Schroeder (2003), and Meesters and Schroeder (2004) investigated the biochemical degradation of PFOA in sewage sludge in laboratory scale reactors. After 25 days under aerobic conditions PFOA (initial concentration 5 mg/l) was not eliminated by metabolic processes, mineralization processes or by adsorption. In a recent study, Wang et al. (2005b) (see review in detail on page 27) report the biological degradation of the fluorinated alkyl group of radiolabeled PFOA (< 0.6% in 28 days).

Conclusion

PFOA is not expected to undergo biodegradation.

2.2.6 Bioaccumulation

Bioconcentration is the process by which a chemical enters an organism and/or is adsorbed on to it as a result of exposure to the chemical in water – it often refers to a condition usually achieved under laboratory and steady state conditions. **Bioaccumulation** is the process by which a chemical enters an organism as a result of uptake through all possible routes of exposure (dietary, dermal, and respiratory). **Biomagnification** is the process in which the chemical concentration in an organism achieves a level that exceeds that in the organism's diet, due to dietary absorption. **Trophic biomagnification** is the phenomenon where chemical concentrations in organisms increase with trophic level resulting in incrementally higher concentrations in upper trophic levels predators than in lower trophic levels.(Gobas et al. 2000)

PFOA does not behave like lipophilic compounds that accumulate in fat tissues. For lipophilic substances, accumulation is expected preferentially in the fat tissues. Due to the perfluorination, the hydrocarbon chains are oleophilic and hydrophobic and the perfluorinated chains are both oleophobic and hydrophobic. In addition, functional groups attached to the perfluorinated chain (e.g., a charged moiety such as an hydroxyl group or sulfonic acid) can impart hydrophilicity to part of the molecule. Hydrophobicity is unlikely to be the sole driving force for the partitioning of perfluorinated substances to tissues because the oleophobic repellency opposes this partitioning process. Perfluorinated substances are also intrinsically polar chemicals because fluorine, a highly electronegative element, imparts polarity. Thus, perfluorinated substances have combined properties of oleophobicity, hydrophobicity, and hydrophilicity over portions of a particular molecule. Due to these properties, the assumption that the traditional hydrophobic and lipophilic interactions between compound and substrate are the main mechanisms governing partitioning may not be applicable for PFOA.

To determine bioconcentration of PFOA, rainbow trout were exposed in a flow-through system for 12 days followed by a depuration time of 33 days in fresh water to determine tissue distribution and bioconcentration (Martin et al., 2003b). For determination of bioconcentration, juvenile fish (5-10 g) were exposed to a concentration of 1.5 µg/l in a flow-through system. At 7 occasions during uptake period and 9 occasions during depuration phase, fish were removed to determine the kinetics of uptake and depuration. Additionally, for the tissue distribution study, four immature trout (30-48 g) were exposed in separate tanks but under the same uptake conditions.

PFOA concentration was highest in blood, kidney, liver and gall bladder and low in the gonads, adipose and muscle tissue. Within the blood, the plasma contained between 94 – 99% of PFOA, with only a minor fraction detectable in the cellular fraction. Recovery from hearts and spleen was low (<10%).

A steady state was reached during uptake time. Visual observation of depuration data indicated possible biphasic depuration in blood, liver and carcass. However, this could not be verified statistically because of the small sample size. The following BCFs are calculated:

$BCF_{carcass}$ = 4.0 (+- 0.6); depuration half-life: 5.2 d (± 0.67)
BCF_{blood} = 27 (+- 9.7); depuration half-life: 4.5 d (± 1.6)
BCF_{liver} = 8.0 (+- 0.59); depuration half-life: 3.9 d (± 0.28)

PFOA occurs mainly in muscle, blood and organs (liver, kidney) but not in lipid tissue and is reported for other species such as birds and mammals by several authors (see Table 20)

Fathead minnows (*Pimephales promelas*) were exposed to PFOA in a static system to a concentration of 25 mg/L for 13 days, followed by a depuration phase of 15 days. A BCF of 1.8 was calculated (3M, 1995).

Daikin (2000) performed a bioaccumulation test according to OECD Guideline 305, with the carp *Cyprinus carpio*. The fish were exposed to PFOA concentrations of 5 and 50 µg/l for 28 days. For the higher concentration of 50 µg/l, the steady state was reached after 16 days and a BCF of 3.2 was calculated. For the lower concentration of 5 µg/l, a BCF of 9.4 was determined after 16 days; this level was reduced to ≤ 5.1 after 28 days. No steady state was reached until end of exposition. Although experiments with fish and other aquatic species provide evidence that PFOA is not highly bioaccumulative, these results should not be extrapolated to other animals. Fish gills may provide an additional mode of elimination and uptake which birds, terrestrial organisms, and marine mammals do not possess (Kelly et al., 2004).

Martin et al. (2003a) exposed juvenile rainbow trout (*Oncorhynchus mykiss*) for 34 d to PFOA in the diet, followed by a 41 day depuration period. During the uptake period, animals were daily fed with spiked food (0.42 mg PFOA/kg food) at a rate of 1.5 % food per fish. Assimilation efficiency (% of PFOA absorbed relative to the amount fed) was 59 %, indicating efficient absorption from food. At 6 occasions during uptake period and during depuration period, fish were removed to determine the kinetics of uptake and depuration. Carcass and liver concentrations were determined by using liquid chromatography-tandem mass spectrometry, and kinetic rates were calculated to determine bioaccumulation parameters.

The carcass uptake curves clearly showed by visual inspection, that the slope of the curve levels off by the end of the uptake period. According to the authors the steady state was reached after 10 days. A depuration half-life time of 3d (± 0.42) and a BAF (Bioaccumulation factor) of 0.038 (± 0.0062) were determined.

Martin et al. (2004a) examined PFOA contents in the food web from Lake Ontario (Canada). Adult lake trouts (top predator) were collected at various years and locations in Lake Ontario. Samples of prey fish (sculpins, smelts and alewifes) and macroinvertebrates (*Mysis* sp., *Diporeia* sp.) were collected at one location in October 2002. Lake trout samples analyzed represented individual whole fish homogenates. The other species were processed as composites of whole individuals. The mean PFOA content in *Diporeia* sp. and sculpin was 90 ng/g and 44 ng/g, respectively. In the other fish samples contents of 1.0 to 2.0 ng/l and in *Mysis* sp. of 2.5 ng/g could be detected. The authors note that *Diporeia* sp. is a benthic invertebrate species, and sculpins feed mainly in the benthic environment. Benthic contamination may therefore be the source of contamination of this food web. As PFOA content in predators is lower than in prey species trophic biomagnification of PFOA in the food web of Lake Ontario is unlikely occur.

Trophic transfer of PFOA and other related perfluorinated compounds was examined in a Great Lakes benthic foodweb including water – algae – zebra mussel – round goby – smallmouth bass. In addition, perfluorinated compounds were measured in livers and eggs of Chinook salmon and lake whitefish, in muscle tissue of carp, and in eggs of brown trout. Similarly, green frog livers, snapping turtle plasma, mink livers, and bald eagle tissues were analyzed to determine concentrations in higher trophic-level organisms in the food chain. Biotic samples were collected from several rivers in Michigan and in the Calumet River in Indiana, USA. PFOA-concentrations in two of the sampling sites, Raisin River and St. Clair River, were 14.7 and 4.5 ng/l, respectively. The concentrations of PFOA in all tissue samples were above detection limit but below the LOQ (see Table 20 Environmental levels of PFOA). Therefore, biomagnification of PFOA in the Great Lakes benthic foodweb is unlikely occur (Kannan et al., 2005).

The bioaccumulation of PFOA in the wild turtles *Trachemys scripta elegans* and *Cinemy reevesii* was reported by Morikawa et al. 2005. Serum concentrations of PFOA from 94 turtles were compared to surface water samples from the site of the turtle capture for several rivers in Japan. In Ai River water concentrations up to 87,100 ng/l were reported. Serum concentrations in turtles collected in Ai River ranged from 47.1 to 115.6 ng/l, the corresponding BCF_{serum} values ranged from 0.9 to 2.9. In Taisyo River water concentrations of 42.3 and 63.4 ng/l (two samples) and 9800 ng/l (one sample) were detected. Serum concentrations of 0.4 and 1.0 ng/l were reported for the turtles collected in low water concentration sides, and 7.6 ng/l were reported for turtles collected in high water concentration sides; corresponding BCF_{serum} of 10-15.8 and 0.8 to 15.8 were reported with surface water concentrations ranging from 21.8 to 87,100 ng/l. However, as the wild turtles' exposure to PFOA was probably not limited to surface water only, the BCFs reported by Morikawa et al (2005) may actually be BAFs.

The biomagnification of PFOA and other perfluoroalkyl compounds in the food web of the bottlenose dolphin *Tursiops truncates* was investigated by Houde et al. (2006). Marine water, surface sediment, atlantic croaker (*Micropogonias undulatus*), pinfish (*Lagodon rhomboides*), red drum (*Sciaenops ocellatus*), spotfish (*Leiostomus xanthurus*), spotted seatrout (Cynoscion *nebulosus*), striped mullet (*Mugil cephalus*) and bottlenose dolphin samples were collected around the Charleston Harbor area (South Carolina). Marine water, surface sediment, zooplankton, sheephead (*Archosargus probatocephalus*), pigfish (*Orthopristis chrysoptera*), pinfish, striped mullet, spotted seatrout and bottlenose dolphin samples were collected at Sarasota Bay (Florida). At both locations seawater and surface sediment samples were collected in 2004. Fish samples were collected in 2002 and 2003 at Charleston and 2004 at Sarasota Bay. Zooplankton samples were collected in Sarasota Bay in 2004. Samples of plasma, skin, and teeth were collected from both locations during capture-release events that took place in the summer of 2004. Additional dolphin tissue samples (i.e., liver, kidney, muscle and other) were collected during the necropsies of recently deceased bottlenose dolphins from Sarasota Bay in 2002 and Charleston in 2003 (n=1 at each site). Also liver and kidney samples collected from stranded bottlenose dolphins were available at Sarasota Bay.

PFOA concentration in water was reported as 9.5 (±13) and 3.6 (±9.2) ng/l for Charleston and Sarasota Bay, respectively. Whole body concentration in fish ranged from <0.5 to 1.8 (±3.2) ng/g for Charleston and <0.5 to 0.3 (±0.5) for Sarasota Bay. PFOA concentration in the plasma of dolphin was 43 (±24) and 3.4 (±3.5) ng/g for Charleston and Sarasota Bay, respectively.

The authors calculated biomagnification factors for several predators and their prey, but it is difficult to understand how they calculated these factors. For example: At Charleston, for seatrout/pinfish a BMF of 7.2 is reported. However, a recalculation from data given in the report, indicates seatrout residues (1.8 ng/g) divided by pinfish residues (<0.5 ng/g) should be 3.6. The BMFs for dolphins and their prey can not be recalculated because they are based on whole body concentration in dolphins. The extrapolation factors from plasma and tissue to whole body is not transparent. In addition the water and biota samples were originating from different years with high variabilities. Keeping these uncertainties in mind, the maximum BMF for dolphinwhole/striped mulletwhole and dolphinwhole/pinfishwhole was 13. As this BMF is >1, it suggests a potential for biomagnification in this predator-prey relationship.

An eastern Arctic food web was analyzed for PFOA and other perfluorinated compounds (Tomy et al., 2004). Liver tissues of beluga whale (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), walrus (*Odobenus rosmarus*), deepwater redfish (*Sebastes mentella*), glaucous gullus (*Larus hyperboreus*), and black-legged kittiwake (*Rissa tridactyla*) and whole organism homogenates of Arctic cod (*Boreogadus saida*), shrimp (*Pandalus borealis; Hymenodora glacialis*), clams (*Mya truncata*; *Serripes groenlandica*), and mixed zooplankton were analyzed. PFOA concentrations in clam and black-legged kittiwake samples (*n*=5) analyzed were below Method Detection Limits (MDLs) of 0.2 ng/g. Mean concentrations of PFOA in zooplankton (*n*=5) were 2.6 ± 0.3 ng/g. PFOA was de-

tected in 3 of 7 shrimp samples at a mean concentration of 0.17 ± 0.06 ng/g. PFOA was detected in only a single Arctic cod (0.47 ng/g) (*n*=5). PFOA was detected in only 2 of 5 the samples of deep-water redfish at a mean concentration of 1.2 ± 0.8 ng/g. Statistical differences in the mean PFOA concentrations were observed between walrus (0.34 ± 0.09 ng/g) and narwhal (0.9 ± 0.1 ng/g; $p < 0.005$) and between walrus and beluga (1.6 ± 0.3 ng/g; $p < 0.05$). Biomagnification of PFOA between individual feeding relationships can be seen but not through the entire food web:

predator	**prey**	**BMFs**
walrus	clam	1.8
narwhal	arctic cod	1.6
beluga whale	arctic cod	2.7
beluga whale	redfish	0.8
black-legged kittiwake	arctic cod	0.3
glaucous gullus	arctic cod	0.6
arctic cod	zooplankton	0.04

Conclusion

The results indicate a low potential for bioaccumulation of PFOA in fish and low biomagnification potential in several aquatic food webs. In some marine and Canadian Arctic mammalian food web studies, a potential for biomagnification has been suggested.

In tests with the rainbow trout *Oncorhynchus mykiss* a bioconcentration factor (BCF) of 0.038 and bioaccumulation factors (BAF) for organs of 27 (blood), 8.0 (liver) and 4.0 (carcass) were obtained. These laboratory studies indicate a low bioaccumulation potential in fish. Some monitoring data suggest a low biomagnification potential in aquatic food webs, while in some marine and Canadian Arctic mammalian food web studies a potential for biomagnification has been suggested. Further elucidation of the mechanisms leading to uptake and accumulation in biota is required.

For substances like PFOA, bioconcentration may not be the most relevant endpoint to consider. For PFOA and other perfluorinated substances, biomagnification appears to be occurring in terrestrial and marine mammals. An assumption of the BAF/BCF approach is that bioaccumulation occurs by the same mechanisms for all chemicals in both water-breathing animals (e.g., fish and aquatic invertebrates) and air-breathing animals (e.g., terrestrial mammals, birds and marine mammals) resulting in a similar bioaccumulation potential between these organism classes for a particular substance. Both Kelly et al. (2004) and Mackay and Fraser (2000) discuss key differences in the bioaccumulation mechanisms between air-breathing animals and water-breathing animals. Furthermore, Kelly et al. (2004), describe how the current BAF/BCF/log K_{ow} paradigm may not be adequate to assess bioaccumulation potential in air-breathing animals. From a scientific perspective, additional measures of bioaccumulation which also address the potential for chemicals to biomagnify include biomagnification factors and trophic magnification factors (Gray 2002; MacKintosh *et al.*, 2004; Martin *et al.*, 2004a; Tomy *et al.*, 2004; and Cabana and Rasmussen, 1994).

2.2.7 Other Information on Environmental Fate

Behaviour in soil

The adsorption-desorption of APFO was studied in 25 ml solutions of ^{14}C-labeled APFO in distilled water with 5 g Brill sandy loam soil for 24 hours at a temperature of 16-19 ºC. The study reported a Kd of 0.21 and a Koc of 14 indicating that PFOA has high mobility in Brill sandy loam soil (3M 1978b). The K_{OC} value, however, is questionable due to the lack of accurate information on the purity of the ^{14}C-labeled test substance (Boyd, 1993a,b).

Moody and Field (1999) conducted sampling and analysis of samples taken from groundwater 1 to 3 meters below the soil surface in close proximity to two fire-training areas with a history of aqueous film forming foam use. Perfluorooctanoate was detected at maximum concentrations ranging from 116 to 6750 µg/l at the two sites many years after its use at those sites had been discontinued. These results suggest that PFOA can leach to groundwater.

An adsorption-desorption test according to OECD guideline 106 was made by APME (2003) at DuPont, Newark sponsored by Plastics Europe. APFO was tested with four soil and one activated sludge samples (equilibration time 24 h). Quantification (analytics: LC-MS/MS) was made using a calibration curve. The K_{OM} values ranged from 28 l/kg to 133 l/kg.

The data from these batch sorption studies are of limited utility in understanding the movement of PFOA released to soil. Batch sorption studies, because of their limited nature, do not provide all the information needed to understand the behavior of PFOA in the environment.

Extensive site specific monitoring of soil and ground water concentrations of PFOA and related substances was conducted by 3M, DuPont Daikin and others. PFOA in soil has been shown to persist for decades and to be a long term source of groundwater and surface water contamination (see for example 3M (2005a), DuPont (2003a)).

At the DuPont Washington Works site soil contaminated by perfluorochemical waste has been shown to contain ppm levels of PFOA 3 decades after application ceased. The underlying groundwater also contains ppm levels of PFOA (DuPont, 1999)

Extensive field monitoring data generated by 3M at the Decatur, AL site have have also shown that PFOA is persistent in soils. Soil samples were collected from a former sludge application area of the 3M Decatur, AL facility also show soil contamination and underlying groundwater contamination up to ppm levels decades after application ceased.

Conclusion

PFOA is not rapidly degraded in soil. PFOA has a very low to weak adsorption to soil organic matter (classification according to Blume and Ahlsdorf (1993) with a soil sorption coefficient Koc between 1 and 100). Therefore a high mobility of PFOA in soils can be assumed. Monitoring data show that PFOA persists in soil and leaches over time. PFOA in soil can be a long term source to underlying groundwater

2.2.8 Environmental Monitoring

Measurements related to Italy, Trissino (Miteni), APFO production site

According to Miteni, information about measurements is not available.

Measurements related to The Netherlands, Dordrecht (DuPont), APFO use site

According to DuPont, no information about measurement of PFOA is available from monitoring of surface water and biota.

Measurements related to Germany, Gendorf (Dyneon and Clariant), use and former production site

Dyneon conducted environmental monitoring measurements in and around the Gendorf site in 2001/2002. Another environmental monitoring campaign with lower detection limits will be completed in 2006. The new sampling campaign includes soil and plants, in addition to the water and fish measurements performed in 2001/2002.

Water

Groundwater wells were sampled in 2002 (locations upstream, under, and downstream of plant site). All measurements gave results below the limit of detection (LOD) (200 µg/l).

All measurements in the receiving stream, river Alz, sampled in 2001 showed results below LOD. The LOD for water was 200 µg/l, for sediment 300 µg/kg and for suspended solids 600 µg/l.

Biota

The sampling results from river Alz biota are described in Table 19.

Table 19 Concentration in Fish sampled at Alz river, in 2001[µg/kg wet weight]

	Eel (n=4)	**Barbel (n=3)**	**Chub (n=3)**
At WWTP outfall, liver	500, 600, 1600, 3200	< LOD	500, 2× < LOD
At WWTP outfall, filet	2 ×< LOD, 1800, 2400	< LOD	< LOD
Upstream of plant, liver & filet	< LOD	< LOD	< LOD

LOD: 500 µg/kg

Measurements related to USA, Alabama, Decatur (3M), APFO use site

Environmental monitoring data on the 3M Decatur, AL facility available to date are summarized in this document. More data are being generated and will be made available to the public through the US EPA Docket in 2007.

Soil

Soil samples were collected from the former sludge application area, formerly investigated areas and background areas of the 3M Decatur, AL facility during February and March, 2006. Samples were collected at 0 - 3 in., 3 - 6 in., 6 - 12 in., several 0.5 foot intervals between one and ten feet and several five foot intervals between ten feet and selected lower competent layers. The range of PFOA concentrations for the sludge application fields was 29-3140 ppb for 0 - 3 in.; 34.2-5690 ppb for 3 - 6 in.; 18.5 - 1870 ppb for 6 - 12 in.; 25.3 - 8780 ppb for 1 - 10 feet; and 14.1-3800 ppb for over 10 feet. Background PFOA levels in the sludge application control and in the non-sludge application background area ranged between 0.2 (non-detect) and 9.92 ppb. The soil samples collected from the borings in the formerly investigated areas had a range of PFOA levels between 0.2 (non-detect) and 810 ppb (3M, 2005b).

Sediment

Sediment samples (12) were collected in the Tennessee River adjacent to the 3M Decatur, AL facility in April, 2006 and analyzed for PFOA concentrations. The range of concentrations of PFOA for 12 sediment samples is between 0.393 and 24.1 ng/g (ppb) (3M, 2005b).

Wastewater treatment effluent

All process wastewaters from the 3M and Dyneon manufacturing operations are treated in this treatment facility which includes both physical-chemical and biological treatment. Water samples were collected prior to discharge to Baker's Creek which then empties into the Tennessee River. This water includes both the wastewater treatment effluent as well as non-contact cooling water

used in the manufacturing process. PFOA was present at an average of 602, 766, 1028, 310, 58, and 88.3 µg/l for samples collected in 1998, 1999, 2000, 2001, January 2003, and May 2003, respectively (3M, 2003b; Santoro, 2003; AR226-1482).

Groundwater

Groundwater concentrations of PFOA were measured in the former sludge application area, the sludge application control and the area wells at the 3M Decatur, AL facility in February and March, 2006. Wells in the sludge application area contained PFOA in the range between 2.27 and 3857 µg/l. The control sludge application wells contained PFOA between 0.220 – 94.7 µg/l. The LOI PFOA levels ranged from 0.025 (non-detect) and1333 µg/l (3M, 2005b).

Surface water

Surface water samples (12) were collected in the Tennessee River and a nearby creek called Baker's Creek adjacent to the 3M Decatur, AL facility in April, 2006. Each sample was analyzed twice for PFOA. Other surface water samples (16) were taken in a longitudinal direction along the length of the river along with a set of 30 samples collected across the river. The range of averaged concentrations of PFOA for the 12 samples is 0.0734 to 0.420 ng/ml (ppb). The range of PFOA concentrations for the longitudinal samples (16) is 0.00492 (non-detect) -0.0260 ng/ml. The range of PFOA concentrations for the transect samples (30) is 0.00266-0.435 ng/ml ((3M, 2005b).

Plants

The 3M Decatur, AL facility collected vegetation in its former sludge application area, sludge application control area and background locations on October 7, 2004. Samples were taken of four vegetative species found in the sludge application areas; broomsedge, pokeweed, curly dock, and goldenrod. PFOA concentrations in the vegetation in the former sludge application fields ranged from 42.3 – 912 ng/g (ppb). In the sludge control and the background area the PFOA concentrations ranged from 1.99 – 9.22 ng/g (ppb) (3M, 2005b).

Invertebrates

Asiatic clams were collected from the same locations as freshwater fish at the 3M Decatur, AL site in December, 2004. PFOA concentrations in clams upriver were non-quantifiable and downriver levels of PFOA ranged from 0.508 ng/g to 1.01 ng/g at Baker's Creek (3M, 2005b).

Freshwater fish

Two species of freshwater fish, channel catfish and large mouth bass were collected from the Tennessee River and Creeks in the vicinity of the 3M Decatur, AL facility during December, 2004. Filet and whole body parts of both species were analyzed for PFOA. The upriver PFOA levels for filet and whole body ranged between non-detect and 5.88 ng/g. Downriver samples of filet and whole body ranged from non-quantifiable to 6.06 ng/g. The 6 ng/g sample was found in a large mouth bass in Baker's Creek adjacent to the 3M facility, a creek that empties into the Tennessee River (3M, 2005b).

Mammals

Small mammal sampling was conducted concurrently with vegetation sampling from October 5-7, 2004 at the 3M Decatur, AL facility. Hispid cotton rat were collected in the same areas as the sampled vegetation. Livers and serum were collected from each rat and analyzed for PFOA. PFOA was present above the 400 ng/ml detection limit in two of the fifteen sampled rat serum samples but below the quantitation limit of 1000 ng/ml. All liver samples measured non-detect for PFOA (3M, 2005b).

Other Media

Surface water samples (12) were collected in the Tennessee River adjacent to the 3M Decatur, AL facility in April, 2006. Each sample was analyzed twice for PFOA. The range of averaged concentrations of PFOA for the 12 samples is 0.0977 to 70.4 ng/ml (ppb) (3M, 2005b).

Measurements related to USA, Alabama, Guin (3M)

Surface Water

Water samples were collected in September 2005 from 2 locations within the 3M Guin facility. Water collected from a surface water location contained concentrations of PFOA ranging from <LOQ (<0.0246 ng/ml) to 10.8 ng/ml (3M, 2005a). Water samples collected from a municipal water pipeline within the 3M Guin facility contained PFOA at concentrations of <LOQ to 9.1 ng/ml (3M, 2005a).

Measurements related to 3M's Cottage Grove, MN (USA)

Soil

Soil samples beneath a "ponded" area at the landfill contained PFOA at concentrations of 22.3 and 31.1 ng/g. Concentrations of PFOA were measured in soil borings at the landfill groundwater pumpout/spray irrigation site at depths from 0 to 26 feet below ground surface PFOA in the range from <0.208 to 21.6 ppb. PFOA concentrations in a background soil ranged from 1.28 to 4.87 ppb. (Oliaei et al., 2006).

Sediment

Sediment cores (collected in 2005) obtained at a depth of 10 cm from the river bottom contained PFOA at 18, 6.62, 1.31, and <0.301 ppb for samples collected at the 3M wastewater treatment discharge site, and 3 sites located progressively downstream, respectively (Oliaei et al., 2006).

Wastewater Treatment effluent and sewage sludge

The MCES wastewater treatment plant in St. Paul, MN receives leachate from the Pine Bend landfill and PFC-containing groundwater from the Oakdale dump. Influent and effluent samples collected in 2005 contained PFOA at concentrations of 46 and 78 ppt, respectively. Primary sludge, secondary sludge, and biosolid samples contained PFOA at 3.79, 21.5, and 11.1 ppb, respectively (Oliaei et al., 2006). The 3M wastewater treatment plant at Cottage Grove, MN discharges from the plant to the Mississippi River. Concentrations of PFOA in effluent of the plant ranged from a high of 1,991 µg/l in January/March 2000 (to a low of 17.7 µg/l in June 2003. (3M, 2003b; Santoro 2003 – AR 226-1482). Influent and effluent PFOA concentrations from this facility in 2005 (single sampling date) were 3.74 and 62.4 µg/l, respectively (Oliaei et al., 2006). The increase in PFOA concentration has been previously attributed to the degradation of PFC precursor compounds to PFOA during the treatment process (Schulz et al., 2005).

Landfill Leachate and gas

Pine Bend landfill received wastewater sludges from the 3M Cottage Grove wastewater treatment plant beginning in 1975. Leachate samples collected in 2005 from the combined leachate tanks contained PFOA ranging from 14.2 ng/ml to 81.8 ng/ml while a gas condensate sample from the landfill's active gas collection system contained PFOA at 83.8 µg/l. (Oliaei et al., 2006) .

Groundwater

3M disposed of fluorochemical wastes from the Cottage Grove site at the unlined Washington county landfill from 1969-1975. Groundwater samples collected in 2004 upgradient and downgradient from the landfill contained PFOA at <0.0027 and 41.6 µg/l, respectively. Groundwater samples collected in 2005 upgradient and downgradient from the Pine Bend landfill contained PFOA at concentrations of 0.008 and 1.6 µg/l, respectively (Oliaei et al., 2006).

Surface water

Surface water samples (collected in 2005) from the 3M Cottage Grove wastewater treatment plant discharge site water, the discharge site river water upper layer and from 3 sites located progressively downstream from this site contained PFOA at 3250, 3650, 35.30, <4.91 and <4.90 ppb, respectively (Oliaei et al., 2006).

Plants

Samples of duckweed collected in 2005 at the site receiving the Cottage Grove wastewater treatment discharge contained PFOA at 18.10 ppb. (Oliaei et al., 2006)

Freshwater Fish

Three sets of freshwater fish were collected in the vicinity of the 3M Cottage Grove facility in 2004 and 2005. Twenty fish were collected in August 2004 from the immediate vicinity of the 3M Cottage Grove wastewater treatment discharge area. Concentrations of PFOA in liver samples from these fish ranged from <0.359 to 0.551 ppb in small mouth bass, <0.374 to 1.27 ppb in white bass, <0.345 to 0.662 ppb in common carp, and <0.357 to 6.51 ppb in walleye. Over 100 fish were collected in October 2005 in a pool downstream and proximate to the 3M Cottage Grove discharge location. Carp blood from this location contained PFOA at concentrations of 0.87 to 15.5 ppb. Blood samples from other fish species generally contained low to non-detectable concentrations of PFOA (Oliaei et al., 2006).

Drinking water

PFOA was measured in water samples collected from the Oakdale municipal well at concentrations ranging from not detected to 0.858 µg/l in December 2004 (2/12 samples below detection limit of 0.025 µg/l) and 0.288 to 0.856 µg/l in August 2005 (0/8 samples below detection limit of 0.025 µg/l) (Bilott, 2005).

Measurements related to DuPont's Chambers Works, Deepwater, NJ (USA)

Wastewater Treatment Plant

Influent concentrations of PFOA to the Chambers Works wastewater treatment plant were 194 and 26 µg/l based on two samples collected on April 21 and May 15, 2003, respectively. Effluent concentrations of PFOA directly from the wastewater treatment plant on April 18 and May 16, 2003 were 475 and 279 µg/l, respectively. Concentrations of PFOA discharged from the wastewater treatment plant following the addition of non-contact cooling water to the Delaware river were 193 and 102 µg/l on April 18 and May 16, 2003, respectively. Sampling at this location on June 10/11, 2003 resulted in PFOA concentrations in water of 76, 144 and 97 µg/l (EPA, 2004b – AR226-2620).

Groundwater

Groundwater from 6 monitoring wells located on the Chambers Works site contained PFOA at concentrations ranging from 191 to 5230 ng/l (all samples were positive; LOQ is 0.050 µg/l; sampling

in May/June 2003) (EPA, 2004b – AR226-2620). Five interceptor wells contained PFOA at concentrations of 354 to 46,800 ng/l (all samples were positive; sampling in May 2003) (EPA, 2004b – AR226-2620).

Surface water

Concentrations of PFOA in Delaware river water samples collected in June 2003 from 30 locations ranged from not quantifiable (LOD of 0.010 µg/l, LOQ of 0.050 µg/l) to 0.577 µg/l (EPA, 2004b – AR226-2620).

Measurements related to DuPont Washington Works site (USA)

Environmental monitoring data on the DuPont, Parkersburg WV facility available to date are summarized in this document. More data are being generated and will be made available to the public through the US EPA Docket in 2006 and 2007.

Air

Air samples were collected with OSHA Versatile Sampling (OVS) method tubes and High-Volume Sampling (HVS) equipment within a two mile radius of the Washington Works, DuPont site in West Virginia from August-October, 2005. The OVS samples ranged from limit of detection (0.09-0.64 ng/tube fraction) to 60.2 ng/m^3 of PFOA. HVS samplers measured PFOA in the range of 0.01 to 75.9 ng/ m^3 . (EPA-HQ-OPPT-0113-0032). Air samples were collected during a ten week period from November 2003-January 2004 along the fence line of the DuPont Washington Works plant in West Virginia. OVS tube monitoring during the ten weeks measured PFOA levels from <120 ng/m^3 to 900 ng/ m^3 (Barton et al., 2006).

Soil

Soil samples were collected on-site at the DuPont Washington Works West Virginia site from soil 3 - 6 in. and 6 - 12 in. below the surface. The soil samples collected from the 3 - 6 in. intervals ranged from 72- 130 ppb PFOA. Those soil samples in the 6 - 12 in. interval contained PFOA in the range of 44-130 ppb PFOA (EPA-HQ-OPPT-0113- DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

Soil samples below the site of former waste management units and anaerobic digestion ponds showed PFOA concentrations ranging from 18 to 48000 ug/kg. The highest levels were from 8-12 foot deep samples (DuPont, 1999).

Groundwater

Groundwater has been monitored at the Washington Works facility in West Virginia from 1996-2006 at groundwater monitoring well locations on the plant site. APFO levels have been measured since 1996 and PFOA levels have been measured since 2004. APFO levels at 70 different groundwater wells at the plant between 1996 and 2006 ranged from < 0.050 to 4.66 x 10^5 ug/l. PFOA levels in groundwater at Washington Works between 2004 and 2006 ranged from 0.537-118 ug/l (EPA-HQ-OPPT-0113-0032).

Groundwater monitoring wells located at three different landfill sites in the vicinity of the DuPont, West Virginia Washington Works plant were monitored for PFOA and APFO between 1996 and 2006. The landfills are the Dry Run landfill, the Local landfill and the Letart landfill. PFOA and APFO levels at the Local landfill ranged from 0.141-69 ug/l and <0.050-91.1 ug/l respectively. For the Dry Run landfill, PFOA and APFO ranged from <0.050-32.6 ug/l and <0.050-39.0 ug/l respectively. PFOA and APFO levels at the Letart landfill were measured at a range between 0.126-20,200 ug/l and 0.058-57,200ug/l, respectively EPA-HQ-OPPT-0113-0032).

Groundwater samples below the site of former waste management units and anaerobic digestion ponds showed PFOA concentrations ranging from 0.1 to 13,600 ug/kg (DuPont, 1999).

Freshwater Fish

Channel catfish and large mouth bass were collected from the Ohio River near the Washington Works, DuPont WVA site and analyzed for APFO and PFOA in filet, viscera and carcass. PFOA levels in viscera samples for both species ranged between 1.23 ng/g and 1440 ng/g; PFOA in carcass samples ranged from 0.412 to 10.8 ng/g; and the filet samples were between 0.234 ng/g and 9.17 ng/g PFOA. Background fish had the following ranges of PFOA: viscera- 0.85-42.6 ng/g; carcass- 0.087-1.59 ng/g; filet- 0.146-1.65 ng/g (EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

Plants

Grass samples collected from on-site and off-site at the Washington Works DuPont West Virginia site were analyzed for PFOA. Samples were also collected at a background location many kilometers away. PFOA was detected in all samples analyzed.

On-site sampled were washed to help evaluate in the PFOA was in the plant tissues or on the surface. On-site unwashed grass samples contained PFOA levels between 154 ppb and 574 ppb on a dry weight basis. Washed grass samples had PFOA levels between 33.5 ppb and 92.8 ppb (dry weight) (EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

For off-site sampling DuPont collected of the above ground plant material and the underlying soil layer. In their report DuPont labeled these samples as 0 - 1 in. and 1 - 3 in. soil/grass samples. The 0 - 1 in. samples contained grass and the underlying thatch layer. The 1 - 6 feet samples contained the underlying soil and root material.

Off-site grass samples were collected during a dry period and following a rainfall event. Grass was sampled from 14 sites within 3.2 km of the facility and a background site far from the facility. Analysis of vegetation and underlying soil from all location sampled had detectable PFOA. The background samples and samples from one location near the facility had levels below quantitation limits (<6.0 ppb dry period and 5.0 ppb wet period). The other 13 locations had PFOA concentrations on grass ranging 6.88 ppb - 118 ppb during the dry period and < 7.0 – 121 ppb following the rain event. The underlying soil layer material collected during the dry period between 1 - 6 in. in depth contained PFOA at <7.0 to 52.8 ppb. Underlying soil following the rain event had PFOA concentrations ranging from <7 ppb to 51.6 ppb. All concentrations were on a dry weight basis (EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

Other Media

DuPont has been sampling public water supply systems in West Virginia and Ohio for APFO since 2002 and for APFO and PFOA since 2004. Twelve systems are sampled in West Virginia and seven systems are sampled in Ohio each year for APFO and PFOA. Additionally, DuPont has sampled twenty-five residential water wells in the vicinity of Washington Works on a quarterly basis for APFO since 2002 and for APFO and PFOA since 2004. The PFOA concentrations in the public supply systems have ranged from a low of 0.001 ug/l (non-detect) to 71.9 ug/l or 71,900 ng/l. The residential well samples have ranged from a low of 0.05 ug/l (non-quantifiable) to 27.1 ug/l or 27,100 ng/l (EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

Rainwater

Precipitation samples were collected at the DuPont Washington Works site, West Virginia August 25-26, 2005 at the same locations where ambient air samples were collected. Precipitation samples had PFOA levels that ranged from < 8.8 ng/l to 1660 ng/l. Out of eleven samples, seven were non-quantifiable, four ranged between 10.2 ng/l and 52.9 ng/l and one measured 1660 ng/l(EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

Environmental Monitoring Analytical Methods

Yamashita et al. (2004a) investigated sources of perfluorooctanoic acid (PFOA) contamination during the analytical pathway. They identified possible contamination caused either by instrumentation or the procedure applied. One source of procedural contamination is fluoropolymers, such as poly(tetrafluoroethylene) and perfluoroalkyloxy compounds, which are present in a variety of laboratory products. Therefore environmental or biological matrixes, samples or extracts are not allowed to come in contact with such fluoropolymers during the analysis. The authors detected PFOA in injections of 10 µL of pesticide-grade methanol taken in an autosampler vial. The HPLC/MS/MS instrument (HPLC tubing, internal fluoropolymer parts and autosampler vial septum) was identified as the possible source of this contamination. PFOA was the most abundant compound, found at approximately 30 pg per 10 µL of methanol injected. PFOA was also detected in polypropylene sample bottles. After extraction with methanol, PFOA was the only target compound detected in polypropylene containers, and the highest concentration found was 27 ng/l. Furthermore, notable amounts of PFOA (46 pg/l) were detected in the Sep-pak cartridge. Three types of purified reagent water were examined for the occurrence of perfluorinated carboxylic acids. Distilled water prepared using the Yamato distillation apparatus contained considerable amounts of target fluorochemicals. Milli-Q and HPLC-grade waters had relatively lower background levels. Based on measurements, the contamination from HPLC-grade water was estimated to be 1.2 pg/l of PFOA. After extraction, extracts often were filtered. Nylon syringe filters contained trace amounts of PFOA.

Boulanger et al. (2004) and So et al. (2004) identified fluoropolymer and fluoroelastomer containing devices as sources of PFOA contamination. Because this contamination cannot be omitted, poor detection limits or recovery rates significantly >100% were observed.

Ellis et al. (2004), and De Silva and Mabury (2004) used GC/MS to avoid instrumental contamination caused by HPLC. However, the drawback of this procedure is the additional derivatization step. Studies which do not provide information on procedural blanks, recoveries or detection limits should be treated with care.

The first worldwide interlaboratory study of PFCs in human and environmental matrices (van Leeuwen et al. 2005) indicates that analytical results from individual laboratories are not directly comparable due to variability in analytical results between individual laboratories.

Air

Only a few studies were available for the determination of PFOA in air. Reagen et al. (2004) developed an analytical procedure using OSHA versatile sampler (OVS) tubes and LC/MS for the determination of PFOA at the work place. A limit of quantitation (LOQ) of 8 µg/m^3, a limit of detection (LOD) of 2.4 µg/m^3, and a recovery rate of 94% were reported for APFO. Kaiser et al. (2005a) obtained a LOQ of 0.474 µg/m³ using electrospray LC/MS or LC/MS/MS and recoveries in the range of 88 and 95.7%. Moriwaki et al. (2003) determined PFOA in dust samples collected in Japanese homes. A LOQ of 50 ng/g and a recovery rate of 73% are reported.

Water

In general, PFOA is analyzed in water samples (surface-, drinking-, sea-, and waste water) using HPLC/MS/MS. Most methods applied have detection limits in the pg/l (e.g. Taniyasu et al. 2005a; Saito et al. 2004b; Berger et al. 2004; Tseng et al. 2006; Caliebe et al. 2004) to low ng/l range; the corresponding limits of quantification are somewhat higher (e.g. Saito et al. 2004; Berger et al. 2004).

Scott et al. (2006b) developed a method for determining very low levels (detection to 0.01ng/l) of PFCAs in large volume samples. Good agreement was shown between this method and the common LC/MS/MS method for samples from sewage treatment plant effluent, Lake Superior samples and precipitation samples in remote sites in Canada. PFOA levels in sewage treatment plant effluent ranged between 27.4 and 34.0 ng/l for the XAD resin and 30.5 to 40.2 for the LC/M/MS method. Samples taken from 260 meters deep in Lake Superior measured 0.26 ng/l on the XAD resin and 0.35 ng/l with the LC/MS/MS method. Precipitation samples in the remote regions of Canada analyzed by GC/MS contained 3.1 and 1.6 ng/l of PFOA for Turkey Lakes and Kejimkujik respectively (Scott et al. 2006a).

Sediment and sewage sludge

Schroeder (2003) used LC/MS and flow injection analysis for the detection of PFOA in sewage sludge (LOD: 10 mg/kg dw). Berger et al. (2004) and Kallenborn et al. (2004) achieved a LOD of 6 ng/g ww and a LOQ of 200 ng/g ww using HPLC/MS for analysis of sediments and sludges. De Voogt and van Roon achieved a LOD of 0.4 ng/g dw for soil samples via HPLC/MS. Higgins et al. (2005) developed a HPLC/MS/MS procedure for the determination of PFOA in sediment and sludge samples. They obtained LODs of 0.011 ng/g dw (sediment) and 1 ng/g dw (sludge); the corresponding recovery rates were 88 and 71%.

Biota

Most of the scientists used the ion-pair extraction procedure of Hansen et al. (2001) for the analysis of biota samples. In most cases HPLC/MS/MS was used for measuring PFOA concentrations. In some cases the LODs and LOQs were relatively high (>30 ng/g ww) due to matrix effects (e.g. Sinclair et al., 2004; Kannan et al., 2002a; Van de Vijver et al. 2005). Taniyasu et al. (2005a) and So et al. (2006a) developed very sensitive analytical methods for the determination of PFOA in tissue of animals; PFOA concentration of 0.01 (=LOD) and 0.2 ng/g ww (LOQ) could be detected.

Other Media

Using LC/MS/MS, Mawn et al. (2005) developed methods to quantify the amount of PFOA extracted from textiles such as apparel, home furnishings and carpet samples through their contact with water, methanol, and sweat and saliva simulants. The LOQ for samples extracted in water and sweat simulant is 1 ppb (ng PFOA/g sample) while the limits of quantitation for samples extracted in saliva simulant and methanol were 3 ppb and 2.5 ppb, respectively.

In SNF (2006) a HPLC/MS method is described for the determination of PFOA in all-weather jackets. The LOD is not reported; the lowest measured value was 6.1 ng/g.

Larsen et al. (2005) determined perfluorooctanoic acid in polytetrafluoroethylene polymers using pressurized solvent, reflux extraction methods and LC/MS/MS.

The available analytical methods are compiled in the Annex.

PFOA in Environmental Samples, temporal trends

Using current analytical methods PFOA might be detected in concentrations in the range of ng/m^3 (air), pg/l (water), and pg/g (ww; biota samples). Improvement of the analytical methods is ongoing. Today, PFOA is quantifiable in environmental matrices where the previous analytical methods failed. Studies using improved analytical sensitivity result in greater detection frequencies.

Only a few studies examined PFOA levels in environmental samples over time (Holmström et al., 2005; Lucaciu et al., 2005; Smithwick et al., 2006; 3M, 2003b). PFOA was not detected in any of the 146 guillemot egg samples collected from 1968 to 2003 in the Baltic Sea, so no trend could be determined. Over this same period, however, perfluorooctane sulfonate (PFOS) levels increased (1968-1997) and then decreased (1997-2003) (Holmström et al., 2005); in many cases PFOS and PFOA are simultaneously detected in environmental samples. Lucaciu et al. (2005) determined PFOA concentrations in suspended sediment samples collected at Niagara-on-the-Lake from the Niagara river. They observed increasing PFOA concentrations from 1981 (ca. 80 pg/g) to 1995 (ca. 220 pg/g). Between 1996 and 2000, PFOA concentrations decreased to ca. 100 pg/g. In the samples taken in 2001 the highest PFOA concentration was measured (ca. 290 pg/g). Smithwick et al. (2006) analyzed archived polar bear liver tissue samples from two geographic locations in the North American Arctic collected from 1972-2002. The eastern group (northern Baffin Island, Canada) comprised 31 samples, the western group (Barrow, Alaska) 27 samples. Between 1972-2002, in the eastern group a significant increase in PFOA concentration was observed (1972: ca. 3 ng/g ww PFOA; 2002: ca. 20 ng/g ww PFOA). Only slight changes in PFOA concentrations were detected in liver tissue samples collected at the western location (1972: ca. 2 ng/g ww PFOA; 2002: ca. 5 ng/g ww PFOA). PFOA was measured in liver tissue from 80 adult female sea otters collected from the California coast. These otters were found freshly dead and beached along the coast. During 1992 – 2002, concentrations of PFOA ranged from <5 to 147 ng.g^{-1} ww (Kannan *et al*., 2006). Concentrations of PFOA also increased significantly from 1992 to 2002 for adult female sea otters. However, PFOA was not found in the adult male sea otters (detection limit of 5 ng.g^{-1}). The reason for this lack of detection is not known. It should be noted that PFOA was found in the procedural blanks and in methanol injections performed between samples, however, as this background PFOA signal was consistent, it was subtracted from the calibration curve and samples (Kannan et al., 2006).Based on the results of 3M (2003b) wastewater effluent concentration collected at Decatur (Alabama, USA), the average concentrations significantly decreased between 1998 (602,000 ng/l) and 2003 (88,300 ng/l).

Air

Oliaei et al. (2006) sampled gas condensates at the Pine Bend Landfill, Inver Grove Heights (USA). The landfill is an operating mixed-municipal solid waste facility where 3M sludges were disposed off. About 80 000 ng/l PFOA were detected in the gas condensate.

Outdoor air measurements of PFOA were available from Japan and the USA. From the 20 samples collected around Kyoto, Japan, the highest result was 920 pg/m^3 and the lowest result was 1.6 pg/m^3 (Harada et al., 2005a). The geometric means for each of the two cities studied also varied widely: 2.0 pg/m^3 (Morioka) and 260 pg/m^3 (Oyamasaki).

In the USA, samples were collected at the fence line of the Washington Works manufacturing site in West Virginia (Barton et al. 2006). Of the 28 samples analyzed, 5 had levels above the reporting limit. The highest result was 900,000 pg/m^3, and the reporting limit was between 120,000 and 170,000 pg/m^3. No vapor-phase PFOA was detected above the detection limit (ca. 70,000 pg/m^3). These values are notably higher than the ambient levels and reporting limits from the Japanese study.

Moriwaki et al. (2003) collected dust via vacuum cleaner in Japanese homes. 16 dust samples from 16 different homes were analyzed. PFOA was found in all samples at concentrations greater than the limit of determination (>50 ng/g). The PFOA concentrations in dust samples ranged from 69 - 3700 ng/g (mean: 380 ng/g, median: 165 ng/g). The source for the occurrence of PFOA could not be identified. Archived dust samples collected by the USEPA for the CTEPP study in July 2000 to November 2001 from 56 homes in Ohio and 52 homes in North Carolina contained PFOA at mean concentrations of 3.155 and 2.977 µg/g dust, respectively (Strynar and Lindstrom, 2005).

Shoeib et al. (2006) measured telomer alcohols in air from the north Atlantic and Canadian Archipelago. They reported 8-2 alcohol concentrations of 5.8 – 26 pg/m^3 and 10-2 alcohol of 1.9-17 pg/m^3.

DuPont measured air concentrations using high volume impact +9samplers and low volume OVS sorption tubes. The reported air concentrations from below detection level (<0.01 ng/m^3) up to 75.9 ng/m^3 in ambient air samples out to two miles from their Washington Works site. (EPA-HQ-2004-0112-0032).

Soil

Limited measurements of PFOA and PFOA precursors in soil have been reported. The most extensive data have been collected by DuPont and 3M near their facilities in Alabama and West Virginia. Two sets were collected near the manufacturing sites in West Virginia and Ohio, and the results ranged from below the reporting limit (0.17 ng/g ww) to 700 ng/g ww (DuPont, 2003c, 2005). The third set was collected at Tomakomai, a Pacific Coast city in southern Hokkaido (Japan). As the result of an earthquake on September 26, 2003, two major fires occurred (26th and 28th September) at an oil storage facility of a refinery located in the west part of Tomakomai. At least 40,000 L of fire fighting foams (FFF) that contained perfluorinated compounds were used to extinguish these fires. A monthly monitoring survey of the environmental levels of perfluoro carboxylic acids (PFCs) in the Tomakomai region was conducted between October and December 2003. Two months after the usage of FFF Yamashita et al. (2004b) detected 2,870 pg/g soil dw. Based on the occurrence of PFOA in snow and soil samples, the authors suggest that significant amounts of PFCs in AFFF were released into air and deposited on land by wet deposition.

De Voogt and van Roon (2005) detected PFOA concentrations of 8.3 ng/g dw in soil samples collected at a contaminated site in The Netherlands (no further information provided). Oliaei et al. (2006) determined PFOA in soil samples collected under the Washington County landfill (USA) and in soils which were not influenced by the landfill. PFOA background concentrations of up to 4.87 ng/g were found. At the contaminated site soils showed PFOA concentrations in the range between <0.208-21.7 ng/g.

Sediment and suspended matter

Measurements of PFOA in sediment samples have been reported for the Nordic countries, the Netherlands, Japan, and the USA. Schrap et al. (2004), and De Voogt and van Roon (2005) detected PFOA in 11 out of 33 sediment and suspended matter samples collected in the Netherlands (LOD = 0.4 ng/g dw); the single highest result was 24 ng/g dw. Only 2 of the 13 samples analyzed from the Nordic countries had levels above the limit of quantitation (0.20 ng/g ww). The values were 0.28 and 0.31 ng/g ww (Kallenborn et al., 2004). Near manufacturing sites in the USA, the results were below the reporting limit of 0.2 ng/g ww (3M, 2001c), whereas results for cities with no manufacturing sites ranged from below the reporting limit to 1.8 ng/g dw. A year later, sediment from the city reporting 1.8 ng/g dw was re-sampled, and the results were below the reporting limit.

In 2005, Oliaei et al. (2006) collected Mississippi river sediment samples in the vicinity of the 3M production plant (USA). The highest PFOA concentration was measured at the point of discharge of

treated wastewater (18 ng/g).Sediment samples collected under ponded water at the Washington County landfill showed PFOA concentrations of up to 31.1 ng/g. In sediment samples collected between 2002 and 2004 in the USA up to 0.625 ng/g dw were detected (Higgins et al. 2005). In suspended sediment samples collected in the Niagara River Lucaciu et al. determined PFOA concentrations of 80 (1982), 100 (1987/88), 180 (1992/93), 220 (1995), 100 (2000), and 290 pg/g (2001). Kurunthachalam et al. (2005) and Nakata et al. (2005) report PFOA concentrations of <0.1-1.2 ng/g dw for sediment samples collected in the Kyoto area and Osaka and of 0.96 ng/g dw for sediment samples collected from the Ariake Sea (Japan).

Sewage Sludge and Effluent

In a sewage sludge sample collected near a manufacturing site in the USA the reported concentration of PFOA in sewage sludge was 244 ng/g dry weight (3M, 2001c). 2 of the 8 samples were below the reporting limit (0.02 ng/g ww). In samples collected away from manufacturing sites in the USA (Port St. Lucie, Florida and Cleveland, Tennessee), 3.1 ng/g dw were determined (3M, 2001c). Similarly, the highest result from the Nordic countries was 1.1 ng/g ww (Kallenborn et al., 2004), while 8 out of 23 samples showed PFOA concentrations below 0.20 ng/g ww. Higgins et al. (2005) analyzed a maximum PFOA concentration of 29.4 ng/g dw in sludge samples from the USA. PFOA was present in 11/13 samples. PFOA wasn't detected in different sludge samples collected in Germany (Schroeder 2003). Oliaei et al. (2006) and Sinclair and Kannan (2006) measured PFOA up to 241 ng/g dw in sludge samples collected at wastewater treatment plants (WWTPs) located in St. Paul (Minnesota) and New York State. The highest value was observed at a WWTP treating domestic, commercial, and industrial waste.

In Japan the highest PFOA concentration in sewage effluent was measured at the Agawam Ryuiki water disposal site (Osaka area); 67,000 ng/l PFOA were detected.

Tseng et al. (2006) detected PFOA concentrations of at maximum 170 ng/l in industrial and municipal WWTPs in Taiwan.

Sewage effluent measurements in the USA show PFOA concentrations up to 2,400 ng/l near a manufacturing site (3M, 2001c). Sewage effluent samples collected during 1998 to 2003 at Cottage Grove and Decatur (3M production plants; USA) showed PFOA concentrations up to 1,991,000 ng/l; measurements conducted with samples collected 2003 showed significantly reduced but still high PFOA concentrations (maximum 110,500 ng/l). Oliaei et al. (2006) investigated the effluent of the 3M Cottage Grove WWTP; they found 62,400 ng/l. At the Washington Works Facility PFOA concentrations of max. 915,000 ng/l (2001), max. 141,000 ng/l (2002), and max. 46,400 ng/l were measured (DuPont 2003a). In the wastewater effluent of a PTFE latex costumer of Solvay Solexis 74,000-75,100 ng/l PFOA were detected (Exygen 2003). A more recent sample from the Decatur Alabama WWTP had > 500 ppb (dw) in process sludge (3M, 2005b).

Alzaga and Bayona (2004) found at maximum 4,300 ng/l PFOA in the effluent of an urban-industrial wastewater treatment plant (WWTP) in Spain. PFOA concentrations ranging from 63-560 ng/l were found in wastewater from a printing plant, electronic production, leather and metal processing, paper production, a photographic laboratory, and textile processing in Austria (Hohenblum et al., 2003). PFOA levels ranging from 1 to 670 ng/l were detected elsewhere in the USA, Canada, and the Nordic countries. (3M 2001c; Scott et al. 2003; Berger et al. 2004; Kallenborn et al. 2004; Sinclair and Kannan 2006; Crozier et al. 2005). Remarkable results observed Oliaei et al. (2006) who found in the effluent of a munical WWTP (St. Paul, Minnesota, USA) higher PFOA effluent concentrations (78 ng/l) than in the influent (46 ng/l). The authors assume that precursor substances were biodegraded to PFOA during WWTP passage.

Boulanger et al. (2005) analyzed influent, effluent, and river water at the point of discharge for a 6 million gallons per day wastewater treatment plant which receives domestic and industrial wastewa-

ter. This WWTP was selected because of the lack of known perfluorooctane surfactant manufacture and production. In the effluent of the WWTP, a PFOA concentration of 22 ng/l was detected (blank: PFOA concentration in HPLC grade water: 2.4 ng/l), in the influent >4 ng/l were found (exact value was not determinable due to the analytical uncertainty encountered for this matrix-poor recovery). PFOA was measured at 8.7 ng/l in river water samples. Boulanger et al. (2005) assume that PFOA and related compounds were introduced into WWTPs through cleaning and care of surface-treated products (from clothing to carpets), through their use in industrial processes, and from the treatment of wastewater influent containing discarded product.

Specific fluorinated analytes, perfluoroalkyl sulfonates, fluorotelomer sulfonates, perfluorocarboxylates and selected fluorinated alkyl sulfonamides were studied at ten municipal wastewater treatment plants across the US. The flow rates in the ten plants ranged from 11,000 m^3 /day to 600,000 m^3 /day. Levels of PFOA in the influent ranged from 1.7 ng/l to 89 ng/l. The effluent concentrations of PFOA ranged from 2.5 ng/l to 97 ng/l (Schultz et.al, 2006).

The effluent waters of six activated sludge wastewater treatment plants in New York State were monitored for PFOA and seven other perfluorinated compounds. All of the six plants received domestic wastewater. All but one of the plants received commercial wastewater and only two plants received industrial wastewater. The levels of PFOA in the effluent of all six plants ranged from 58 ng/l to 1050 ng/l. Average concentrations of PFOA in the effluent of the two high capacity plants were 135 ng/l and 239 ng/l at the 25 and 35 MGD plants respectively. The smaller plant average effluent levels ranged from 67 ng/l to 697 ng/l. Concentrations of PFOA in the sludge samples at the two large plants were 69-241 ng/g and 18-89 ng/g (Sinclair and Kannan, 2006).

Landfill Effluent

From various manufacturing sites in the USA, the concentrations of PFOA in landfill effluent ranged from not detected (3M, 2001c) to 3,200,000 ng/l (DuPont, 2003a). The range of concentrations measured in landfill effluent from a non-manufacturing site in the USA was 940 to 1,000 ng/l (3M, 2001c), while the range in the Nordic countries was 91 to 516 ng/l (Kallenborn et al., 2004); Berger et al. 2004). Oliaei et al. (2006) detected max. PFOA concentrations of 81,800 ng/l in the landfill leachate sampled at the Pine Bend Landfill, Inver Grove Heights, Dakota County (USA).

Ground Water

At various manufacturing sites in the USA, the levels of PFOA in ground water ranged from < LOD to 3,400,000 ng/l (Santoro 2003). In ground water potentially impacted by previous fire-fighting activities in the USA, the measurements ranged from below the reporting limit (which decreased from 36,000 ng/l in 1999 to 3,000 ng/l in 2003) to 6,570,000 ng/l (Moody and Field 1999; Moody et al., 2003). Ground water samples collected at Washington Co. Landfill (USA) contained PFOA concentrations ranging from 1,300-70,000 ng/l (MDH 2004). Ground water samples collected at private wells in the vicinity of the aforementioned landfill showed PFOA concentrations < 1,000 ng/l (PFOA detected but not quantified; no further information; MDH, 2004).

DuPont (2003b,c) detected PFOA concentrations up to 51,000 ng/l at Chambers Works in Deepwater and Washington County (USA). Investigations of 3 M (2003b) revealed PFOA concentrations in ground water taken at the 3M production facilities at Decatur and Cottage Grove of max. 3,433,000 ng/l. Oliaei et al. (2006) analyzed ground water samples collected at the Washington County Landfill and the Pine Bend Landfill, Inver Grove Heights (USA). PFOA concentrations ranged from 8-41,600 ng/l

Fresh Water

PFOA in fresh water samples (e.g. lakes, rivers, streams, rain water, tap water) has been measured in the Nordic countries, Germany, The Netherlands, Japan, Taiwan, South Korea, Canada and the USA. The highest measurement from a creek after a spill of fire retardant foam was 11,300 ng/l, while the upstream maximum concentration was 33 ng/l (Moody et al., 2002). Near manufacturing facilities in the USA, the highest reported concentration was 598 ng/l (Hansen et al., 2002), while results for some samples were below the reporting limit. In further investigations of freshwater sampled at or near 3M production facilities concentrations up to 10,800 ng/l were detected (3M 2005; Oliaei et al. 2006). Away from spills and manufacturing sites, the highest reported concentration of PFOA in surface water was 760 ng/l, which was from Port St. Lucie, Florida, USA (3M, 2001c). In German rivers (Rhine, Pfinz, Neckar, Enz, Main, Kinzig, Mosel, Sieg, Erft, Ruhr, and Danube) PFOA concentrations ranging from <1 ng/l to 8 ng/l were detected (Lange et al. 2004). Skutlarek, Exner, and Färber (2006) detected <2-48 ng/l in the river Rhein (Germany; Swiss) and its most important tributaries. In the river Ruhr and its tributaries concentrations up to 3640 ng/l PFOA were detected (Skutlarek, Exner, and Färber 2006). In 2005, De Voogt and van Roon (2005) detected PFOA (160 ng/l) in freshwater samples taken from a canal after receiving wastewater from a fire incident where fire fighting foam has been used.

In rain water, PFOA has been detected up to 90 ng/l in a sample from Delaware, USA (Scott et al., 2006). Some rain water and surface water results were below detection limits. In the Nordic countries PFOA concentrations up to 17 ng/l were measured in rain water (Berger et al., 2004; Kallenborn et al., 2004). According to Berger et al. (2004) and Kallenborn et al. (2004) lake water sampled in the Nordic countries contained PFOA concentrations up to 8.2 ng/l.

Rainwater samples were collected in Winnipeg, Manitoba and analyzed for PFCAs. However, no PFCAs (including PFOA) were detected (method detection limit of 0.0072 µg.L-1) (Loewen et al., 2005). Loewen et al. (2005) suggested that this may be due insufficient atmospheric concentrations of PFCAs and a relatively high method detection limit.Scott et al. (2005) determined PFOA in precipitation from 4 American and 3 Canadian locations; the highest value was 85 ng/l determined at Delaware (USA).

DuPont measured PFOA in rain at 9 locations within two miles of their Washington Works in West Virginia in fall 2005. They detected PFOA in all samples ranging from below quantitation limit (8.8 ng/l) to 1,660 ng/l . They also measured concentrations at a background site in Maryland at below quantitation level (11 ng/l)(EPA-HQ-2004-0112-0032). Other researchers are working on measuring rain water in North America. When their research becomes available there will likely be a better understanding of the occurrence of PFOA in rain water.

Saito et al. (2004) found 0.12–40 ng/l PFOA (geometric mean) in tap water samples from Osaka and the Tohoku area (Japan). Morikawa et al. (2005) detected 87,100 ng/l PFOA in a Japanese river. PFOA concentrations ranging from 0.9 to 62 ng/l were measured in streams of the Shihwa industrial zone and Lake Shihwa in South Korea (Rostkowski et al. 2005). In Taiwanese rivers PFOA concentrations of 113-181 ng/l were measured (Tseng et al. 2006).

Scott et al. (2006b) measured PFOA and other perfluoroacids in rain from 9 sites in North America. They reported PFOA concentrations of <0.1-6.1 ng/L in remote location samples and 0.6-89 ng/L in more urban locations.

Salt Water

Samples from oceans, seas and coastal waters from various regions have been analyzed for PFOA. Caliebe et al. (2004) analyzed sea water samples collected in the German Bight and determined the concentrations and the distribution of perfluorinated chemicals. In March 2003 at the mouth of the

river Elbe, PFOA concentrations of about 20 ng/l were detected. Along the Elbe plume towards the north, the concentration of PFOA decreased to ca. 6 ng/l and 4 ng/l (near Sylt); in the open sea the concentration dropped to ca. 1 ng/l. In the southern German Bight, PFOA concentrations of about 7 (north of Norderney) and 15 ng/l (west of Borkum) were detected. Especially at these locations it is obvious that, in contradiction to the other measurements, the PFOA concentrations were significantly higher than the perfluorooctanesulfonate (PFOS) concentrations, indicating a different source of PFCs than at other sampling sites where PFOA and PFOS concentrations were comparable. Samples collected in July/August 2003 again showed the highest PFOA concentration (ca. 16 ng/l) at the mouth of the river Elbe. PFOA was detected at elevated concentrations near Sylt (4 ng/l PFOA) and in the southern German Bight (ca. 10 ng/l PFOA west of Borkum). Between the English and Dutch coasts (Ipswich-Rotterdam) PFOA concentrations of about 1 ng/l were found. At sampling sites in the open sea PFOA concentrations were about 0.5 ng/l. PFOA concentrations in the range between 1 and 2 ng/l were detected at the Norwegian coast.

Within the framework of a UBA research program, Theobald and Caliebe (2005) analyzed sea water samples collected in the German Bight and in the North Atlantic-North Polar Sea in 2004. In the region of the German Bight, the highest PFOA concentrations were detected in the river Elbe and at its mouth (Stade: 6.821 ng/l; near Cuxhaven: 3.791 ng/l). Decreasing PFOA concentrations were observed along the Elbe plume towards Sylt (Eider: 2.248 ng/l; Amrum: 2.083 ng/l; Sylt: 1.355; 1.972; and 2.182 ng/l). At one sampling station located in the direct vicinity of Sylt, the samples showed elevated PFOA concentrations in relation to the other two sampling points near Sylt (2.182 ng/l). Elevated PFOA concentrations were also detected north of the island Schiermonnikoog (2.567 ng/l). In the open sea PFOA concentrations of < 0.2 ng/l were detected (detection limit: 0.013 ng/l). The samples collected during the cruise of the Polarstern in June/July 2004 in the North Atlantic-North Polar Sea showed PFOA concentrations ranging from 0.035 to 0.097 ng/l (concentrations blank value corrected; Theobald and Caliebe, 2005).

The highest PFOA levels were observed in Asia. PFOA concentrations up to 450 ng/l were detected offshore of Japan (Saito et al., 2004). In the Atlantic Ocean even at a depth of 5,300 meters PFOA could be detected (0.05 - 0.1 ng/l; Scott et al. 2005). Background concentrations in the open oceans range from 0.06 to 0.2 ng/l (Caliebe et al., 2004; Theobald and Caliebe, 2005; Yamashita et al., 2004a; Taniyasu et al., 2004). In coastal waters from Jun-Wen estuary and Chi-Ku lagoon (Taiwan) Tseng et al. determined 130-270 ng/l PFOA.

Plants

Data on PFOA uptake and occurrence in plants is very limited. Ongoing studies will provide additional information as results are finalized.

Researchers at the University of Pennsylvania measured blood serum PFOA concentrations in a stratified random sample of people residing in near the DuPont Washington Works facility. The reported a statistically significant correlation between servings of home grown fruit and vegetable eaten and blood levels. Additionally, there was an increase in serum PFOA with servings of meat or game grown or harvested locally (P=0.005) (Emmett et al., 2006). Analysis of the concentration levels in food produce items is underway.

Grass samples collected from on-site and off-site at the Washington Works DuPont West Virginia (USA) site were analyzed for PFOA. Samples were also collected at a background location many kilometers away. They detected PFOA in all samples analyzed.

On-site sampled were washed to help evaluate in the PFOA was in the plant tissues or on the surface. On-site unwashed grass samples contained PFOA levels between 154 ppb and 574 ppb on a dry weight basis. Washed grass samples had PFOA levels between 33.5 ppb and 92.8 ppb (dry weight).

For off-site sampling DuPont collected of the above ground plant material and the underlying soil layer. In their report DuPont labeled these samples as 0 - 1 in. and 1 - 3 in. soil/grass samples. The 0 - 1 in. samples contained grass and the underlying thatch layer. The 1-6 feet samples contained the underlying soil and root material.

Off-site grass samples were collected during a dry period and following a rainfall event. Grass was sampled from 14 sites within 3.2 km of the facility and a background site far from the facility. Analysis of vegetation and underlying soil from all location sampled had detectable PFOA. The background samples and samples from one location near the facility had levels below quantitation limits (<6.0 ppb dry period and 5.0 wet period). The other 13 locations had PFOA concentrations on grass ranging 6.88 ppb - 118 ppb during the dry period and < 7.0 – 121 ppb following the rain event. The underlying soil layer material collected during the dry period between 1 -6 in. in depth contained PFOA at <7.0 to 52.8 ppb. Underlying soil following the rain even had PFOA concentrations ranging from <7 ppb to 51.6 ppb. All concentrations were on a dry weight basis (EPA-HQ-OPPT-0113- DuPont Quarterly MOU Status Report #2 Text, Tables and Figures).

At the 3M Decatur, AL (USA) facility vegetation was collected in the former sludge application area, sludge application control area and background locations during October, 2004. Samples were taken of four vegetative species found in the sludge application areas; broomsedge, pokeweed, curly dock, and goldenrod. PFOA concentrations in the vegetation in the former industrial sludge application fields ranged from 42.3 – 912 ppb. In the sludge control and the background area that did not receive PFOA application the PFOA concentrations ranged from 1.99 – 9.22 ppb (3M, 2005b).

Samples of duckweed collected in 2005 at the site receiving the wastewater treatment discharge from the 3M Cottage Grove, MN facility contained PFOA at 18.10 ppb. (Oliaeie et al., 2006).

In benthic algae collected at the Raisin River, the St. Clair River, and Calumet River (USA) PFOA could not be detected at a detection limit of 0.2 ng/g ww (Kannan et al., 2005).

Oliaei et al. (2006) analysed 18.1 ng/g PFOA in floating Lemna minor samples collected from the river cove that receives the 3M Cottage Grove WWTP discharge.

Invertebrates

Two species of invertebrates collected in the wild – mysis and diporeia – were studied by Martin et al. (2004a), and the mean PFOA concentrations ranged from 2.5 to 90 ng/g ww. Kannan et al. (2005) analysed tissue samples of amphipods, zebra mussels, and crayfish collected at the Raisin River, the St. Clair River, and Calumet River (USA). PFOA could not be detected in any of the samples at LODs of 0.2 - 5 ng/g ww.

Asiatic clams were collected from the same locations as freshwater fish at the 3M Decatur, AL site in December, 2004. PFOA concentrations in clams upriver were non-quantifiable and downriver levels of PFOA ranged from 0.508 ng/g to 1.01 ng/g at Baker's Creek (3M, 2005b).

So et al. (2005a; 2006a) detected PFOA concentrations up to 0.660 ng/g ww in mussel and oyster tissue collected from Tokyo Bay and the east coast of China. Worm samples (whole body) collected from Ariake Sea (western Japan) revealed PFOA concentrations of 82 ng/g ww (Nakata et al. 2005).

Freshwater Fish

Over 300 samples of freshwater fish from the Nordic countries, Poland, the Czech Republic, the Netherlands, Canada and the USA have been analyzed for PFOA (Kannan et al. 2005; Kallenborn et al. 2004; Martin et al. 2004a,b; Schrap 2004; Sinclair et al. 2004; Giesy and Kannan 2001; Moody et al. 2002). Many of the results were below the respective reporting limits of these studies;

however, the relative high detection and quantification limits must be taken into account (LOQ up to 72 ng/g ww). The highest detected concentration, 91 ng/g ww, was from the liver of a fish downstream of a spill of fire retardant foam (Moody et al., 2002). More typical levels in whole samples of freshwater fish range from 1.0 to 44 ng/g wet weight (Martin et al., 2004a). PFOA was detected only occasionally (in 2 out of 30 samples) in fish liver collected in the Nordic countries. The highest concentration is given as 1.4 ng/g ww (Kallenborn et al. 2004). Actual findings with improved analytical methods (reporting limits <2 ng/g ww) reveal that PFOA concentrations in fish (liver) are in the pg/l to low ng/g ww range (Oliaei et al. 2006; Sinclair et al. 2006).

Plankton and Shellfish

Whole samples of zooplankton, shrimp and clams from the Canadian eastern Arctic had maximum levels of PFOA of 3.4, 0.5 and < 0.2 ng/g ww, respectively (Tomy et al., 2004). All of the 77 oysters sampled from the Gulf of Mexico and the Chesapeake Bay, USA, had PFOA levels below the limit of quantification (< 19 ng/g ww; Giesy and Kannan, 2001). Tseng et al. (2006) detected 130-160 ng/g dw and 130-80 ng/g dw in muscle and viscous samples from oyster collected from culture sites in coastal areas of Taiwan. Nakata et al. (2005) found PFOA concentrations up to 9.5 ng/g ww in soft tissue of oyster, mussel, and clam collected at tidal flat from Ariake Sea (Japan).

Saltwater Fish

PFOA has been measured in hundreds of saltwater fish from the Baltic Sea, the Mediterranean Sea, the North Pacific Ocean, from the coasts of the Canadian Arctic, Taiwan, Japan, Belgium, the Netherlands, Nordic Countries; Poland, Colombia and Antarctica. Olivero-Verbel et al. (2005) determined PFOA concentrations up to 370 ng/ml in bile. In fish samples collected in the Ariake Sea (Japan) liver tissue contained PFOA concentration of at maximum 15 ng/g ww (Nakata et al. (2005). Liver and muscle samples of coastal fish collected in Taiwan revealed PFOA concentrations of 120-340 ng/g dw and 100 ng/g dw, respectively (Tseng et al. 2006). The highest level detected in a liver sample from a fish from a Nordic country was 5.4 ng/g ww (Kallenborn et al., 2004), while the highest level detected in a liver sample from a fish from the Canadian Arctic was 5.3 ng/g ww (Tomy et al., 2004). PFOA concentrations up to 46 ng/g ww were determined in muscle tissue from fish sampled in Belgium (Giesy and Kannan, 2001).

Reptiles and amphibians

Wild turtles that rank high in the food chain that were captured in a river in Japan in 2003 contained serum levels of PFOA up to 115.6 ug/l and were found to bioconcentrate the PFOA in the surface waters in which they lived by an average (geometric mean) factor of 3.2, with a range of BCFs from 0.8 to 15.8. Serum concentrations of PFOA from 94 turtles ranged from 0.3 to 115.6 ug/l. Surface water concentrations in the area where the turtles lived ranged from .021 to 87.1 ug/l of PFOA. (Morikawa et.al, 2005).

Plasma samples from sea turtles along the southeastern coast of the USA were analyzed for PFOA (Keller et al., 2004; 2005). The mean results were between 3 and 4 ng/ml. Kannan et al. (2005) investigated tissue samples of turtles and frogs collected in the USA. At LODs of 2.5 (turtle) and 72 ng/g ww (frog) PFOA could not be detected. Morikawa et al. (2005) analyzed serum samples of turtles caught in a Japanese river. PFOA concentrations ranging from <0.2 to 870 ng/ml were detected.

Birds

The highest PFOA concentration was detected in a liver sample from a cormorant from the Mediterranean Sea (450 ng/g ww; Corsolini and Kannan, 2004; Kannan et al., 2002b). It should be noted though that this value appears to qualify as an outlier as the concentration was 4.5 times greater than

the standard deviation of the mean. In liver samples from glaucous gull, black-headed gull, and black-eared kite PFOA was found occasionally in maximum concentrations of 21 ng/g ww (Tomy et al., 2004; Kannan et al., 2002a; Berger and Haukas 2005; Nakata et al. 2005). Double-crested cormorant (blood and yolk samples) and sea eagle (blood samples) showed PFOA concentrations up to 245 ng/ml (Giesy and Kannan, 2001).

In spleen of brown pelicans collected from Colombia PFOA concentrations of 182 ng/g ww were found (Olivero-Verbel et al. 2005). Hölmström et al. (2005) analysed guillemot egg samples collected between 1968 and 2003 in the Baltic Sea; PFOA could not be detected at a reporting limit of 3 ng/g ww.

Osprey nesting areas in the Baltimore Harbor, the Patapsco River, the Anacostia and the middle Potomac Rivers, the Elizabeth River of Maryland and Virginia (USA) and what was thought to be a reference site were monitored for a suite of contaminants included perfluorinated chemicals in 2000 and 2001. The C9, C10, C11, and C12 perfluorocarboxcylic acids were detected in all egg samples monitored in these areas but only quantifiable in a few (values were <30 ng/g wet weight). PFOA was not detected in any samples (LOD = 30 ng/g) (Rattner et al. 2004).

Mammals

Over 1,000 samples of terrestrial (e.g., mice, mink) and marine (e.g., seals, whales, dolphins) mammals have been analysed for PFOA. The highest result for liver samples from mink was 108 ng/g ww (Giesy and Kannan, 2001), and the highest result for liver samples from otters was 19 ng/g ww (Hoff et al., 2004). For seals, the highest result for a liver sample was 41 ng/g ww (Giesy and Kannan, 2001). The highest mean result for liver samples from polar bears, 55.8 ng/g ww, was from South Baffin Island (Smithwick et al., 2005a).

Smithwick et al. 2006 analyzed archived polar bear liver tissue samples from two geographic locations in the North American Arctic collected from 1972-2002. The eastern group (northern Baffin Island, Canada) comprised 31 samples, the western group (Barrow, Alaska) 27 samples. Between 1972-2002, in the eastern group a significant increase in PFOA concentration was observed (1972: ca. 3 ng/g ww PFOA; 2002: ca. 20 ng/g ww PFOA). Only slight changes in PFOA concentrations were detected in liver tissue samples collected at the western location (1972: ca. 2 ng/g ww PFOA; 2002: ca. 5 ng/g ww PFOA).

The highest level of PFOA measured in liver samples from dolphin, 20 ng/g wet weight, was from the Gulf of Mexico, and the highest blood level obtained for samples collected on the east coast of USA was 163 ng/g ww (Houde et al. 2005) and 4 ng/ml for dolphin from the Mediterranean Sea (Giesy and Kannan, 2001). Van de Vijver et al. (2004) analysed 62 ng/g ww PFOA in the tissue of harbor porpoises sampled as by-caught from coastal waters from Iceland, Norway, Denmark, and the German Baltic Sea.

In liver samples of whale PFOA was only occasionally detected at concentrations in the pg/g to low ng/g ww range (Bossi et al. 2005; Corsolini and Kannan 2004; Kallenborn et al. 2004; Tomy et al. 2004; van de Vijver et al. 2003; Kannan et al. 2002b). Guruge et al. (2005a) analysed serum and liver samples of domestic animals (cattle, chicken, pig, and horse). In serum samples up to 965 pg/ml (chicken) while in liver samples up to 300 pg/g (pig) were detected.

De Silva and Mabury (2004) isolated isomers of perfluorocarboxylates in polar bears from two geographical locations, the southeastern Hudson Bay region of Canada (2002) and central eastern Greenland (1999 - 2001). According to the study authors hypothesis branched PFOA isomers were found in all seven Greenland bear samples. Conversely, the PFOA in all eight Canadian bears consisted solely of the linear isomer. These contrasting patterns indicate a difference in the source of

PFOA. A complete absence of branched PFOA in the Canadian bears suggests a non-ECF input of PFOA to this location.

In the Greenland polar bear samples, the average branched PFOA isomer composition was 5.0% of the total PFOA. This value is much less than the 22% found in the ECF standard. Volatile telomer compounds may be responsible for the additional linear PFOA determined in the samples as PFOA and perfluorononanoic acid (PFNA) are the major products of the photochemically induced degradation of 8:2 fluorotelomer alcohol. The authors suggest that the difference of perfluorocarboxylic acids (PFCs) occurrence is due to the air current. Whereas the impact in North America is due to releases there, Greenland can be influenced from both North American and European sources. This may account for the differences in the consistent and distinct pattern of PFCA isomer distribution between Canadian and Greenland polar bear samples. This implies that there are differences in production and/or application of fluorinated materials between these two regions.

The environmental distribution and the biomagnification of a suite of perfluoroalkyl compounds (PFCs), was investigated in the food web of the bottlenose dolphin. Dolphin tissue samples and surficial seawater were collected at Sarasota Bay, Florida and Charleston Harbor, South Carolina. Dolphin plasma PFOA concentrations for Sarasota averaged 3.4 ng/l (for discussion of biomagnification and bioaccumulation see text above “2.2.6 Bioaccumulation”) (Houde et al., 2006).

PFOS and PFOA levels in serum of the giant panda and the red panda were taken in animals captured in zoos and animals parks from six provinces in China. The PFOA level ranged from 0.33 to 8.20 ug/l for the red panda and from 0.32 to 1.56 ug/l for the giant panda. Greater concentrations of the fluorochemicals were found in panda sera for those individuals collected from zoos near urbanized or industrialized areas than other areas (Dai et al., 2006).

Prevedouros et al. (2006) suggested that patterns in chain lengths and the branching of perfluorinated substances in environmental samples may provide clues to their sources. For instance, they note that branched materials are only known to arise from the ECF production processes. However, associating chain length patterns in environmental samples to patterns in sources is complicated because patterns may become altered as a result of different partitioning and uptake/clearance rates in biota for the various perfluorinated substances. Clearance rates of linear and branched PFOA in rats are different, with increased clearance of the branched material as compared to linear (Loveless et al, 2006). Hence, occurrence of only linear PFOA in biota does not seem to provide definitive information on sources.

The environmental levels of PFOA are compiled in Table 20. As this is a hazard assessment and not a risk assessment, this table contains a list of published data of PFOA emissions found in the environment without interpretation to relevance.

Other Media

The US EPA collected archived dust samples for PFOA in July 2000 to November 2001. The samples were obtained from 56 homes in Ohio and 52 homes in North Carolina. The samples from the 56 homes in Ohio contained PFOA at a mean concentration of 3.155 µg/g. Samples from the 52 homes in north Carolina contained PFOA at a mean concentration of 2.977 µg/g (Strynar and Lindstrom 2005).

Archived EPA house dust samples(n=112) were sieved to 150 um and analyzed for select PFCs. Telomer alcohols (6:2 , 8:2, 10:2 FTOH) were analyzed by GC/MS after sonic extraction & SPE cleanup. PerF carboxlic acids(C6-12) and sulfonates (PFOS, PFHS, PFBS) were analyzed by LC/MS/MS. Mean concentrations ranged from 0.357ug/g (PFDA) to 11.23 ug/g (PFHS). Maximum ranged from 2.42 ug/g (6:2 FTOH) to 357.0 ug/g (PFHS) (Strynar and Lindstrom, 2005).

Drinking water from surface water: Billot (2005) detected PFOA in drinking water samples (Oakdale Municipal Well (USA) in concentrations of <0.2-858 ng/l. Skutlarek, Exner, and Färber (2006) investigated drinking water sampled in the Ruhr area in Germany; PFOA was present in the samples in concentrations ranging from <0.2 to 519 ng/l. The water supplies are mainly based on bank filtration from surface water (see above page 58).

PFOA was measured in polar ice caps from three areas in the high Arctic (Melville Ice Cap, Northwest Territories; Agassiz Ice Cap, Nunavut; and Devon Ice Cap, Nunavut) (Young *et al*., 2005). PFOA concentrations ranged from 0.00245 to 0.00315 $\mu g.L^{-1}$ suggesting that contamination may be a result of atmospheric input. However, it should be noted that these values were not blank-corrected due to the unavailability of blanks at the time of analysis. Therefore, actual results may be lower than reported. A flux calculation was performed using a total area of the Canadian Arctic and sub-Arctic as 3.38 E6 km^2 and precipitation and snow density data from the Melville Ice Cap. A concentration of 0.001 $\mu g.L^{-1}$ PFOA was assumed in the Melville Ice Cap samples. The authors based this concentration on the assumption that the estimated flux falls in the range of atmospheric chemistry models based on the oxidation chemistry of FTOH alcohols in the atmosphere. These models estimated the total flux of PFCAs to the Arctic on the order of 0.1 to 1 tonne/year. The resulting atmospheric flux was 450 kg/year, which is comparable to values calculated from atmospheric modeling

Table 20 Environmental levels of PFOA

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Outdoor Air					
EPA-HQ-OPPT-0113-0032	Site	No data	0.09 ng/tube	OVS samples limit of detection (0.09-0.64 ng/tube fraction) to 60.2 ng/m^3 HVS samples 0.01 to 75.9 ng/m^3	2005; Air samples were collected with OSHA Versatile Sampling (OVS) method tubes and High-Volume Sampling (HVS) equipment within a two mile radius of the Washington Works, DuPont site in West Virginia; USA
Oliaei et al., 2006	Site	NR	NR	About 80,000 ng/l	2005; gas condensate collected from the condensation of water vapor in the gas; condensates sampled at the Pine Bend Landfill, Inver Grove Heights, Dakota County, Minnesota, USA; the landfill is an operating, mixed-municipal solid waste facility where 3M sludges were disposed off
Barton et al., 2006	Site	5/28	120,000 – 170,000 pg/m^3	< RL – 900,000 pg/m^3	2003 – 2004; fence line monitoring at Washington Works, Parkersburg, West Virginia, USA
Harada et al., 2005a	Amb	8/8	0.46 pg/m^3	geom. mean = 2.0 pg/m^3 (1.6 – 2.6 pg/m^3)	Morioka, Japan
	Amb	12/12	0.33 pg/m^3	geom. mean = 260 pg/m^3 (72 – 920 pg/m^3)	Oyamasaki, Japan, which has a major truck route passing through; corres-ponding concentration in airborne dust geom. mean = 3,400 ng/g
Indoor Air					
Moriwaki et al., 2003	Site	16/16	50 ng/g	69 - 3,700 ng/g dust (mean = 380 ng/g; median 165 ng/g	Vacuum cleaner dust samples from 16 different homes in Japan
Strynar and Lindstrom, 2005	Amb	No data/108	No data	mean concentrations 3.155 and 2.977 µg/g dust	200-2001; archived dust samples collected at 56 homes in Ohio and 52 homes in North Carolina, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Soil					
EPA-HQ-OPPT-0113-DuPont Quarterly MOU Status Report #2 Text, Tables and Figures	Site	No data	No data	Soil samples collected from the 3"-6" intervals 72-130 ng/g (ppb) soil samples in the 6"-12" interval 44-130 ng/g (ppb)	Soil samples were collected on-site at the DuPont Washington Works West Virginia site (USA) from soil 3"-6" and 6"-12" below the surface
Oliaei et al. 2006	Site	NR	0.208 ng/g	<0.208-21.7 ng/g	Soil samples collected under the Washington County landfill at different depths (0-26 ft), USA
	Amb	NR	0.208 ng/g	1.28-4.87 ng/g	Background soils were samples from an area upgradient of the groundwater direction of flow and out of the direct influence of the area where groundwater was spray irrigated at the Washington County landfill (depths: 0-8 ft), USA
De Voogt and van Roon, 2005	Site	NR	0.4 ng/g dw	8.3 ng/g dw	2004-2005 ; soil samples collected from a contaminated site ; The Netherlands
3M, 2005b	Site	No data	0.2 ng/g (ppb)	29-3140 ng/g (ppb; 0-3") 34.2-5690 ng/g (ppb; 3-6") 18.5-1870 ng/g (ppb; 6-12") 25.3-8780 ng/g (ppb; 1-10") 14.1-3800 ng/g (ppb; >10")	2006; soil samples collected at depth of 0-12"' from former sludge application area, formerly investigated areas and background areas of the 3M Decatur facility, AL; USA
	Amb	No data	0.2 ng/g (ppb)	0.2-9.92 ng/g (ppb; background level)	
	Site	No data	0.2 ng/g p(pb)	0.2-810 ng/g (ppb; formerly investigated areas)	
DuPont, 2005b	Site	9/9	2.1 – 21 ng/g dw	2.2 – 700 ng/g dw	2003; 0 – 1 foot deep; West Virginia, USA
Yamashita et al., 2004b	Site	2/2	NR	mean = 2780 pg/g dw	2003; two months after fire fighting foam used at refinery fire; Tomakomai Bay, Japan
DuPont, 2003c	Site	11/22	0.17 – 0.19 ng/g ww	< RL – 170 ng/g ww	Two borings up to 56 feet deep; Washington County, Ohio, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Sediment					
3M, 2005b	Site	No data/12	No data	0.393 and 24.1 ng/g	2006; sediment samples collected in the Tennessee River adjacent to the 3M Decatur, AL facility; USA
Oliaei et al., 2006	Site	NR	<0.298-0.301 ng/g	18 ng/g (sediment cove; point of discharge of treated wastewater) 6.62 ng/g (immediately downstream of the cove) <0.301-1.31 ng/g (concentration decresed with increasing distance from the discharge location1) <0.298 ng/g (upstream of point of discharge)	2005; Mississippi river sediment samples collected at a depth of 10 cm in the vicinity of the 3M production plant;USA
Oliaei et al. 2006	Site	NR	NR	22,3-31.1 ng/g	Sediment samples collected under ponded water at the Washington County landfill, USA
De Voogt and van Roon, 2005	Site	NR	0.4 ng/g dw	7.0 ng/g dw	2004-2005 ; sediment samples collected from a contaminated site ; the Netherlands
Higgins et al., 2005	Amb	15/17	0.011 ng/g dw	RL – 0.625 ng/g dw	2002-2005 sediments collected from San Francisco Bay area, Palo Alto Mud Flats, Hayward, CA, Baltimore, MD, and Corvallis, OR, USA
Kuruntha-chalam et al., 2005	Amb	NR	0.1 ng/g dw	<0.1-1.2 ng/g dw	2003-2005; sediment samples collected from rivers in the Kyoto area and from Osaka; Japan
Lucaciu et al., 2005	Amb	NR	NR	Ca. 80 pg/g (1982) Ca. 100 pg/g (1987/88) Ca. 180 pg/g (1992/3) Ca. 220 pg/g (1995) Ca. 100 pg/g (2000) Ca. 290 pg/g (2001)	1981-2001; suspended sediment samples collected at Niagara-on-the-Lake in the Niagara River
Nakata et al., 2005	Amb	5/5	0.09-0.10 ng/g dw	0.96 ng/g dw	Sediment samples collected from the Ariake Sea, western Japan

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Sediment (continued)					
Kallenborn et al., 2004	Amb	2/13	0.20 ng/g ww	< RL – 0.31 ng/g ww	2003/2004; sediment samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Schrap et al., 2004; De Voogt and van Roon, 2005	Amb	11/33	0.4 ng/g dw	< RL – 24 ng/g dw	2004; sediment and suspended matter from fresh water and marine locations; the Netherlands
3M, 2001c	Amb	6/6	0.2 ng/g ww	0.30 – 1.8 ng/g dw	1999; Port St. Lucie, Florida, USA
	Amb	0/6	0.2 ng/g ww	< RL	2000; Port St. Lucie, Florida, USA
	Amb	0/6	0.2 ng/g ww	< RL	Cleveland, Tennessee, USA
	Site	0/24	0.2 ng/g ww	< RL	Manufacturing sites (Decatur, Alabama; Mobile, Alabama; Columbus, Georgia; Pensacola Florida), USA
Sewage Sludge					
Oliaei et al., 2006	Site	NR	NR	3.79 ng/g dw (primary sludge) 21.5 ng/g dw (secondary sludge) 11.1 ng/g dw (dewatered sludge prior to incineration)	2005; sludge samples collected at metro WWTP a municipal wastewater treatment located in St. Paul, Minnesota; USA
Sinclair and Kannan, 2006	Site	10/10	10 ng/g dw	69-241 ng/g dw (D,C, I) 18-89 ng/g dw (D,C)	2004-2005; sludge samples collected from two WWTPs located in New York State, USA; WWTP treating: domestic (D), commercial (C) and industrial waste (I)
Higgins et al., 2005	Amb	11/13	1 ng/g dw	< RL – 29.4 ng/g dw	1998-2004; Pacific NW, West, West North Central, West South Central, and Northeast; USA
Kallenborn et al., 2004	Amb	15/23	0.20 ng/g ww	< RL – 1.1 ng/g ww	2003/2004; sewage samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Sewage Sludge (continued)					
Schroeder, 2003	Site	NR	10 mg/kg dw	< RL	sludge samples collected in NRW, Germany
3M, 2001c	Amb	2/6	0.2 ng/g ww	< RL – 3.1 ng/g dw	Port St. Lucie, Florida, and Cleveland, Tennessee; USA
	Site	6/8	0.2 ng/g ww	< RL – 244 ng/g dw	Manufacturing sites (Decatur, Alabama; Mobile, Alabama; Columbus, Georgia; Pensacola Florida), USA
Sewage Effluent					
Oliaei et al., 2006	Site	NR	NR	78 ng/l (effluent) 46 ng/l (influent)	2005; influent and effluent of metro WWTP a municipal wastewater treatment located in St. Paul, Minnesota; USA
Oliaei et al., 2006	Site	NR	NR	62,400 ng/l	2005; effluent of 3M Cottage Grove WWTP treating process wastewater generated from the production facility; after passing three different treatment systems the combined effluents are discharged to the Mississippi River; USA
Schultz et.al, 2006	Site	No data	No data	Influent 1.7-89 ng/l effluent 2.5-97 ng/l	Sewage effluent at ten municipal wastewater treatment plants across the USA
Sinclair and Kannan 2006	Site	45/45	2.5 ng/l	142-398 ng/l (D,C, I) 66-202 ng/l (D,C) 435-851 ng/l (D,C) 361-1050 ng/l (D,C) 132-196ng/l (D,I) 58-78 ng/l (D,C)	2004-2005; WWTP effluent samples collected from six plants located in New York State, USA; WWTP treating: domestic (D), commercial © and industrial waste
Tseng et al. 2006	site	2/2	0.8 ng/l	Not detected-39 ng/l	Industrial effluents of textile and dyeing manufacturers in Chung-Li, Taiwan
Tseng et al. 2006	site	2/2	0.8 ng/l	36-170 ng/l	effluents of municipal WWTPs near Taipei and Tainan, Taiwan
Boulanger et al., 2005	Site	NR/3	< 2 ng/l	Mean: 22 ng/l	2004; WWTP effluent in Iowa City; USA
	Site	NR/3	< 2 ng/l	Mean: 8.7 ng/l	2004; river water concentration at point of discharge; Iowa City, USA
Crozier et al. 2005	Site	NR	1 ng/l 0.1 ng/g dw	10-34 ng/l (stp effluent) 0.7-13 ng/g dw (biosolids)	Sewage treatment plant final effluent and biosolids

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Sewage Effluent (continued)					
Alzaga and Bayona, 2004	Site	NR/15	100 ng/l	Mean: < 100 – 1,400 ng/l (range: 100 – 4,300 ng/l)	2003; effluent of an urban-industrial WWTP; Spain
	Site	1/5	100 ng/l	150 ng/l	2003; 5 locations in Barcelona harbor; Spain
Berger et al., 2004	Amb	5/5	NR	Median: 20.5 ng/l	samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
EPA, 2004b – AR226-2620	Site	2/2	No data	Influent 26,000 and 194,000 ng/l	2003; Influent concentrations of PFOA to the Chambers Works wastewater treatment plant; USA
	Site	2/2	No data	Effluent 279,000 and 475,000 ng/l,	2003; effluent concentrations of PFOA directly from the Chambers Works wastewater treatment plant; USA
	Site	No data	No data	102,000 and 193,000 ng/l (April 18/ May 16) 76,000, 144,000 and 97,000 ng/l (June 10/11)	2003; PFOA discharged from the Chambers Works wastewater treatment plant (USA) following the addition of non-contact cooling water to the Delaware river
Kallenborn et al., 2004	Amb	7/7	0.06 ng/l	1.3 – 23 ng/l	2003/2004; samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faroe Islands
Saito et al., 2004	Site	NR	0.1 ng/l	67,000 ng/l	2003; sewage effluent at Aigawa Ryuiki water disposal site; Osaka area, Japan
3M, 2003b; Santoro 2003 AR226-1482	Site	NR	NR	1,991,000 ng/l (Jan.-March 2000) 216,000 ng/l (Sept.-Oct. 2000) 180,000 ng/l (Dec, 2002) 17,700-110,500 ng/l (average values for Jan.-June 2003)	2000-2003; effluent wastewater samples collected at the plant outfall on the Mississippi River; 3M production plant Cottage Grove, Minnesota, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Sewage Effluent (continued)					
3M, 2003b	Site	NR	0.082 ng/l	602,000 ng/l (1998) 766,000 ng/l (1999) 1,028,000 ng/l (2000) 310,000 ng/l (2001) 58,000-88,300 ng/l (Jan./May 2003) all values refer to average concentrations	1998-2003; effluent wastewater samples collected at Decatur, Alabama, USA
DuPont 2003a	Site	14/14	50 ng/l	750-46,400 ng/l (2003)	2001-2003; six outfalls were sampled at Washington Works Facility; USA
	Site	83/84	50 ng/l	<50-141,000 ng/l (2002)	
	Site	26/26	50 ng/l	118-915,000 ng/l (2001)	
Exygen 2003	Site	2/2	50 ng/l	74,000-75,100 ng/l	2003; wastewater of a PTFE latex costumer of Solvay Solexis; USA
Hohenblum et al., 2003	Site	4/15	50 ng/l	63 – 560 ng/l	wastewater from printing plant, electronic production, leather processing, metal processing, paper production, photo-graphic laboratory, textile production; Austria
Scott et al., 2003	Amb	7/7	NR	3 – 30 ng/l	3 Great Lakes
3M, 2001c	Amb	6/6	25 ng/l	40 – 670 ng/l	Port St. Lucie, Florida, and Cleveland, Tennessee; USA
	Site	8/8	25 ng/l	84 – 2,400 ng/l	Manufacturing sites (Decatur, Alabama; Mobile, Alabama; Columbus, Georgia; Pensacola Florida), USA
Landfill Effluent					
Oliaei et al., 2006	Site	NR	NR	61,650 ng/l (average; range: 14,200-81,800 ng/l)	2005; Landfill leachate sampled at the Pine Bend Landfill, Inver Grove Heights, Dakota County, Minnesota, USA; the landfill is an operating, mixed-municipal solid waste facility where 3M sludges were disposed off
Berger et al., 2004	Amb	6/6	NR	Median: 297 ng/l	samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Landfill Effluent (continued)					
Kallenborn et al., 2004	Amb	9/9	0.06 ng/l	91 – 516 ng/l	2003/2004; samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
DuPont, 2003a	Site	168/168	50 ng/l	61 – 3,200,000 ng/l	1994–2003 monitoring; Parkersburg, West Virginia, USA
3M, 2001c	Amb	4/4	25 ng/l	940 – 1,000 ng/l	Port St. Lucie, Florida, USA
	Site	3/8	25 ng/l	< RL – 48,000 ng/l	Manufacturing sites (Decatur, Alabama; Mobile, Alabama; Columbus, Georgia; Pensacola Florida), USA
Ground Water					
3M, 2005b	Amb/ Site	No data	25 ng/l	2270-3,857,000 ng/l (sludge application area) 220–94,700 ng/l (control sludge application wells) 25 (non-detect) - 1,333,000 ng/l (LOI PFOA levels)	2006; groundwater samples collected from the former sludge application area, the sludge application control and the area wells at the 3M Decatur, AL facility; USA
EPA-HQ-OPPT-0113-0032).	Site	No data/70	No data	<0.050 to 3.22 x 10^5 µg/l (APFO 1996-2006) 537-118,000 ng/l (PFOA 2004-2006	1996-2006; groundwater wells (on the plant site) has been monitored at the Washington Works facility in West Virginia (USA)
EPA-HQ-OPPT-0113-0032).	Site	No data	50 ng/l (APFO)	PFOA and APFO levels at the Local landfill 141-69,000 ng/l (PFOA) <50-91,100 ng/l (APFO) Dry Run landfill <50-32,600 ng/l (PFOA) <50-39,000 ng/l (APFO) PFOA and APFO levels at the Letart landfill 126-20,200,000 ng/l (PFOA) 58-57,200,000 ng/l (APFO)	1996-2006; Groundwater monitoring wells located at three different landfill sites in the vicinity of the DuPont, West Virginia Washington Works plant were monitored
Oliaei et al., 2006	Site	NR	NR	41,600 ng/l	Ground water at the Wahington County Landfill, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Ground Water (continued)					
Oliaei et al., 2006	Site	NR	NR	8-1,600 ng/l	2005; ground water from monitoring wells sampled at the Pine Bend Landfill, Inver Grove Heights, Dakota County, Minnesota, USA; the landfill is an operating, mixed-municipal solid waste facility where 3M sludges were disposed off
Skutlarek, Exner, and Färber, 2006	Amb	NR	2 ng/l	<0.2-519 ng/l	Ca. 2005; drinking water samples collected in the Ruhr area, Germany
Billot, 2005	Amb	10/12	25 ng/l	<25-858 ng/l (average values)	2004; drinking water samples collected at Oakdale Municipal Well; USA
Billot, 2005	Amb	8/8	25 ng/l	288-856 ng/l (average values)	2005; drinking water samples collected at Oakdale Municipal Well; USA
EPA, 2004b – AR226-2620	Site	No data	50 ng/l	191 to 5230 ng/l	2003; groundwater from 6 monitoring wells located on the Chambers Works site
	Site	No data	50 ng/l	354 to 46,800 ng/l	2003; Groundwater from 5 interceptor wells
MDH, 2004	Site	NR	NR	1,300 – 70,000 ng/l	2003/2004; Ground water samples collected at Washington Co. landfill, USA
	Amb	7/32	NR	< 1,000 ng/l (PFOA detected but not quantified)	2003/2004; ground water from private wells in the vicinity of the Washington Co. landfill, USA
3M, 2003b	Site	NR	82 ng/l	87-3,433,000 ng/l (2001; average) <LOD-2,386,000 ng/l (2003; average)	2001/2003; groundwater well samples collected at Decatur, Alabama, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Ground Water (continued)					
3M, 2003b	Amb	NR	NR	174,000 ng/l (2002; eastern/downgradient of site industrial activities)	2001-2002; groundwater samples collected at and near 3M production plant (Cottage Grove, Minnesota), USA
	Amb	NR	NR	4,630-6,410 ng/l (2001;western/ downgradient of site industrial activities)	
	Site	NR	NR	5,290-6,330 ng/l (2001; central to site industrial activities	
	Site	NR	NR	1,185-40,100 (2001; site-wide ground water from the production wells)	
3M, 2003b	Amb	NR	NR	314 ng/l (upgradient of site industrial activities)	2003; groundwater samples collected at and near 3M production plant (Cottage Grove, Minnesota), USA
	Site	NR	NR	10,200 ng/l (central to site industrial activities	
	Amb	NR	NR	4,810 ng/l (western/ downgradient of site industrial activities)	
	Amb	NR	NR	136,000 ng/l (eastern/downgradient of site industrial activities)	
	Site	NR	NR	27,900 ng/l (site-wide ground water from the production wells)	
DuPont, 2003b	Site	14/14	10 ng/l	200 – 47,000 ng/l	Chambers Works in Deepwater, New Jersey, USA
DuPont, 2003c	Site	85/87	10 ng/l	< RL – 51,000 ng/l	2001–2003; Washington County, Ohio, USA
Moody et al., 2003	Site	9/10	3,000 ng/l	< RL – 105,000 ng/l	5 years after fire-fighting activity; Wurtsmith Air Force Base, Michigan, USA
Santoro, 2003	Site	14/14	NR	310 – 174,000 ng/l	2001–2003; Cottage Grove, Minnesota, USA
	Site	14/16	NR	< RL – 3,400,000 ng/l	2001, 2003; Decatur, Alabama, US

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Ground Water (continued)					
DuPont, 2002	Site	43/62	10-50 ng/l	< RL – 8,700 ng/l max. concentation in drinking water: 6,500 ng/l max, concentration in unused water: 8,700 ng/l	2002; well, cistern, and spring water samples collected within a two-mile radius of the Washington Works facility; Washington County, Ohio, USA
Moody and Field, 1999	Site	8/14	36,000 ng/l	means = < RL – 6,570,000 ng/l	Naval Air Station Fallon, Nevada, USA
	Site	9/11	36,000 ng/l	means = < RL – 116,000 ng/l	Tyndall Air Force Base, Florida, USA
Fresh Water					
3M, 2005b	Site	No data/12	No data	73.4 to 420 ng/l	2006; surface water samples collected in the Tennessee River and a nearby creek called Baker's Creek adjacent to the 3M Decatur, AL facility; USA
3M, 2005b	Site	No data/16 No dat/30	4.92 ng/l	4.92-26 ng/l (longitudinal) 2.66-435 ng/l (transect samples)	2006; surface water samples (collected in longitudinal direction along the length of the river, n=16; collected across the river, n=30) collected in the Tennessee River and a nearby creek called Baker's Creek adjacent to the 3M Decatur, AL facility; USA
EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures	Amb	No data	1 ng/l	public supply 1-71,900 ng/l residential well samples 5-27,100 ng/l	2002/2004; DuPont has been sampling public water supply systems in West Virginia (n=12) and Ohio (n=7) for APFO and PFOA since 2004. Additionally, DuPont has sampled twenty-five residential water wells in the vicinity of Washington Works on a quarterly basis for APFO and PFOA
EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures EPA-HQ-2004-0112-0032	Site	11/11	8.8 ng/l	<8.8-1,660 ng/l (seven samples were non-quantifiable, four ranged between 10.2 and 52.9 ng/l and one measured 1660 ng/l)	2005; precipitation samples were collected at the DuPont Washington Works site, West Virginia, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Fresh Water (continued)					
EPA-HQ-2004-0112-0032	Amb	No data	11 ng/l	<11 ng/l	2005; DuPont measured PFOA in rain at a background site in Maryland; USA
Oliaei et al., 2006	Site	NR	4.9 ng/l	3,250 ng/l (at 3M discharge location; cove water) 3,650 ng/l (at 3M discharge location; cove water top surface) 35.3 ng/l (immediately downstream of the 3M discharge) <4.9 ng/l (other downstream locations)	2005; Mississippi river samples collected 2 ft below the river surface in the vicinity of the 3M production plant in Minnesota, USA
Sinclair et al., 2006	Amb	51/51	2.5 ng/l	10-173 ng/l	2004; water samples collected from nine major water bodies of New York State, USA
Skutlarek, Exner, and Färber, 2006	Amb	NR	2 ng/l	<2 – 48 ng/l	Ca. 2005; surface water samples collected in the river Rhine (Schaffhausen, CH till Wesel, Niederrhein, Germany) and its most important tributaries (Neckar, Main, Nahe, Lahn, Mosel, Ahr, Sieg, Wupper, Erft, Ruhr, Emscher, and Lippe: sample collection at the mouth of the rivers)
Skutlarek, Exner, and Färber, 2006	Amb	NR	2 ng/l	<0.2-3640 ng/l	Ca. 2005; surface water samples collected in the river Ruhr and its tributaries, Germany
Tseng et al., 2006	Amb	3/3	0.8 ng/l	113 ng/l (mean)	Water samples from Tour-Chyan river, Taiwan
	Amb	1/1	0.8 ng/l	181 ng/l	Water samples from Nan-Kan river, Taiwan
3M, 2005a	Site	3/5 3/5	Ca. 25 ng/l	<25-10,800 ng/l <25-9,100 ng/l	2005; surface water sampled within the 3M Guin (USA) facility at two locations
De Voogt and van Roon, 2005	Site	2/2	80 ng/l	160 ng/l	2004-2005; freshwater samples taken from a canal after receiving wastewater from a fire incident where fire fighting foam has been used; the Netherlands
Furdui et al. 2005	Amb	30/33	1-4 ng/l	2-7 ng/l	2004; water samples collected from the Great Lakes (11 locations)

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Fresh Water (continued)					
Kannan et al., 2005	Amb	NR	NR	4 – 14.7 ng/l	2001; water samples from Raisin River and St. Clair River, Michigan, USA
Kuruntha-chalam et al., 2005	Amb	NR	7.9 ng/l	7.9-110 ng/l	2005; river water samples collected in the Kyoto area ; Japan
Loewen et al., 2005	Amb	NR	7.2 ng/l.	<7.2 ng/l.	2004; rain water samples collected in Winnipeg; Canada
Morikawa et al., 2005	Amb	NR	0.1 ng/l	16.7-87,100 ng/l	2003; surface water samples from a Japanese river
Rostkowski et al., 2005	Site	27/27	0.9 ng/l	0.9-62 ng/l	2004; water samples were collected from streams of the Shihwa industrial zone and Lake Shihwa, South Korea
Scott et al., 2005	Amb	NR	NR	Max. 85 ng/l (Delaware)	1998-1999 (American locations); precipitation samples from 4 American sites (New York, Delaware, Maryland, and Vermont-urbanized locations) and 3 Canadian sites (Saturna Island, Algoma and Kejimkujik-rural locations)
Simcik and Dorweiler, 2005	Amb	36/36	0.29-0.58 ng/l	0.14-0.66 ng/l (remote lakes) 0.45-19 ng/l (urban surface waters) 0.28-3.4 ng/l (Lake Michigan)	Surface water samples collected in the mid-western US (Minneapolis, remote areas of Minnesota, shore of Lake Superior, and Voyageurs National Park along the US_Canadian boder)
Berger et al., 2004	Amb	4/4	NR	median: 7.8 ng/l	lake water samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
	Amb	5/5	NR	13.1 ng/l	rain water, samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Boulanger et al., 2004	Amb	8/8	13 ng/l	21 – 47 ng/l	Lake Erie, Canada/USA
	Amb	8/8	13 ng/l	15 – 70 ng/l	Lake Ontario, Canada/USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Fresh Water (continued)					
Kallenborn et al., 2004	Amb	6/6	0.06 ng/l	8.2 – 17 ng/l	2003/2004; rain water samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
	Amb	5/5	0.06 ng/l	4.8 – 8.2 ng/l	Lake water; samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
EPA, 2004b – AR226-2620	Amb	No data	10 ng/l	<10-577 ng/l	2003; surface water from Delaware river
Lange et al., 2004	Amb	NR	1 ng/l	< RL – 8 ng/l	German rivers Rhine, Pfinz, Neckar, Enz, Main, Kinzig, Mosel, Sieg, Erft, Ruhr and Danube
Saito et al., 2004	Amb	79/79	0.1 ng/l	0.1 – 460 ng/l	Rivers in 6 districts throughout Japan
	Amb	NR/30	0.1 ng/l	geom. mean: 0.12 - 40 ng/l	2003; tap water; Osaka and Tohoku area, Japan
Sinclair et al., 2004	Amb	NR/44	8 ng/l	< RL – 36 ng/l	2001-2003; 9 locations throughout Michigan, USA
	Amb	NR	8 ng/l	< RL – 16 ng/l	Background
Yamashita et al., 2004b	Site	2/2	NR	mean = 160 ng/l	2003; one month after fire fighting foam used at refinery fire; runoff into Tomakomai Bay, Japan
	Site	4/4	NR	mean = 18 ng/l	2003; two months after fire fighting foam used at refinery fire; snow near Tomakomai Bay, Japan
DuPont, 2003b	Site	NR	NR	max. = 89 ng/l	Deepwater, New Jersey, USA
Scott et al., 2003	Amb	NR/43	0.5 ng/l	< RL – 90 ng/l	Rain water archived from 1998; New York, Maryland and Delaware, USA
	Amb	NR/16	0.5 ng/l	< RL – 4 ng/l	Rain water from 2002; Algoma, Canada
	Amb	14/14	0.5 ng/l	1 – 10 ng/l	2001; tributaries to the Great Lakes
	Amb	NR	0.5 ng/l	< RL – 1 ng/l	Mid depth; 4 Great Lakes

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Fresh Water (continued)					
Hansen et al., 2002	Amb	0/20	25 ng/l	< RL	Upstream from fluorochemical manufacturing facility; Tennessee River, Decatur, Alabama, USA
	Site	18/20	25 ng/l	< RL – 598 ng/l	Downstream from fluorochemical manufacturing facility; Tennessee River, Decatur, Alabama, USA
Moody et al., 2002	Site	48/51	9 ng/l	< RL – 11,300 ng/l	2000/2001; spill of fire retardant foam; Etobicoke Creek, Toronto, Canada
	Amb	8/8	9 ng/l	mean = 20 ng/l (8 – 33 ng/l)	2000/2001; upstream of spill
3M, 2001c	Amb	4/4	25 ng/l	97 – 760 ng/l	Port St. Lucie, Florida, USA
	Site	3/8	25 ng/l	< RL – 63 ng/l	Manufacturing sites (Decatur, Alabama; Mobile, Alabama; Columbus, Georgia; Pensacola Florida), USA
Salt Water					
Houde et al., 2006	Amb	No data	No data	3.6 ng/l (Sarasota sea; averaged) 9.5 ng/l (Charleston; averaged)	Sea water samples collected from the Sarasota Bay and Charleston; USA
Tseng et al., 2006	Amb	2/2	0.8 ng/l	130-270 ng/l	Coastal waters collected from Jun-Wen estuary and Chi-Ku lagoon, Taiwan
Taniyasu et al., 2005	site	2/2	ca. 3 ng/l	62.4-63.7 ng/l	2003; 3 months after fire fighting foam was used; Tomakomai Bay (Japan)
	site	2/2	ca. 3 ng/l	34.8-35.1 ng/l	2004; Tokyo Bay (Japan)
Scott et al., 2005	Amb	NR	NR	0.050 – 0.10 ng/l	Atlantic Ocean; depths to 5,300 meters
Theobald and Caliebe, 2005	Amb	14/14	0.013 ng/l	0.140 – 6.82 ng/l	2004; Elbe River estuary and German Bight
	Amb	12/12	0.008 ng/l	0.035 – 0.097 ng/l	2004; North Atlantic, North Polar Sea
Nakata et al., 2005	Amb	5/5	0.8-10 ng/l	50 ng/l	water samples collected from the Ariake Sea, western Japan
Berger et al., 2004	Amb	11/11	NR	median: 5.2 ng/l	2003/2004; water samples collected in industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Caliebe et al., 2004	Amb	18/19	0.5 ng/l	approx. 0.5 – 20 ng/l	2003; Elbe estuary and German Bight (North Sea)

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Salt Water (continued)					
Kallenborn et al., 2004	Amb	15/15	0.06 ng/l	3.5 – 8.5 ng/l	2003/2004; sea water samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Saito et al., 2004	Amb	6/6	0.1 ng/l	1.9 – 450 ng/l	2003; Japan (Sea of Kushiro, Mutsu Bay, Honjo Marina, Funabashi Coast, Pacific Ocean, Koshien Coast)
So et al., 2004	Amb	31/31	0.02 ng/l	0.24 – 320 ng/l	2003/2004; coastal waters of Hong Kong, South China and Korea
Theobald and Caliebe, 2005	Amb	14/14	0.013 ng/l	0.140 – 6.82 ng/l	2004; Elbe River estuary and German Bight
	Amb	12/12	0.008 ng/l	0.035 – 0.097 ng/l	2004; North Atlantic, North Polar Sea
Yamashita et al., 2004a; Taniyasu et al., 2004 Yamashita et al., 2005	Amb	3/3	approx. 0.01 ng/l	154 – 192 ng/l	2002; Tokyo Bay; depth 0 – 2 meters
	Amb	NR/4	approx. 0.01 ng/l	0.137 – 1.060 ng/l	Offshore of Japan
	Amb	NR/12	approx. 0.01 ng/l	0.673 – 5.450 ng/l	Coastal area of Hong Kong
	Amb	NR/14	approx. 0.01 ng/l	0.243 – 15.300 ng/l	Coastal area of China
	Amb	NR/10	approx. 0.01 ng/l	0.239 – 11.300 ng/l	Coastal area of Korea
	Amb	3/3	approx. 0.01 ng/l	0.10 – 0.15 ng/l	2002; Mid Atlantic Ocean; depth 0 – 2 meters
	Amb	NR/9	approx. 0.01 ng/l	0.100 – 0.439 ng/l	Mid Atlantic Ocean
	Amb	5/5	approx. 0.01 ng/l	0.076 – 0.51 ng/l	2002; Sulu Sea; depth 10 – 3,000 meters
Yamashita et al., 2004a Taniyasu et al., 2004 Yamashita et al., 2005	Amb	2/2	approx. 0.01 ng/l	0.16 – 0.42 ng/l	2002; South China Sea; depth 10 meters
	Amb	2/2	approx. 0.01 ng/l	0.136 – 0.142 ng/l	2002; Western Pacific Ocean; depth 0 – 2 meters
	Amb	5/5	approx. 0.01 ng/l	0.015 – 0.056 ng/l	Central to Eastern Pacific Ocean; depth 0 – 4,400 meters
	Amb	NR/9	approx. 0.01 ng/l	0.160 – 0.338 ng/l	North Atlantic Ocean
Yamashita et al., 2004b	Site	NR/6	NR	mean = 4.15 ng/l	2003; one month after fire fighting foam used at refinery fire; Tomakomai Bay, Japan
	Site	NR/8	NR	mean = 2.67 ng/l	2003; two months after fire fighting foam used at refinery fire; Tomakomai Bay, Japan

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Plants					
3M, 2005b	site	No data	No data	42.3–912 ng/g (former sludge application area)	2004; 3M Decatur, AL facility collected vegetation in its former sludge application area, sludge application control area and background locations (plants sampled: broomsedge, pokeweed, curly duck, goldenrod)
	Amb	No data	No data	1.99–9.22 ng/g (sludge application control area and background locations)	
EPA-HQ-OPPT-0113-DuPont Quarterly MOU Status Report #2 Text, Tables and Figures	Site	No data	6 ng/g dw (ppb; dry period) 5.0 ng/g dw (ppb; wet period)	On-site unwashed grass samples 154-574 ng/g dw Washed grass samples 33.5-92.8 ppb dw.	Grass samples collected from on-site and off-site at Washington Works DuPont West Virginia site, USA Off-site grass samples were collected during a dry period and following a rainfall event; grass was sampled from 14 sites within 3.2 km of the facility and a background site far from the facility
	Amb	No data	6 ng/g dw (ppb; dry period) 5.0 ng/g dw (ppb; wet period)	background samples and samples from one location near the facility <6.0 ng/g dw (dry period) <5.0 ng/g dw (wet period) PFOA concentrations at the other 13 locations -on grass 6.88-118 ng/g dw (dry period) < 7.0 – 121 ng/g dw (following the rain event) -underlying soil layer material collected during the dry period <7.0 - 52.8 ng/g dw (1”-6” in depth) -Underlying soil following the rain event <7 - 51.6 ng/g dw all concentrations were on a dry weight basis	
Oliaei et al., 2006	Site	NR	NR	18.1 ng/g	2005; floating Lemna minor samples collected from the river cove that receives the 3M Cottage Grove wastewater treatment plant discharge, Minnesota; USA
Kannan et al., 2005	Amb	NR	0.2 ng/g ww	< RL	1999; benthic algae sampled at the Raisin River, the St. Clair River and Calumet River; USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Invertebrates					
3M, 2005b	Site	No data	No data	Upriver non-quantifiable downriver 0.508-1.01 ng/g	2004; asiatic clams were collected from locations (Baker's Creek) at the 3M Decatur plant, AL; USA
So et al., 2005a; So et al., 2006a	Amb	NR	0.204 ng/g ww	<204-328 ng/g ww	2004; mussel tissue samples collected from six locations along the east coast of China
So et al., 2005a; So et al., 2006a	Amb	NR	0.204 ng/g ww	660 ng/g ww	2004; oyster tissue samples collected from Tokyo Bay, Japan
Kannan et al., 2005	Amb	NR	0.2/5 ng/g ww	< RL	1998/1999; tissue samples of amphipods, zebra mussels, and crayfish collected at the Raisin River, the St. Clair River and Calumet River; USA
Nakata et al., 2005	Amb	5/5	3 ng/g ww	82 ng/g ww	Worm samples (whole body) collected from Ariake Sea, western Japan
Martin et al., 2004a	Amb	NR	2 ng/g ww	means = 2.5 – 90 ng/g ww	2001; whole body homogenates of Mysis and diporeia; Lake Ontario, Canada/USA
Freshwater Fish					
3M, 2005b	Site	No data	No data	Upriver non-detect - 5.88 ng/g (filet and whole body) Downriver non-quantifiable - 6.06 ng/g (filet and whole body)	2004; two species of freshwater fish, channel catfish and large mouth bass were collected from the Tennessee River and Creeks in the vicinity of the 3M Decatur plant, AL; USA
EPA-HQ-OPPT-0113-0032 DuPont Quarterly MOU Status Report #2 Text, Tables and Figures	Site	No data	No data	PFOA levels in viscera samples for both species 1.23-1440 ng/g PFOA in carcass samples 0.412-10.8 ng/g PFOA in filet samples 0.234-9.17 ng/g	Channel catfish and large mouth bass were collected from the Ohio River near the Washington Works, DuPont WVA site (USA)
	Amb	No data	No data	PFOA in background fish -viscera 0.85-42.6 ng/g carcass 0.087-1.59 ng/g filet 0.146-1.65 ng/g	

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Freshwater Fish (continued)					
Oliaei et al., 2006	Site	13/20	0.345-0.374 ng/g ww	<0.345-1.27 ng/g ww	2004; fish liver samples (small mouth bass, white bass, common carp, walleye) collected in the Mississippi river in the immediate vicinity of the 3M Cottage Grove discharge; USA
Oliaei et al., 2006	Site	0/20	0.417-0.668 ng/g ww	<0.417-<0.668 ng/g ww	2004; fish fillet samples (small mouth bass, white bass, common carp, walleye) collected in the Mississippi river in the immediate vicinity of the 3M Cottage Grove discharge; USA
Oliaei et al., 2006	Site	NR/>100	0.236-0.944 ng/ml	<0.236-15.50 ng/ml	2005; fish blood samples (small mouth bass, white bass, common carp, walleye, small mouth buffalo, gizzard shad, emerald shiner) collected in the Mississippi river in the immediate vicinity of the 3M Cottage Grove discharge and in the Lake Pepin area; USA
Sinclair et al., 2006	Amb	ca. 60/66	1.5 ng/g ww	<1.5-7.7 ng/g ww	2001-2003; liver samples from smallmouth and largemouth bass collected in New York State, USA
Kannan et al., 2005	Amb	NR	0.2/2 ng/g ww	< RL	1998/1999; tissue samples of round gobies and smallmouth bass collected at the Raisin River, the St. Clair River and Calumet River; USA
	Amb	NR/26	18/72 ng/g ww	< RL	Tissue samples of various fish (salmon, trout, carp, Lake whitefish) from Michigan waters, USA
Kallenborn et al., 2004	Amb	2/30	1 ng/g ww	< RL – 1.4 ng/g ww	2003/2004; various species; liver samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Martin et al., 2004a	Amb	NR	2 ng/g ww	means = 1.0 – 44 ng/g ww	2001; alewife, rainbow smelt, slimy sculpin and lake trout; whole homo-genate samples; Lake Ontario, Canada/USA
Martin et al., 2004b	Amb	0/5	2.0 ng/g	< RL	Trout and lake whitefish; liver samples; Canadian Arctic

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Freshwater Fish (continued)					
Schrap, 2004	Amb	0/NR	3 ng/g ww	< RL	Eel filet samples (fresh water); the Netherlands
Sinclair et al., 2004	Amb	0/87	72 ng/g ww	< RL	1999/2000; liver and muscle samples from various fish; Michigan, USA
Giesy and Kannan, 2001; as sum-marized in APME, 2002	Amb	1/7	8.0 ng/g ww	< RL – 13.7 ng/g ww	Various species; egg samples; Michigan, USA
	Amb	3/103	8.0 ng/g ww	< RL – detected (not quantified)	Various species; muscle samples; Michigan, USA
	Amb	0/159	72 ng/g ww	< RL	Various species; whole, liver, muscle, egg, blood and testes samples; Michigan, USA, Poland and Czech Republic
Moody et al., 2002	Site	8/8	1.2 ng/g ww	6.0 – 91 ng/g ww	2000/2001; after spill of fire retardant foam; common shiner; liver samples; Etobicoke Creek, Toronto, Canada
	Amb	1/1	1.2 ng/gww	88 ng/g ww	2000/2001; upstream of spill; common shiner; liver samples; Etobicoke Creek, Toronto, Canada
Plankton and Shellfish					
Tseng et al., 2006	Amb	3/3 3/3	10 ng/g dw	130 ng/g dw (mean) 160 ng/g dw (mean)	Muscle samples from oysters (Crassostrea gigas) collected from oyster culture sites in Chi-Ku coastal area; Taiwan
	Amb	3/3 3/3	10 ng/g dw	130 ng/g dw (mean) 180 ng/g dw (mean)	Viscus samples from oysters (Crassostrea gigas) collected from oyster culture sites in Chi-Ku coastal area; Taiwan
Nakata et el., 2005	Amb	16/16	3 ng/g ww	6.0-9.5 ng/g ww	Soft tissue of oyster, mussel, and clam collected at tidal flat from Ariake Sea, western Japan
Tomy et al., 2004	Amb	5/5	0.2 ng/g ww	mean = 2.6 ng/g ww (1.7 – 3.4 ng/g ww)	2002; zooplankton; whole samples; Canadian eastern Arctic
	Amb	3/7	0.2 ng/g ww	mean = 0.17 ng/g ww (< RL–0.5 ng/g ww)	2000/2001; shrimp; whole samples; Canadian eastern Arctic
	Amb	0/5	0.2 ng/g ww	< RL	2002; clams; whole samples; Canadian eastern Arctic
Giesy and Kannan, 2001; as sum-marized in APME, 2002	Amb	0/77	19 ng/g ww	< RL	American oyster; whole samples; Gulf of Mexico and Chesapeake Bay, USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Saltwater Fish					
Bossi et al., 2005	Amb	0/20	12 ng/g ww	< RL	Shorthorn sculpin; liver samples; Greenland
Tseng et al., 2006	Amb	6/6	10 ng/g dw	120 ng/g dw (tilapia; mean) 340 ng/g dw (seaperch; mean)	Liver samples of coastal fish (tilapia; Japanese seaperch); Taiwan
	Amb	3/3	10 ng/g dw	100 ng/g dw (mean)	Muscle samples of coastal fish (tilapia); Taiwan
Gulkowska et al., 2005	Amb	NR/18	0.05 ng/ml	<0.05-0.73 ng/ml	2002-2003; cod samples (blood) collected from the Gulf of Gdansk, Baltic Sea (Poland)
Kuruntha-chalam et al., 2005	Amb	NR	2.5 ng/g ww	<2.5-10 ng/g ww	2005 ; fish (scad, jack mackerel, sand fish, trout, sardine) were purchased in retail shopping markets and liver samples investigated; Japan
Nakata et al., 2005	Amb	NR/21	3 ng/g ww	<3-15 ng/g ww	Liver samples of fish (filefish, sea bream, red sea bream, right eye flunder, hammerhead sharkcollected from shallow water at Ariake Sea, western Japan
Nakata et al., 2005	Amb	-/10	3 ng/g ww	<3 ng/g ww	Liver of herbivore mudskipper collected from tidal flat at Ariake Sea, western Japan
	Amb		3 ng/g ww	7.8 ng/g ww	Liver of omniivore mudskipper collected from tidal flat at Ariake Sea, western Japan
Olivero-Verbel et al., 2005	Amb	NR/47	50 ng/ml	<50-370 ng/ml (bile)	2003; mullet (mugil incilis) were collected along the north coast of Colombia (3 locations) and tissue and organs investigated
Corsolini and Kannan, 2004	Amb	0/15	72 ng/g ww	< RL	Northern blue fin tuna and swordfish; liver and blood samples; Mediterranean Sea
Johnson et al., 2004	Amb	NR	NR	a	Mullet; bile samples; Cartagena Bay, Colombia
Kallenborn et al., 2004	Amb	2/14	1 ng/g ww	< RL – 5.4 ng/g ww	Various species; liver samples; samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Martin et al., 2004b	Amb	0/5	2.0 ng/g	< RL	Arctic sculpin, northern pike and white sucker; liver samples; Canadian Arctic

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Saltwater Fish (continued)					
Schrap, 2004	Amb	0/NR	3 ng/g ww	< RL	Flounder and cod filet samples; the Netherlands
Tomy et al., 2004	Amb	1/6	0.2 ng/g ww	mean = 0.16 ng/g ww (< RL – 0.5 ng/g ww)	2000/2001; arctic cod; whole samples; Canadian eastern Arctic
	Amb	2/7	0.2 ng/g ww	mean = 1.2 ng/g ww (< RL – 5.3 ng/g ww)	2000/2001; deepwater redfish; liver samples; Canadian eastern Arctic
Kannan et al., 2002b	Amb	0/35	72 ng/g ww	< RL	1999, swordfish, tuna, Atlantic salmon; liver samples; Baltic and Mediterranean Seas
Giesy and Kannan, 2001; as sum-marized in APME, 2002	Amb	8/26	7.5 ng/g ww	< RL – 46 ng/g ww	Various species; muscle samples; Belgium
	Amb	0/65	72 ng/g ww	< RL	Various species; whole, liver and blood samples; North Pacific Ocean, Baltic Sea, Mediterranean Sea and Antarctica
Reptiles and amphibians					
Kannan et al., 2005	Amb	NR/5	2.5 ng/g ww	< RL	Snapping turtle tissue samples collected from Macomb County, Michigan, USA
	Amb	NR/4	72 ng/g ww	< RL	Green frog tissue samples collected from Kalamazoo, Michigan, USA
Morikawa et al., 2005	Amb	91/94	0.2 ng/ml	<0.2-870 ng/ml	2003-2004; serum samples of turtles caught in a Japanese river
Keller et al., 2004; Keller et al., 2005	Amb	73/73	NR	0.49-8.14 ng/ml (mean = 3.2 ng/ml)	Juvenile loggerhead sea turtles; plasma samples; south-eastern coast USA
	Amb	6/6	NR	2.77-4.25 ng/ml (mean = 3.6 ng/ml)	Juvenile Kemp's ridley sea turtles; plasma samples; south-eastern coast USA
Birds					
Berger and Haukas 2005	Amb	NR	0.128 ng/g ww	<0.128 ng/g ww	2004; glaucous gull liver samples collected from the marginal ice zone in the Barents Sea; Norway
Bossi et al., 2005	Amb	0/38	12 ng/g ww	< RL	Black guillemot and fulmar; liver samples; Greenland and Faeroe Islands
Holmström et al., 2005	Amb	0/146	3 ng/g ww	< RL	Guillemot; egg samples from 1968–2003; Baltic Sea

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Birds					
Kannan et al., 2005	Amb	0/NR	19/38 ng/g ww	< RL	2000; Bald eagle samples of liver, kidney, gall bladder, muscle, testes, ovary; Upper Peninsula, Michigan, USA
Nakata et al., 2005	Amb	-/13	3 ng/g ww	<3 ng/g ww	Liver of mallard and black-headed gull sampled from shallow water at Ariake Sea, western Japan
Olivero-Verbel et al., 2005	Amb	1/5	30 ng/g ww	182 ng/g ww (spleen)	2004; brown pelicans were collected in Cartagena Bay (Colombia) and tissue and organs investigated
Corsolini and Kannan, 2004	Amb	12/12	2.5 ng/g ww	29 – 450 ng/g ww	1997; common cormorant; liver samples; Mediterranean Sea
Kallenborn et al., 2004	Amb	0/2	1 ng/g ww	< RL	2002; fulmar; egg samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Martin et al., 2004b	Amb	0/15	2.0 ng/g ww	< RL	Common loon, northern fulmar and black guillemot; liver samples; Canadian Arctic
Tomy et al., 2004	Amb	0/4	0.2 ng/g ww	< RL	Black-legged kittiwake; liver samples; Canadian eastern Arctic
	Amb	1/5	0.2 ng/g ww	mean = 0.14 ng/g ww (< RL – 0.3 ng/g ww)	Glaucous gulls; liver samples; Canadian eastern Arctic
Kannan et al., 2002a	Amb	4–8/83	19 ng/g ww (Japan) 36 ng/g ww (Korea)	< RL – 21 ng/g ww	Black-headed gull, black-eared kite; liver samples; Japan and Korea
Kannan et al., 2002b	Amb	12/12	1–72 ng/g ww	29 – 450 ng/g ww	Cormorant; liver samples; Baltic and Mediterranean Seas

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Birds (continued)					
Giesy and Kannan, 2001; as sum-marized in APME, 2002	Amb	15/18	19 ng/g ww	< RL – 444 ng/g ww	Various fish-eating water birds; liver samples; Mediterranean Sea, Midway Atoll and Japan
	Amb	3/88	30 ng/ml	< RL – 49 ng/ml	Double crested cormorant and sea eagle; blood samples; Great Lakes, Japan and Mediterranean Sea
	Amb	2/4	180 ng/ml	< RL – 245 ng/ml	Double crested cormorant; yolk samples; Lake Winnipeg
	Amb	0/349	37 ng/g ww	< RL	Various fish-eating water birds; liver, kidney, muscle, ovary, testes, gall bladder, blood, serum, plasma and egg samples; USA, Poland, Baltic Sea, Mediterranean Sea, Japan and India
Mammals					
3M, 2005b	Site	2/15 0/15	400 ng/ml -	<1000 ng/ml serum) non-detect (liver)	2004; small mammal Hispid cotton rat: livers and serum) sampling was conducted concurrently with vegetation sampling at the 3M Decatur, AL facility; USA
Dai et al., 2006	Amb	No data	No data	330-8200 ng/l (red panda) 320-1560 ng/l (giant panda)	PFOA levels in serum of the giant panda and the red panda were taken in animals captured in zoos and animals parks from six provinces in China
Smithwick et al., 2006	Amb	58/58	2.3 ng/g ww	Eastern group: ca. 3 ng/g ww (1972) ca. 20 ng/g ww 2002) Western group: ca. 2 ng/g ww (1972) ca. 5 ng/g ww (2002)	1972-2002; polar bear liver tissue samples collected from two geographic locations in the North American Arctic (eastern group: northern Baffin Island, Canada; western group: Barrow, Alaska
Guruge et al., 2005a	Amb		49 pg/ml sera 49 pg/g liver	Serum: 50 pg/ml (cattle) <49-965 pg/ml (chicken) 176 pg/ml (pig) <49 pg/ml (horse) liver: <49-239 pg/g (cattle) <49 pg/g (chicken) <49-300 pg/g (pig)	Serum and liver samples of domestic animals (cattle, chicken, pig, and horse); Japan
Houde et al., 2006	Amb	No data	No data	Dolphin plasma 3.4 ng/l (Sarasota; averaged) 43 ng/l (Charleston; averaged)	Dolphin tissue samples were collected at Sarasota Bay, Florida and Charleston Harbor, South Carolina; USA

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Mammals (continued)					
Houde et al., 2005					2003, blood samples from bottlenose dolphin collected in the Gulf of Mexico and the Atlantic Ocean along the coast of the USA
	Amb	13/13	0.5 ng/g ww	0.7-26 ng/g ww	-Sarasota Bay
	Amb	2/2	0.5 ng/g ww	0.6-0.9 ng/g ww	-Bermuda
	Amb	42/42	0.5 ng/g ww	1-70 ng/g ww	-Indian River Lagoon
	Amb	47/47	0.5 ng/g ww	4.6-163 ng/g ww	-Charleston
	Amb	5/5	0.5 ng/g ww	20-115 ng/g ww	-Delaware Bay
Nakata et al., 2005	Amb	5/5	3 ng/g ww	9.1 ng/g ww	Liver of finless porpoise sampled from shallow water at Ariake Sea, western Japan
Smithwick et al., 2005a	Amb	> 75%	2.3 ng/g ww	mean = 2.4 – 36 ng/g ww min = < 2.3 – 20 ng/g ww max = 9.04 – 55.8 ng/g ww	2002; polar bear samples collected from 5 locations in North American Arctic and 2 locations in European Arctic
Taniyasu et al., 2005 ; Taniyasu et al., 2005a	Amb	4/4	0.01-0.08 ng/g ww	0.28-0.29 ng/g ww	Beaver liver ; Poland
van de Vijver et al., 2005	Amb	-/82	62 ng/g ww	<62 ng/g ww	2002; harbor seals stranded along the coastline of the Wadden Sea were collected and organs and tissues analysed (liver, kidney, blubber, muscle, tracheo-branchial muscle, and spleen); The Netherlans
Bossi et al., 2005	Amb	0/10	12 ng/g ww	< RL	Polar bear; liver samples; Greenland
	Amb	0/35	12 ng/g ww	< RL	Mink whale and long-finned pilot whale; liver samples; Greenland and Faeroe Islands
Kannan et al., 2005	Amb	NR/8	2 ng/g ww	6.3 – 40 ng/g ww	Mink liver samples; Kalamazoo, Michigan, USA
Smithwick et al., 2005b	Amb	NR/29	2 ng/g ww	mean = 10 ng/g ww	1999-2001; polar bear; liver samples; Greenland
	Amb	0/30	12 ng/g ww	< RL	Ringed seal; liver samples; Greenland
Corsolini and Kannan, 2004	Amb	<4/19	2.5 ng/g ww	< RL – 3.8 ng/g ww	Dolphin and whale; liver, muscle and blood samples; Mediterranean Sea

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Mammals (continued)					
Ellis et al., 2004	Amb	NR	NR	10 – 110 ng/g ww (value refers to PFOA and/or PFNA; not further specified)	Polar bear; liver
De Silva and Mabury 2004	Amb	NR/15	NR	Greenland>Canada	1999-2002; polar bear; liver samples; Greenland and Canada
Guruge et al., 2004	Amb	12/15	0.010 ng/ml	< RL – 0.10 ng/ml	Black beef cattle followed from age 9 to 27 months; plasma samples; Japan
Hoff et al., 2004	Amb	0/42	110 ng/g ww	< RL	Wood mice; liver samples; Belgium
Kallenborn et al., 2004	Amb	8/8	1 ng/g ww	0.3 – 5.6 ng/g ww	Seal; liver; samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
	Amb	0/5	1 ng/g ww	< RL	Mink whale; liver; samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
	Amb	4/4	1 ng/g ww	0.35 – 1.7 ng/g ww	Pilot whale; liver; samples collected near industrialized or urban areas of Finland, Sweden, Norway, Denmark, Iceland, Faeroe Islands
Martin et al., 2004b	Amb	0/39	2.0 ng/g ww	< RL	Mink, fox and ringed seal; liver samples; Canadian Arctic
	Amb	7/7	2.0 ng/g ww	mean = 8.6 ng/g ww (2.9 – 13 ng/g ww)	Polar bear; liver samples; Canadian Arctic
Tomy et al., 2004	Amb	NR/5	0.2 ng/g ww	mean = 0.34 ng/g ww (< RL – 0.7 ng/g ww)	1998; walrus; liver samples; Canadian eastern Arctic
	Amb	5/5	0.2 ng/g ww	mean = 0.9 ng/g ww (0.7 – 1.1 ng/g ww)	2000; narwhal whale; liver samples; Canadian eastern Arctic
	Amb	5/5	0.2 ng/g ww	mean = 1.6 ng/g ww (1.0 – 2.8 ng/g ww)	1996; Beluga whale; liver samples; Canadian eastern Arctic
Van de Vijver et al., 2004	Amb	1/41	62 ng/g ww	62 ng/g ww	Harbor porpoises liver tissue; sampled as by-caught from coastal waters; Iceland, Norway, Denmark, German Baltic Sea

Table 20 Environmental levels of PFOA (continued)

Reference	Site or Amb.	Detection Frequency	Reporting Limit	PFOA Concentration	Comments
Mammals (continued)					
Van de Vijver et al., 2003	Amb	0/184	110 ng/g ww	< RL	1995–2000; porpoise, seal, dolphin and whale; kidney and liver samples; stranded along Dutch, Belgian and French North Sea coasts
Kannan et al., 2002b	Amb	<10/52	19 ng/g ww	< RL–39 ng/g ww	Seal; liver samples; Baltic Sea
	Amb	0/15	72 ng/g ww	< RL	1991–1999; dolphin and whale; liver and muscle samples; Baltic and Mediterranean Seas
	Amb	<4/4	2.5 ng/ml	mean = 3.1 ng/ml (< RL – 3.8 ng/ml)	1997; bottlenose dolphin; blood samples; Mediterranean Sea
Kannan et al., 2002c	Amb	23/112	4.5 ng/g ww	< RL – 27 ng/g ww	1995–2000; mink; liver samples; Illinois, Massachusetts, South Carolina and Louisiana, USA
	Amb	<11/20	7.5 ng/g ww	< RL – 19 ng/g ww	1997/1998; river otter; liver samples; Washington and Oregon, USA
Giesy and Kannan, 2001; as summarized in APME, 2002	Amb	18/31	19 ng/g dw	< RL – 108 ng/g dw	Mink; liver samples; Massachusetts, USA
	Amb	3/87	19 ng/g ww	< RL – 41 ng/g ww	California sea lion and ringed seal; liver samples; California, USA, and Baltic Sea
	Amb	11/62	5.0 ng/ml	< RL – 23 ng/ml	Seal; blood and serum samples; Baltic, Caspian and Mediterranean Seas
Giesy and Kannan, 2001; as summarized in APME, 2002	Amb	0/51	36 ng/g ww	< RL	Mink and river otter; liver samples; 5 states in USA
	Amb	0/215	72 ng/g ww	< RL	Various marine species; liver, brain, kidney and blood samples; USA, Canada and Antarctica
	Amb	1/3	7.5 ng/g ww	< RL – 20 ng/g ww	Dolphin; liver samples; Gulf of Mexico
	Amb	3/4	2 ng/ml	< RL – 4 ng/ml	Dolphin; blood samples; Mediterranean Sea
	Amb	0/31	38 ng/g ww	< RL	Dolphin and whale; liver and muscle samples; India, Mediterranean Sea, Gulf of Mexico and Atlantic Ocean

a = concentration reported as “several hundred ng/ml”

Amb = ambient environmental samples (not collected near a site of high contamination)

APME = Association of Plastic Manufacturers in Europe

dw = dry weight

geom. = geometric

LOQ = limit of quantitation

NR = not reported

RL = reporting limit, which is the detection limit in some studies and the limit of quantitation in other studies (the value for the reporting limit is included in parenthesis)

Site = samples collected near a manufacturing site or a highly contaminated site (e.g., where fire fighting foam was previously used)

ww = wet weight

2.3 Human Exposure

2.3.1 Human Biomonitoring Data

PFOA has been measured in human serum in relatively small numbers of samples from the general population in several countries throughout the world and in serum of workers occupationally exposed to APFO. Most of the biomonitoring data on PFOA have been collected in the U.S. and in Japan. In general, reported mean serum PFOA concentrations in the general U.S. population have been lower than those reported in U.S. workers exposed to PFOA. However, data recently submitted to US EPA have reported substantially higher PFOA concentrations in U.S. residents living near a facility that uses PFOA than those that have been reported thus far in the general population. Small numbers of blood samples have also been collected, and PFOA has been detected, in presumably non-occupationally exposed subjects in Canada, Columbia, Poland, Belgium, India, Korea, Sri Lanka, Japan, and Sweden. Mean concentrations in these countries are generally similar to those in the U.S., with the exception of Korea where PFOA concentrations were much higher. However, it is currently difficult to compare or interpret PFOA concentrations in human blood across the globe without nationally representative samples and standard analytical methods. The mechanisms and pathways leading to the presence of PFOA in human blood are not well characterized, although PFOA has been detected in wildlife, air, water, soil, indoor dust, sludge, and food.

2.3.2 Occupational Exposure

3M and DuPont have been the primary U.S. producers and users of perfluorinated compounds. Both companies offer voluntary medical surveillance programs to workers at plants that produce or use perfluorinated compounds. 3M discontinued manufacturing PFOA from 2000 to 2002.

2.3.2.1 3M Occupational Data

3M has been offering voluntary medical surveillance to workers at plants that produce or use perfluorinated compounds since 1976. Serum PFOA levels in 3M workers have been measured since 1993 (Olsen et al., 2003a; 2003b; 2003c; 2001a; 2001b; 2001c; 2000; 1999; 1998c). Prior to this time, analytical capabilities precluded the accurate measurement of any specific fluorochemical analyte and only total organic fluorine was measured. PFOA analysis differed slightly in each surveillance program year and different laboratories were used to assay PFOA in each year. The samples were analyzed for PFOA using high performance liquid chromatography mass spectrometry (HPLC/MS), but the extraction methods differed slightly each year (Olsen, et al., 2001f; 2003a, 2003b).

Serum PFOA concentrations for workers participating in 3M's biomonitoring program have been reported for 3 plants: Cottage Grove, Minnesota; Decatur, Alabama; and Antwerp, Belgium. The

same workers were not necessarily common to each sampling period. The highest serum PFOA concentrations have been reported in workers at 3M's Cottage Grove facility in Minnesota. The mean PFOA concentration for Cottage Grove workers in 2002 was 4.3 ug/ml (range, 0.07 – 32.6) (Olsen, et al., 2003a).

A summary of the occupational data collected at the 3M facilities are presented in Table 21.

Table 21 Summary of 3M Biomonitoring Data for Occupational Exposures (serum concentrations, µg/ml)*

Plant	Arith-metic Mean	Range	Geometric Mean	95% CI of Geometric Mean	Reference
Cottage Grove					
2002 (n=38)	4.3	0.07 - 32.6	1.7	1.02-2.72	Olsen, et al., 2003a
2000 (n = 148)	NR	NR	NR	NR	Olsen, et al., 2003f
Male (n = 131)	4.51	.007-92.03	0.85	0.64- 1.22	
Female (n = 17)	0.85	.04 - 4.73	0.42	0.23 - 0.79	
1997 (n =74)	6.4	0.1 - 81.3	NR	NR	Olsen, et al., 2000
1995 (n = 80)	6.8	0 - 114.1	NR	NR	Olsen, et al., 2000
1993 (n = 111)	5.0	0 - 80.0	NR	NR	Olsen, et al., 2000
Decatur					
2002 (n =54)	1.497	0.025-4.81	0.713	0.483-1.055	Olsen, et al., 2003b
2000 (n = 263)	1.78	0.04-12.70	1.13	0.99 - 1.30	Olsen, et al., 2001a
Male (n = 215)	1.90	0.04-12.70	NR	NR	Olsen et al., 2003e
Female (n = 48)	1.23	NR	NR	NR	Olsen et al., 2003e
1998 (n = 126)	1.54	0.02 - 6.76	0.90	0.72 -1.12	Olsen, et al., 1999
Male (n =102)	1.735	0.02 - 6.76	1.142	NR	
Female (n=24)	0.691	NR	0.326	NR	
1997 (n = 84) (males only)	1.40	NR	NR	NR	Olsen, et al., 1998c
1995 (n = 90) (males only)	1.72	NR	NR	NR	Olsen, et al., 1998c
Antwerp					
2003 (n = 30)	2.63	0.92 – 5.69	2.35	1.96 – 2.82	Olsen et al., 2003c
2000 (n = 258)	0.84	0.01 – 7.04	0.33	0.27 – 0.40	Olsen, et al., 2001c
1995 (n = 93)	1.13	0.00 – 13.2	*	*	

* µg/ml = ppm NR not reported

2.3.2.2 DuPont Occupational Data

Dupont has been measuring PFOA in workers' blood serum since 1981 (Dupont, 2001a, 2001b). Prior to 1981, total blood fluoride levels were reported. All of Dupont's operations in the U.S. that use PFOA with significant exposure potential are concentrated at the Washington Works plant in Washington, West Virginia. PFOA is used as a fluoropolymer reaction aid at this plant. The data

presented in Table 22 reflect serum concentrations of volunteer workers in the plant who had potential PFOA exposure. With the exception of the 2000 sampling period, the same workers were common to all of the other sampling periods, with additional workers participating in 1995. The data available at this time are limited to what is presented here.

Table 22 Summary of DuPont Occupational PFOA Biomonitoring (serum concentrations, µg/ml)*

Year	Arithmetic Mean	Range	Reference
Washington Works			
2004 (n = 259)	0.494[a]	0.0174 - 9.55	DuPont, 2004
2000 (n= 72)	1.53	0.02 - 9.0	DuPont, 2001a
1995 (n = 80)	1.56	0.12 - 4.5	DuPpont, 2001b
1989-90 (n =22)	1.96	0.06 - 11	DuPont, 2001b
1985 (n = 22)	2.34	0.06 - 18	DuPont, 2001b
1984 (n = 19)	3.21	0.07 - 24	DuPont, 2001b

* µg/ml = ppm [a] median (Teflon workers only)

2.3.3 General Population Exposures

Limited data on PFOA concentrations in the general population of various countries throughout the world are currently available. Most of the data presented here are the first to be collected on human populations, and most of the sample sizes are fairly small. Data on both pooled and individual samples are available. PFOA has been detected in human blood (plasma, serum and whole blood), liver, seminal plasma, and breast milk. Based on the data currently available, the ranges of PFOA concentrations indicate that the interindividual variability can be quite large in some populations and that human serum concentrations can vary between countries, as well. In addition, preliminary US reports indicate that individuals living near a facility that uses PFOA may have much higher PFOA serum concentrations than the levels previously reported for US populations (Emmett et al., 2006).

Very little data on trends in human populations are currently available. Blood samples collected in western Maryland in 1974 and 1989 (n = 178 for each time period) indicate that median PFOA concentrations have more than doubled over this time period (Olsen, et al., 2005). However, other samples from different study participants (n=108) collected in the same region in 2001 did not indicate that the geometric mean was statistically significantly different between 1989 and 2001. On the other hand, a recent report has shown that PFOA serum concentrations in Japanese residents have steadily increased over the past 25 years (Harada, et al., 2004).

Pooled Serum Data in the General Population

Pooled serum PFOA data from US residents have been reported for 1998, 1999, 1990-1998, 2002 and 2001-2002. Reported means and ranges for pooled blood samples collected in the US in 1998 (n = 18 lots, 340-680 donors) and 1999 (n = 35 lots) were 3 and 17 ppb (µg/l), respectively, with ranges of 1-13 ppb and 12-22 ppb (3M 1999a, 1999b).

In another study, 23 archived serum pools consisting of samples from various US locations were analyzed for PFOA and other perfluorinated compounds. The samples were collected from 1990-1998 and in 2002 (Calafat, et al., 2005). Fifteen pools were prepared from the 1990-1998 pools and were comprised of serum from 40 – 200 residents of the southeastern US. One of the 2002 pools

was comprised of serum from 15 Louisville, KY residents; another 2002 pool was from 15 residents of Philadelphia, Memphis, and Miami. Six serum pools of samples collected from 1999-2001 were each made up of serum of 6-8 Seattle residents. In 2003, blood samples were also collected from 44 residents of Trujillo, Peru (25 men, 19 pregnant women) and analyzed for perfluorinated chemicals. PFOA was detected in 100% of the US samples, while it was only detected in 25% of the samples collected in Peru. In the US pooled samples, the geometric mean of PFOA was 9.6 µg/l (range 2.8 – 23.7) (median 11.6). When the samples were analyzed by geographic region, the mean PFOA concentration was 5.8 µg/l (s.d. 0.6) for the Seattle samples and 14.1 µg/l (s.d. 7.6) for the pooled samples collected in the eastern U.S. The 95th percentile serum concentration was 0.3 µg/l for the samples collected in Peru.

A recent study comprised of 54 pooled serum samples collected from 1833 participants 12 years and older of the 2001-2002 NHANES showed mean PFOA serum concentrations highest for non-Hispanic whites, followed by non-Hispanic blacks, and Mexican Americans. Females had lower mean PFOA concentrations than males, and adolescents aged 12-19 had higher concentrations than adults 20 years of age and older (Calafat AM, 2006).

Individual Serum Data in the General Population

Results of analyses on individual human biological samples have been reported in the literature for several countries. With the exception of 3 studies 3M conducted on fairly large cohorts in the U.S., but that are now 5-10 years old, most of the sample sizes are small. However, based on these data which are available thus far, it is indicated that there are geographic differences both between and within countries in exposure to PFOA.

Data currently available on worldwide PFOA concentrations in various biological matrices in individual samples in human populations are presented in Table 23. The concentrations reported for whole blood have been converted to serum concentrations by doubling the whole blood levels (Kannan, et al., 2004). Based on a recent analysis of 12 production workers occupationally exposed to PFOA, plasma and serum concentrations were very similar in each individual but were approximately 51% higher than whole blood concentrations; therefore, using a factor of 2 to estimate serum concentrations from whole blood seems reasonable (Ehresman et al., 2005a). In addition, analytical laboratory techniques are not reflected in Table 23, and various methods can be used to analyze perfluorochemicals. A recent interlaboratory study on perfluorinated compounds has concluded that the overall concordance between laboratories for PFOA analysis is “satisfactory”; however, the variability for PFOA analysis approximated as much as 50% (Van Leeuwen, et al., 2005). Details of the three largest biomonitoring studies on perfluorinated chemicals are provided following Table 23.

Table 23 Summary of PFOA Individual Biomonitoring Data in Various Countries (serum concentrations, ng/ml)*

Location	Age	Matrix	Year of Collection	N	Average[1,2,3]	Range	Reference
American Red Cross Blood Banks(6 US locations)	adults (20-69)	serum	2000	645	5.6[1]	1.9 – 52.3	Olsen et al., 2003
Seattle, WA	elderly (65-96)	serum	2000	238	4.2[2]	1.4 – 16.7	Olsen et al., 2004
23 US states	children (2-12)	serum	1995	598	5.6[1]	1.9 – 56.1	Olsen et al., 2004

Location	Age	Matrix	Year of Collection	N	Average[1,2,3]	Range	Reference
Central Michigan	female adults male adults	serum	2000	46 29	4.7[1] 5.7[1]	<3 - 7.3 <3 – 14.7	Kannan et al., 2004
Murray, KY	female adults male adults	whole blood[4]	2002	11 19	23[1] 41.6[1]	15 - 39 11 - 88	Kannan et al., 2004
New York, NY	unknown	plasma	2002	70	27.5[1]	14 - 56	Kannan et al., 2004
Atlanta, GA	adults	serum	2003	20	4.89[1]	0.2 – 10.4	Kuklenyik et al., 2004
Samples from biological supply company (US)	unknown	serum	unknown	65	6.4	<5 - 35.2	Hansen et al., 2001
Sera and liver tissue from IAM	5 – 74 years	serum liver	NR	23 pairs	-- 90% < LLOQ	<0.0030 - 0.0070 <0. – 0.0147	Olsen et al., 2003
Hagerstown, MD	adults	serum plasma	1974 1989	178 178	2.1[2] 5.5[2]	1.9 - 2.25 5.2 - 5.95	Olsen et al., 2005
Ottawa, Ontario, Gatineau, Quebec	adults	serum	2002	56	3.4[3]	<1.2 – 7.2	Kubwabo et al., 2004
Cartagena, Columbia	female adults male adults	whole blood	2003	25 31	6.1 6.2	3.7 – 9.2 3.9 – 12.2	Kannan et al., 2004
Gdansk, Poland	female adults male adults	whole blood	2003	15 10	21.9 20.5	9.7 - 34 11 – 40	Kannan et al., 2004
Flanders, Wallonia, Belgium	female adults male adults	plasma	1998, 2000	4 16	4.1 5.0	< 1 – 7.6 1.1 – 13	Kannan et al., 2004
Coimbatore, India	female adults male adults	serum	2000	11 34	< 3 (0% detected) 3.5 (3%)	< 3 <3 – 3.5	Kannan et al., 2004
Daegu, Korea	female adults male adults	whole blood	2003	25 25	88.1 (19%) 35.5 (25%)	15 – 256 < 15 – 71.4	Kannan et al., 2004
Colombo, Talawakele, Haldummulla Sri Lanka	male adults	serum seminal plasma	2003	30 0.323	6.38 <0.072 – 0.621	0.23 – 23.5	Guruge, et al., 2005
Kyoto, Japan	male adults female adults	serum	2004	20 20	12.96-12.41 7.89-12.63	5.2 – 15.8 7.6 - 20.6	Harada, et al., 2005

Location	Age	Matrix	Year of Collection	N	Average[1,2,3]	Range	Reference
Kyoto, Japan	female adults male adults	serum	2003	20 14	7.1[2] 12.4[2]	2.8 – 12.4	Harada, et al., 2004
Miyagi, Japan	female adults male adults female adults	serum serum serum	2003 2003 1977	23 32 39	2.8[2] 3.3[2] 0.2[2]	NR NR NR	Harada, et al., 2004
Yokohama, Tsukuba Japan	female adults male adults	serum	2002	13 25	12.3 (8%) <6.8 (0%)	< 6.8 – 12.3 < 6.8	Kannan et al., 2004
Akita, Japan	female adults male adults female adults female adults male adults	serum serum serum serum serum	2003 2003 1995 1991 1991	50 66 40 60 16	2.5[2] 3.4[2] 1.9[2] 1.8[2] 2.2[2]	NR NR NR NR NR	Harada, et al., 2004
Kyoto, Japan 10 locations Female adults male adults	Adults	serum	1983 1999 2003	100 200	2.2[2] 9.7[2] 3.5[2] 4.2[2]	0.4 – 52.2	Saito, et al., 2005
Sweden	female adults	serum	NR	108	2.0[1]	NR (SD = 0.8)	Holmstrom et al., 2005
Zhoushan, China	female adults	breast milk	2004	19	NR	47 – 210[5]	So et al., 2006b
Germany, environmental specimen bank	male, female 20-29	plasma	2004	16	6.5	4.1-33.2	UBA, 2004

* ng/ml = ppb; NR – not reported
1 Mean
2 Geometric Mean
3 16 samples above LOQ
4 whole blood results are estimates of serum concentrations
5 ng/L (ppt)

Individual blood samples from 3 different age populations were analyzed for PFOA and other fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMS) (Olsen et al., 2002a, 2002b, 2002c). The studies' participants included adult blood donors, an elderly population participating in a prospective study in Seattle, WA, and children from 23 states participating in a clinical trial. Overall, the PFOA geometric means were similar across all 3 populations (4.6, 4.2 , and 4.9 ng/ml, respectively). The geometric means and 95% tolerance lim-

its (the exposure below which 95% of the population is expected to be found) and their upper bounds were comparable across all 3 studies. However, the upper ranges for the children and adults were much higher than for the elderly population. It is not clear whether this is the result of geographic differences in PFOA levels or some other factor. It should be noted that PFOS and PFOA were highly correlated in all three studies ($r = .63$, $r = .70$, and $r = .75$) and that PFOA did not meet the criteria for a log normal distribution based on the Shapiro-Wilk test in any of the studies. The authors suggest that it may be due to the greater proportion of subjects with values less than the lower limit of quantitation (LLOQ); however, only 12 of the 1481 total samples were below the LLOQ. In those instances where a sample was measured below the LLOQ, the midpoint between zero and the LLOQ was used for calculation of the geometric mean. The details of each study are provided below.

Blood samples from 645 U.S. adult blood donors (332 males, 313 females), ages 20-69, were obtained from six American Red Cross blood banks located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD (Olsen et al., 2002a). Each blood bank was requested to provide approximately 10 samples per 10-year age intervals (20-29, 30-39, etc.) for each sex. The only demographic factors known for each donor were age, gender, and location.

The geometric mean serum PFOA level was 4.6 ng/ml. The range was <lower limit of quantitation (1.9 ng/ml) to 52.3 ng/ml. Only 2 samples were less than the LLOQ. Males had significantly higher ($p < .05$) geometric mean PFOA levels than females (4.9 ng/ml vs. 4.2 ng/ml). Age was not an important predictor of adult serum fluorochemical concentrations. When stratified by geographic location, the highest geometric mean for PFOA was in the samples from Charlotte, NC (6.3 ng/ml, range: 2.1 – 2.9 ng/ml) and the lowest from Portland (3.6 ng/ml, range: 2.1 – 16.7 ng/ml). The highest individual value was reported in Hagerstown (52.3 ng/ml).

Serum PFOA levels were reported for 238 (118 males and 120 females) elderly volunteers in Seatle participating in a study designed to examine cognitive function in adults aged 65-96 (Olsen et al., 2002b, 2004a). Age, gender and number of years' residence in Seattle were the only data available on the participants. Most of the participants were under the age of 85 and had lived in the Seattle area for over 50 years.

The geometric mean of PFOA for all samples was 4.2 ng/ml. The range was 1.4 – 16.7 ng/ml. Only 5 samples were less than the LLOQ of 1.4 ng/ml. There was no significant ($p < .05$) difference in geometric means for males and females. In simple linear regression analyses, age was negatively ($p < .05$) associated with PFOA in elderly men and women. PFOS and PFOA were highly correlated ($r = .75$) in this study.

A sample of 598 children, ages 2-12 years old, participating in a study of group A streptococcal infections, was analyzed for serum PFOA levels (Olsen et al., 2002c, 2004b). The samples were collected in 1994-1995 from children residing in 23 states and the District of Columbia. PFOA did not meet the criteria for a log normal distribution based on the Shapiro-Wilk test. The authors suggest that it may be due to the greater proportion of subjects with values < LLOQ for PFOA; however, only 5 samples were less than the LLOQ of 1.9 ng/ml. The geometric mean of PFOA for all of the participants was 0.0049 ng/ml, and the range was 1.9 to 56.1 ng/ml. Male children had significantly ($p<.01$) higher geometric mean serum PFOA levels than females: 5.2 ng/ml and 4.7 ng/ml, respectively. In simple linear regression analyses, age was significantly ($p < .05$) negatively associated with PFOA in both males and females. When stratified by age, the geometric mean of PFOA was highest at age 4 (5.7 ng/ml) and lowest at age 12 (3.5 ng/ml). Although the data were not reported, a graphical presentation of log PFOA levels for each state by gender looked similar across the states. However, it is difficult to interpret these data without analyzing them and the sample sizes were limited for each gender/location subgroup. PFOS and PFOA were highly correlated ($r = .70$) in this

study. PFOA and PFHS (perfluorohexanesulfonate) were also correlated, although not as strongly (r = .48).

The above 3 studies indicate similar geometric means and ranges of PFOA among sampled adults, children, and an elderly population. However, an unexpected finding was the level of PFHS and M570 (N-methyl perfluorooctanesulfonamidoacetate) in children (Olsen, 2002c, 2004b). These serum levels were much higher in the sampled children than in the sampled adults or elderly. It is not clear why this occurred, but it is probably due to a different exposure pattern in children.

In another study, the PFOA concentration was analyzed in human sera and liver samples using HPLC/ESMS (Olsen et al., 2001d). Thirty-one donor samples were obtained from 16 males and 15 females. The average age of the male donors was 50 years (SD 15.6, range 5-69) and the average age of the female donors was 45 years (SD 18.5, range 13-74). The causes of death were intracranial hemorrhage (n = 16 or 52%), motor vehicle accident (n = 7 or 23%), head trauma (n = 4 or 13%), brain tumor (n = 2 or 6%), drug overdose (n = 1 or 3%) and respiratory arrest (n = 1 or 3%). Both serum and liver tissue were obtained from 23 donors; 7 donors contributed liver tissue only and 1 donor contributed serum only. Resulting serum values for PFOA ranged from < LOQ (<3.0 - 7.0 ng/ml). Assuming the midpoint value between zero and LOQ serum value for samples <LOQ, the mean serum PFOA level was calculated as 0.0031 ng/ml with a geometric mean of 0.0025 ng/ml. No liver to serum ratios were provided because more than 90% of the individual liver samples were <LOQ.

Conclusions

Data are available on PFOA blood serum levels in both occupationally- and non-occupationally-exposed populations. Although the sample sizes have been relatively small, and it is currently difficult to compare or interpret PFOA concentrations without nationally representative samples and standard analytical methods, PFOA has been detected in samples of human blood (plasma, serum and whole blood), liver, seminal plasma, and breast milk from several countries throughout the world, including the US, Canada, Columbia, Poland, Belgium, India, Korea, Sri Lanka, Japan, Sweden, and Germany. Mean blood serum concentrations in most of these countries seem to be generally similar to those in the US, where most of the human biomonitoring data have been collected thus far. However, preliminary US reports indicate that individuals living near a facility that uses PFOA have much higher PFOA serum concentrations than the levels previously reported for US populations.

2.3.4 Consumer Exposure

In a collaboration between The Norwegian, Swedish and Danish Societies for Nature Conservation, textile samples were taken from a range of all-weather jackets for children in the Nordic Market. Alltogether 6 jackets from 5 different brands were collected from shops or warehouses in Sweden and Norway. The jackets investigated came from Norwegian, Swedish and foreign manufacturers. Common to the textile was that they were so-called breathing synthetic textiles, with the exception of one cotton anorak material. Samples (10 x 10 cm; 1.04-1.91 g) were extracted with ethyl acetate and methanol and analysed via GC/MS and HPLC/MS. PFOA was detected in concentrations ranging from 0.80 to 24.6 µg/m² and 6.1 to 107 ng/g. According to the analysis results the cotton anorak contained 127 µg/m² and 416 ng/g PFOA (SNF 2006).

According to Kuklenyik et al. (2004) PFOA is used primarily in the production of fluoropolymers, such as poly(tetrafluoroethylene) (PTFE) and poly(vinylidine fluoride), and fluoroelastomers. These polymers have numerous uses in many industrial and consumer products, including soil-, stain-, grease-, and water-resistant coatings on textiles and carpet, personal care products, and nonstick

coatings on cookware, and uses in the automotive, mechanical, aerospace, chemical, electrical, medical, and building and construction industries. Consumer exposure to PFOA due to impurities in the finished product can not be excluded. Washburn et al. (2005) investigated the potential exposure towards PFO(A) during consumer use of certain articles. Certain consumer articles are manufactured with either fluorotelomers or fluoropolymers. Fluorotelomer-based products are typically manufactured through telomerization of tetrafluoroethylene with perfluoroethyl iodide. Trace amounts of PFO may be generated during this process as an unintended by-product. Fluorotelomer-based products are used to treat a variety of consumer articles (e.g., apparel and carpeting), primarily to impart stain and soil resistance. Fluoropolymers are films (e.g., on nonstick cookware) or membranes (e.g., in outerwear). The ammonium perfluorooctanoate (APFO) is an essential processing aid in the formulation of such fluoropolymers; residual PFO(A) may be present in fluoropolymer films and membranes used in manufacturing certain consumer articles. The authors analysed (LC/MS/MS) fluorotelomer-based product formulations and finished consumer articles manufactured with fluoropolymers or fluorotelomer-based products for the occurrence of PFOA. The analytical test results are summarized in Table 24. As the analytical test results are based on the extraction of new articles the values obtained are representing worst case situations. Although PFO(A) was not detected in the fluorotelomer-based product applied to the upholstery PFO(A) was detected in extraction tests on treated upholstery. According to the opinion of the authors this may result from unrelated background sources of PFO(A) on upholstery. A simple one-compartment model was developed to estimate contributions of potential consumer exposures to PFO concentrations in serum. Based on a simple compartmental model estimation, the hypothetical aggregate exposures to the consumer articles investigated correspond to serum concentrations ranging from approximately 0.05 to 0.25 ppb for the residential adolescent and adult and from about 0.05 to 0.25 ppb for the professional and therefore less than the current quantification limit for PFO(A) in serum (ca.0.5 ppb). In adults of the general U.S. population the geometric mean of PFO(A) is reported to be ca. 5 ppb. Based on this estimation an explanation for the PFO(A) concentrations found in humans can not be given.

Table 24 PFOA concentration in fluorotelomer and fluoropolymer articles

Articles	Concentration in Fluorotelomer product (mg PFO(A)/l)	Calculated total concentration in finished article (mg PFO(A)/kg)	Extraction of finished article (ng PFO(A)/cm2 article)*
Fluorotelomer-Containing Articles			
mill-treated carpeting (n>60)	30-80	0.2-0.6	<0.2 (l.o.d.) to 23
carpet-care solution-treated carpeting (n=14)	1-50	0.2-2	28-50
treated apparel (n >100)	<1 (l.o.d.) to 40	<0.02 (l.o.d.) to 1.4	<0.01 (l.o.d.) to 12
treated upholstery (n=3)	<1 (l.o.d.)	<0.034 (l.o.d.)	0.4-4
treated home textiles	<1(l.o.d.)) to 40	<0.02(l.o.d.) to 1.4	Not tested
treated technical textiles	<1(l.o.d.)	<0.034(l.o.d.)	Not tested
treated nonwoven medical garments (n=6)	<1(l.o.d.)	<0.034(l.o.d.)	<0.02(l.o.d.)
stone, tile, and wood sealants	<1(l.o.d.)	<0.1(l.o.d.)	Not tested
industrial floor waxes and wax removers	5-120	0.0005-0.06	Not tested

Articles	Concentration in Fluorotelomer product (mg PFO(A)/l)	Calculated total concentration in finished article (mg PFO(A)/kg)	Extraction of finished article (ng PFO(A)/cm2 article)*
latex paint	50-150	0.02-0.08	Not tested
home and office cleaners	50-150	0.005-0.05	Not tested
Fluoropolymer-Containing Articles			
membranes for apparel (n=20)	Not applicable	Not applicable	0.008-0.07
non-stick cookware (n=40)**	Not applicable	Not applicable	<0.1(l.o.d.)
thread seal tape (n=20)	Not applicable	Not applicable	0.02-0.08

* simulation of human exposure (extractants: water, saliva, perspiration)

** manufacture of nonstick cookware includes a high temperature step which reduces residual PFO(A) prior to article use by consumers

According to a study performed in contract for a journal for consumer protection (without peer review process), PFOA was detected in all 5 jackets tested at concentrations ranging from 0.08 to 0.60 mg/kg. In 2 out of 3 impregnation sprays 0.24 and 0.48 mg/kg PFOA were detected (Öko-Test 2005). Samples were taken from the outside of jackets, extracted with methanol and analysed using HPLC-MS/MS negative uib electrospray (Eurofins, 2005).

Larsen et al. (2005) determined perfluorooctanoic acid in polytetrafluoroethylene polymers. In one polymer which was an intermediate of a commercial lot a PFOA concentration of 1040 ppb was detected, in the other polymer obtained from a commercial lot the corresponding PFOA concentration was 59 ppb. Based on the results the authors concluded that the finishing step significantly reduced PFOA concentration in the commercial polymer.

Powley et al. (2005a) developed an analytical method for the determination of PFOA released from the surface of commercial cookware. The cookware was extracted with water and water/ethanol mixtures at 100 and 125°C and the extracts analyzed via LC/MS/MS. PFOA could not be detected in any of the samples at a detection limit of 100 pg/cm^{-2}.

Powley et al. (2005b) examined fry pans purchased from retail for extractable PFOA. The pans were heated with water for 30 minutes. The water was cooled and analysed via HPLC/MS/MS. Further more fry pans were cut into pieces and placed in a pressurized fluid extraction apparatus and extracted with etanol/water (1:9 and 19:1) to simulate watery and acidic foods and fatty or oily foods. None of the fluoropolymer-treated pans showed any detectable levels of PFOA.

Ellis et al. (2001) investigated the thermal decomposition of fluoropolymers at 360 and 500°C. The thermolysis of PTFE produced perfluoroalkyl acids with C atoms ranging from 3-14. The amount of PFOA resulting from the thermolysis of the fluoropolymer is not stated; the sum of PFCs (C3-C14) is given to >0.01%. PFOA was not detected as thermal degradation product of other fluoropolymers (CPTFE: chloropolytrifluoroethylene, ECTFE: ethylene-chlorotrifluoro-ethylen, PFEPE).

Boulanger et al. (2005) analysed a 1994 Scotchgard sample (0.832 g was sprayed into a HDPE bottle, dissolved in methanol and analyzed using HPLC/MS/MS). The PFOA concentration found corresponded to 1.34 x 10^{-3}% (=82% of perfluorinated compounds). The authors assume that the presence of perfluorinated compounds (e.g. PFOA) was the result of residual monomers in the formula.

Based on the analysis of human blood sera and seminal plasma collected from volunteers in Sri Lanka Guruge et al. (2005) concluded that the greater exposure levels found in Colombo are indica-

tive of sources from various industries and frequent use of products containing PFCs such as paper, packing products, carpet spray, stain-resistent textiles, cosmetics, electronics and fire fighting foams.

In the frame of a Multi-City Study, a market basket sampling was conducted. In a total over 200 samples taken from green beans, apples, pork muscle, cow milk, chicken muscle, chicken eggs, bread, hot dogs, catfish, and ground beef were analysed for PFOA. Measurable quantities of PFOA, ranging up to 2.35 ng/g, were found in two ground beef samples, two bread samples, two apple samples, and one green bean sample (3M 2001c)

Begley et al. (2005) analysed PTFE cookware for PFOA content. Food cooked using PTFE cookware was also analysed for PFOA. Results of migration tests show mg/kg amounts of perfluoro paper additives/coatings transfer to food oil. Analysis of PTFE cookware shows residual amounts of PFOA in the low μg/kg range. PFOA was detected in microwave popcorn bag paper in concentrations up to 300 μg/kg.

Kubwabo et al. (2005) investigated house dust samples for the occurrence of perfluoro chemicals (PFOS, PFOA, perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHS) and perfluorooctane sulfonamide (PFOSA). The data revealed a correlation between the concentrations of PFCs and the percentage of carpeting in the house; older houses tended to have less carpeting, hence lower levels of these perfluorinated compounds in their dust.

3 HUMAN HEALTH HAZARDS

3.1 Metabolism and Pharmacokinetics

3.1.1 Metabolism and Pharmacokinetic Studies in Humans

Little is known about the metabolism and pharmacokinetics of PFOA in humans. One report notes the presence of PFOA in the cord blood of some pregnant workers suggesting that PFOA can cross the placenta (U.S. EPA, 2003). However, PFOA was not detected in cord blood in a study of 15 pairs of maternal and cord blood samples in non-occupationally exposed women and infants in Japan (Inoue et al., 2004). PFOA has also been detected in seminal plasma in tea workers in Sri Lanka (Guruge et al., 2005) and in very low concentrations in breast milk in a small sample of women in China (So et al., 2005).

There are limited data on the half-life of PFOA in humans. With the exception of a 1980 study in which total organic fluorine in blood serum was measured in one worker (Ubel et al., 1980), no other data were available until June 2000. 3M has conducted a half-life study on 26 retired fluorochemical production workers from their Decatur, Alabama (n = 24) and Cottage Grove, Minnesota (n = 3) plants in which serum samples were drawn every 6 months over a 5-year period. Two interim reports describing the results thus far were submitted to EPA (Burris et al., 2000; Burris et al., 2002). Final results have not yet been published, however, preliminary data have been presented which indicate that 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). Some of the details of this study are provided below.

Blood was collected from the subjects between 1998 and 2004, depending on the facility at which the subject had worked. Responses on questionnaires determined whether any of the retirees had any occupational exposures after retirement. One subject was excluded from the analysis based on his response. The average number of years that this sample 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 these 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. 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. (2005) 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. (2005) 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.

3.1.2 Metabolism and Pharmacokinetic Studies in Non-Human Primates

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 APFO daily via oral capsule at dose rates of 0, 3, 10, or 20 mg/kg (30 mg/kg for 12 days, reduced to 20 mg/kg when dosing reinitiated on test day 22) for six months. 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 weeks in all dose groups. Steady-state concentrations of PFOA in serum were 81 ± 40, 99 ± 50, and 156 ± 103 µg/ml for the 3, 10, and 20 mg/kg dose groups, respectively. The mean serum concentration of PFOA in control monkeys was 0.134 ± 0.113 µg/ml. 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 10 mg/kg recovery monkeys, serum PFOA elimination half-life was 19.5 (R^2=0.98) days and indicated first-order elimination kinetics. For three 20 mg/kg monkeys, serum PFOA elimination half-life was 20.8 days (R^2=0.82) and also indicated first-order elimination kinetics. Urine PFOA concentrations reached steady-state after 4 weeks at 53 ± 25, 166 ± 83, and 181 ± 100 µg/ml in the 3, 10, and 20 mg/kg dose groups, respectively. Within two weeks of recovery, urine PFOA concentrations were <1% of the previous value measured during treatment and decreased slowly thereafter. Fecal PFOA concentrations were 6.8 ± 5.3, 28 ± 20, and 50 ± 33 µg/ml 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. 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 the two 20 mg/kg monkeys were 16.0 and 83.3 µg/g. Liver PFOA concentrations in two 10 mg/kg-day monkeys at the end of the recovery period were 0.08 and 0.15 µg/g.

Butenhoff et al. (2004b) also conducted an intravenous pharmacokinetic study in cynomolgus monkeys. Three male and three female monkeys were administered a single iv dose at 10 mg/kg of potassium PFOA. Blood samples were collected prior to dose administration; at 0.5, 2, 4, 8, and 24 hr; and on Days 2, 4, 7, 11, 14, 21, 28, 57, 79, 87, and 123 post-dose. Urine was collected in metabo-

lism cages for 24-hr intervals on the following days: prior to dose administration, Day 1 (0-24 hr post-dose), and on Days 2, 7, 14, 21, and 28. Serum PFOA concentrations at 0.5 hr post-dose ranged from 91 to 97 µg/ml in the male monkeys and from 89 to 96 µg/ml in the females. At 48 hr, serum PFOA concentrations were between 49 and 66 µg/ml in the males and 40 and 62 µg/ml in the females. After 48 hours, serum PFOA concentrations in two of the three males decreased at a faster rate than that observed in the third male monkey and the three female monkeys. On day 28, serum PFOA concentrations ranged from 1.9 to 27 µg/ml in the male monkeys and from 7.1 to 34 µg/ml in the females. Substantial amounts of PFOA were excreted in the urine, ranging from 3 to 19 % of the dose on Day 1 and from 1.3 to 14.1 % on Day 7. Urinary excretion of PFOA was prolonged, evidenced by 0.1 to 1 % of the dose in urine on Day 28. Pharmacokinetic parameters were calculated from serum PFOA concentrations using a non-compartmental analysis (Table 25). AUC0-infinity values ranged from 571 to 2501 µg/•day/ml (mean: 1235 µg/•day/ml) for male monkeys and from 1094 to 3224 µg/•day/ml (mean: 2293 µg/•day/ml) for female monkeys. The terminal half-lives of PFOA in serum were 13.6, 13.7, and 35.3 days in the three male monkeys and 26.8, 29.3, and 41.7 days in the three females. The total body clearances were 4.0, 15.8, and 17.5 ml/day/kg for males and 3.1, 3.9, and 9.1 ml/day/kg for females. Volumes of distribution of PFOA at steady state were 168, 184, and 192 ml/kg in males and 133, 190, and 270 ml/kg in females, suggesting distribution primarily in the extracellular space.

Table 25 Pharmacokinetic Parameters Calculated from Serum Concentrations of PFOA in Cynomolgus Monkeys

Parameter	Males					Females				
	2052	2054	2211	Mean	S.D.	2058	2059	2061	Mean	S.D.
Cmax (µg/ml)[a]	101	98.4	91.6	97.0	4.9	105	93.0	103	100	6.0
$T_{½}$ (day)[b]	35.3	13.7	13.6	20.9	12.5	26.8	41.7	29.3	32.6	8.0
$AUC_{0\text{-last}}$ (µg•day/ml)[c]	1999	569	633	1067	808	3083	2314	1056	2151	1023
$AUC_{0\text{-infinity}}$ (µg•day/ml)[d]	2501	571	634	1235	1097	3224	2560	1094	2293	1090
Cl (ml/day/kg)[e]	4.0	17.5	15.8	12.4	7.7	3.1	3.9	9.1	5.4	3.3
Vd_{ss} (ml/kg)[f]	192	168	184	181	12	133	190	270	198	69

(from Butenhoff et al., 2004b)

a Maximum concentration in serum
b Half-life of the terminal elimination phase
c Area under the serum concentration versus time curve calculated from 0 to the last time point
d Area under the serum concentration versus time curve calculated from 0 to infinity
e Total body clearance
f Volume of distribution at steady state

3.1.3 Metabolism and Pharmacokinetic Studies in Adult Rats

3.1.3.1 Absorption Studies

Studies in rats have shown that PFOA is absorbed following oral, inhalation, and dermal exposures.

3.1.3.1.1 Oral Exposure

Gibson and Johnson (1979) administered a single dose of ^{14}C-APFO 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 the total carbon-14 was absorbed; the elimination half-life of carbon-14 from the plasma was 4.8 days.

Ophaug and Singer (1980) administered 2 ml of an aqueous solution of 2 mg PFOA to female Holtzman rats. Ionic fluoride, nonionic fluorine, and total fluorine were measured. Within 4.5 hr, 37% of the total fluorine in the administered dose was recovered in the urine. The quantity of nonionic fluorine recovered in the urine increased to 61%, 76% and 89% at 8, 24 and 96 hr, respectively, after administration. Within 4.5 hr, serum from treated rats had a nonionic fluorine level of 13.6 ppm. The nonionic fluorine level in the serum decreased to 11.2 ppm at 8 hr, 0.35 ppm at 24 hr, and 0.08 ppm at 96 hr. Despite the large decrease in nonionic fluorine in the serum, the ionic fluoride level was only 0.03 ppm and remained at that level throughout the experiment. Prior to administration of PFOA, the ionic and nonionic fluorine background levels in serum were 0.032 and 0.07 ppm, respectively. The authors concluded that PFOA is rapidly absorbed from the gastrointestinal tract and then rapidly cleared from the serum.

3.1.3.1.2 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 pre-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 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, while the male Cmax occurred from the end of the exposure period up to six hours after exposure. 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 dose study, Hinderliter (2003) exposed male and female rats (5/sex/group) to the same aerosol concentrations of PFOA for 6 hrs/day, 5 days/week for 3 weeks. Blood was collected immediately before and after the daily exposure period three days per week. The aerosol sizes were 1.3 - 1.9 µm MMAD 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 carryover in males. Male rats apparently reached steady state plasma levels by three weeks with plasma concentrations of 8, 21, and 36 µg/ml for the 1, 10

and 25 mg/m3 groups, respectively. In females, the post-exposure plasma levels were 1, 2, and 4 μg/ml for the 1, 10, and 25 mg/m3 groups, respectively. When measured immediately before the daily exposure, plasma levels had returned to baseline in females.

3.1.3.1.3 Dermal Exposure

No specific dermal absorption studies have been conducted in rats. However, Kennedy (1985) treated rats dermally with a total of 10 applications of APFO 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.

The results of in-vitro percutaneous absorption studies of APFO through rat and human skin in vitro have been reported (Fasano et al, 2005). The permeability coefficient for APFO 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.

3.1.3.2 Serum Pharmacokinetic Parameters in Adult Rats

Serum pharmacokinetic parameters of PFOA have been evaluated in adult Sprague-Dawley rats following gavage administration, and in Wistar rats following i.v. administration.

3.1.3.2.1 Oral and Intravenous Exposure in Sprague-Dawley Rats

Kemper (2003) examined the plasma concentration profile of PFOA following gavage and intravenous 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, and intravenously at a dose rate of 1 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 26 and Table 27). Comparison of AUC for the oral and intravenous 1 mg/kg doses indicated that oral bioavailability of PFOA was approximately 100%. Plasma elimination curves were linear with respect to time in male rats at all dose levels, while elimination kinetics were biphasic in females at the 5 mg/kg and 25 mg/kg dose levels. In males, plasma elimination half-lives were independent of dose level and ranged from approximately 138 hours to 202 hours. In females, terminal elimination half-lives ranged from approximately 2.8 hours at the lowest dose to approximately 16 hours at the high dose. To further characterize plasma elimination kinetics, particularly in male rats, 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 (24 hours and 2016 hours in females and males, respectively). Estimated plasma elimination half-lives in this experiment were approximately 277 hours in males and 3.4 hours in females (Table 26, Table 27).

Table 26 Pharmacokinetic Parameters in Male Sprague-Dawley Rats Following Administration of PFOA by Gavage (Mean (SD))

Parameter	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)

Table 27 Pharmacokinetic Parameters in Female Sprague-Dawley Rats, Following Administration of PFOA by Gavage (Mean (SD))

Parameter	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)

3.1.3.2.2 Intravenous Exposure in Wistar Rats

Kudo et al. (2002) examined the plasma concentration profile of PFOA following i.v. administration in 9-week old Wistar rats. Male and female rats were injected intravenously with 48.63 μmol/kg body weight PFOA, and blood samples were collected at 5 min., 1, 6, 12 hrs, and 1, 2, 3, 4, 6, 8, 10 and 12 days after injection. A two-compartment open model was used to estimate serum pharmacokinetic parameters (Table 28). The half-life of PFOA in male rats was found to be 70 times longer (5.7 days versus 1.9 hours) than in females. In female rats, there appears to be biphasic elimination of PFOA; the fast phase occurs with a half-life of approximately 1.9 hours while the slow phase occurs with a half-life of approximately 24 hours. The total clearance of PFOA in female rats was 44 times higher than that in male rats.

Table 28 Pharmacokinetic Parameters of PFOA in Wistar Rats after i.v. Administration

Parameters	Male	Female
Total clearance (ml/min per kg)	0.035 ± 0.010	1.551 ± 0.559*
$T_{1/2}$ (day)	5.68 ± 0.99	0.08 ± 0.03**
Volume of distribution (ml/kg)	345.6 ± 57.3	211.2 ± 28.2*

(from Kudo et al., 2002)

Values are means ± S.D. for three to four rats. Differences were statistically significant between male and female rats (*, P<0.01: **, P<0.001).

3.1.3.3 Distribution Studies in Adult Rats

The distribution of PFOA has been examined in tissues of adult rats following administration by gavage and by i.v. and intraperitoneal (i.p.) injection. PFOA distributes primarily to the liver, plasma, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. The distribution of PFOA is predominantly extracellular.

3.1.3.3.1 Oral Exposure

Kemper (2003) examined the distribution and clearance 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 (Tmax) and at the time 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. $T_{max/2}$ was calculated as T_{max} + $T_{1/2}$. 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 29 and Table 30 for males and females, respectively. Liver, kidney and blood were the primary tissues for distribution of 14C-PFOA. In males, the fraction of the dose found in liver increased from T_{max} to $T_{max/2}$, 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 Tmax in males were greater than 1, and increased between T_{max} and $T_{max/2}$. Kidney-to-blood concentration ratios at T_{max} in females were approximately 2 at all dose levels and remained relatively constant between T_{max} and $T_{max/2}$.

Table 29 Distribution of PFOA in Male Sprague-Dawley Rats after Oral Exposure (Percent of dose recovered at Tmax and Tmax/2 in tissues)

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$
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)
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.

Table 30 Distribution of PFOA in Female Sprague-Dawley Rats after Oral Exposure (Percent of dose recovered at Tmax and Tmax/2 in tissues)

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$
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

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$	T_{max}	$T_{max/2}$
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)

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.

3.1.3.3.2 Intravenous Exposure

Gibson and Johnson (1980) examined the tissue distribution in 10-week old male CD rats following a single i.v. dose of 13.1 mg/kg ^{14}C-APFO. The animals were sacrificed 36 days after dosing. The results are presented in Table 31. PFOA distributed mainly to the liver, plasma and kidney.

Table 31 Distribution of PFOA in Male CD Rats After i.v. Exposure

Tissue	Mean ± SD[a]
Liver	7.97 ± 4.02
Plasma	3.19 ± 1.72
Kidney	2.33 ± 1.13
Lung	0.79 ± 0.51
Red Blood Cells	0.77 ± 0.45
Skin	0.40 ± 0.28
Spleen	0.33 ± 0.19
Bone marrow	0.33 ± 0.26
Subcutaneous Fat	0.28 ± 0.18
Muscle	0.20 ± 0.09
Brain	0.11 ± 0.04
Abdominal Fat	< 0.12

(from Gibson and Johnson, 1980)

a Total 14C concentration expressed as µg equivalents of 14C-PFOA.

3.1.3.3.3 Intraperitoneal Exposure

Ylinen et al. (1990) studied the distribution and elimination of PFOA in a limited sample of tissues following a single i.p. dose of 50 mg/kg in 10 week old Wistar rats (20/sex). Samples were collected from 2 animals per sex for analysis of PFOA at 12, 24 - 168 (in 24 hour intervals), 224 and 336 hours after dosing. 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 half-life of PFOA was estimated from the linear regression of time and concentration of PFOA in a semilogarithmic plot. No PFOA was detected in the adipose tissue. In both sexes at 12 hours after administration, the highest concentration of PFOA was found in the serum, followed by the liver, kidney, spleen and brain. In the females, the concentration of PFOA in the serum, liver and kidney decreased in a discontinuous fashion indicating distinct phases. The half-life of PFOA in the serum, liver, kidney and spleen is presented in Table 32; the values are generally much lower in females than in males.

Table 32 Mean Half-life (hours) of PFOA in Wistar Rats After i.p. Exposure

Tissue	Males	Females
Serum	105	24
Liver	210	60
Kidney	130	145
Spleen	170	73

(from Ylinen et al., 1990)

Vanden Heuvel et al. (1991) examined the distribution of PFOA in the serum and a limited number of tissues following administration of 9.4 µmol/kg ^{14}C-PFOA by i.p. injection to 6-week old male and female Harlan Sprague-Dawley rats. At various time-points for 28 days following treatment, four rats per sex were sacrificed; blood was collected by cardiac puncture, and tissues were removed and frozen. ^{14}C-PFOA-derived radioactivity was quantitated using a Liquid Scintillation Analyzer. Assuming first order kinetics, tissue elimination rates were calculated as $T_{½} = \ln 2/k_e$. The distribution and elimination of PFOA-derived ^{14}C in selected tissues is summarized in Table 33. In the male rats, 21% of the administered dose was present in the liver, and the next highest concentrations were found in the plasma and kidney. Far lower PFOA concentrations were found in the heart, testis, fat, and gastrocnemius muscle. In females, the highest concentrations of PFOA were found in the plasma followed by the kidney, liver and ovaries. In males, PFOA was eliminated from the liver at a slower rate than the other tissues; the $T_{½}$ for liver was 11 days compared to 8-9 days in most extrahepatic tissues. The rates of elimination were much faster in the female rats than in the male rats.

The high concentration of PFOA in the male liver was further examined using a liver perfusion technique. Liver was infused with 0.08 µmol ^{14}C-PFOA/min over a 48 min period for a total of 3.84 µmol ^{14}C. Approximately 11% of the cumulative dose of ^{14}C-PFOA infused was extracted by the liver in a first pass. At 2 min, the cumulative percent of PFOA extracted by the liver was 33%; that was substantially greater than the 11% cumulative dose of ^{14}C that was extracted after 48 min indicating that first-pass hepatic uptake of PFOA may be saturable.

Table 33 Distribution and Elimination of PFOA in Selected Tissues in Harlan Sprague-Dawley Rat after i.p. Exposurea

Tissue	% Dose[a]				Elimination Rate[b]		Half-life	
	Male (2 hrs)	Female (2 hrs)	Male (24 hrs)	Female (24 hrs)	Male (day^{-1})	Female ($hour^{-1}$)	Male (day)	Female (hour)
Liver	2.03 (0.10)	1.53 (0.25)	2.08 (0.14)	0.06 (0.02)	0.062 (0.005)	0.185 (0.011)	11.3	3.8
Plasma	1.99 (0.03)	2.39 (0.44)	1.63 (0.11)	0.02	0.077 (0.006)	0.242 (0.007)	9.0	2.9
Kidney	0.95 (0.08)	2.00 (0.38)	0.74 (0.03)	0.06 (0.02)	0.074 (0.005)	0.214 (0.012)	9.4	3.2
Heart	0.42 (0.02)	ND	0.39 (0.03)	ND	0.066 (0.006)	ND	10.4	ND
Gastrocnemius	0.26 (0.01)	ND	0.21 (0.01)	ND	0.076 (0.007)	ND	9.2	ND
Fat[c]	0.32 (0.02)	ND	0.27 (0.04)	ND	0.087 (0.008)	ND	8.0	ND
Testis/ Ovary	0.33 (0.01)	0.53 (0.10)	0.27 (0.03)	0.05 (0.01)	0.078 (0.006)	ND	9.0	ND

(from Vanden Heuvel et al., 1991)

ND Not determined

a Values are percent dose of PFOA-derived 14C per gram tissue; mean (SEM); n=4; values with no SEM indicate that SEM < 0.01

b Mean (SEM); n=32 for males and n=16 for females

c Epididymal fat pad

3.1.3.4 Metabolism Studies in Adult Rats

Several studies have examined metabolism of PFOA. However, no studies show clear evidence of metabolism. Ophaug and Singer (1980) found no change in ionic fluoride 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.

Vanden Heuvel et al. (1991) investigated the metabolism of PFOA in Harlan Sprague-Dawley rats administered ^{14}C-PFOA (9.4 µmol/kg, i.p.). Pooled daily urine samples (0-4 days post-treatment) and bile extracts analyzed by HPLC contained a single radioactive peak eluting identically to the parent compound. Tissues were taken from rats treated 4, 14, and 28 days after treatment to determine the presence of PFOA-containing lipid conjugates. Only the parent compound was present in rat tissues; no PFOA-containing hybrid lipids were detected. Fluoride concentrations in plasma and urine before and after PFOA treatment were unchanged, indicating that PFOA does not undergo defluorination.

3.1.3.5 Elimination Studies in Adult Rats

In adult rats, there is evidence of enterohepatic circulation of PFOA. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in

male rats. There are gender differences in the elimination of PFOA in rats. The rapid excretion of PFOA by female rats is due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to change the rate of elimination in rats.

3.1.3.5.1 Enterohepatic Circulation

Johnson et al. (1984) investigated the effect of feeding cholestyramine to rats on the elimination of APFO. Since APFO exists as an anion at physiologic pH, it would be expected to complex with cholestyramine. Ten male Charles River CD rats, 12 weeks of age, were given a single i.v. injection of 13 mg/kg ^{14}C-APFO. Five rats were given 4% cholestyramine in feed. Urine and feces samples were collected at intervals for 14 days, at which time the animals were sacrificed and liver samples were collected. At 14 days post dose, the mean percentage of PFOA eliminated in the feces of cholestyramine-treated rats was 9.8-fold the mean percentage eliminated in the feces of rats that did not receive cholestyramine. Excretion in urine was 41% of the administered dose for cholestyramine treated rats and 67% for rats that did not receive cholestyramine. ^{14}C in the liver equaled 4% or 12.1 ± 2.1 µg eq/g in cholestyramine treated rats and 8 % or 22.3 ± 6.2 µg eq/g in rats that did not receive cholestryamine. In plasma, the levels were 5.1 ± 1.7 µg eq/ml in cholestyramine treated rats and 14.7±6.8 µg eq/ml in rats that did not receive cholestyramine. In red blood cells, the levels were 1.8 ± 0.7 µg eq/ml in cholestryamine treated rats and 4.2 ± 2.4 µg eq/ml in rats that did receive cholestryamine. The high concentration of ^{14}C-APFO in the liver at 2 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of ^{14}C nearly 10-fold suggests that there is enterohepatic circulation of PFOA.

3.1.3.5.2 General Elimination Studies

3.1.3.5.2.1 Oral Exposure

Kemper (2003) investigated the elimination of PFOA in male and female Sprague-Dawley rats (4 rats/sex/group) administered a single dose of ^{14}C-PFOA by oral gavage at dose levels of 1, 5, and 25 mg/kg. Urine and feces were collected for 28 days in males and 7 days in females. Urine was the primary route of excretion of ^{14}C in both sexes, accounting for 43-62% of the administered dose in males and 76-84% of the administered dose in females. Cumulative recovery of ^{14}C in the urine increased gradually over the 28 days in male rats, but was essentially complete in female rats within the first 72 hours. Fecal excretion of ^{14}C accounted for 6-14% of the dose in males and 2-6% of the dose in females. Pilot experiments demonstrated that ^{14}C was not eliminated as either $^{14}CO_2$ or volatile organic compounds in ^{14}C-PFOA-treated rats. Pretreatment of rats with 1 mg/kg-day PFOA for 14 days had little or no effect on the excretion of a challenge dose of 1 mg/kg ^{14}C-PFOA.

3.1.3.5.2.2 Intravenous Exposure

Gibson and Johnson (1980) examined the excretion of total ^{14}C in male and female CD rats after a single i.v. dose of ^{14}C-APFO. The mean dose for females was 16.7 mg/kg while that for males was 13.1 mg/kg. Female rats excreted essentially all of the administered dose via the urine in the 24 hours after treatment. During the same time period, male rats excreted only 20% of the total dose. Male rats excreted 83% of the total dose via the urine and 5.4% via the feces by 36 days post dose. No radioactivity was detected in tissues of female rats at 17 days post dose; 2.8% of the total dose was detected in the liver of male rats and 1.1% in the plasma at 36 days post dose with lower levels equaling < 0.5% of the total dose in other organs.

3.1.3.5.3 Elimination Studies in the Pregnant Rat

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-APFO (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-APFO. 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.

3.1.3.5.4 Studies on the Mechanism of the Gender Difference in Elimination in Adult Rats

Several studies have been conducted to elucidate the cause of the gender difference in rats in the elimination of PFOA. Hanhijarvi et al. (1982) conducted a series of studies to examine the effect of probenecid, which inhibits the renal active secretion system for organic acids, on the elimination of PFOA in male and female Holtzman rats. In the first study, 4 male and 6 female Holtzman rats were administered 2 mg of nonionic fluorine as PFOA by gavage. Seven female rats were administered 2 ml distilled water as controls. The animals were then placed in metabolism cages and urine was collected until the animals were sacrificed at 24 hr by cardiac puncture. Serum was collected. Ionic fluoride and total fluorine content of serum and urine were determined, and nonionic fluorine was calculated as the difference. Twenty-four hours after oral administration of PFOA, female rats had excreted 76 ± 2.7% of the dose in the urine and had a mean serum nonionic fluorine level of 0.35 ± 0.11 µg/ml, while male rats had excreted only 9.2 ± 3.5% of the dose and had a mean serum nonionic fluorine level of 44.0 ± 1.7 µg/ml. A mean of 97.5 ± 0.25% of the PFOA was bound in the plasma of both male and female rats.

In the second study, Hanhijarvi et al. (1982) examined the effect of probenecid on the clearance of PFOA and inulin. Holtzman rats were anesthetized and the femoral artery was cannulated for continuous infusion of 5% mannitol in isotonic saline, while the femoral artery was cannulated for drawing blood samples. The urinary bladder was also cannulated for serial collections of urine. When the urine and serum collections for the clearance study were complete, 65-68 mg/kg probenecid was administered by i.p. injection and after 20 - 30 minutes, additional 10 minute clearance tests were performed. Administration of probenecid reduced the PFOA/inulin clearance ratio in females from 14.5 to 0.46. PFOA clearance was reduced from 5.8 to 0.11 ml/min/100g. Net PFOA excretion was reduced from 4.6 µg/min/100g to 0.13 µg/min/100g. In male rats, however, the PFOA/inulin clearance ratio and the net excretion of PFOA were virtually unaffected by probenecid. In the males, PFOA clearance was 0.17 ml/min/100g, the PFOA/inulin clearance ratio was 0.22, and net PFOA excretion was 0.17 µg/min/mg.

Finally, Hanhijarvi et al. (1982) examined the cumulative excretion of PFOA over a 7-hour period. Holtzman rats were dosed i.v. with a mixture of 10%-20% radiolabeled-PFOA and 80-90% unlabeled PFOA. Mannitol (5%) was infused and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65-68 mg/kg by i.p. injection at least 30 min prior to the administration of PFOA. Female rats 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.

Ylinen et al. (1989) studied the urinary excretion of PFOA in male Wistar rats after castration and estradiol administration. Twenty male rats were castrated at 28 days of age and were used in tests of PFOA excretion 5 weeks later. Ten castrated and 10 intact males were given 500 µg/kg estradiol valerate by s.c. injection every second day for 14 days before administration of PFOA. PFOA was

administered as a single i.p. injection at 50 mg/kg. Urine was collected in metabolism cages for 96 hr after PFOA administration. Blood samples were collected by cardiac puncture. Six female rats were also included in the experiment. Castration and administration of estradiol to the male rats had a significant stimulatory effect on the urinary excretion of PFOA. During the first 24 hours, female rats excreted 72 ± 5% of the administered dose of PFOA, whereas the intact males excreted only 9 ± 4%. After the estradiol treatment, both the intact and castrated males excreted PFOA in amounts similar to females, 61 ± 19% and 68 ± 14%, respectively. The castrated males without estradiol treatment excreted 50 ± 13% of the administered dose of PFOA in the urine. This was faster than the intact males but less than the females and the estrogen treated males. At the end of the test, the concentration of PFOA in the serum of intact males was 17- 40 times higher than the concentration PFOA in the serum of other groups. There were no statistically significant differences in the serum concentrations between the other groups. PFOA was similarly bound by the proteins in the serum of males and females.

Vanden Heuvel et al. (1992a) investigated whether androgens or estrogens are involved in the marked sex-differences in the urinary excretion of PFOA. Castrated Harlan Sprague-Dawley male rats were given 9.4 µmol/kg ^{14}C-PFOA by i.p. injection. Castration increased the elimination of PFOA in the urine (36% of the dose was eliminated in 4 days versus 16% in controls), suggesting that a factor produced by the testis is responsible for the slow elimination of PFOA in male rats. Castration plus 17B-estradiol had no further effect on PFOA elimination whereas castration plus testosterone replacement at the physiological level reduced PFOA elimination to the same level as rats with intact testis. Thus, in male rats, testosterone exerts an inhibitory effect on renal excretion of PFOA. In female rats, neither ovariectomy or ovariectomy plus testosterone affected the urinary excretion of PFOA, demonstrating that the inhibitory effect of testosterone on PFOA renal excretion is a male-specific response. Probenecid, which inhibits the renal transport system, decreased the high rate of PFOA renal excretion in castrated males but had no effect on male rats with intact testis.

Kudo et al. (2002) examined the role of sex hormones on the renal clearance (CL_R) of PFOA and the renal mRNA levels of specific organic anion transporters in male and female Wistar rats. 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, which was reduced by estradiol treatment. Treatments of female rats with testosterone reduced the CL_R of PFOA. Treatment with probenecid, a known inhibitor of organic anion transporters, markedly reduced the CL_R of PFOA in male rats, castrated male rats, and female rats. 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. The level of organic anion transporter 2 (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 OATP1 was mentioned as a possible factor in male rat retention of PFOA.

3.1.4 Metabolism and Pharmacokinetic Studies in Immature Rats

No studies have been conducted to specifically examine the absorption, metabolism or elimination of PFOA in the developing rat. However, recent studies have been conducted to examine the concentrations of PFOA in the developing Sprague-Dawley rat, and to determine when the gender dif-

ference in elimination of PFOA becomes apparent. In addition, several studies have examined the serum and tissue distribution of PFOA in newly weaned Wistar rats. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. During lactation and immediately after weaning, the elimination of PFOA is similar in males and females. In the male rat between 4-5 weeks of age, the factor(s) responsible for the gender difference develop, and the rats assume the adult male elimination profile. In addition, distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

3.1.4.1 PFOA Levels During Pregnancy and Lactation

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

Maternal PFOA levels during gestation and lactation are presented in Table 34. 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). The concentration of PFOA in the milk was also fairly similar throughout lactation and was approximately 1/10th of the PFOA levels in the plasma; the mean values were 1.1, 2.8, and 6.2 µg/ml in the 3, 10, and 30 mg/kg-day groups, respectively.

Table 34 Maternal PFOA Levels (µg/ml) During Gestation and Lactation[a]

Exposure Period	Sample Time	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
	- milk	1.07 ± 0.26	2.03 ± 0.33	4.97 ± 1.20
GD 4 - LD 7	LD 7 - plasma	10.09 ± 2.90	25.83 ± 2.07	66.91 ± 11.82
	- milk	0.94 ± 0.22	2.74 ±0.91	5.76 ±1.26
GD 4 - LD 14	LD 14 - plasma	9.69 ± 0.92	23.79 ± 2,81	54.65 ± 11.63
	- milk	1.15 ± 0.06	3.45 ± 1.18	6.45 ± 1.38
GD 4 - LD 21	LD 21 - plasma	9.04 ± 1.01	28.84 ± 5.15	64.13 ± 1.45
	- milk	1.13 ± 0.08	3.07 ± 0.51	7.48 ± 1.63
NA	Average plasma	11.19 ± 2.76	26.84 ± 4.21	66.64 ± 9.80
	Average milk	1.07 ± 0.09	2.82 ± 0.60	6.16 ± 1.06

(from Hinderliter et al., 2005 and Mylchreest, 2003)

[a] mean ± SD; samples were from 5 dams/group/time point and were collected 2 hrs post-dosing

PFOA levels in the placenta, amniotic fluid, embryo, fetus, and pup plasma are presented in Table 35. 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. The concentration of PFOA in the plasma of the day 21 fetus were approximately half the levels observed in the maternal plasma; the mean values were 5.9, 14.5, and 33.1 µg/ml in the 3, 10, and 30 mg/kg-day groups, respectively. Pup plasma levels decreased until lactation day 7, and were thereafter similar to the levels observed in the milk.

Table 35 PFOA Concentrations (µg/ml) During Gestation and Lactation in Sprague-Dawley Rats[a]

Exposure Period	Tissue	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
	- 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

Exposure Period	Tissue	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
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)

a mean ± SD; samples were pooled by litter and were collected 2 hrs post-dosing

3.1.4.2 PFOA Levels in the Postweaning Sprague-Dawley Rat

Two studies have been conducted to examine PFOA levels in the postweaning Sprague-Dawley rat to determine the ontogeny of the gender difference in elimination. Han (2003) administered four to eight week old rats (10/sex/age) a single dose of 10 mg/kg-day APFO by oral gavage. Blood samples were collected 24 hours after dosing and the plasma concentration of PFOA was measured by HPLC-MS. At four weeks of age, the concentration of plasma PFOA was approximately 2.7 times higher in males than in the females (Table 36). In females, the concentration of plasma PFOA decreased by 2.7 fold between 4 and 5 weeks of age, and thereafter remained fairly steady. In males, the concentration of plasma PFOA increased by 5.4 fold between 4 and 5 weeks of age, and thereafter remained fairly steady. Between 5 and 8 weeks of age, the PFOA plasma concentrations were 34.7 - 65.1 fold higher in males than in females of the same age. Thus, it appears that maturation of the factor(s) responsible for the gender difference in elimination of PFOA occurs between the ages of 4 and 5 weeks in the rat.

Table 36 Plasma PFOA Concentrations (µg/ml) in Postweaning Sprague-Dawley Rats[a]

Age (weeks)	Males	Females
4	7.32 ± 1.01	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) investigated the relationship between age and both plasma and urine PFOA concentrations in male and female Sprague-Dawley rats. Immature rats 3, 4, or 5 weeks of age were administered APFO 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/group were sacrificed and blood samples were collected. The remaining 5 rats/sex/group were placed in metabolism cages for 24-hour urine collection. These rats were sacrificed at 24 hours and blood samples were collected. In 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 and five weeks of age ($p<0.01$) which showed the lowest PFOA level (Table 37). PFOA plasma concentrations following a 30 mg/kg dose were 2-3 times higher than those following a 10 mg/kg dose.

Table 37 Plasma PFOA Concentrations in Male Rats Dosed via Oral Gavage

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 female rats, plasma PFOA concentrations were significantly lower at 5 weeks of age than at 3 or 4 weeks of age for both sample times and doses, except at 2 hours post-dose in the 10 μg/kg dose group ($p<0.01$, Table 38). Plasma PFOA concentrations were significantly lower at 24 hours post dosing as compared to 2 hours post dosing in all ages and doses tested. 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.

Table 38 Plasma PFOA Concentrations in Female Rats Dosed via Oral Gavage

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 ($p<0.01$).

The results of the prior study (Han, 2003, Table 15) are consistent with the current study for plasma PFOA concentrations of both sexes at 5 weeks of age but not at 4 weeks of age (3 week old animals were not previously tested) when rats were administered 10 mg/kg APFO and plasma was collected at 24 hours post-dose. The only known variation between the two studies is that in the previous study, rats were fasted overnight for approximately 12 hours before dosing with APFO. In a supplemental study to determine the effect of fasting (Hinderliter, 2004), 4 week old rats, 4 rats/sex, were administered 10 mg/kg APFO via oral gavage. Two animals/sex were fasted overnight for 12

hours before dosing with APFO. 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 5 week old rat plasma PFOA concentrations, the authors concluded there may be an unknown physiological variability in rats younger than 5 weeks which impacts the pharmacokinetics of PFOA.

Urine PFOA concentrations differed significantly with age, dose and sex ($p<0.01$, Table 39). Female rats had higher urine PFOA concentrations than males, and the female urine PFOA concentrations increased with age. In male rats, urine PFOA concentrations decreased with age. 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. No attempt was made to quantify urinary output (volume) or standardize for creatinine levels.

Table 39 Urine PFOA Concentrations in Male and Female Rats Dosed via Oral Gavage

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)

3.1.4.3 Serum and Tissue Distribution in Immature Wistar Rats Following Oral Exposure

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 40. 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 and tissues were generally lower in the 30 mg/kg-day group than in the 10 mg/kg-day group due to increased urinary elimination in the 30 mg/kg-day group.

Table 40 Tissue Distribution of PFOA in Wistar Rats after 28 Days of Treatment

Tissue	Males[a]			Females[a]		
	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day	3 mg/kg-day	10 mg/kg-day	30 mg/kg-day
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

b N=3, no SD

c below the limit of quantitation

Hanhijarvi et al. (1987) administered PFOA by gavage to 48 newly-weaned Wistar rats (6/sex/group) at 0, 3, 10, and 30 mg/kg-day for 28 consecutive days and determined the serum levels of PFOA. At the end of the study, blood was collected via cardiac puncture; PFOA levels were determined by gas chromatography. At each dose level, the mean PFOA concentrations in the plasma of the male rats were significantly higher than those of the female rats. The mean plasma PFOA concentrations for the male rats were 48.6 ± 26.5 µg/ml, 83.1 ± 24.7 µg/ml, and 53.4 ± 11.2 µg/ml, respectively for the 3, 10 and 30 mg/kg-day dose levels. The corresponding figures for female rats were 2.43 ± 5.96 µg/ml, 11.3 ± 8.59 µg/ml, and 9.06 ± 8.80 µg/ml respectively, for the 3, 10 and 30 mg/kg-day dose levels.

3.1.5 Comparative Studies of Protein Binding in Humans, Non-Human Primates, and Rats

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 of rats, humans or monkeys to gain understanding of its absorption, distribution and elimination, and species and gender differences.

Protein binding in plasma from cynomolgus monkeys, rats, and humans was tested with PFOA (Kerstner-Wood et al., 2003). The results are summarized in Table 41. Rat, human, and monkey plasma all bind PFOA at 97- 100% at tested concentrations ranging from 1-500 ppm. PFOA was found to bind primarily to human serum albumin compared to other protein components of human plasma at physiological concentrations.

Table 41 % 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 BQL<6.25 ng/ml

Han et al. (2003) investigated the binding of PFOA to rat and human plasma proteins in vitro. Male and female rats treated in vivo showed no gender difference in the binding of PFOA to serum, though the persistence of PFOA in vivo is much greater in male than female rats. The authors conclude that there is 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 NMR allowed the calculation of disassociation constants for PFOA binding to rodent and human serum albumin. No significant differences between binding to the two proteins was detected (Table 42).

Table 42 Dissociation Constants (K_d) of Binding between PFOA and Rodent Serum Albumin (RSA) and Human Serum Albumin (HSA) and the Number of PFOA Binding Sites (n) on RSA and HSA

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
N	micro-SEC[b]	7.8 ± 1.5	7.2 ± 1.3

(from Han et al., 2003)
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.

3.1.6 Metabolism and Pharmacokinetic Studies in Other Test Species

There is limited information on the metabolism and pharmacokinetics of PFOA in mice, rabbits and dogs. No specific pharmacokinetic studies have been conducted in mice. However, toxicology studies in mice indicate that PFOA is absorbed, and furthermore, there does not appear to be a gender difference in elimination. For example, Sohlenius et al. (1992) exposed male and female C57B1/6 mice to dietary levels of 0.02% PFOA for one week. There was a significant decrease in mean body weight and a significant increase in absolute liver weight; the response was similar in male and female mice.

In rabbits, there is no information available on the metabolism or elimination of PFOA by any route of exposure. No specific studies of the absorption of PFOA have been conducted following oral or

inhalation exposure. However, there is evidence that PFOA is absorbed following dermal exposure. O'Malley and Ebbens (1981) treated male and female New Zealand White rabbits dermally with doses of 100, 1,000 and 2,000 mg/kg APFO for 14 days. Mortality was 100% (4/4) in the 2,000 mg/kg group, 75% (3/4) in the 1,000 mg/kg group and 0% (0/4) in the 100 mg/kg group. Similarly, Kennedy (1985) treated rabbits dermally with a total of 10 applications of APFO 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.

In addition, limited information is available on the serum and liver distribution of PFOA in rabbits following i.v. administration. Johnson (1995a) administered individual female rabbits intravenous doses of 0, 4, 16, 24 and 40 mg/kg tetrabutyl ammonium salt of PFOA. The animal given 40 mg/kg died within 5 minutes of treatment. All other animals appeared normal throughout the study. Serum samples were analyzed for total organic fluorine at 2, 4, 6, 8, 12, 24, and 48 hours post dose. At 2 hrs, serum organic fluorine levels in the rabbits that received 0, 4, 16, and 24 mg/kg were 1.25 µg/ml, 4.09 µg/ml, 14.9 µg/ml, and 41.0 µg/ml, respectively. There was a rapid decrease of total organic fluorine in the serum with time; it was essentially eliminated at 24 hr and was non-detectable at 48 hr. The biological half-life was on the order of 4 hours. The total organic fluorine levels in whole liver at 48 hr post dose for the rabbits that received 0 mg/kg, 4 mg/kg, 16 mg/kg, and 24 mg/kg were 20 µg, 43 µg, 66 µg, and 54 µg, respectively.

There is no information on the specific absorption, metabolism or distribution of PFOA in dogs by any route of exposure. One study examined the elimination of PFOA in dogs following i.v. administration. Hanhijarvi et al. (1988) administered beagle dogs (3/sex) an i.v. injection of 30 mg/kg of PFOA followed by continuous infusion with 5% mannitol. Urine and blood were collected at 10 minute intervals for 60 min. Probenecid was then administered by i.v. injection, and urine and blood samples were collected as before. Renal clearance of PFOA was calculated for the before and after probenecid injection periods. Four additional dogs (2/sex) were given 30 mg/kg of PFOA by i.v. injection. These dogs were kept in metabolism cages, and blood samples were collected intermittently for 30 days. The renal clearance rate was approximately 0.03 ml/min/kg. Probenecid significantly reduced the PFOA clearance rate in both sexes, indicating an active secretion mechanism for PFOA. The plasma half-life of PFOA was 473 hr before probenecid administration and 541 hr after in male dogs, and 202 hr before probenecid and 305 hr after in the female dogs.

Conclusions

Little information is available concerning the pharmacokinetics of PFOA and its salts in humans. Preliminary results of a 5-year half-life study in 26 retired workers indicate that the mean serum elimination half-life of PFOA in these workers was 3.8 years (1378 days, 95% CI, 1131-1624 days) and the range was 1.5 - 9.1 years. Metabolism and pharmacokinetic studies in non-human primates has been examined in a study of 3 male and 3 female cynomolgus monkeys administered a single i.v. dose of 10 mg/kg potassium PFOA. In male monkeys, the average serum half-life was 20.9 days. In female monkeys, the average serum half-life was 32.6 days. In addition, 4-6 male cynomolgus monkeys were administered APFO daily via oral capsule at 10 or 20 mg/kg-day for six months, and the elimination of PFOA was monitored after cessation of dosing. For the two 10 mg/kg-day recovery monkeys, serum PFOA elimination half-life was 19.5 days, and the serum PFOA elimination half-life was 20.8 days for the three 20 mg/kg-day monkeys.

Studies in adult rats have shown that the ammonium salt of PFOA (APFO) is absorbed following oral, inhalation and dermal exposure. Serum pharmacokinetic parameters and the distribution of PFOA has been examined in the tissues of adult rats following administration by gavage and by i.v. and i.p. injection. PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the

major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats.

There are gender differences in the elimination of PFOA in adult rats following administration by gavage and by i.v. and i.p.injection. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8 to 16 hours, while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138 to 202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic anion transporters); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

Several recent studies have been conducted to examine the kinetics of PFOA in the developing Sprague-Dawley rat. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. The gender difference in elimination is developmentally regulated; between 4-5 weeks of age, elimination assumes the adult pattern and the gender difference becomes readily apparent. Distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

3.2 Epidemiology Studies

3M and Dupont have conducted several epidemiology and medical surveillance studies of the workers at their plants in various cities of the U.S. No remarkable health effects that can be directly attributed to PFOA exposure have been reported in fluorochemical production workers described in the studies below.

3.2.1 Mortality and Cancer Incidence Studies in Workers

3M and Dupont have both conducted mortality and cancer incidence studies in workers exposed to POSF-based fluorochemicals. Mortality studies have been undertaken at 3M's Cottage Grove plant in Minnesota while Dupont carried out mortality and cancer incidence studies on workers employed at their Washington Works plant in West Virginia.

A retrospective cohort mortality study was performed on employees at the 3M Cottage Grove, MN plant which produces APFO (Gilliland and Mandel, 1993). At this plant, APFO production was limited to the Chemical Division. The cohort consisted of workers who had been employed at the plant for at least 6 months between January 1947 and December 1983. Death certificates of all of the workers were obtained to determine cause of death. There was almost complete follow-up (99.5%) of all of the study participants. The exposure status of the workers was categorized based on their job histories. If they had been employed for at least 1 month in the Chemical Division, they were considered exposed. All others were considered to be not exposed to PFOA. The number of months employed in the Chemical Division provided the cumulative exposure measurements. Of the 3537 (2788 men and 749 women) employees who participated in this study, 398 (348 men and 50 women) were deceased. Eleven of the 50 women and 148 of the 348 men worked in the Chemical Division, and therefore, were considered exposed to PFOA.

Standardized Mortality Ratios (SMRs), adjusted for age, sex, and race were calculated and compared to U.S. and Minnesota white death rates for men. For women, only state rates were available. The SMRs for males were stratified for 3 latency periods (10, 15, and 20 years) and 3 periods of duration of employment (5, 10, and 20 years).

For all female employees, the SMRs for all causes and for all cancers were less than 1. The only elevated (although not significant) SMR was for lymphopoietic cancer, and was based on only 3 deaths. When exposure status was considered, SMRs for all causes of death and for all cancers were significantly lower than expected, based on the U.S. rates, for both the Chemical Division workers and the other employees of the plant.

In all male workers at the plant, the SMRs were close to 1 for most of the causes of death when compared to both the U.S. and the Minnesota death rates. When latency and duration of employment were considered, there were no elevated SMRs. When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI .55 - 4.59). This was based on 4 deaths (1.97 expected). There was also a statistically significant (p = 0.03) association with length of employment in the Chemical Division and prostate cancer mortality. Based on the results of proportional hazard models, the relative risk for a 1-year increase in employment in the Chemical Division was 1.13 (95% CI 1.01 to 1.27). It rose to 3.3 (95% CI 1.02 -10.6) for workers employed in the Chemical Division for 10 years when compared to the other employees in the plant. The SMR for workers not employed in the Chemical Division was less than expected for prostate cancer (.58).

An update of this study was conducted to include the death experience of employees through 1997 (Alexander, 2001a). The cohort consisted of 3992 workers. The eligibility requirement was increased to 1 year of employment at the Cottage Grove plant, and the exposure categories were changed to be more specific. Workers were placed into 3 exposure groups based on job history information: definite PFOA exposure (n = 492, jobs where cell generation, drying, shipping and packaging of PFOA occurred throughout the history of the plant); probable PFOA exposure (n = 1685, other chemical division jobs where exposure to PFOA was possible but with lower or transient exposures); and not exposed to fluorochemicals (n = 1815, primarily non-chemical division jobs).

In this new cohort, 607 deaths were identified: 46 of these deaths were in the PFOA exposure group, 267 in the probable exposure group, and 294 in the non-exposed group. When all employees were compared to the state mortality rates, SMRs were less than 1 or only slightly higher for all of the causes of death analyzed. None of the SMRs were statistically significant at p = .05. The highest SMR reported was for bladder cancer (SMR = 1.31, 95% CI = 0.42 – 3.05). Five deaths were observed (3.83 expected).

A few SMRs were elevated for employees in the definite PFOA exposure group: 2 deaths from cancer of the large intestine (SMR = 1.67, 95% CI = 0.02 – 6.02), 1 from pancreatic cancer (SMR = 1.34, 95% CI = 0.03 – 7.42), and 1 from prostate cancer (SMR = 1.30, 95% CI = 0.03 – 7.20). In addition, employees in the definite PFOA exposure group were 2.5 times more likely to experience cerebrovascular disease mortality (5 deaths observed, 1.94 expected; 95% CI = 0.84 – 6.03).

The excess mortality in cerebrovascular disease noted in employees in the definite exposure group was further analyzed based on number of years of employment at the plant. Three of the 5 deaths occurred in workers who were employed in jobs with definite PFOA exposure for more than 5 years but less than 10 years (SMR = 15.03, 95% CI = 3.02 – 43.91). The other 2 occurred in employees with less than 1 year of definite exposure. The SMR was 6.9 (95% CI = 1.39 – 20.24) for employees with greater than 5 years of definite PFOA exposure. In order to confirm that the results regarding cerebrovascular disease were not an artifact of death certificate coding, regional mortality rates were used for the reference population. The results did not change. When these deaths were further analyzed by cumulative exposure (time-weighted according to exposure category), workers with 27 years of exposure in probable PFOA exposed jobs or those with 9 years of definite PFOA exposure were 3.3 times more likely to die of cerebrovascular disease than the general population. A dose-response relationship was not observed with years of exposure.

In the probable exposure group, 3 SMRs were elevated: cancer of the testis and other male genital organs (SMR = 2.75, 95% CI = 0.07 – 15.3); pancreatic cancer (SMR = 1.24, 95% CI = 0.45 – 2.70); and malignant melanoma of the skin (SMR = 1.42, 95% CI = 0.17 – 5.11). Only 1, 6, and 2 cases were observed, respectively. The SMR for prostate cancer in this group was 0.86 (95% CI = 0.28 – 2.02) (n = 5).

There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs. Four cases were observed and only 1.89 were expected (95% CI = 0.58 – 5.40).

It is difficult to interpret the results of the prostate cancer deaths between the first study and the update because the exposure categories were modified in the update. Only 1 death was reported in the definite exposure group and 5 were observed in the probable exposure group. All of these deaths would have been placed in the chemical plant employee exposure group in the first study. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported. This is important because even 1 prostate cancer death in the definite PFOA exposure group resulted in an elevated SMR for the group. Therefore, if any of the employees' exposures were misclassified, the results of the analysis could be altered significantly. This issue has become more apparent, given the results of a biomonitoring study that took place at the Cottage Grove plant in 2000 in which PFOA concentrations were not correlated with years worked in the Chemical Division but instead were associated with the specific area of the plant where APFO was produced (Olsen, et al., 2003f).

The exposure categories were modified between the first and second Cottage Grove mortality studies; therefore, comparison of the results is difficult. Although the potential for exposure misclassification was certainly more likely in the first study, it may still have occurred in the update as well. It is difficult to judge the reliability of the exposure categories that were defined without measured exposures. Although serum PFOA measurements were considered in the exposure matrix developed for the update, they were not directly used. In the second study, the chemical plant employees were sub-divided into PFOA-exposed groups, and the film plant employees essentially remained in the "non-exposed" group. This was an effort to more accurately classify exposures; however, these new categories do not take into account duration of exposure or length of employment. Another limitation to this study is that 17 death certificates were not located for deceased employees and therefore were not included in the study. The inclusion or exclusion of these deaths could change the analyses for the causes of death that had a small number of cases. Follow-up of worker mortality at Cottage Grove needs to continue. Although there were more than 200 additional deaths included in this analysis, it is a small number and the cohort is still relatively young. Given the results of studies on fluorochemicals in both animals and humans, further analysis is warranted.

Limited data are available on mortality and cancer incidence at Dupont's Washington Works Plant in Parkersburg, WV. These studies were periodically undertaken as part of a medical surveillance program at the plant. The most recent report is summarized here. 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 other data, such as employee exposure information, lifestyle factors, employee demographics, or other chemicals used at the plant, are available in this report.

In the cancer incidence 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 [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 ele-

vated SIRs but which were 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).

In the mortality report, when all causes of death were reported, SMRs, adjusted for age and gender, were statistically significant (p = 0.05) for rheumatic heart disease (SMR = 3.55; 95% CI, 1.14-8.30) and atherosclerosis and aneurysm (SMR = 1.98; 95% CI, 1.17-3.14).

Two separate analyses of leukemia incidence were conducted prior to the above studies at Dupont's Washington Works plant (Walrath and Burke, 1989; Karns and Fayerweather, 1991). The initial study reported a statistically significant (p = 0.10) 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, 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 OR were also reported based on the area of the plant where the cases worked; however, no statistically significant (p = 0.10) OR were reported.

The mortality data reported above do not show any statistically significant (p = 0.10) elevations in leukemia deaths (all of the cases in the case-control study were dead at the time of the mortality report), possibly because the number of cases was very small and divided among different types of leukemias. 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.

3.2.2 Hormone Study in Male Workers

Endocrine effects have been associated with PFOA exposure in animals; therefore, medical surveillance data, including hormone testing, from male employees only of the Cottage Grove, Minnesota plant were analyzed (Olsen et al., 1998a). PFOA serum levels were obtained for volunteer workers in 1993 (n = 111) and 1995 (n = 80). Sixty-eight employees were common to both sampling periods. In 1993, the range of PFOA was 0-80 ppm and 0-115 ppm in 1995 using thermospray mass spectrophotometry assay. Eleven hormones were assayed from the serum samples. They were: cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, follicle stimulating hormone (FSH), 17 gamma-hydroxyprogesterone (17-HP), free testosterone, total testosterone, luteinizing hormone (LH), prolactin, thyroid-stimulating hormone (TSH) and sex hormone-binding globulin (SHBG). 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.

PFOA was not highly correlated with any of the hormones or with the following covariates: age, alcohol consumption, body mass index (BMI), or cigarettes. Most of the employees had PFOA serum levels less than 10 ppm. In 1993, only 12 employees had serum levels > 10 ppm, and 15 in 1995. However, these levels ranged from approximately 10 ppm to over 114 ppm. There were only 4 employees in the >30 ppm PFOA group in 1993 and only 5 in 1995. Therefore, it is likely that there was not enough power to detect differences in either of the highest categories. The mean age

of the employees in the highest exposure category was the lowest in both 1993 and 1995 (33.3 years and 38.2 years, respectively). Although not significantly different from the other categories, BMI was slightly higher in the highest PFOA category.

When the mean values of the various hormones were compared by exposure categories, there was a statistically significant ($p = .01$) elevation in prolactin in 1993 only for the 10 workers whose serum levels were between 10 and 30 ppm compared to the lower 2 exposure categories. In addition, TSH was significantly ($p = .002$) elevated in the same exposure category for 1995 only (mean blood serum 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 only in this same exposure category, as well; however did not reach statistical significance ($p = .09$).

Estradiol levels in the >30 ppm group in both years were 10% higher than the other PFOA groups; however, the difference was not statistically significant ($p < 0.05$). These results were confounded by estradiol being highly correlated with BMI ($r = .41$, $p < .001$ in 1993, and $r = .30$, $p < .01$ in 1995). In 1995, all 5 employees with PFOA levels > 30 ppm had BMIs > 28, although this effect was not observed in 1993. 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.

The authors did not report a negative association between PFOA serum levels and testosterone. There were no statistically significant trends ($p < 0.05$) noted for PFOA and either bound or free testosterone. However, 17-HP, a precursor of testosterone, was highest in the >30 ppm PFOA group in both 1993 and 1995. In 1995, PFOA was significantly associated with 17-HP in regression models adjusted for possible confounders. However, the authors state that this association was based on the results of one employee (data were not provided in the report). Free testosterone was highly correlated with age in both 1993 and 1995 ($r = -.48$, $p < .001$; $r = -.40$, $p < .001$, respectively).

There are several design issues that should be noted when evaluating the results of this study. First, although there were 2 study years (1993 and 1995), the populations were not independent. Sixty-eight employees participated in both years. Second, there were 31 fewer employees who participated in the study in 1995, thus reducing the power of the study. There were also very few employees in either year with serum PFOA levels greater than 10 ppm. Third, the cross-sectional design of the study does not allow for analysis of temporality of an association. Since the half-life of PFOA is at least 1 year, the authors suggest that it is possible that there may be some biological accommodation to the effects of PFOA. Fourth, only one sample was taken for each hormone for each of the study years. In order to get more accurate measurements for some of the hormones, pooled blood taken in a short time period should have been used for each participant. Fifth, some of the associations that were measured in this study were done based on the results of an earlier paper that linked PFOA with increased estradiol and decreased testosterone levels. However, total serum organic fluorine was measured in that study instead of PFOA, making it difficult to compare the results. Finally, there may have been some measurement error of some of the confounding variables.

3.2.3 Occupational Study on Episodes of Care

In order to gain additional insight into the effects of fluorochemical exposure on workers' health, an "episode of care" analysis was undertaken at the Decatur plant to screen for morbidity outcomes that may be associated with long-term, high exposure to fluorochemicals (Olsen et al., 2001g; 2004c). An "episode of care" is a series of health care services provided from the start of a particu-

lar disease or condition until solution or resolution of that problem. Episodes of care were identified in employees' health claims records using Clinical Care Groups (CCG) software. All inpatient and outpatient visits to health care providers, procedures, ancillary services and prescription drugs used in the diagnosis, treatment, and management of over 400 diseases or conditions were tracked.

Episodes of care were analyzed for 652 chemical employees and 659 film plant employees who worked at the Decatur plant for at least 1 year between January 1, 1993 and December 31, 1998. Based on work history records, employees were placed into different comparison groups: Group A consisted of all film and chemical plant workers; Group B had employees who only worked in either the film or chemical plant; Group C consisted of employees who worked in jobs with high POSF exposures; and Group D had employees who worked in high exposures in the chemical plant for 10 years or more prior to the onset of the study. Film plant employees were considered to have little or no fluorochemical exposure, while chemical plant employees were assumed to have the highest exposures.

Ratios of observed to expected episodes of care were calculated for each plant. Expected numbers were based on 3M's employee population experience using indirect standardization techniques. A ratio of the chemical plant's observed to expected experience divided by the film plant's observed to expected experience was calculated to provide a relative risk ratio for each episode of care (RREpC). For each RREpC, 95% confidence intervals were calculated. Episodes of care that were of greatest interest were those which had been reported in animal or epidemiologic literature on PFOS and PFOA: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, disorders of the liver and biliary tract, and reproductive disorders.

The only increased risk of episodes for these conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers (RREpC = 8.2, 95% CI = 0.8 - 399). One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding should be noted because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study when there were only 2 exposure categories (chemical division employees and non-chemical division employees). The update of the study sub-divided the chemical plant employees and did not corroborate this finding when exposures were divided into definitely exposed and probably exposed employees.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. The limitations include: 1) episodes of care are reported, not disease incidence, 2) the data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease, 3) many of the risk ratios for episodes of care had very wide confidence intervals, thereby providing unstable results, 4) the analysis was limited to 6 years, 5) the utilization of health care services may reflect local medical practice patterns, 6) individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications, 7) episodes of care may include the same individual several times, 8) not all employees were included in the database, such as those on long-term disability, 9) the analysis may be limited by the software used, which may misclassify episodes of care, 10) the software may assign 2 different diagnoses to the same episode, and 11) certain services, such as lab procedures may not have been reported in the database.

3.2.4 Other 3M Medical Surveillance Studies

3.2.4.1 Antwerp and Decatur Plants–Cross-Sectional and Longitudinal Studies

A cross-sectional analysis of the data from the 2000 medical surveillance program at the Decatur and Antwerp plants was undertaken to determine if there were any associations between PFOA and hematology, clinical chemistries, and hormonal parameters of volunteer employees (Olsen et al., 2001e). The data were analyzed for all employees from both plant locations. Mean PFOA serum levels were 1.03 ppm for all male employees at the Antwerp plant and 1.90 ppm for all male employees at the Decatur plant. Male production employees at the Decatur plant had significantly higher ($p < .05$) mean serum levels (2.34 ppm) than those at the Antwerp plant (1.28 ppm). Non-production employees at both plants had mean levels below 1 ppm. PFOA serum levels were higher than the PFOS serum values at both plants, especially the Decatur plant where serum levels are higher overall. In addition, values for total organic fluorine were even higher than the PFOA levels.

Multivariable regression analyses were conducted to adjust for possible confounders that may affect the results of the clinical chemistry tests. The following variables were included: production job (yes or no), plant, age, BMI, cigarettes/day, drinks/day and years worked at the plant. A positive significant association was reported between PFOA and cholesterol ($p = .05$) and PFOA and triglycerides ($p = .002$). 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. When both PFOA and PFOS were included in the analyses, neither reached statistical significance in the cholesterol model, while PFOA remained significant ($p = .02$) in the triglycerides model. High-density lipoprotein (HDL) was negatively associated with PFOA ($p = .04$) and

remained significant (p = .04) when both PFOA and PFOS were included in the model. A positive association (p = .01) between T3 and PFOA was also observed and remained statistically significant (p = .05) when PFOS was included in the model. BMI, cigarettes/day, alcohol/day were also significant in the model. None of the other clinical chemistry, thyroid or hematology measures were significantly associated with PFOA in the regression model.

A longitudinal analysis of the above data and previous medical surveillance results was performed to determine whether occupational exposure to fluorochemicals over time is related to changes in clinical chemistry and lipid results in employees of the Antwerp and Decatur facilities (Olsen et al., 2001f). The clinical chemistries included: cholesterol, HDL, triglycerides, alkaline phosphatase, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin. 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 = .0008) and triglycerides (p = .0002) over time. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees (p = .005) and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years (p = .001). However, the association between PFOA and triglycerides was also statistically significant (p = .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, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

There are several limitations to the 2000 cross-sectional and longitudinal studies including:
1) serum PFOA levels were significantly higher at the Decatur plant than at the Antwerp plant, 2) all participants were volunteers, 3) there were several consistent differences in clinical chemistry profiles and demographics between employees of the Decatur and Antwerp plants (Antwerp employees as compared to Decatur employees had lower PFOA serum levels, were younger, had lower BMIs, worked fewer years, had higher alcohol consumption, higher mean HDL and bilirubin values, lower mean triglyceride, alkaline phosphatase, GGT, AST, and ALT values, and mean thyroid hormone values tended to be higher), 4) PFOS and other perfluorinated chemicals are also present in these plants, 5) in the cross-sectional study, plant populations cannot be compared because they were placed into quartiles based on PFOS serum distributions only which were different for each subgroup and not applicable to PFOA, 6) only one measurement at a certain point in time was collected for each clinical chemistry test, and 7) PFOA serum levels overall have been increasing over time in these employees. In addition, in the longitudinal study only a small number of employees participated in all 3 sampling periods (24%), different labs and analytical techniques for PFOA were used each year, and female employees could not be analyzed because of the small number of participants.

3.2.4.2 Cottage Grove Plant–Cross-sectional Studies of Clinical Chemistries and CCK

A voluntary medical surveillance program was offered to employees of the Cottage Grove, Minnesota plant in 1993, 1995, and 1997 (n = 111, 80 and 74 employees, respectively) (Olsen et al., 1998b, 2000). The clinical chemistry parameters (cholesterol, hepatic enzymes, and lipoprotein levels) used in the longitudinal and cross-sectional studies of the Antwerp and Decatur plants were also used in this study. In addition, in 1997 only, cholecystokinin-33 (CCK) was also measured at the Cottage Grove plant. CCK levels were observed because certain research has suggested that pancreas acinar cell adenomas seen in rats exposed to PFOA may be the result of increased CCK levels (Obourn et al., 1997).

Only male employees involved in PFOA production were included in the study. Sixty-eight employees were common to the 1993 and 1995 sampling periods, 21 were common between 1995 and 1997, and 17 participated in all three surveillance years. Cottage Grove has the highest serum PFOA levels of the 3 plants studied.

Employees' serum PFOA levels were stratified into 3 categories (<1, 1- <10, and ≥10 ppm), chosen to provide a greater number of employees in the ⊃10 ppm category. As employees' mean serum PFOA levels increased, no statistically significant ($p < 0.05$) abnormal liver function tests, hypolipidemia, or cholestasis were observed in any of the sampling years. Multivariable regression analyses controlling for potential confounders (age, alcohol consumption, BMI, and cigarettes smoked) yielded similar results. The authors also reported that renal function, blood glucose, and hematology measures were not associated with serum PFOA levels; however, these data were not provided in the paper.

The mean CCK value reported for the 1997 sample was 28.5 pg/ml (range 8.8 - 86.7 pg/ml). The means in the 2 serum categories < 10 ppm were at least 50% higher than in the ⊃ 10 ppm category. A statistically significant ($p = .03$) negative association between mean CCK levels and the 3 PFOA serum categories was observed. A scatter plot of the natural log of CCK and PFOA shows that all but 2 CCK values are within the assay's reference range of 0 - 80 pg/ml. Both of these employees (CCK values of 80.5 and 86.7 pg/ml) had serum PFOA levels less than 10 ppm (0.6 and 5.6 ppm, respectively). 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.

The cross-sectional design is a limitation of this study. Only 17 subjects were common to all 3 sampling years. In addition, the medical surveillance program is a voluntary one. The participation rate of eligible production employees decreased from approximately 70% in 1993 to 50% in 1997. Also, the laboratory reference range changed substantially for ALT in 1997. Finally, different analytical methods were used to measure serum PFOA. Serum PFOA was determined by electrospray high-performance liquid chromatography/mass spectrometry in 1997, but by thermospray in 1993 and 1995.

3M used data collected in their 2000 medical surveillance program to determine whether serum PFOA levels greater than 5 ppm were associated with changes in hepatic, lipid and thyroid function in workers (Olsen, et al., 2003f). Clinical chemistries, including thyroid function tests, were performed for 131 male and 17 female workers at the plant. Serum samples were extracted and quantitatively analyzed for PFOA using HPLC/EMSS. All of the samples were above the lower limit of quantitation (LLOQ) (see Table 24 in Section 4.1.1 for biomonitoring data).

Fifteen percent (n = 20) of male employees had serum concentrations that exceeded 5 ppm, and none of the female employees were above 5 ppm. Number of years worked in the Chemical Division of the plant were not correlated with PFOA serum measurements, but were correlated with specific production areas. When the male employees were separated into 3 groups based on serum PFOA levels (< 1 ppm, 1- 4.9 ppm, and >5 ppm), there were no statistically significant ($p < .05$)

differences in mean lipid and hepatic test results or in thyroid hormone levels, between the 3 groups both before and after adjusting for potential confounders (eg. BMI, smoking status, and alcohol consumption). The elimination of employees receiving cholesterol-reducing drugs (n = 9) from the analysis did not alter these findings. Similar to findings at the Decatur and Antwerp plants, triglyceride levels were higher in employees with the highest PFOA serum concentrations, although not statistically significant ($p < .05$). In simple linear regression analyses, a weak negative association between T4 and serum PFOA concentrations was reported ($p = .07$). However, all of the serum PFOA concentrations were within the T4 reference range, the statistical association explained minimal variation in the model ($r^2 = .03$), there was no increase in serum TSH or T3 levels, and no negative association between free T4 levels and PFOA. No statistically significant ($p < 0.05$) associations between PFOA and clinical chemistries or thyroid test results for the small group of female employees was reported.

An earlier medical surveillance study on workers who were employed in the 1980's was conducted at the Cottage Grove plant; however, total serum fluorine was measured instead of PFOA (Gilliland and Mandel, 1996). Based on animal studies that reported that animals exposed to PFOA develop hepatomegaly and alterations in lipid metabolism, a cross-sectional, occupational study was performed to determine if similar effects are present in workers exposed to PFOA. In a PFOA production facility, 115 workers were studied to determine whether serum PFOA affected their cholesterol, lipoproteins, and hepatic enzymes. Forty-eight workers who were exposed to PFOA from 1985-1989 were included in the study (96% participation rate). Sixty-five employees who either volunteered or were asked to participate, were included in the unexposed group. These employees were assumed to have little or no PFOA exposure based on their job description. However, when serum levels were analyzed, it was noted that this group of workers had PFOA levels much greater than the general population. Therefore, instead of job categories, total serum fluorine was used to classify workers into exposure groups.

Total serum fluorine was used as a surrogate measure for PFOA. Serum PFOA was not measured, due to the cost of analyzing the samples. Blood samples were analyzed for total serum fluorine, serum glutamyl oxaloacetic transaminase (SGOT or AST), serum glutamyl pyruvic transaminase (SGPT or ALT), GGT, cholesterol, low-density lipoproteins (LDL), and HDL. All of the participants were placed into five categories of total serum fluorine levels: <1 ppm, 1-3 ppm, >3 - 10 ppm, >10 - 15 ppm, and > 15 ppm. The range of the serum fluorine values was 0 to 26 ppm (mean 3.3 ppm). Approximately half of the workers fell into the > 1 - 3 ppm category, while 23 had serum levels < 1 ppm and 11 had levels > 10 ppm.

There were no significant differences between exposure categories when analyzed using univariate analyses for cholesterol, LDL, and HDL. In the multivariate analysis, there was not a significant association between total serum fluorine and cholesterol or LDL after adjusting for alcohol consumption, age, BMI, and cigarette smoking. There were no statistically significant differences among the exposure categories of total serum fluorine for AST, ALT and GGT. However, increases in AST and ALT occurred with increasing total serum fluorine levels in obese workers (BMI = 35 kg/m^2). This result was not observed when PFOA was measured directly in serum of workers in 1993, 1995, or 1997 surveillance data of employees of the Cottage Grove plant (Olsen et al., 2000).

Since PFOA was not measured directly and there is no exposure information provided on the employees (e.g. length of employment/exposure), the results of the study provide limited information. The authors state that no adverse clinical outcomes related to PFOA exposure have been observed in these employees; however, it is not clear that there has been follow-up of former employees. In addition, the range of results reported for the liver enzymes were fairly wide for many of the exposure categories, indicating variability in the results. Given that only one sample was taken from each employee, this is not surprising. It would be much more helpful to have several samples taken over time to ensure their reliability. It also would have been interesting to compare the results of the

workers who were known to be exposed to PFOA to those who were originally thought not to be exposed to see if there were any differences among the employees in these groups. There were more of the "unexposed" employees (n = 65) participating in the study than those who worked in PFOA production (n = 48).

Conclusions

Several epidemiology and medical surveillance studies have been conducted on workers employed at various APFO manufacturing sites in the U.S. Most of the studies were cross-sectional and focused primarily on males. A retrospective cohort mortality study demonstrated a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures PFOA. However, in an update to this study in which more specific exposure measures were used, a significant association for prostate cancer was not observed. Other mortality studies lacked adequate exposure data which could be linked to health outcomes. A study which examined hormone levels in workers reported an increase in estradiol levels in workers with the highest PFOA serum levels; however, these results may have been confounded by body mass index. Cholesterol and triglyceride levels in workers were positively associated with PFOA exposures, which is inconsistent with the hypolipidemic effects observed in rat studies. A statistically significant positive association was reported for PFOA and T3 levels in workers but not for any other thyroid hormones.

3.3 Mutagenicity Studies

APFO 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 at one dose level in S. typhimurium TA1537 when tested without metabolic activation was not reproducible. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without metabolic activation. APFO 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 APFO 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, 1996d) tested APFO twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO 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, APFO induced significant increases in chromosomal aberrations and in polyploidy (Murli, 1996b).

APFO was tested in a cell transformation and cytotoxicity assay conducted in C_3H $10T_{½}$ mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation potential. There was no evidence of transformation at any of the dose levels tested in either the colony or foci assay methods (Garry & Nelson, 1981).

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

Conclusions

APFO is not mutagenic. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without metabolic activation. APFO 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. APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO 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, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in C_3H $10T_{½}$ mouse embryo fibroblasts and in the mouse micronucleus assay.

3.4 Acute Toxicity Studies in Animals

Dean and Jessup (1978) reported an oral LD50 of 680 mg/kg and 430 mg/kg for male and female CD rats, respectively. Glaza (1997) reported an oral LD50 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 LD50 of less than 1000 mg/kg for male and female Sherman-Wistar rats. Rusch (1979) reported no mortality in male or female Sprague-Dawley rats following inhalation exposure to 18.6 mg/l for one hour. The dermal LD50 in New Zealand White rabbits was determined to be greater than 2000 mg/kg (Glaza, 1995). Kennedy (1985) determined a dermal LD50 of 4300 mg/kg for rabbits, 7000 mg/kg for male rats, and 7500 mg/kg for female rats. APFO 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 APFO to be a skin irritant in rabbits, while Gabriel (1976b) did not.

Conclusions

In acute toxicity studies in animals, the oral LD50 values for CD rats were >500 mg/kg for males and 250-500 mg/kg for females, and <1000 mg/kg for male and female Wistar rats. There was no mortality following inhalation exposure of 18.6 mg/l for one hour in rats. The dermal LD50 in rabbits was determined to be greater than 2000 mg/kg. The dermal LD50 is 4300 mg/kg for rabbits, 7000 mg/kg for male rats, and 7500 mg/kg for female rats. APFO is a primary ocular irritant in rabbits, while the data regarding potential skin irritancy are conflicting.

3.5 Repeat Dose Studies in Animals

3.5.1 Subchronic Studies in Non-Human Primates

Goldenthal (1978b) administered rhesus monkeys (2/sex/group) doses of 0, 3, 10, 30 or 100 mg/kg-day APFO by gavage for 90 days. Animals were observed twice daily and body weights were recorded weekly. Blood and urine samples were collected once during the control period, and at 1 and 3 months of the study 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 during the study. The first death occurred during week 2; all animals were dead by week 5. Signs and symptoms which first appeared during week 1 included anorexia, frothy emesis which was sometimes brown in color, pale face and gums, swollen face and eyes, slight to severe decreased activity, prostration and body trembling. Three monkeys from the 30 mg/kg-day group died during the study; one male died during week 7 and the two females died during weeks 12 and 13. Beginning in week 4, all four animals showed slight to moder-

ate and sometimes-severe decreased activity. One monkey had emesis and ataxia, swollen face, eyes and vulva, as well as pallor of the face and gums. Beginning in week 6, two monkeys had black stools and one monkey had slight to moderate dehydration and ptosis of the eyelids. 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; frothy emesis was also occasionally noted in this group.

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 (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, the following changes in absolute and relative organ weight changes were noted: absolute and relative weight of the hearts in females from the 10 mg/kg-day group were significantly decreased; absolute brain weight of females from this same group were also significantly decreased and relative group mean weight of the pituitary in males from the 3 mg/kg-day group 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 before the end of the study, 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.

Only one male in the 30 mg/kg-day group survived until terminal sacrifice, and this male also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. No treatment related lesions were seen in the organs of animals from the 3 and 10 mg/kg-day groups.

The levels of PFOA in the serum and liver of these animals are presented below in Table 43. Individual values are presented so there are double entries for most dose levels.

Table 43 Levels of PFOA in the Serum and Liver of Surviving Rhesus Monkeys

Dose	Serum (µg/ml)		Liver (µg/ml)		Liver total (µg)	
	Male	Female	Male	Female	Male	Female
0	ND	1	0.05	0.07	3	5
3	53	65	3	7	250	350
3	48	50	ND	ND	ND	ND
10	45	79	9	ND	600	ND
10	71	71	ND	10	ND	750
30	145	Dead	61	Dead	4000	Dead

ND - Not Determined
Source: Goldenthal 1978b

Under the conditions of this study the LOAEL is 3 mg/kg-day and no NOAEL was established.

Thomford (2001a, b) conducted a range-finding and a 6-month toxicity study in male cynomolgus monkeys. In the range-finding study, Thomford (2001a) administered male cynomolgus monkeys an oral capsule containing 0, 2, or 20 mg/kg-day APFO for 4 weeks. There were 3 monkeys in the 2 and 20 mg/kg-day groups and one monkey in the control group. The monkeys weighed 2.1 to 3.6 kg at the start of treatment. 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 clinical hematology and clinical chemistry analyses, and hormone and PFOA level. Blood for clinical chemistry was also collected from each animal on day 2 (approximately 24 hours after the first dose). Samples were analyzed for estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin.

At scheduled necropsy, samples of the right lateral lobe of the liver were collected from each animal and analyzed for palmitoyl CoA oxidase activity. Representative samples of liver, right and left testes, and pancreas were collected from each animal for cell proliferation evaluation using proliferation cell nuclear antigen. Bile was collected from each animal for bile acid determination. A sample of liver was collected from each animal for PFOA concentration analysis. The adrenals, liver, pancreas, spleen, and testes from each animal were examined microscopically, and the remaining tissues were preserved for possible future examination.

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 estradiol, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin. Estrone levels were notably lower for males given 2 and 20 mg/kg-day APFO. 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.

In the 26-week study, male cynomolgus monkeys were administered APFO by oral capsule at doses of 0, 3, 10 or 30 mg/kg-day for 26 weeks (Thomford 2001b; Butenhoff et al., 2002). At study initiation the monkeys weighed 3.2 to 4.5 kg. There were 4 monkeys in the 3 mg/kg-day group and 6 monkeys in each of the other groups. Dosing of animals in the 30 mg/kg-day dose group was stopped from days 11–21 because of toxicity. When dosing was resumed on day 22, animals received 20 mg/kg-day and this group was designated the 30/20 mg/kg-day group. At the end of the 26-week treatment period, 2 animals in the control and 10 mg/kg-day groups were observed for a 13-week recovery period.

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 done before initiation of treatment and during weeks 26 and 40. Body weights were recorded weekly and food consumption was assessed qualitatively. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis before the start of treatment and on days 11, 31, 63, 91, 182, 217, 245 and 275. Blood samples were also taken for hormone determinations; samples were analyzed for estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, total and free thyroxin, testosterone, and cholecystokinin (CCK). Blood, urine and feces were collected during week 2 and every 2 weeks thereafter during treatment and recovery for PFOA concentration analyses.

At scheduled necropsy, liver samples were taken for determination of PFOA levels. The right lateral lobe of the liver was collected from each animal for palmitoyl CoA oxidase activity analyses, and representative samples of liver, right and left testes, and pancreas were collected from each animal for cell proliferation evaluation using proliferation cell nuclear antigen. All available bile was col-

lected for bile acid determination. Weights of the adrenal glands, brain, epididymis, kidney, liver, pancreas, testis, and thyroid with parathyroid were recorded. The following tissues were collected for histopathology: adrenal (2), aorta, brain, cecum, colon, duodenum, epididymis (2), esophagus, eyes [preserved in Davidson's fixative (2)], femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, jejunum, kidneys (2), lesions, liver, lung, mesenteric lymph node, mammary gland, pancreas, pituitary, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis [(2) preserved in Bouin's solution], thymus, thyroid (2) with parathyroid, trachea and urinary bladder.

Two animals, one male from the 30/20 mg/kg-day dose group and one male from the 3 mg/kg-day dose group, were sacrificed in moribund condition during the study. The male in the 30/20 mg/kg-day dose group was sacrificed on day 29. This animal exhibited signs of hypoactivity, weight loss, few or no feces, low or no food consumption and the entire body was cold to the touch before death. Necropsy revealed esophageal and gastric lesions that were indicative of an injury that occurred during dosing and liver lesions that were presumed to be treatment related. The animal from the 3 mg/kg-day dose group was sacrificed on day 137. This animal showed clinical signs of limited use and paralysis of the hind limbs, ataxia and hypoactive behavior, few feces and no food consumption. The cause of death was not determined, but APFO treatment could not be ruled out.

Males given 30 mg/kg-day from days 1-11 had clinical signs of few feces and low food consumption and they lost weight during week 1 of treatment. Based on these signs, treatment was stopped on day 11 and was not resumed until day 22. When treatment was resumed, the dose was lowered to 20 mg/kg-day; this group was then designated the 30/20 mg/kg-day group. Of the remaining animals in this group, only 2 tolerated this dose level for the remaining 23 weeks of treatment. Treatment of three males given 30/20 mg/kg-day was halted on days 43 (week 7), 66 (week 10), and 81 (week 12) respectively. Clinical signs in these animals included thin appearance, few or no feces, low or no food consumption, and weight loss. The animals appeared to recover from compound-related effects within 3 weeks after cessation of treatment.

Mean body weight changes were notably lower during weeks 1 and 2 for males receiving 30 mg/kg-day. During week 2, this change was statistically significant. Treatment was stopped on day 11 and when it was resumed at 20 mg/kg-day on day 21, mean body weight changes were significantly lower than controls during weeks 7, 9 and 24. Overall mean body weight changes through week 27 were notably lower for the males in the 30/20 mg/kg-day group. There was an increased incidence of low or no food consumption for animals in the 30/20 mg/kg-day group that was considered to be treatment related.

There were no consistent or clearly dose-related effects on estrone, estradiol, estriol, testosterone, or CCK levels in treated groups over time. Similarly, thyroid stimulating hormone and free and total thyroxine levels remained relatively constant and were not significantly altered throughout the study. Mean individual values for free and total triiodothyronine levels were statistically significantly decreased during the dosing period at the 30/20 mg/kg/day dose group compared to controls, although the biological significance of this decrease is unclear.

At terminal sacrifice at 26 weeks, there were statistically significant increases in mean absolute liver weights and mean liver-to-body weight percentages in all dose groups. In addition, there was a positive dose-response trend towards an increased relative liver-to-body weight, with statistical significance at the high dose group of 30/20 mg/kg/day. The increased liver weights were considered to be treatment-related. The increased liver weight was thought to be due, in part, to hepatocellular hypertrophy (as demonstrated by decreased hepatic DNA content) which in turn may be due to mitochondrial proliferation (as demonstrated by increased succinate dehydrogenase activity).

Since administration of APFO to rats results in liver, Leydig cell and pancreatic acinar cell tumors, Butenhoff et al (2002) specifically looked for markers of tumor formation in the monkeys. In the liver, there was only a two-fold increase in hepatic palmitoyl CoA oxidase activity in the 30/20 mg/kg-day group, which is consistent with reports for other species that are not particularly responsive to PPAR•-agonists. Replicative DNA synthesis in the liver, an indication of cell proliferation, was not altered in the treated animals. Similarly, it has been proposed that changes associated with the pancreatic acinar cell tumors in rats include increased serum CCK concentrations and indications of cholestasis, including alkaline phosphatase, bilirubin, and bile acids; none of these changes were noted in the cynomolgus monkeys. Finally, in the rat, it has been proposed that the Leydig cell tumors are due to a sustained increase in estradiol resulting from aromatase induction. In the treated cynomolgus monkeys there were no significant changes in estradiol, estriol, or testosterone. In addition, there was no change in replicative DNA synthesis in the pancreas or testes.

At the recovery sacrifice, there were no treatment-related effects on terminal body weights or on absolute or relative organ weights indicating that the liver weight changes seen at terminal sacrifice were reversible over time. There were no treatment-related macroscopic or microscopic changes at the recovery sacrifice.

Serum and liver concentrations of PFOA did not increase in a dose-related manner. This may have been due to saturation or attaining steady state levels in the first several weeks of the study. In addition, there was a great deal of variability in the PFOA levels. This may have been due to the method of dosing (by capsule), the timing of dosing relative to blood sample collection and gall bladder emptying, or the analytical method ($\pm$ 30% for interassay, intra-assay and system). Since steady state appeared to have been reached by 4-6 weeks of dosing, the study authors calculated the mean serum levels for the period following 6 weeks; steady state serum levels were 77 $\pm$ 39, 86 $\pm$ 33 and 158 $\pm$ 100 µg/ml for the 3, 10 and 30/20 mg/kg-day groups, respectively. In the control animals, 2/3 of the serum samples contained PFOA and averaged a level of 0.203 $\pm$ 0.154 µg/ml. There was not a statistically significant difference in the mean serum levels of the 3 and 10 mg/kg-day groups, but the mean serum level in the 30/20 mg/kg-day group was significantly higher than the 3 and 10 mg/kg-day groups. At terminal sacrifice, the levels of PFOA in the liver were similar in the 3 and 10 mg/kg-day groups, and ranged from 6.29 - 21.9 µg/g. The two monkeys in the 30/20 mg/kg-day group had liver concentrations of 16 and 83.3 µg/g. After the recovery period, the serum and liver PFOA levels in the 10 mg/kg-day group had returned to baseline.

Under the conditions of the study, the LOAEL was 3 mg/kg-day (increased liver weight and possibly mortality) and a NOAEL was not established.

Conclusions

Repeat-dose studies have been conducted in non-human primates. In a 13-week study with Rhesus monkeys, exposure to doses of 30 mg/kg-day or higher resulted in death. Clinical signs of toxicity were noted at doses as low as 3 mg/kg-day. Under the conditions of this study the LOAEL is 3 mg/kg-day and no NOAEL was established. In a 6-month study of male cynomolgus monkeys, there was a steep dose response curve for mortality. Increases in liver weight were noted at doses as low as 3 mg/kg-day, but there was no evidence of peroxisome proliferator-activated receptor alpha activity (PPARα). Under the conditions of the study, the LOAEL was 3 mg/kg-day (increased liver weight and possibly mortality) and a NOAEL was not established.

3.5.2 Subchronic Studies in Rodents

Christopher and Marias (1977) administered ChR-CD mice (5/sex/group) dietary concentrations of 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm of APFO for 28 days. The animals were observed daily and body weights and food consumption were recorded weekly. At necropsy, the organs were weighed, examined for gross pathology and preserved for histopathology. All animals in the 1000 ppm and higher groups died before the end of day 9. All animals in the 300 ppm group died within 26 days except one male. One animal in each of the 30 and 100 ppm groups died prematurely. Clinical signs were observed in mice exposed to 100 ppm and higher doses of APFO. At 100 ppm some animals exhibited cyanosis on days 10 and 11 of testing, but appeared normal throughout the rest of the study. Animals fed 300 ppm exhibited roughed fur and muscular weakness as well as signs of cyanosis after 9 days of treatment. Animals fed 1000 ppm exhibited similar effects after 6 days and those receiving 3000 ppm or greater doses exhibited effects after 4 days. There was a dose-related reduction in mean body weight in all treated groups. Relative and absolute liver weights were increased in mice fed 30 ppm or more APFO. Treatment-related changes were observed in the livers among all APFO treated animals including enlargement and/or discoloration of 1 or more liver lobes. Histopathologic examination of all surviving treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver (panlobular hypertrophy) accompanied by focal to multifocal cytoplasmic lipid vacuoles of variable size which were random in distribution.

Metrick and Marias (1977) administered ChR-CD rats (5/sex/group) dietary concentrations of 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm APFO for 28 days. The animals were observed daily and body weights and food consumption were recorded weekly. At necropsy, the organs were weighed, examined for gross pathology and preserved for histopathology. All animals in the 10,000 and 30,000 ppm groups died before the end of the first week. Gross pathologic examination revealed white foci in the cortex and medulla in the kidneys of a 10,000 ppm female. Pelvic dilation was evident in the kidneys of a control male and a 30,000 ppm female. There were no premature deaths or unusual behavioral reactions in the other groups. Food consumption was reduced in the 1000 and 3000 ppm groups. Body weight gain was reduced as dose increased. The reduction in body weight gain was statistically significant for males at 1000 ppm and males and females at 3000 ppm. Absolute liver weights were increased in males fed 30 ppm or more and females fed 300 ppm or more. Treatment-related morphologic changes were observed in the livers of all test animals. Focal to multifocal cytoplasmic enlargement of hepatocytes in the centrilobular to midzonal areas was noted in animals fed 30 to 300 ppm, and multifocal to diffuse enlargement of hepatocytes throughout the liver lobules (panlobular) was noted in animals fed 1000 ppm or higher. The hypertrophy of hepatocytes was accompanied by acidophilic degeneration and/or necrosis of scattered liver cells with no lobular distribution. The severity and degree of tissue involvement were more pronounced in males than in females.

Goldenthal (1978a) administered ChR-CD rats (5/sex/group) dietary levels of 0, 10, 30, 100, 300, and 1000 ppm APFO for 90 days. These dose levels are equivalent to 0.056, 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 and 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. One female in the 100 and one female in the 300 ppm group died during collection of blood. These deaths were not considered to be treatment related. All other animals survived until scheduled sacrifice. There was a decrease in body weight gain for male rats at the 300 and 1000 ppm dose level. 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 urine 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. 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) and the NOAEL is 300 ppm (22.4 mg/kg-day).

In a dietary study, male ChR-CD rats (45-55 per group) were administered concentrations of 1, 10, 30, or 100 ppm APFO for 13 weeks (Palazzolo 1993; Perkins et al., 2004). These doses are equivalent to 0.06, 0.64, 1.94, and 6.50 mg/kg-day. Two control groups (a nonpair-fed control group and a control group pair-fed to the 100 ppm dose group) were fed basal diet during that period. 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 APFO 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 APFO. No treatment-related clinical signs were noted. At 100 ppm, significant reductions in body weights 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 100 ppm, mean body weight gains were significantly higher than the pair-fed control group during week 1 and significantly lower than the nonpair-fed control group during weeks 1-13. 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 in the serum 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. There was no evidence of any degenerative changes or abnormalities associated with the hypertrophy. 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, none of the liver effects were observed, indicating that these treatment-related liver effects were reversible.

Serum levels from the male rats in this study are shown below in Table 44.

Table 44 Summary of Serum Values (µg/ml) in Male Rats Treated with APFO for 90 Days

	Dose Group (mg/kg-day)			
Week	0.06	0.64	1.94	6.50
4	6.5 ± 1.0[a] (8)	55 ± 8.1 (9)	104 ± 14 (8)	159 ± 30 (10)
7	7.5 ± 1.3 (9)	46 ± 16 (10)	87 ± 28 (10)	149 ± 35 (10)
13	7.1 ± 1.2 (10)	41 ± 13 (10)	70 ± 16 (10)	138± 34 (10)
21	1.2 (1)	1.1± 1.3 (3)	1.6 ± 0.9 (3)	2.5± 0.9 (2)

a Mean ± SD, (N)
Source Perkins et al., 2004

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).

Conclusions

Repeat-dose studies in rats and mice demonstrated that the liver is the primary target organ. Due to gender differences in elimination, adult male rats exhibit effects at lower administered doses than adult female rats. In a 90-day dietary study of APFO in rats, the LOAEL was 10 ppm (0.64 mg/kg-day) based on increases in absolute and relative liver weights with hepatocellular hypertrophy, and the NOAEL was 1.0 ppm (0.06 mg/kg-day).

3.6 Carcinogenicity Studies in Animals

3.6.1 Chronic and Carcinogenicity Studies in Rats

The chronic toxicity and carcinogenic potential of PFOA has been investigated in two dietary studies in rats. In the first study (Sibinski, 1987), groups of 50 male and 50 female Sprague-Dawley (Crl:CD BR) rats were fed diets containing 0, 30 or 300 ppm APFO for two years. Groups of 15 additional rats per sex were fed 0 or 300 ppm APFO and evaluated at the one year interim sacrifice. In males. the mean test article consumption was 1.3 and 14.2 mg/kg-day for the 30 and 300 ppm groups, respectively; in females, the mean test article consumption was 1.6 and 16.1 mg/kg-day for the 30 and 300 ppm groups, respectively. All animals were observed daily throughout the two year

dosing period. Body weights and feed consumption were recorded once per week for the first six months, and then once every two weeks for the remainder of the study. Clinical pathological examinations including hematology, serum chemistry and urinalysis were conducted on samples obtained from 15 rats per sex from each group at 3, 6, 12, 18 and 24 months. Macroscopic postmortem examinations were performed on all animals that died during the study and those which were terminated at the one year interim and two year necropsies. The weights of the kidneys, liver, testes, brain, heart, spleen, adrenal glands and uterus were recorded for 15 randomly selected rats/sex at the interim termination from both the control and high-dose groups, and from the control and both treated groups at the two year necropsy. Microscopic evaluation was performed on all tissues from all of the control and high-dose rats.

There was a dose-related decrease in body weight gains in the male rats and to a lesser extent, in the female rats as compared to the controls; the decreases were statistically significant in the high-dose groups of both sexes. In the high-dose (300 ppm) males, there was a 21% decrease in body weight gain compared to the control males by week 6 and the body weight gains were decreased over 10% compared to the control males through 66 weeks of the study. This difference was statistically significant until week 98. In the low-dose (30 ppm) males, a 5% decrease in body weights was observed at week 6, however, there was little additional decrease thereafter. Mean body weights were only slightly decrease in the treated females compared to the control values through the first 18 months of the study but reached a maximum of 11% decrease in the high-dose females at 92 weeks. The body weight changes are treatment related since feed consumption was actually increased (rather than decreased).

There were no differences in mortality between the treated and untreated groups; the survival rates at the end of 104 weeks for the male control, low-, and high-dose groups were 70%, 72% and 88%, respectively; in females, the survival rates were 50%, 48% and 58% for the control, 30 and 300 ppm groups, respectively. The only clinical sign observed was a dose-related increase in ataxia in the female rats; which was most commonly associated with moribund animals; the incidences in the control, low- and high-dose groups were 4%, 18% and 30%. 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 ($P< 0.05$) in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) in both treated male groups from 3-18 months, but only in the high-dose males at 24 months. Slight (up to about 10%) increases in absolute or relative liver and kidney weights were noted in both high-dose male and female rats at the 1 year interim sacrifice and at the terminal necropsy; however, only the relative liver weight (vs. body weight or brain weight) increases in the high-dose males were statistically significant ($P<0.05$).

Histologic evaluations showed lesions in the liver, testis and ovary. In the liver, the increased incidence of lesions reached statistical significance only in the high-dose male group. At the 1 year interim sacrifice, diffuse hepatomegalocytosis (12/15 animals), portal mononuclear cell infiltration (13/15 animals) and hepatocellular necrosis (6/15 animals) were seen in the high-dose males while incidences in the control group were, 0/15, 7/15 and 0/15, respectively. Hepatocellular vacuolation was seen in 11/15 high-dose females as compared to an incidence of 5/15 in the control group. At the 2-year sacrifice, megalocytosis was found at an incidence of 0%, 12% and 80% in the males, and 0%, 2% and 16% in the females from the control, low-, and high-dose groups, respectively. Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, was observed in 14% and 56% of the low- and high-dose males, as compared to a control incidence of 8%. The incidence of hyperplastic nodules, a localized proliferation of hepatic parenchymal cells, was slightly increased in the high-dosed males with an incidence of 6% as compared to 0% in the control males.

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. Furthermore, marked aspermatogenesis was found in 2/15 high-dose males but none in the control males. At the 2-year sacrifice, vascular mineralization of the testes occurred in 18% of the high-dosed male and 6% of the low-dosed males, but was not seen in the controls. These testicular effects reached statistical significance in the high-dose group.

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. Recently, however, slides of the ovaries from that study were re-evaluated, with particular emphasis placed on the proliferative lesions of the ovary (Mann and Frame, 2004). 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 45. 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 45 Incidence of Ovarian Stromal Hyperplasia and Adenoma in Rats

Group	Control	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)

The non-cancer effects in this study, the LOAEL for male rats is 300 ppm and the NOAEL is 30 ppm based on a decrease in body weight gain, increase in liver and kidney weights and toxicity in the hematological and hepatic systems. The LOAEL for female rats is 300 ppm based on a decrease in body weight gain and hematologic effects and the NOAEL is 30 ppm.

At the termination of the study, there was a significant increase ($P<0.05$) in the incidence of testicular (Leydig) cell adenomas in the high-dose male rats. The incidence of the Leydig cell tumors (LCT) 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 LCT in 2-year old Sprague-Dawley rats in other studies was reported to be approximately 5% (cited in Clegg et al., 1997).

The report also indicated a significant increase ($P<0.05$) in the incidence of mammary fibroadenomas in both groups of female rats. The incidence of the mammary fibroadenoma was 21% (10/47), 40% (19/47) and 43% (21/49) in the control, 30, and 300 ppm groups, respectively. 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 (Chandra et al., 1992). The investigators 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, which is considered an inappropriate historical reference. When the mammary fibroadenoma incidences were compared to the historical control incidence (37%) in 947 female rats in the Haskell Laboratory, however, there does not appear to be any compound related effect (Sykes,1987).

The mammary gland findings were re-examined recently by a Pathology Working Group (Hardisty, 2005) using 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 or total malignant neoplasms of the mammary glands between control and treated animals using Fischer's Exact Test for pair-wise comparision. 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 which the PWG classified as fibroadenoma, mostly in the control group. According to the PWG, the incidence of mammary fibroadenoma in the control, low- and high-dose groups were: 32% (16/50), 32% (16/50), and 40% (20/50), respectively.

The induction of Leydig cell tumors was confirmed in a follow-up 2-year mechanistic study of PFOA toxicity in male Sprague-Dawley rats at a dietary level of 300 ppm (Cook et al., 1994; Biegel et al., 2001). There were 156 animals in the treatment group and 80 animals in the control group. Cage-side examinations were conducted at least once daily throughout the study. Rats were weighed once a week during the first 3 months and once every other week for the remainder of the study. Rats were euthanized at interim time points of 1, 3, 6, 9, 12, 15, 18, and 21 months. At each time point, the liver and testis from 6 rats/group were weighed and evaluated for cell proliferation. Another 6 rats/group were selected for peroxisome proliferation, and 10 rats/group for serum hormone (estradiol, testosterone, LH, FSH, and prolactin) analysis. All rats surviving the 24-month test period were necropsied for microscopic examination of various organs: e.g., kidneys, liver, testes, brain, heart, spleen.

In the treated group, relative liver weights and hepatic β-oxidation activity were statistically significantly increased at all of the sampling time points when compared to the controls. Absolute testis weights were increased only at 24 months. No hepatic or Leydig cell proliferation was observed at any of sampling times. There were no significant differences in serum testosterone, FSH, LH, or prolactin in the PFOA-treated rats when compared to the controls. There were, however, significant increases in serum estradiol concentrations in the treated rats at 1, 3, 6, 9, and 12 months.

There was a significant increase in the incidence of LCT in the treated rats 11% (8/76) as compared to the controls (0%, 0/80). In addition, the treated group had a significant increase in the incidences of liver adenomas and pancreatic acinar cell tumors (PACT). The incidences of liver adenomas in the control and treated groups were 3% (2/80) and 13% (10/76), respectively, whereas those for the pancreatic acinar cell adenomas were 0% (0/80) and 9% (7/76). There was one pancreatic acinar cell carcinoma in 76 of the treated rats and none in 80 controls. The incidence of combined pancreatic acinar cell adenoma/carcinoma in the treated rats (11%, 8/76) was significantly increased as compared with the controls (0%, 0/80).

In the first carcinogenicity study (Sibinski, 1987), there was no reported increase in the incidence of PACT, 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 indicates 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 second study compared to the first study. The difference between pancreatic acinar hyperplasia (reported in Sibinski, 1987) and adenoma (reported in Cook et al., 1994; Biegel et al. 2001) in the rat is a reflection of arbitrary diagnostic criteria and nomenclature by different pathologists. The basis for the quantitative difference in the lesions observed in not known but was believed to be due most likely to difference in the diets used in the two laboratories (Frame and McConnell, 2003).

Conclusions

Two carcinogenicity studies of PFOA have shown that PFOA induced liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumors in male Sprague-Dawley rats. PFOA has also been shown to promote liver carcinogenesis in rodents (Abdellatif et al.,1991; Nilsson et al., 1991). The evidence for mammary fibroadenomas in the female rats is equivocal since the incidences were comparable to some historical background incidences and a recent Pathology Working Group (Hardisty, 2005) concluded that there were no statistically significant differences in the incidence of fibroadenoma or other neoplasms of the mammary glands between control and treated animals.

3.6.2 Mode of Action Studies

The modes of carcinogenic action of PFOA-induced LCTs and PACT have not been fully elucidated (Klaunig et al., 2004). However, as described below, there is ample evidence that the liver tumors in rats are due to a PPARα-agonist mode of action (MOA).

3.6.2.1 Mode of Action Analysis of Liver Adenomas in Rats

There are a number of possible modes of action for hepatocarcinogenesis of chemicals. The weight of evidence of short-term genotoxicity assays on PFOA suggest that PFOA is not a DNA-reactive compound.

As mitochondria play a major role in cell signaling and apoptotic modes of cell death, and several structurally related perfluorinated compounds have been shown to manifest their toxicity by interfering with mitochondria biogenesis and bioenergetics, the effects of PFOA on mitochondrial biogenesis and bioenergetics were investigated. A number of studies have shown that mitochondria biogenesis in liver was increased following treatment of rats with PFOA (*e.g.*, Berthiaume and Wallace, 2002). PFOA has also been demonstrated to uncouple oxidative phosphorylation in mitochondria of the liver from rats exposed to PFOA in the 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 the surface-active property of the compound (Starkov and Wallace, 2002). Treatment with 200 µm PFOA was found to cause a dramatic increase in the cellular content of reactive oxygen species (ROS) in human hepG2 cells. The activation of caspase-9 and apoptosis by PFOA observed was postulated to be the result of the disruption of mi-

tochondria membrane and accumulation of ROS (Panaretakis et al., 2001). Further research is needed, however, to elucidate how apoptosis is involved in tumorigenesis of PFOA.

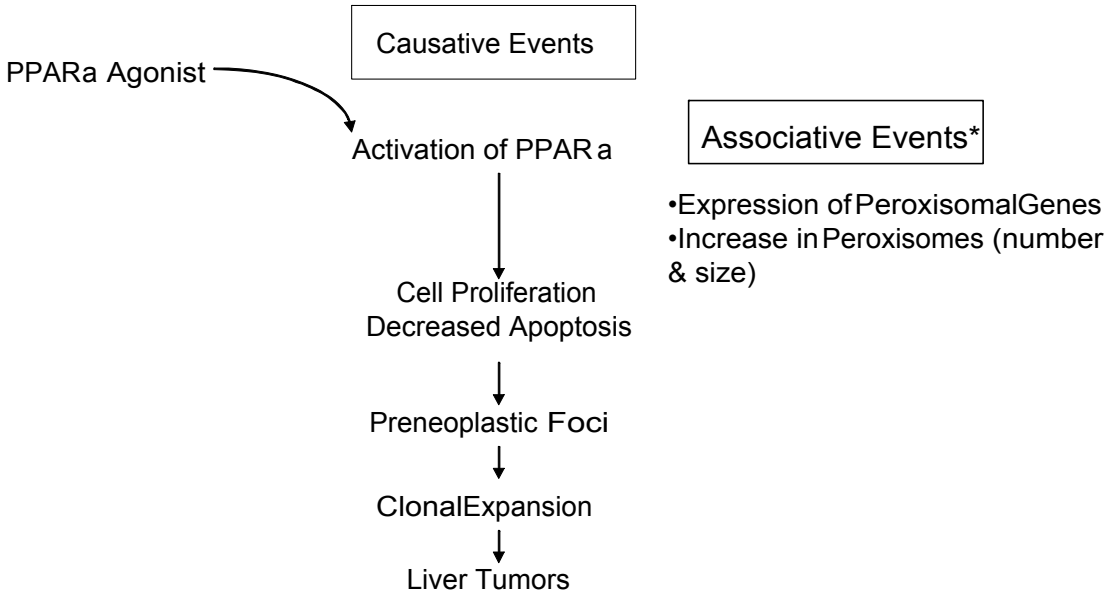
Gap junctional intercellular communication (GJIC), a process by which cells exchange ions, second messages, and other small molecules, is important for normal growth, development, and differentiation, as well as maintenance of homeostasis in muticellular organisms. Because tumor formation requires loss 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). Since inhibition of GJIC is a widespread phenomenon, and the effect by PFOA was neither species nor tissue specific and was reversible, the significance of GJIC inhibition in regard to the mode of carcinogenic action of PFOA is unknown.

Estrogen has been shown to promote hepatocarcinogenesis in rats (Yager and Yager, 1980; Cameron et al., 1982). However, more research is needed to support the involvement of this MOA in the hepatocarcinogenesis of PFOA.

Low and sporadic incidences of liver necrosis were noted in both control and treated rats in the subchronic and chronic toxicity studies of PFOA. Liver necrosis and regenerative cell proliferation may play a role in promoting pre-initiated cells in carcinogenesis. The involvement of necrosis in the liver tumor induction by PFOA in rats remains to be investigated.

While available data are not sufficient to support any of the above MOAs for the liver tumor induction by PFOA, there is strong evidence to conclude that the liver adenomas that are observed in rats following exposure to PFOA result from a PPARα-agonist MOA. As described in Klaunig et al. (2003), the MOA of PPARα-agonist induced liver tumors involves four causal key events which are shown in Figure 4. The first key event is activation of PPARα (which regulates the transcription of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism). Activation of PPARα leads to an increase in cell proliferation and a decrease in apoptosis, which in turn leads to preneoplastic cells and further clonal expansion and formation of liver tumors. Of these key events, only PPARα activation is highly specific for this MOA while cell proliferation/apoptosis and clonal expansion are common to other modes of action. As depicted in Figure 4, there are also several "associative" events that are markers of PPARα agonism but are not directly involved in the etiology of liver tumors. These include peroxisome proliferation (a highly specific indicator that this MOA is operative) and peroxisomal gene expression. Peroxisomal proliferation may also result in hepatocyte oxidative stress which may contribute to the mode of action by causing indirect DNA damage and leading to mutations, or by stimulating cell proliferation. However, increases in oxidative damage to DNA have not been unambiguously demonstrated for PPARα agonists. Oxidative stress is a general phenomenon, and thus does not represent a highly specific marker for PPARα-agonist induced liver carcinogenesis.

Information that would help establish that a chemical is inducing liver tumors via a PPARα agonist MOA includes *in vitro* evidence of PPARα agonism (*i.e.*, evidence from an in vitro receptor assay), *in vivo* evidence of an increase in number and size of peroxisomes, increases in the activity of acyl CoA oxidase, and hepatic cell proliferation. The *in vivo* evidence should be collected from studies designed to provide the data needed to show dose-response and temporal concordance between precursor events and liver tumor formation. Other information that is desirable and may strengthen the weight of evidence for demonstrating that a PPARα agonist MOA is operative includes data on hepatic CYP4A1 induction, palmitoyl CoA activity, hepatocyte hypertrophy, increase in liver weights, decrease in the incidence of apoptosis, increase in microsomal fatty acid oxidation, and enhanced formation of hydrogen peroxide.



*Although there are other biological events (e.g., Kupffer cell mediated events, inhibition of gap junctions), the measurements of peroxisome proliferation and peroxisomal enzyme activity (in particular acyl-CoA) are widely used as reliable markers of PPAR a activation.

Figure 4 Key events in the mode of action for PPARα-agonis induced rodent liver tumors

There is sufficient information to demonstrate the key events for a PPARα agonist MOA following exposure to PFOA in rodents. It has been well documented that PFOA is a potent peroxisome proliferator, inducing peroxisome proliferation in the liver of rats and mice (*e.g.*, Ikeda et al., 1985; Pastoor et al., 1987; Sohlenius et al., 1992). Maloney and Waxman (1999) demonstrated that PFOA activates PPARα using COS1 cells transfected with a luciferase reporter gene. Maximal transcriptional activity with PFOA was seen at 10 M in the mouse PPARα (and at 20 M in human PPAR). Like many other peroxisome proliferators, PFOA has also been shown to cause hepatomegaly (an early biomarker of peroxisome proliferator hepatocarcinogenesis) in rats (Takagi et al., 1992; Cook, 1994) and mice (Kennedy, 1986), and induce oxidative DNA damage in liver of rats (Takagi et al., 1991).

Several studies have been conducted to examine the dose-response and temporal relationships among key endpoints. 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). The results are summarized in Table 46. 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. There was no evidence of hepatocellular necrosis.

Table 46 Summary of Liver Effects in Male Sprague-Dawley Rats Fed APFO for 90 Days

Parameter	Week	Dose (mg/kg-day)[1]					
		0 [a]	0 [b]	0.06	0.64	1.94	6.5
Palmitoyl CoA	4	8 (0.5)	5 (0.4)	9 (1.7)	14 (3.8)[c]	24 (11.4)[c]	37 (14.8)[cd]
Oxidase (IU/G)	7	7 (1.5)	7 (1.5)	7 (0.8)	18 (5.5)	32 (12.2)[c]	54 (35.3)[cf]
	13	8 (0.9)	5 (1.1)	8 (1.9)	10 (2.1)	14 (3.4)[c]	17 (4.5)[cd]
Hepatocellular	4	0/15	0/15	0/15	12/15	15/15	14/15
Hypertrophy	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	4	0/15	1/15	0/15	0/15	1/15	2/15
Necrosis,	7	0/15	0/15	0/15	0/15	0/15	1/15
Coagulative	13	0/15	0/15	1/15	0/15	1/15	0/15
Absolute Liver	4	16.34	15.83	15.45	17.89	23.23	25.44
Weight (g)		(2.14)	(1.13)	(1.71)	(2.13)	(2.83)[c]	(1.89)[cd]
	7	17.78	16.91	17.68	19.42	27.76	27.76
		(2.12)	(2.22)	(??)	(2.10)	(3.51)[c]	(3.51)[cd]
	13	19.73	16.30	18.03	20.44	22.74	26.78
		(2.01)	(1.62)	(2.81)	(2.87)	(4.21)	(5.47)[cd]
Liver/Body	4	3.97	4.07	3.73	4.48	5.77	6.73
Weight (%)		(0.37)	(0.27)	(0.23)	(0.32)[d]	(0.60)[d]	(0.49)[de]
	7	3.75	3.76	3.64	4.12	5.14	6.06
		(0.29)	(0.37)	(0.33)	(0.37)	(0.53)[c]	(0.59)[cd]
	13	3.53	3.23	3.24	3.69	4.21	5.49
		(0.28)	(0.23)	(0.30)[c]	(0.32)	(0.56)[c]	(0.84)[cd]

1- Mean (SD)
a - non-pair-fed controls
b - pair-fed controls
c - statistically significant at $p < 0.05$ with the non-pair-fed control
d - statistically significant at $p < 0.05$ with the pair-fed control
e - calculated using the non-pair-fed control
f - calculated using the pair-fed control

Liu et al. (1996) characterized the dose-response relationships of several key endpoints in male CD rats exposed to doses of 0.2, 2, 20, and 40 mg/kg-day PFOA for 14 days. These endpoints included liver weight, hepatic β-oxidation, hepatic aromatase (P450 19A1), and hepatic total cytochrome P450. The NOEL for these endpoints was found to be 0.2 mg/kg-day with significant changes observed at $\geq$ 2 mg/kg-day for all endpoints. Thus, the studies of Palazzolo (1993) and Liu et al. (1996) demonstrate evidence of peroxisome proliferation at doses close to and below doses that result in liver adenomas following chronic exposures (1.5 and 15 mg/kg-day).

Biegel et al. (2001) examined the temporal relationship between relative liver weights, hepatic β-oxidation, and hepatic cell proliferation, and hepatic adenomas were evaluated in CD rats following PFOA exposure for 1, 3, 6, 9, 12, 15, 18, 21, and 24 months. Relative liver weights and hepatic β-oxidation were increased at all time points. Hepatic cell proliferation was numerically increased relative to the pair-fed control at 9, 15, 18, and 21 months. The liver endpoints (weight, β-oxidation,

and cell proliferation) were all elevated well before the first occurrence of liver adenomas, which occurred after 12 months of treatment.

Significant increases in liver weight were also observed in wild-type mice exposed to PFOA, or Wy-14,643 (a benchmark peroxisome proliferator) in their diet at 0.02% and 0.125% (w/w), respectively, for 7 days (Yang et al., 2002a). This response was totally absent upon treatment of PPARα-null mice with Wy-14,643, but, surprisingly, virtually unaltered in null mice exposed to PFOA. The finding appears to suggest that hepatomegaly may be induced by PFOA independent of PPARα in mice. Since this is only a short-term study in mice, further research is required to demonstrate if the hepatomegaly induced by PFOA in rat is PPARα-independent.

Conclusions

The data clearly demonstrate that PFOA induces liver toxicity and adenomas via a PPARα agonist MOA in rats. PFOA activates the PPARα and the requisite dose-response and/or temporal associations of the key events for the PPARα mode of action with the liver adenomas have been characterized.

3.7 Prenatal Developmental Toxicity Studies in Animals

Several prenatal developmental toxicity studies of APFO have been conducted. These include two oral studies in rats, one oral study in rabbits, one oral study in mice, and one inhalation study in rats.

Gortner (1981) administered time-mated Sprague-Dawley rats (22 per group) doses of 0, 0.05, 1.5, 5, and 150 mg/kg-day APFO in distilled water by gavage on gestation days (GD) 6-15. Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning. The remaining fetuses were subjected to skeletal examination using alizarin red.

Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15 at the high-dose group of 150 mg/kg-day. Mean maternal body weight on GD 20 continued to remain lower than controls, although the difference was not statistically significant. Other signs of maternal toxicity that occurred only at the high-dose group included ataxia and death in three rat dams. No other effects were reported. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract of the dams. Under the conditions of the study, a NOAEL of 5 mg/kg-day and a LOAEL of 150 mg/kg-day for maternal toxicity were indicated.

A significantly higher incidence in fetuses with one missing sternebrae was observed at the high-dose group of 150 mg/kg-day; however this skeletal variation also occurred in the controls and the other three dose groups (at similar incidence but lower than the high-dose group) and therefore was not considered to be treatment-related. No significant differences between treated and control groups were noted for other developmental parameters that included the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights. Likewise, a fetal lens finding initially described as a variety of abnormal morphological changes localized to the area of the embryonal nucleus, was later determined to be an artifact of the free-hand sectioning technique and therefore not considered to be treatment-

related. Under the conditions of the study, a NOAEL for developmental toxicity of 150 mg/kg-day (highest dose group) was indicated.

Staples et al. (1984) also conducted a developmental toxicity study of APFO in rats. The study design consisted of an inhalation and an oral portion, each with two trials or experiments. In the first trial the dams were sacrificed on GD 21; while in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35-post partum. For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to 0, 0.1, 1, 10, and 25 mg/m^3 APFO by whole-body dust inhalation for 6 hours/day, on GD 6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg-day APFO in corn oil by gavage on GD 6-15. For both routes of administration, females were mated on an as-needed basis and when the number of mated females was bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. Finally, two additional groups (six dams per group) were added to each trial that was pair-fed to the 10 and 25 mg/m^3 groups.

For trial one, 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.

For trial two, in which the dams were allowed to litter, the procedure was the same as that for trial one up to 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 Day 1 PP. Dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. On Day 23 PP all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. The eyes of the pups were also examined on Days 15 and 17 PP for the inhalation portion and on Days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on Day 35 PP and examined for visceral and skeletal alterations.

In trial one of the inhalation study, 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 both 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 groups ($p = 0.002$) and in the control group pair-fed 25 mg/m^3 ($p = 0.001$). However, 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 trial two of the inhalation study, 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 Day 1 PP (6.1 g at 25 mg/m^3 vs. 6.8 g in controls). On Days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant (Day 4 PP: 9.7 g at 25 mg/m^3 vs.10.3 in controls; Day 22 PP: 49.0 g at 25 mg/m^3 vs. 50.1 in controls). No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Again, interpretation of these effects is problematic given the high incidence of maternal mortality. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

In trial one of the oral study, three out of 25 dams died during treatment of 100 mg/kg APFO during gestation (one death on GD 11; two on GD 12). Clinical signs of maternal toxicity in the dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Likewise, no significant differences between treated and control groups were noted for fetal weights, or in the incidences of malformations and variations; nor were there any effects noted following microscopic examination of the eyes.

In trial two of the oral study, similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted.

An oral prenatal developmental toxicity study was conducted in rabbits (Gortner, 1982). Based on the results of a range-finding study, an upper dose level of 50 mg/kg-day was set for the definitive study in which four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg-day APFO in distilled water by gavage on gestation days 6-18. Pregnancy was established in each sexually mature female by i.v. injection of pituitary lutenizing hormone in order to induce ovulation, followed by artificial insemination with 0.5 ml of pooled semen collected from male rabbits; the day of insemination was designated as day 0 of gestation. A constant dose volume of 1 ml/kg was administered. Individual body weights were measured on GD 3, 6, 9, 12, 15, 18, and 29. The does were observed daily on GD 3-29 for abnormal clinical signs. On GD 29, the does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in a 37^0 C incubator for a 24-hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities. A blood sample was taken from six does prior to dosing and then on GD 18 and 29; a liver sample was taken from the same animals on GD 29. All samples were sent to the sponsor for analysis. This information was unavailable at the time of this review.

Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD 12-29. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contents of the does. No clinical or other treatment-related signs were reported. Under the conditions of the study, a NOAEL of 50 mg/kg-day, the highest dose tested, for maternal toxicity was indicated.

No significant differences were noted between controls and treated groups for the number of male and female fetuses, dead or live fetuses, or fetal weights. Likewise, there were no significant differences reported for the number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate, or the 24-hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13^{th} rib, with statistical significance at the high-and mid-dose groups (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). The incidence of 13^{th} rib at the low-dose group occurred at a slightly higher rate, but was not statistically significantly different from controls. Therefore, under the conditions of the study, a LOAEL for developmental toxicity of 5 mg/kg-day was indicated.

Lau et al. (2005) recently conducted a developmental toxicity study of APFO in mice in order to ascertain whether there was a sex difference in the bioaccumulation of APFO in the mouse, and to evaluate the effects of APFO 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 APFO daily by oral gavage on GD 1-17. Maternal weight was monitored during gestation. Dams were divided into two groups. In the first group, dams were sacrificed on GD 18 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 APFO serum concentration. APFO 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 APFO was administered on GD 18. Dams were allowed to give birth on GD 19. The day following parturition was designated as postnatal day (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.

In order to compare the body burdens of APFO between rat and mouse after subchronic exposure, a separate study was conducted in which 0 mg/kg/day, 10 mg/kg (for 20 days) and 20 mg/kg (for 17 days) APFO was administered by gavage to adult male and female rats and mice. Twenty-four hours after the last treatment, the animals were sacrificed and blood samples collected for subsequent APFO serum analyses.

Serum concentrations of PFOA for controls and treated animals were analyzed by high-performance liquid chromatography with mass spectrometry by spiking aliquots of thawed serum sample with ^{13}C-PFOA internal standard.

Signs of maternal toxicity were observed following exposure to APFO during pregnancy. Slight, but statistically significant ($p \leq 0.05$), increases in the average time to parturition were observed at 10 and 20 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. Statistically significant ($p \leq 0.05$) dose-related increases in maternal liver weight were also observed, beginning at 1 mg/kg/day.

Signs of developmental toxicity were observed following in utero exposure to APFO. Statistically significant ($p \leq 0.05$) increases in full-litter resorptions were reported at doses of ≥ 5 mg/kg/day,

with complete loss of pregnancies at the highest dose group of 40 mg/kg/day. However, the number of implantations in treated groups was comparable to controls, which according to the authors, may indicate the pregnancy losses may have occurred shortly after implantation. In litters with live fetuses at term, statistically significant ($p \leq 0.05$) increases in prenatal loss were observed in the 20 mg/kg/day dose group. 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. Statistically significant ($p \leq 0.05$) increases in delayed or reduced ossification of various bones were observed at doses of 10 and 20 mg/kg/day. 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. A 20% reduction ($p \leq 0.05$) in live fetal body weight at term was reported at 20 mg/kg/day. At doses $\geq$ 3mg/kg/day, a trend in growth retardation ($p \leq 0.05$), as manifested by reductions (25-30%) in body weights, was observed in the neonates at weaning. Body weights returned to control levels by 6 weeks of age for females, and by 13 weeks of age for males, with body weights exceeding those of controls by 10% as the animals continued to age. 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 20mg/kg/day in females; which was in contrast to significant accelerations ($p \leq 0.05$) in sexual maturation observed in males, with preputial separation occurring 4 days earlier than controls at the 1 mg/kg/day; and 2-3 days earlier in the 3 through 10 dose groups; but not at the 20 mg/kg/day dose group, which was slightly delayed compared to controls.

Serum analyses in adult male and female rats following subchronic exposures indicated differences in the accumulation of APFO. Following subchronic exposures of 20 days, serum concentration of APFO in male rats exposed to 10 mg/kg APFO was 111 µg/ml, while only 0.69 µg/ml was detected in female rats. This is in contrast to what was observed in adult mice; no significant differences in serum APFO concentrations were observed between the genders. In fact, a steady-state in APFO serum concentration in mice was reached by 7 days and remained fairly constant for the rest of the exposure period of 17 days. Serum levels in mice exposed to 20 mg/kg APFO recorded at 7 and 17 days of treatment were the same for both males and females, approximately 180-200 µg/ml. Serum analysis for pregnant mice revealed a dose-dependent increase in accumulation of APFO at term. Based on the results observed in the study, the authors calculated various benchmark dose values for maternal and developmental toxic endpoints:

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.

For developmental toxicity, BMD_5 and $BMDL_5$ estimates for the incidence of full-litter resorptions and neonatal mortality (determined by survival to weaning) observed at the 5 mg/kg/day dose group 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.

According to the study authors, the results of the current study in mice suggest significant differences in the developmental toxicity profile than that previously reported in rats. The authors concluded that these differences in toxicity could be due, in part, to the differential pharmacokinetic handling and disposition of APFO between rats and mice.

Conclusions

In an oral prenatal developmental toxicity study in rats, the LOAEL and NOAEL for maternal toxicity were 150 mg/kg-day and 5 mg/kg-day, respectively. There was no evidence of developmental toxicity after exposure to doses as high as 150 mg/kg-day. In a rat inhalation developmental toxicity study, the NOAEL and LOAEL for maternal toxicity were 1 and 10 mg/m^3, respectively. The NOAEL and LOAEL for developmental toxicity were of 10 and 25 mg/m^3, respectively. In a rabbit oral prenatal developmental toxicity study there was a significant increase in skeletal variations after exposure to 50 mg/kg-day APFO. There was no evidence of maternal toxicity at 50 mg/kg-day, the highest dose tested. In a mouse oral developmental toxicity study, there was evidence of maternal toxicity (decreased weight gain and increased liver weight; the BMD_5 and $BMDL_5$ estimates for decreases in maternal weight gain were 6.76 and 3.58 mg/kg, respectively, and the BMD_5 and $BMDL_5$ estimates for increases in maternal liver weight were 0.20 mg/kg and 0.17 mg/kg, respectively. There was also evidence of developmental toxicity in the mouse study and the authors calculated benchmark doses for a variety of endpoints. The BMD_5 and $BMDL_5$ estimates for the incidence of full-litter resorptions and neonatal mortality (determined by survival to weaning) observed at the 5 mg/kg/day dose group 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.

3.8 Reproductive Toxicity Studies in Animals

An oral two-generation reproductive toxicity study of APFO in rats was conducted (York, 2002; Butenhoff et al., 2004a). Five groups of 30 Sprague-Dawley rats per sex per dose group were administered APFO by gavage at doses of 0, 1, 3, 10, and 30 mg/kg-day six weeks prior to and during mating. Treatment of the F0 male rats continued until mating was confirmed, and treatment of the F0 female rats continued throughout gestation, parturition, and lactation.

The F0 animals were examined twice daily for clinical signs, abortions, premature deliveries, and deaths. Body weights of F0 male rats were recorded weekly during the dosage period and then on the day of sacrifice. Body weights of F0 female rats were recorded weekly during the pre- and cohabitation periods and then on gestation days (GD) 0, 7, 10, 14, 18, 21, and 25 (if necessary) and on lactation days (LD) 1, 5, 8, 11, 15, and 22 (terminal body weight). Food consumption values in F0 male rats were recorded weekly during the treatment period, while in F0 female rats, values were recorded weekly during the precohabitation period, on GDs 0, 7, 10, 14, 18, 21, and 25 and on LDs 1, 5, 8, 11, and 15.

Estrous cycling was evaluated daily by examination of vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until confirmation of mating by the presence of sperm in a vaginal smear or confirmation of a copulatory plug. On the day of scheduled sacrifice, the stage of the estrous cycle was assessed.

For mating, one male rat and one female rat per group were cohabitated for a maximum of 14 days. Female rats with evidence of sperm in a vaginal smear or copulatory plug were designated as GD 0. Parental females were evaluated for length of gestation, fertility index, gestation index, number and sex of offspring per litter, number of implantation sites, general condition of the dam and litter during the postpartum period, litter size and viability, viability index, lactation index, percent survival, and sex ratio. Maternal behavior of the dams was recorded on LDs 1, 5, 8, 15, and 22.

F0 generation animals were sacrificed by carbon dioxide asphyxiation (day 106 to 110 of the study for male rats, i.e., after completion of the cohabitation period; and LD 22 for female rats), necropsied, and examined for gross lesions. Gross necropsy included examination of external surfaces and orifices, as well as internal examination of tissues and organs. Individual organs were weighed and organ-to-body weight and organ-to-brain weight ratios were calculated for the brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary, uterus with oviducts and cervix, left epididymis (whole and cauda), right epididymis, prostate and seminal vesicles, (with coagulating glands and with and without fluid). Tissues retained in neutral buffered 10% formalin for possible histological evaluation included the pituitary, adrenal glands, vagina, uterus, with oviducts, cervix and ovaries, right testis, seminal vesicles, right epididymis, and prostate. Histological examination was performed on tissues from 10 randomly selected rats per sex from the control and high dosage groups. All gross lesions were examined histologically. All F0 generation rats that died or appeared moribund were also examined.

Histological examination of the reproductive organs in the low- and mid-dose groups was conducted in rats that exhibited reduced fertility by either failing to mate, conceive, sire, or deliver healthy offspring; or for which estrous cyclicity or sperm number, motility, or morphology were altered. Sperm number, motility, and morphology were evaluated in the left cauda epididymis of F0 generation male rats; testicular spermatid concentrations were evaluated in the left testis. The number and distribution of implantation sites were recorded in F0 generation female rats. Rats that did not deliver a litter were sacrificed on GD 25 and examined for pregnancy status. Uteri of apparently nonpregnant rats were examined to confirm the absence of implantation sites. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Female rats without a confirmed mating date that did not deliver a litter were sacrificed on an estimated day 25 of gestation.

At scheduled sacrifice, after completion of the cohabitation period in F0 male rats and on LD 22 in F0 female rats, blood samples (10 males and 10 females each for the 10 and 30 mg/kg-day dose groups; 3 males and 3 females for the control group) were collected for analysis of PFOA.

The F1 generation pups in each litter were counted once daily. The litter sizes were not standardized on day 4 or at anytime during lactation. Physical signs (including variations from expected lactation behavior and gross external physical anomalies) were recorded for the pups each day. Pup body weights were recorded on LDs 1, 5, 8, 15 and 22. On LD 12, all F1 generation male pups were examined for the presence of nipples. Pups that died before examination of the litter for pup viability on LD 1 were evaluated for vital status at birth. Pups found dead on LDs 2 to 22 were examined for gross lesions and for the cause of death. All F1 generation rats were weaned on LD 22 based on observed growth and viability of these pups.

At weaning (LD 22), two F1 generation pups per sex per litter per group (60 male and 60 female pups per group) were selected for continued evaluation, resulting in 600 total rats (300 rats per sex) assigned to the five dosage groups. At least two male pups and two female pups per litter, when possible, were selected. F1 generation pups not selected for continued observation for sexual maturation were sacrificed. Three pups per sex per litter were examined for gross lesions. Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. The brain, spleen and thymus from one of the three selected pups per sex per litter were weighed and the brain, spleen, and thymus from the three selected pups per sex per litter were retained for possible histological evaluation. All remaining pups were discarded without further examination.

The F1 generation rats were given the same dosage level of the test substance and in the same manner as their respective F0 generation sires and dams. Dosages were given once daily, beginning at weaning and continuing until the day before sacrifice. F1 generation female rats were examined for age of vaginal patency, beginning on day 28 postpartum (LD 28). F1 generation male rats were

evaluated for age of preputial separation, beginning on day 39 postpartum (LD 39). Body weights were recorded when rats reached sexual maturation.

Following sexual maturation, a table of random units was used to select one male and one female per litter per group for continuation through mating to produce the F2 generation. The remaining F1 animals were sacrificed.

Estrous cycling was evaluated daily by examination of vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until confirmation of mating by the presence of sperm in a vaginal smear or confirmation of a copulatory plug. On the day of scheduled sacrifice, the stage of the estrous cycle was assessed.

A table of random units was used to assign F1 generation rats to cohabitation, one male rat per female rat. If random assignment to cohabitation resulted in the pairing of F1 generation siblings, an alternate assignment was made. The cohabitation period consisted of a maximum of 14 days.

Body weights of the F1 generation male rats were recorded weekly during the postweaning period and on the day of sacrifice. Body weights of the F1 generation female rats were recorded weekly during the postweaning period to cohabitation, and on GDs 0, 7, 10, 14, 18, 21 and 25 (if necessary) and on LDs 1, 5, 8, 11, 15 and 22. Food consumption values for the F1 generation male rats were recorded weekly during the dosage period. Food consumption values for the F1 generation female rats were recorded weekly during the postweaning period to cohabitation, on GDs 0, 7, 10, 14, 18, 21 and 25 and on LDs 1, 5, 8, 11 and 15. Because pups begin to consume maternal food on or about LD 15, food consumption values were not tabulated after LD 15.

At scheduled sacrifice, the F1 animals were subjected to gross necropsy, and selected organs were weighed and examined histologically as described above for the F0 animals. Sperm analyses were also conducted as described for the F0 animals.

F2 generation litters were examined after delivery to identify the number and sex of pups, stillbirths, live births and gross alterations. Each litter was evaluated for viability at least twice each day of the 22-day postpartum period. Dead pups observed at these times were removed from the nesting box. Anogenital distance was measured for all live F2 generation pups on LDs 1 and 22, and F2 male pups were examined for the presence of nipples on LD 12.

3.8.1 F0 Generation

3.8.1.1 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 (emaciation, cold-to-touch, and decreased motor activity). Necroscopic examination in that animal revealed a pale and tan liver and red testes. All other F0 generation male rats survived to scheduled sacrifice. 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 mating and fertility parameters assessed, including numbers of days to inseminate, numbers of rats that mated, fertility index, numbers of rats with confirmed mating dates during the first and second week of cohabitation, and numbers of pregnant rats per rats in cohabitation. At necropsy, none of the sperm parameters evaluated (sperm number, motility, or morphology) were affected by treatment at any dose level.

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; $p \leq 0.05$). Absolute weights of the left and right epididymides, left cauda epididymis, seminal vesicles (with and without fluid), prostate, pituitary, left and right adrenals, spleen, and thymus were also statistically significantly reduced at 30 mg/kg-day. These absolute organ weight reductions are probably due to reductions in body weight and not a reflection of target organ toxicity since the organ-to-body weight ratios were either normal or significantly increased. The biological significance of the weight changes for the adrenals, however, is unclear since dose-related histopathological changes were observed. The absolute weight of the seminal vesicles without fluid was significantly reduced in the 10 mg/kg-day dose group. The absolute weight of the liver was statistically significantly increased in all dose-groups. 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. The biological significance of the weight changes observed in the liver and kidney is unknown since no histopathology was conducted on these organs. 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.

No treatment-related effects were seen at necropsy or upon microscopic examination of the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolation of the cells of the adrenal cortex in 2/10 males in the 10 mg/kg-day group and 7/10 males in the 30 mg/kg-day dose group.

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.

3.8.1.2 F0 Females

No treatment-related deaths or adverse clinical signs were reported in parental females at any dose level. No treatment-related effects were reported for body weights, body weight gains, and absolute and relative food consumption values.

There were no treatment-related effects on estrous cyclicity, mating or fertility parameters. None of the natural delivery and litter observations were affected by treatment, that is, the numbers of dams delivering litters, the duration of gestation, the averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, dams with all pups dying, liveborn and stillborn pups viability index, pup sex ratios, and mean birth weights were comparable to controls among all treated groups.

Necropsy and histopathological evaluation were also unremarkable. Terminal body weights, organ weights, and organ-to-terminal body weight ratios were comparable to control values for all treated

groups, except for kidney and liver weights. The absolute weights of the left and right kidney, and the ratios of these organ weights-to-terminal body weight and of the left kidney weight-to-brain weight were significantly reduced at the highest dose of 30 mg/kg-day. The biological significance of these weight changes is not known since histopathology was not conducted on the kidney. The ratio of liver weight-to-terminal body weight was significantly reduced at 3 and 10 mg/kg-day, but there were no effects observed at 30 mg/kg-day or in absolute liver weight.

Serum PFOA levels were analyzed from the control, 10 and 30 mg/kg-day groups on LD 22. The samples were collected 24 hours after dosing. In the 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.

3.8.2 F1 Generation

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage of male pups, litter size and average pup body weight per litter at birth. At 30 mg/kg-day, one pup from one dam died prior to weaning on lactation day 1 (LD1). Additionally, on lactation days 6 and 8, statistically significant increases in the numbers of pups found dead were observed at 3 and 30 mg/kg-day. According to the study authors, this was not considered to be treatment related because they did not occur in a dose-related manner and did not appear to affect any other measures of pup viability including numbers of surviving pups per litter and live litter size at weighing. An independent statistical analysis was conducted by US EPA (2002b). No significant differences were observed between dose groups and the response did not have any trend in dose.

The authors did not present the mean pup body weights for the male and female pups separately. Pup body weight on a per litter basis (sexes combined) was reduced throughout lactation in the 30 mg/kg-day group, and statistical significance ($p \leq 0.01$) was achieved on days 1 (9.5%), 5 (9.6%), and 8 (10.5%). Of the pups necropsied at weaning, no statistically significant, treatment-related differences were observed for the weights of the brain, spleen and thymus and the ratios of these organ weights to the terminal body weight and brain weight.

3.8.2.1 F1 Males

Significant increases in treatment-related deaths (5/60 animals) were reported in F1 males in the high dose group of 30 mg/kg-day between days 2-4 postweaning. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning.

Statistically significant increases in clinical signs of toxicity were also observed in F1 males during most of the entire postweaning period. These signs included an increased incidence of annular constriction of the tail at all doses, with statistical significance at the 1, 10, and 30 mg/kg-day; a significant increase at 10 and 30 mg/kg-day in the number of male rats that were emaciated; and a significant increase in the incidence of urine-stained abdominal fur, decreased motor activity, and abdominal distention at 30 mg/kg-day.

Statistically significant reductions in body weight gain were observed at 10 and 30 mg/kg-day during days 8-15, 22-29, 29-36, 43-50, and 50-57 postweaning. Body weight gains were also significantly reduced in the 30 mg/kg-day group on days 1-8, 15-22, 36-43, 57-64, and 64-70 postweaning. Body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. In the 3 mg/kg-day group,

mean body weight gain was significantly reduced on days 43-50 and 57-64 postweaning, and mean body weights were significantly reduced on days 106 and 113 postweaning. In the 1 mg/kg-day group, mean body weight gain was significantly reduced on days 15-22 and 43-50 postweaning, and mean body weights were significantly reduced on days 50, 57, 64, 70, 99, 106 and 113 postweaning. 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 ($p \leq 0.01$) delays in sexual maturation (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).

No apparent effects were observed on any of the mating or fertility parameters including fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), the number of days to inseminate, the number of rats that mated, and the number of rats with confirmed mating dates during the first week. No statistically significant, treatment-related effects were observed on any of the sperm parameters (motility, concentration, or morphology).

Necroscopic examination revealed statistically significant treatment-related effects at 3, 10, and 30 mg/kg-day ranging from tan areas in the lateral and median lobes of the liver to moderate to slight dilation of the pelvis of one or both kidneys.

Statistically significant, dose-related decreases in terminal body weights were observed in the 1, 3, 10, and 30 mg/kg-day dose groups(6%, 6%, 11%, and 22% decrease from controls, respectively; $p \leq 0.01$ at 1 and 3 mg/kg-day, $p \leq 0.05$ at 10 and 30 mg/kg-day). The absolute and relative weights of the liver were statistically significantly increased in all treated groups ($p \leq 0.01$) and was accompanied by histopathological changes. 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. Organ weight-to-terminal body weight and brain weight ratios for the kidney were statistically significantly increased in all treated groups. The biological significance of the effects on kidney weight is unknown since histopathology was not conducted on that organ. All other organ weight changes observed (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes, and epididymis) are probably due to decrements in body weight and not a reflection of target organ toxicity since the absolute weights of these organs was significantly reduced while the relative weights were either normal or significantly increased. However, the biological significance of the weight changes observed in the adrenal is unclear since histopathological changes were also observed.

Histopathologic examination of the reproductive organs was unremarkable; however, 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). No other treatment-related effects were reported.

3.8.2.2 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 for any dose level during any time of the study period.

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 type of calculation can be used as a screen for effects on the estrous cycle, but a more detailed analysis should then be conducted to determine whether there is truly an effect. 3M (2002) recently completed an analysis that showed there were no effects on the estrous cycle; there were no differences in the number of females with $\geq$ 3 days of estrus or with $\geq$ 4 days of diestrus in the control and high dose groups. Analyses conducted by the US EPA (2002a) also demonstrated that there were no differences in the estrous cycle among the control and high dose groups. 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 is due to the way in which the calculation was done, and is not biologically meaningful.

No effects on any of the mating and fertility parameters (numbers of days in cohabitation, numbers of rats that mated, fertility index, rats with confirmed mating dates during the first week of cohabitation and number of rats pregnant per rats in cohabitation).

All natural delivery observations were unaffected by treatment at any dose level. Numbers of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, dams with all pups dying and liveborn and stillborn pups were comparable among treated and control groups.

The terminal body weights and absolute and relative pituitary weights are shown in Table 47 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.

Table 47 Summary of Body and Pituitary Weights of F1 Females

Group	Terminal Body Weight (g)[a]	Pituitary Weight (g)[a]	Pituitary/ Body Weight (%)[a]	Pituitary/Brain Weight (%)[a]
Control	322.9 ± 23.4	0.017 ± 0.004	5.46 ± 1.6	0.84 ± 0.21
1 mg/kg-day	321.7 ± 24.2	0.016 ± 0.003	5.0 ± 0.86	0.76 ± 0.14
3 mg/kg-day	329.2 ± 21.5	0.015 ± 0.003*	4.65 ± 0.86*	0.74 ± 0.14*
10 mg/kg-day	325.1 ± 23.5	0.015 ± 0.002*	4.72 ± 0.90*	0.72 ± 0.12*
30 mg/kg-day	315.7 ± 20.9	0.015 ± 0.003*	4.75 ± 1.03*	0.72 ± 0.16**

a- Mean ± SD
* Significantly different from control at $p \leq 0.05$
** Significantly different from control at $p \leq 0.01$

3.8.3 F2 Generation

No treatment-related adverse clinical signs were observed at any dose level. Dead or stillborn pups were noted in both the control and treated groups. The deaths occurred on lactation days 1-18 with the majority occurring on days 1-6. However, there was no dose-response relationship and therefore were unlikely related to treatment. Statistically significant increases ($p \leq 0.01$) in the number of pups found dead were observed on lactation day 1 in the 3 and 10 mg/kg-day groups. According to the study authors, this was not considered to be treatment related because they did not occur in a dose-related manner and did not appear to affect any other measures of pup viability including numbers of surviving pups per litter and live litter size at weighing. An independent statistical analysis was conducted by US EPA (2002b). No significant differences were observed between dose groups and the response did not have any trend in dose.

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage of male pups, litter size and average pup body weight per litter when measured on LDs 1, 5, 8, 15, or 22. Anogenital distances measured for F2 male and female pups on LDs 1 and 22 were also comparable among the five dosage groups and did not differ significantly.

Terminal body weights in F2 pups were not significantly different from controls. Absolute weights of the brain, spleen and thymus and the ratios of these organ weights-to-terminal body weight and to brain weight were also comparable among treated and control groups. There were no treatment-related effects following necroscopic examination, with the exception of no milk in the stomach of the pups that were found dead.

3.8.4 Conclusions of the Reproductive Toxicity Study

Dosing with APFO at 30 mg/kg-day resulted in a delay in the onset of sexual maturation in both male and female F1 offspring. The authors of the study contend that the delays in sexual maturation (preputial separation or vaginal patency) observed in high-dose animals are due to the fact that these animals have a decreased gestational age, a variable which they have defined as the time in days from evidence of mating in the F0 generation until evidence of sexual maturation in the F1 generation. The authors state that gestational age appeared to be decreased in high-dose animals at the time of acquisition (the time when sexual maturation was reached), which they believe meant the animals in that group were younger and more immature than the control group, in which there was no significant difference in sexual maturation.

In order to test this hypothesis, the authors covaried separately the decreases in body weight and in gestational age with the delays in sexual maturation in order to determine whether or not body weights and gestational age were a contributing factor. When the body weight was covaried with the time to sexual maturation, the time to sexual maturation showed a dose related delay that was statistically significant at $p \leq 0.05$. This suggests that the delay in sexual maturation was partly related to body weight, but not entirely. When gestational age was covaried with the time to sexual maturation, there was no significant difference in the time of onset of sexual maturation between controls and high-dose animals. This indicates that the effect of delayed sexual maturation could possibly be attributed to decreased gestational age.

While it is known and commonly accepted that changes in the body weights of offspring can affect the time to sexual maturation, whether or not gestational age, as defined by the authors, also affects the time of sexual maturation is purely speculative, especially since there were no data provided by the authors to support this relationship. Additionally, covaring gestational age with time to sexual maturation is problematic from a statistical standpoint. Since there was no significant change in the length of gestation at 30 mg/kg-day, based on the authors' definition of 'gestational age', the decreases in gestational age would have to be due mostly to changes in time to sexual maturation. Therefore, sexual maturation is essentially being covaried with itself. Still, even if a relationship between gestational age and time to sexual maturation were shown, it merely offers an explanation for the observed delays in sexual maturation in high-dose animals, but does not diminish its significance.

A variety of endpoints are evaluated throughout different lifestages in a two generation reproductive toxicity study. Therefore, some of the endpoints may be indicative of developmental/reproductive toxicity, while others may be indicative of adult toxicity. The selection of developmental endpoints (and the appropriate LOAELs and NOAELs) for this study was based on the Agency's Developmental Toxicity Risk Assessment Guidelines (EPA, 1991) and the Agency's Reproductive Toxicity Risk Assessment Guidelines (EPA, 1996). According to the guidelines, the period of exposure for developmental toxicity is prior to conception in either parent, through prenatal development and continuing until sexual maturation. In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism. For selection of the developmental endpoints from the two generation reproductive toxicity study, attention was focused on effects that were noted during the period of developmental exposure. Thus, only effects that occurred up to sexual maturation were considered relevant for assessing developmental toxicity. Effects occurring after sexual maturation were considered relevant for assessing adult toxicity since it was not possible to determine whether the effects were due to developmental and/or adult exposures.

Therefore, 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 weight. A NOAEL for the F0 parental males could not be determined since the increases in liver weight were seen at all doses tested. The NOAEL for F0 parental females is 30 mg/kg-day, the highest dose tested.

A variety of developmental/reproductive effects were noted in the F1 generation. During lactation, there was a significant reduction in F1 mean body weight on a litter basis (sexes combined) in the 30 mg/kg-day group. F1 males in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day beginning on postweaning day 8. F1 females in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 males and females in the 30 mg/kg-day group. For F1 males, the LOAEL for developmental/reproductive toxicity was 10 mg/kg-day, and the

NOAEL was 3 mg/kg-day. For F1 females, the LOAEL for developmental/reproductive toxicity was considered to be 30 mg/kg-day, and the NOAEL was 10 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 the F1 males could not be determined since these effects were seen at all doses tested.

The NOAEL and LOAEL for adult systemic toxicity in the F1 females are 10 and 30 mg/kg-day, respectively, based on statistically significant decreases in body weight and body weight gains.

The NOAEL for developmental/reproductive toxicity in the F2 offspring was 30 mg/kg-day. No treatment-related effects were observed at any doses tested in the study. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation.

Testicular and ovarian effects were noted in the 2-year rat study (Sibinski, 1987; see section 3.6.1).

3.9 Immunotoxicity Studies in Animals

Four immunotoxicity studies of PFOA have been conducted in mice. In the first study, Yang et al. (2000) administered 0.02 % PFOA 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. The effect of PFOA administration on the cellularity, cell surface phenotype, and cell cycle of thymocytes and splenocytes was determined. In addition, effects of exposure of thymocytes and splenocytes to PFOA in vitro were examined. Administration of 0.02% PFOA for 2, 5, 7, or 10 days resulted in a significant increase, relative to controls, in liver weight, even at the earliest time point. Also, a decrease in body weight was observed. Following five days of administration, significant decreases in thymus and spleen weight were noted. After administration of 0.02% PFOA for 7 days, significant decreases (85% and 80%, respectively) in the total number of thymocytes and splenocytes were observed. In addition, the number of thymocytes expressing both CD4 and CD8 decreased by 95%; the number expressing neither CD4 and CD8 decreased by 57%; and the number expressing either CD4 or CD8 decreased by 64% and 72%, respectively. For the splenocytes, both T cells (CD3) and B cells (CD19) decreased by 75% and 86%, respectively. Also, significant decreases in both CD4 helper and CD8 cytotoxic splenic T cells were observed. Upon administration of 0.02% PFOA to mice for 7 days, thymocyte proliferation was also inhibited, as detected by cell cycle flow cytometry analyses. In vitro studies showed that there was spontaneous apoptosis occurring in splenocytes and thymocytes after 8 or 24 hours of culturing in the presence of varying concentrations (50, 100, or 200 M) of PFOA. However, PFOA did not significantly alter the cell cycle under these conditions.

In order to examine the dose dependency of the effects, Yang et al. (2001) administered C57Bl/6 mice diets consisting of 0.001%-0.05% PFOA (w/w) for 10 days. For examining the time-course, a diet containing 0.02% PFOA was given for 2, 5, 7 or 10 days. Effects of withdrawal of PFOA were also studied. The results showed that, at higher doses, a significant decrease, relative to controls, in body weight was observed, although no other apparent signs of toxicity such as sores, lethargy, and poor grooming were noticed. However, a significant decrease in total water intake was observed. Mice receiving dietary PFOA for 10 days experienced significant increases in liver weight and peroxisome proliferation, as measured by induction of acyl-CoA oxidase with lauroyl-CoA or palmitoyl-CoA as substrate. These increases started at the lowest dose and reached their maximal values at a dose of 0.003-0.01%. In contrast, the weight decreases of the spleen and thymus began at a

higher dose (0.01%) with no maximum reached with the doses given. The time course studies showed that increased liver weights and peroxisome proliferation were evident at the earliest time point examined. In contrast, significant thymus and spleen weight decreases required PFOA administration for a period of at least 5 days, following which the spleen weight remained constant while the thymus weight continued to decrease. However, upon prolonged treatment for one month, no further decreases in thymus and spleen weights were observed. In another set of experiments, animals received 0.02% PFOA for 7 days, and then they received normal chow for a period of 10 days. These recovery experiments showed that the animals rapidly recovered the body weight the second day after withdrawal of PFOA. However, the liver weight did not return to normal even after 10 days of recovery. Thymus recovery started on day 2 and was completed by day 10. The spleen weights returned to normal by day 2 post-withdrawal. In addition, the changes in thymus and spleen weight upon PFOA treatment and withdrawal paralleled the changes in total thymocyte and splenocyte counts. Furthermore, flow cytometry cell cycle experiments showed that the decrease in thymocyte number caused by PFOA treatment is due mainly to inhibition of thymocyte proliferation. In contrast, PFOA treatment caused no changes in the cell cycle of splenocytes.

A third feeding study (Yang et al. 2002a) was designed to examine the possible involvement of the peroxisome proliferator-activated receptor alpha (PPAR) in the immunomodulation exerted by PFOA. This study made use of transgenic PPAR null mice, which are homozygous with regards to a functional mutation in the PPAR gene. These mice do not exhibit peroxisome proliferation or hepatomegaly and hepatocarcinogenesis even after exposure to peroxisome proliferators. These mice were fed a diet consisting of 0.02% PFOA (w/w) 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 PPAR null mice PFOA resulted in no significant decrease in body weight. As expected, peroxisome proliferation, as measured by fatty acid oxidation, was totally lacking in PPAR 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. At the same time, the decrease in weight and cellularity of the thymus was attenuated, but not totally eliminated in the PPARα null 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, which reflects inhibition of proliferation, observed in wild type mice administered PFOA were much less extensive in PPARα null mice. Finally, in contrast to wild type mice, PFOA treatment caused no significant change in splenocyte proliferation in response to mitogens in PPARα null mice.

A fourth feeding study (Yang et al. 2002b) was designed to examine the effects of PFOA on specific humoral immune responses in mice. For this study, 0.02 % PFOA was administered to male C57Bl/6 mice for 10 days. Then the animals were examined, 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 with 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 M) 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.

Conclusions

In these studies there is evidence that PFOA may be immunotoxic in mice. Feeding C57Bl/6 mice a diet containing 0.02% PFOA resulted in adverse effects to both the thymus and spleen. In addition, this feeding regimen resulted in suppression of the specific humoral immune response to horse red blood cells, and suppression of splenic lymphocyte proliferation. The suppressed mice recovered their ability to generate a humoral immune response when they were fed a diet devoid of PFOA. Studies using transgenic mice showed that the PPARα was involved in causing the adverse effects to the immune system. Additional studies using conventional protocols are underway to confirm the results of these studies.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Concerning the acute toxicity of PFOA for aquatic organisms, experimental data are available for three trophic levels (cf. Table 48). The tests were performed with ammonium perfluorooctanoate (APFO), the ammonium salt of PFOA.

A 96 h-LC_{50} of 707 mg/l for *Oncorhynchus mykiss* was evaluated in a static test according to OECD TG 203 (CIT, 2003b). No analytical monitoring of the test item was carried out as the substance was expected to be stable in water under the test conditions. Animals were exposed to nominal test concentrations between 31.3 and 1000 mg/l. Transient sub-lethal effects (color change and abnormal respiratory rates) were observed in animals lying at the bottom of the aquarium at 250 and 500 mg/l. For both concentrations, these effects were only observed from 24 to 72 hours but not subsequently.

An acute toxicity test with *Daphnia magna* was performed according to OECD TG 202 (CIT, 2003a). The daphnids were exposed to nominal APFO concentrations between 100 and 1000 mg/l in a static test system. No analytical monitoring of the test item was carried out as the substance was expected to be stable in water under the test conditions. A 48 h-EC_{50} of 480 mg/l for immobilization was calculated.

The toxicity of APFO was evaluated with the green algae *Pseudokirchneriella subcapitata* in a static test system according to OECD TG 201 (CIT, 2004a). The stability of the test substance was experimentally determined by ion chromatography with electrochemical detection (conductimetry) at the beginning and at the end of the test. Recovery rates were within ±20%, therefore the results are expressed as nominal values. Nominal APFO concentrations were in the range of 5.25 to 400 mg/l. The 72h and 96h-EC_{50} for both biomass and growth rate were > 400 mg/l. NOECs for both biomass and growth rates were 200 mg/l after 72h, and 12.5 mg/l after 96h.

Additional but less reliable tests with fish (96 h-LC_{50} values from 300 to >1000 mg/l), daphnids (48 h-EC_{50} values from 240 to 584 mg/l) and algae (72 h E_rC_{50} = 3330 mg/l) are available. The results are presented in Table 48.

Chronic Toxicity Test Results

Concerning prolonged and chronic toxicity of PFOA towards aquatic species, experimental data are available for two trophic levels (cf. Table 48). The tests were conducted with ammonium perfluorooctanoate (APFO), the ammonium salt of PFOA. For both tests the stability of the test substance in the medium was experimentally determined by ion chromatography with electrochemical detection (conductimetry). Analytical recovery rates were less than 80% so the results were expressed as effective values.

The prolonged toxicity of APFO to fish (*Oncorhynchus mykiss*) was tested under flow-through conditions in an ELS test according to OECD TG 210 (CIT, 2004b). The test started immediately after fertilization and was terminated 60 days after hatching in the control at day 85. For the endpoint mortality of embryos (post hatch), larvae, and juveniles a NOEC of 40.0 mg/l (the highest concentration tested) was obtained. Also a NOEC of 40 mg/l for sub-lethal endpoints as mean weight and mean length was determined.

A 21-d-reproduction study with *Daphnia magna* according to OECD TG 211 was conducted with APFO in a semi static test system (CIT, 2003c). Test solutions were renewed three times a week. A 21 d-NOEC of 20 mg/l and a LOEC of 44 mg/l for reproduction capacity (number of living neonates per parent alive) was obtained. The 21 d-NOEC based on length of parent animals was 44 mg/l and based on survival of parent animals was 88.6 mg/l.

Table 48 Toxicity of APFO to aquatic species

Species	Test type	Parameter	Effects	Reference	IUCLID
Oncorhynchus mykiss	Static	96 h-LC_{50}	= 707 mg/l (n)	CIT, 2003b	4.1
Oncorhynchus mykiss	Static	96 h-LC_{50}	= 800 mg/l (e)	DuPont, 1999	4.1
Lepomis macrochirus	Static	96 h-LC_{50}	= 634 mg/l (n)	DuPont, 1994	4.1
Pimephales promelas	Static	96 h-LC_{50}	140 mg/l (n) *	3M, 1996d	4.1
Pimephales promelas	Static	96 h-LC_{50}	> 301 mg/l (n)	3M, 1987	4.1
Pimephales promelas	Static	96 h-LC_{50}	= 766 mg/l (n)	3M, 1980	4.1
Pimephales promelas	Static	96 h-LC_{50}	> 1000 mg/l (n)	3M, 1990c	4.1
Pimephales promelas	Static	96 h-LC_{50}	2470 mg/l (n)	3M, 1996a	4.1
Oncorhynchus mykiss (early life stage)	Flow-through	Mortality of embryos, larvae, juveniles 85 d-NOEC 85 d-LOEC	 = 40 mg/l (e) > 40 mg/l (e)	CIT, 2004b	4.5.1
Pimephales promelas (eggs, larvae)	Flow-through	Hatching success, 30 d post-hatch success of larvae 30 d-NOEC 30 d-LOEC	 = 100 mg/l (n) > 100 mg/l (n)	EG & G, Bionomics, 1978	4.5.1

Species	Test type	Parameter	Effects	Reference	IUCLID
Daphnia magna	Static	Immobilisation 48 h-EC_{50}	 = 480 mg/l (n)	CIT, 2003a	4.2
Daphnia magna	Static	48 h-EC_{50}	= 584 mg/l (n)	3M, 1990b	4.2
Daphnia magna	Static	48 h-EC_{50}	= 240 mg/l (n)	3M, 1996b	4.2
Daphnia magna	Static	48 h-EC_{50}	= 360 mg/l (n)	3M, 1996c	4.2
Daphnia magna	Semi-static	Reproduction 21 d-NOEC 21 d-LOEC	 = 20 mg/l (e) = 44 mg/l (e)	CIT, 2003c	4.5.2
Pseu-dokirchneriella subcapitata	Static	Biomass, growth rate 72 h-EC_{50} 72 h-NOEC 96 h-EC_{50} 96 h-NOEC	 > 400 mg/l (n) = 200 mg/l (n) > 400 mg/l (n) = 12.5 mg/l (n)	CIT, 2004a	4.3
Selenastrum capricornutum	Static	72 h-E_rC_{50}	=3330 mg/l (n)	3M, 1996e	4.3

(e): effective concentration
(n): nominal concentration
* : low pH values in high test concentrations may have had an adverse effect on survival

Toxicity to Microorganisms

The toxicity of APFO to activated sludge was evaluated in several respiratory inhibition tests. The results of the toxicity tests with microorganisms are listed in Table 49.

Table 49 Toxicity of APFO to microorganisms

Species	Parameter	Effects	Reference
activated sludge	NOEC (7 min)	= 1000 [1*]	3M, 1980a; US EPA, 2002
	EC_{50} (30 min)	> 1000 [1]	US EPA, 2002
	EC_{50} (30 min)	> 500 [2]	
	EC_{50} (30 min)	> 664 [3]	
	EC_{50} (3 h)	> 1000	3M, 1987; 3M, 1990
	EC_{50} (3 h)	> 3320	3M, 1996

1 Test substance 100% APFO
* Test documentation insufficient for assessment, report lacks record of methodology and analytical measurement of test substance concentrations in the test solutions.
2 Test substance APFO in 50% water, value adjusted to 100% active ingredient
3 Test substance APFO in 80% water, value adjusted to 100% active ingredient

4.2 Terrestrial Effects

Acute Toxicity Test Results

Tominaga et al. (2004) conducted tests with the soil dwelling nematode *Caenorhabditis elegans*. 20 worms each were exposed to different nominal concentrations (0.01 to 5.0 mM [4.14 to 2070 mg/l)

of PFOA on NGM agar plates. The test solutions were poured on plates before the worms were added. After 1, 2, 3, 4, 24 and 48 hours the total number of worms and the number of active worms on each plate was counted. These experiments were carried out in triplicate and repeated at least 3 times. A 24 h-EC_{50} of 2.75 mM (1138 mg/l) and a 48 h-EC_{50} on activity of the worm of 2.35 mM (973 mg/l) was calculated using the PROBIT analysis.

Chronic Toxicity Test Results

In a multi-generation test the effects of PFOA on the fecundity of the soil dwelling nematode *Caenorhabditis elegans* was investigated (Tominaga et al., 2004). The animals were exposed to concentrations of 100 pM, 1 nM, 10 nM and 100 µM [equivalent to $4.1x10^{-5}$, $4.1x10^{-4}$, $4.1x10^{-3}$, 41.4 mg/l, respectively]. L1-larvae of the first generation were added to NGM agar plates. When worms grew to L4 larvae, one worm was picked up and transferred to a new plate of the same composition and incubated at 16°C. The number of worms and eggs were counted in 24 h-intervals. The second- generation worms were allowed to grow to L4 larvae on the same plate, and then again one worm was picked up and transferred to a new plate of the same composition. These steps were repeated until the fifth generation was cultured. These experiments were carried out in triplicate and repeated at least 3 times.

At the highest concentration tested (100 µM = 41.4 mg/l) the worm abundances from first to second generation showed an 'extreme' decrease, so sub-culturing was discontinued at this exposure level. In all other concentrations worm abundances and egg numbers during the first generation did not differ significantly from controls. In the forth generation a significant decrease in worm abundances and egg production was observed at 10 nM (= $4.1x10^{-3}$ mg/l). For the endpoints worm abundance and egg production a NOEC of 1 nM ($4.1x10^{-4}$ mg/l) and a LOEC of 10 nM ($4.1x10^{-3}$ mg/l) was maintained.

In the acute and multi-generation study with *Caenorhabditis elegans* the culture conditions and the exposure to PFOA on NGM agar plates are not relevant for the environment.

Conclusion

The acute toxicity of PFOA to *Caenorhabditis elegans* is low. After exposure for 4 generations the most sensitive sublethal endpoints were abundance and egg productions with NOEC values about $2.3x10^{-6}$ lower than acute the EC_{50}-value.

4.3 Other Environmental Effects

Toxicity to Sediment Dwelling Organisms

In a range-finding test with PFOA with the sediment dwelling organism *Chironomus tentans* a 10 d-EC_{50} and LC_{50} > 100 mg/l (the highest concentration tested) was obtained (McDonald et al., 2004). No significant impacts on survival and growth were observed. The test was conducted according to US EPA-600-R99-064 and ASTM E-729-96 guidelines. The animals were exposed via overlaying water. Test solutions were renewed every 48 h beginning on day 2. No analytical monitoring was conducted in this range finding test.

Microcosm Studies

The impact of sodium perfluorooctanic acid (CAS No. 335-95-5) on the structure of the zooplankton community was evaluated in indoor microcosms over a period of 35 days (Sanderson et al., 2002, 2003, 2004). PVC aquariums with a volume of 30 l were randomly filled with sediment, animals, phytoplankton (ca. 10^3 cells / ml) and clean water from the field. The zooplankton community consisted of the following representative species: *Cyclops diaptomus*, *Cyclops strenuous*, *Cyclops*

canthocampus staphylinus, *Daphnia magna*, *Keratella quadrata*, *Phyllopoda* sp., *Echninorhynchus* sp., *Ostracoda* sp., and *Rotifera* sp. Also occasional macrophytes (*Elodea canadensis*, *Myriophyllum spicatum*) and larger invertebrates (*Ephemeroptera* sp., *Asselus aquaticus*) were present. Single application of 1, 10, 20, 30, 70 mg/l (nominal; based on the anion) in five replicates for each concentration and controls were tested.

Only temporary statistically significant structural fluctuations were observed. The fluctuations were always followed by rapid recovery and therefore, there are no ecological significant effects in this study.

Daphnia magna seemed to be the most sensitive species of the pelagic compartment with a LOEC of 20 mg/l (NOEC = 10 mg/l) 24 h post-treatment. In contrast, the smaller and more robust species of *Rotifera* sp. increased in abundance throughout the study at the two highest treatment concentrations of 30 mg/l and 70 mg/l.

Total zooplankton abundance showed no significant trends. Zooplankton abundance trends observed in the 70 mg/l-microcosms were consistently lower than those found in all other concentrations throughout the study. Total zooplankton abundance was significantly ($p<0,01$) increased at 1, 10, 20 and 30 mg/l, but not at 70 mg/l.

Species richness was highest in low treatment microcosms and lowest in high treatment microcosms. The overall species richness was significantly ($p<0,05$) reduced at 10, 20, 30 and 70 mg/l, indicating a simplification of community structure. A shift from a more diverse community with more total zooplankton species towards less richness dominated by smaller zooplankton species like *Rotifera* sp. could be observed.

A $NOEC_{community}$ was not determined because of high within-treatment variability and low statistical power due to high inter-replicate variance and relatively small effect sizes. A $LOEC_{species\ richness}$ of 10 mg/l can be deduced. The authors of the study presented a tentative order of descriptor sensitivity as follows: *Daphnia magna* > richness > *Cyclops canthocampus staphylinus* > *Cyclops diaptomus* > total zooplankton > *Rotifera* sp.

Oakes et al. (2004) investigated the reproductive impairment and biochemical changes in fathead minnow (*Pimephales promelas*) exposed for 39 days to varying concentrations of perfluorooctanoic acid (PFOA) under outdoor microcosm conditions. Test concentrations used were 0.3, 1.0, 30 and 100 mg/l (0.27, 0.65, 23.9 and 74.1 mg/l as TWA [time-weighted averages] of measured conentrations).

No mortality was associated with PFOA exposure. Significant declines in circulating plasma steroids (testosterone) were observed in both sexes, but these were accompanied by only limited increases in time to first oviposition and decreases in overall egg production in females. Only modest changes were observed in condition factor (females) and in relative liver size (in males); gonad sizes were unchanged in both sexes. Peroxisome proliferation, as quantified by fatty acyl-CoA oxidase (FAO) activity, was elevated with low PFOA concentrations, but attenuated with exposure to higher PFOA doses. Little evidence was seen for differential induction of peroxisome- associated enzyme activity with sex. Oxidative stress, as quantified by the 2-thiobarbituric acid reactive substances (TBARS) assay, was only modestly influenced by PFOA exposure and is not a significant consequence of FAO activity in the fathead minnow.

The NOEC for survival, was > 100 mg/l (nominal); the NOEC for time to first oviposition was 24 mg/l (effective); the NOEC for male plasma 11-ketotestosterone and testosterone was 0.27 mg/l (effective).

The toxicity of PFOA to the two macrophyte species *Myriophyllum spicatum* and *M. sibiricum* was evaluated in an outdoor microcosm study (Hanson et al., 2005). Replicate microcosms (n=3) were treated with 0.3, 1.0, 30 and 100 mg/l PFOA as sodium salt (0.27, 0.65, 23.9 and 74.1 mg/l as TWA (time-weighted averages) of measured concentrations) and assessed at regular intervals over 35 days. At each sampling interval (at day 7, 14, 21 and 35) two plants of each species were randomly removed from the microcosms and examined for several endpoints. These were growth (plant length), biomass (wet mass/dry mass), root number (primary roots from the plant stem), primary root length (total and longest), number of nodes. Pigment concentrations (chlorophyll a/b and carotenoid concentrations) were also examined.

The two species were similar in their sensitivity to PFOA under the simulated field conditions. No statistically significant differences or concentration-response trends were noted after 7 days of exposure to PFOA in both species. A 35d-NOEC of 23.9 mg/l and a 35d-LOEC of 74.1 mg/l was determined. For *M. spicatum* a 35d-EC_{10} of 5.7 mg/l and for *M. sibiricum* a 35d-EC_{10} of 8.4 mg/l was calculated. Effect values are based on TWA of measured concentrations.

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ANNEX

Analytical Methods

Table 50 Analysis of air samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Air	Adsorption on Amberlite XAD-2 cartridges; elution with methanol or ethyl acetate	GC/MS LC/MS/MS ^{19}F NMR	NR	Ellis et al. 2004
Air	Collection of air samples via OSHA versatile sampler consisting of glass or quartz fiber filter followed by a polystyrene resin sorbent; samples were collected at a flowrate of 1 L/min; collection times 8 h; sample divided into three fractions (plastic retaining ring and glass or quartz fiber filter; first section of resin beads and first polyurethane foam; second section of resin beads and the back PUF filter); a. fractions were agitated with C_{10} solution and methanol; extracts were transferred to other vials and analysed b. fraction were placed in glass tubes and methanol added; the mixture was shaken for 60 min; an aliquot was filtered and transferred to a amber glass vial; 1,2-^{13}C-perfluorooctanoic acid as internal standard	Electro-spray LC/MS or LC/MS/MS	LOQ ; 0.474 µg/m³// 88-95.7%	Kaiser et al., 2005a
Air (particulate and vapor samples from workplace air)	Air collected on OVS (OSHA versatile sampler) tubes containing glass fiber filter, XAD and polyurethane foam; extraction with acetone	LC/MS	LOQ: 8 µg/m^3 LOD: 2.4 µg/m^3// 94% ($C_8F_{15}OONH_4$)	Reagen et al. 2004
Dust	Samples were extracted with methanol in a centrifuge tube using ultrasonic agitation; after centri-fugation the supernatant was filtered; for analysis, the internal standard in methanol was added to an aliquot of the filtrate and then analyzed	LC/MS/MS	LOD: 0.5 ng/ml LOQ: 50 ng/g// 73%	Moriwaki et al. 2003

Table 51 Analysis of water samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Surface water, drinking water	Water sample was extracted via SPE at pH 7-8; after drying the cartridge was extracted with aceton/acetonitrile; the extracts were dried; the residues were dissolved in methanol/water/ ammonium acetate	HPLC/MS/ MS	LOD: 2 ng/l// 50-95%	Skutlarek, Exner, and Färber 2006
Surface water	Water samples were extracted using a weak anion exchange cartridge; the analytes were eluted with methanol and 0.1% ammonium methanol	HPLC/MS/ MS	LOQ : 0.1 ng/l// NR	Falandysz et al., 2005
Surface water	Water samples were mixed with an equal volume of methanol containing internal standards (^{13}C-PFOA and $^{13}C_2$-PFDA); the mixture was filtered	HPLC/MS/ MS	LOD: 1-4 ng/l// NR	Furdui et al., 2005
Surface water	Extraction of water samples was carried out using Oasis HLB and Oasis WAX cartridges; cartridges were preconditioned with methanol and water (HLB) and NH_4OH in methanol, methanol and water (WAX); samples were passed through the cartridges at a rate of 1 drop/sec; cartridges were then washed and analytes eluted	HPLC/MS/ MS	LOD: 0.01-1 ng/l// >80% (HLB) 85-107% (WAX)	Taniyasu et al., 2005a
River water	Samples were thoroughly mixed; 40 ml of the sample was transferred to a polypropylene centrifuge tube; extraction was conducted using SPE cartridges; analytes were eluted with 100% methanol	HPLC/MS/ MS	LOD: 25 ng/l LOQ : 25–50 ng/l// 112–114%	Hansen et al. 2002
Surface water	Filtration of the water sample; the water was pushed through a solid phase extraction cartridge; elution of the cartridges with methanol	HPLC/MS	LOD: 6 ng/l LOQ: 13 ng/l// 102–113%	Boulanger et al. 2004

Table 51 Analysis of water samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Surface water	Water samples were spiked with perfluorooctanesulfonate as inter-nal standard; the samples were extracted using Oasis HLB cartridges; elution was carried out with methanol; the eluate was reduced to 0.2–0.5 ml under a gentle stream of nitrogen	HPLC/MS-MS	LOQ: 0.02 ng/l// 160–190%	So et al. 2004
water	Enrichment on SPE cartridge; elution with methanol; solution concentrated under argon	HPLC/conductometric detection/MS andGC/MS	LOD: 200 µg/l// 100%	Hori et al. 2004a, b
Water (river, coastal sea water, tap water)	Samples filtered through glass and membrane filter; enrichment on cartridges; elution with methanol; concentrated under stream of nitrogen	HPLC/MS	LOD: 0.06 ng/l LOQ: 0.1 ng/l// 92–99%	Saito et al. 2004
Water (well, stream, spring, tap, Omnisolve, and type I water)	Enrichment on SPE cartridges; elution with 100% methanol	LC/MS/MS	LOQ: 25 ng/l// 70–130%	Risha et al. 2005
Surface Water	Samples were extracted using SPE cartridges; the analytes were eluted with methanol	LC/MS/MS	LOQ: 9 ng/l// 80–109%	Moody et al. 2002 Moody et al. 2001
Lake water	Thawed samples were allowed to settle; aliquots were decanted into polypropylene bottles and spiked with PFBS recovery standard; extraction was carried out via SPE cartridges; cartridges were eluted with methanol and the eluent concentrated	HPLC-MS/MS	LOD: ca. 8 ng/l LOQ: 25 ng/l// NR	Sinclair et al. 2004

Table 51 Analysis of water samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Water samples (river water, effluent of industrial/ municipal WWTP; and coastal water)	Water samples were adjusted to pH 2-3; preconcentrated with RP-C_{18} SPE cartridges; the cartridge was washed with deionised water; elution was carried out using tert-butyl ether/methanol ((1:1), MTBE/methanol (1:4), and methanol alone; extracts were evaporated to less than 100 µl by a stream of nitrogen; addition of deionised water resulting in a final volume of 1 ml; addition of internal standard (PFDoA)	HPLC/MS	LOD: 0.8 ng/l LOQ: 2 ng/l// 52-103%	Tseng et al., 2006
Water (effluent of WWTP)	Samples were allowed to settle; an aliquot of 200 ml was decanted into a polypropylene bottle; addition of internal standard (PFBS and ^{13}C-PFOA); the mixture was passed through cartridge; after this extraction the cartridge was washed with 20% aqueous methanol and Milli-Q water; after drying of the cartridge the target compounds were eluted with methanol; the extract was concentrated and filtered	HPLC/MS/ MS	LOQ : 2.5 ng/l// 70-130% (for different fluorochemicals e.g. PFOA)	Sinclair & Kannan 2006
Water samples	Samples were acidified with formic acid and filtered; a. the filtered particles were spiked with recovery standard and extracted via ASE (see sediment and sludge samples) b. ammonium acetate and internal standard were added to the water phase; the water samples were extracted using SPE cartridges; the cartridges were then rinsed with methanol/water (40:60) and eluted with methanol; after concentrating the methanol extract, recovery standard and aqueous buffer was added	HPLC/MS	LOD: 0.06 ng/l LOQ: 3.0 ng/l// NR	Berger et al. 2004 Kallenborn et al. 2004

Table 51 Analysis of water samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Water (effluent of urban/industrial WWTP and seawater)	Ion-pair SPME	GC/MS	LOD: 0.1 ug/l LOQ: 0.34 ug/l// 73–90%	Alzaga & Bayona 2004
STP effluent and biosolids	a. Biosolids were air dried and reconstituted with distilled water; after addition of internal standard, sodium carbonate and tetrabutylammonium hydrogensulfate the samples were homogenized; slurries were extracted with MTBE; samples were evaporated to dryness and reconstituted in methanol; the final extract was filtered b. effluent samples were diluted with methanol containing internal standards; the solution was filtered	HPLC/MS/MS	LOD: 1 ng/l and 0.1 ng/g dw// NR	Crozier et al., 2005
wastewater	Water sample was treatedwith sodium thiosulfate solution; 40 ml sample was transferred to a C_{18}-SPE cartridge; cartridge was washed with 40% methanol/water; analytes were eluted with 100% methanol.	HPLC/MS/MS	LOQ : 50 ng/l// 98-100%	Exygen 2006
Plant effluent	Samples filtered through glass fiber filter; SPE on C18 column; column dried in N2-stream; elution with methanol; extract reduced on a rotation vacuum concentrator; redissolved in methanol; addition of internal standard (1H,1H, 10H,10H-hexadecafluoro-1,10-decandiol)	HPLC/MS	LOD: 25 ng/l LOQ: 50 ng/l// 103%	Hohenblum et al. 2003
Seawater, open ocean water	Samples were spiked with perfluorooctanesulfonate as internal standard; water samples were extracted using SPE cartridges; elution was conducted with methanol; the solvent was removed by a gentle stream of nitrogen; particles in the final solution were removed by filtration	HPLC/MS-MS	LOD: 5.2 pg/l LOQ: 1–100 ng/l// 147%	Yamashita et al. 2004a Taniyasu et al. 2004

Table 51 Analysis of water samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Sea water	Water samples collected in 5 m water depth; the water phase was acidified with hydrochloric acid to pH 2–3; addition of internal standard (d_6-diuron and d_3-mecoprop); the aqueous solution was pumped through a cartridge filled with Chromabond or Cromafix HR-P resin; the cartridges were washed with pure water and dried under N2-stream; elution with methanol or methanol ammonium acetate, and acetic acid in reversed direction; the extract was concentrated and then analyzed	HPLC/MS/ MS	LOD: 0.05–0.5 ng/l// 97–106%	Caliebe et al. 2004 Theobald and Caliebe, 2005
Sea water	Samples were extracted using HLB and WAX cartridges; the cartridges were washed and the target analytes eluted	HPLC/MS-MS	ca. 3 ng/l// >80%	Taniyasu et al., 2005
Water	Samples were extracted by C_{18}-SPE; cartridges were eluted with methanol; clean up via adsorption chromatography	HPLC/MS	LOD: 80 ng/l// NR	De Voogt and van Roon 2005
Surface water	Sample was transferred to a clean flask, residual chlorine was reduced by adding sodium thiosulfate; ^{13}C-PFOA was added as internal standard; the samples were shaken and passed through a cartridge; cartridge was eluted with methanol; the extract was concentrated to 1 ml and then filtered	HPLC/MS/ MS	LOQ : 900 ng/l// 91-114%	Kurunthachalam et al., 2005
Sea water	Samples were spiked with perfluorooctanesulfonate as internal standard; water samples were extracted using HLB AND WAX cartridges; elution was conducted with methanol; the solvent was removed by a gentle stream of nitrogen; particles in the final solution were removed by filtration	HPLC/MS-MS	0.08 ng/l >90%	Taniyasu et al., 2005

Table 52 Analysis of sediment and sludge samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Sludge	Air-dried sludge was spiked with PFBS and ^{13}C-PFOA as internal standards; the sludge was sonicated at 60°C for 20 min in 7.5 ml acetic acid; the supernatant was removed by centrifugation; the residue was resuspended in methanol/1% acetic acid and was sonicated at 60°C for 20 min; the extracts were combined; the extraction procedure was repeated three times; after addition of 1% acetic acid the extract was passed through cartridge; the cartridge was washed with 20% methanol and dried completely under vacuum; target compounds were eluted with methanol; after concentrating to 1 ml the extract was filtered	HPLC/MS/MS	LOQ: 10 ng/g dw// 70-130% (for different fluorochemicals, e.g. PFOA)	Sinclair & Kannan, 2006

Sediment and sludge samples	Homogenized, air dried samples were washed with acetic acid and vortexed; extraction of the samples via a mixture of methanol and 1% acetic acid (90:10); the wash and extraction was carried out in a heated (60°C) sonication bath; mixture was centrifuged and the acetic acid solution separated; the extracts were passed through C_{18} SPE cartridges; the fluorochemi-cals were eluted from the cartridges using methanol; after concentrating in a gentle stream of nitrogen 0.01% aqueous ammonium hydroxide solution was added; finally internal standard was added	HPLC/MS/MS	LOD: 0.011 ng/g dw (sediment)//
			88%
			LOD: 1 ng/g dw (sludge)//
			71%
				Higgins et al., 2005

Table 52 Analysis of sediment and sludge samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Sediment and sludge samples	Samples were spiked with 7-H-perfluoroheptanoic acid as internal standard and extracted using acclerated solvent extraction (ASE); the extraction was performed three times with methanol; then the extracts were concentrated; recovery standard (3,5-bis(trifluoromethyl) phenyl acetic acid) and aqueous ammonium acetate were added; the solution was treated in an ultrasonic bath and filtered	HPLC/MS	LOD: 6 ng/g ww LOQ: 200 ng/g ww// NR	Berger et al. 2004 Kallenborn et al. 2004
Sewage sludge	Sludges were extracted via pressurized liquid extraction with ethylacetate-dimethylformamide and methanol-phosphoric acid as extraction solvents; the extracts were reduced in volume; no further clean-up step was necessary	Flow injection analysis combined with MS LC/MS	LOD: 10 mg/kg dw// NR	Schröder 2003
Sediment and soil	Samples were homogenized; addition of internal standard, tetrapropylammonium acid (pH 10), and Na_2CO_3/$NaHCO_3$ buffer; tubes were closed; addition of MTBE and Cu powder; mixture was shaken and sonicated; centrifugation; MTBE extract was separated; solvent evaporated; after addition of methanol the mixture was filtered	HPLC/MS	LOD: 0.4 ng/g dw// NR	De Voogt and van Roon 2005
Sediment	To the samples surrogate standard, sodium carbonate, and tetrabutylammonium hydrogen sulfate was added ; the sediment samples were extracted with MTBE; MTBE layer was separated using a centrifuge; the extracts were reduced in volume and 1,1,1,3,3,3-hexafluoro-2-propanol added; the extracts were then filtered and evaporated to dryness; extracts were reconstituted in methanol	HPLC/MS	LOQ/LOD: NR// 50-80%	Lucaciu et al., 2005

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Biota (polar cod and glaucous gill liver)	Liver was homogenized: a. addition of internal standard (7-H-PFHpA), methanol/water and ammonium acetate; sample was then vortexed and sonicated; the mixture was filtered and recovery standard was added b. . addition of internal standard (7-H-PFHpA), water, buffer and tetra –n-butylammonium hydrogen sulfate; pH adjusted to 10; extraction performed using tert-butyl methyl ether; extracts were evaporated to dryness; residue suspended in methanol/water and recovery standard was added; the extract was filtered	HPLC/MS	LOD: 1.28 ng/g ww// 83-84 %	Berger and Haukas, 2005
Biota (e.g. muscle and liver tissue)	Tissue samples were agitated with KOH in methanol and the mixture shaken for 16 h; the extract was diluted with distilled water; the extract was passed through a WAX cartridge; the cartridge was washed and the analytes eluted	HPLC/MS/MS	LOD: 0.01-0.08 ng/g ww// NR	Taniyasu et al., 2005a
Biota (narwhal and burbot liver)	Homogenized samples were; extracted using methanol, vortexing the mixture and centrifuging; the extraction was repeated 2 more times; the supernatant methanol phases were separated; the methanol phases were combined and reduced in volume; the residue was transferred to a microcentrifuge vial and ultracentrifuged; the methanol extract was removed and transferred to a HPLC injection vial; the mixture was spiked with internal standards; for comparison a ion-pair extraction was performed with homogenized burbot liver according to Hansen et al., 2001	HPLC/MS/MS	LOD/LOQ: NR// >85%	Tomy, Halldorson, and Tittlemier, 2005

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Biota (organs and tissue of harbor seals)	Tissue was homogenized on ice and in the presence of Milli-Q water; homogenate, internal standard, tetrabutylammonium hydrogen sulfate (pH 10), and carbonate buffer were mixed; MTBE was added and the mixture was shaken; the layers were separated via centrifugation; an aliquot was removed from the aqueous layer and reduced in volume; the extract was resuspended in methanol and filtered	HPLC/MS/MS	LOQ: 62 ng/g ww// NR	Van de Vijver et al., 2005
Fresh water fish: liver	Liver was homogenized in water; tetrabutylammonium hydrogen sulfate, (pH10) and Na_2CO_3/ $NaHCO_3$ buffer and MTBE were then added; samples were shaken and centrifuged; an aliquot of the organic layer was transferred to a polypropylene tube; the solution was dried under a gentle nitrogen stream; residue was resuspended in methanol; extract was filtered through nylon mesh filter	HPLC/MS/MS	LOD: 72 ng/g ww// NR	Sinclair et al. 2004
Fresh water fish: liver	liver homogenized in sodium carbonate and TBAS (tetrabutyl-ammonium hydrogen sulfate); the aqueous homogenate was shaken twice for 10 min with MTBE (methyl tert-butyl ether); the combined MTBE extracts were dried in a gentle stream of N_2; then water/methanol (1:1) was added and the sample filtered	LC/MS/MS	LOD: 1.2 ng/g ww// >80%	Moody et al. 2002

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Fresh water fish and invertebrates: whole body homogenates	Invertebrate samples required no sample preparation Fish were homogenized; a small amount of homogenate was weighed and centrifuged in the presence of Na_2CO_3, water, ion-pairing agent (tetrabutylammonium hydrogensulfate; pH 10), and internal standard, PFHpA (per-fluoroheptanoic acid); for extraction methyl tert-butyl ether was added; organic phase was separated via centrifugation; the MTBE super-natant was collected; this extraction process was repeated once more and the supernatants combined; the solution was dried under a gentle stream of nitrogen; the analytes were taken up in methanol and the concentrates were filtered	LC/MS/MS	LOD: 2 ng/g (fish)// > 68%	Martin et al. 2004b
Fish and oyster	Ground sample was added to deionised water in a polyethylene tube and mixed; to 1 ml of the mixture TBA solution and Na_2CO_3 buffer were added; extraction was performed using MTBE; after centrifugation the MTBE layer was separated; the solution was then reduced in volume	HPLC/MS	LOD: 10 ng/g dw LOQ: 20 ng/g dw// 78-120%	Tseng et al., 2006

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Mussel and oyster tissue	The frozen tissue samples were thawed and homogenized; 1 g of homogenized tissue was trans-ferred to PP centrifuge tube and a mixture of perfluorinated com-pounds (external standard) and 30 ml of 0.01 N KOH/methanol were added; the mixture was shaken at ca. 25°C for 16 h; afterwards 1 ml of the solution was added to a PP bottle containing distilled water and shaken thoroughly; extraction was carried out using Oasis HLB cartridges; elution of the cartridge was performed via 40% methanol and 100% methanol; after volume reduction the solution was filtered and finally the filter washed with methanol; the eluate was then reduced in a gentle stream of nitrogen	HPLC/MS/MS	LOQ: 0.204 ng/g ww// mean: 100.5%	So et al., 2006
Biota (fresh and salt water fish, marine mammals)	Sample preparation according to the method of Hansen et al. (2001): samples were homogenized with water; ammonium carbonate buffer and tetrabutylammonium hydrogen sulfate (pH 10) were added; extraction of the resulting solution was carried out using tert-butyl methyl ether; extracts were combined and evaporated to dryness; the residue was dissolved in methanol and filtered	HPLC/MS	LOD: 0.4 ng/g ww// 40–72%	Berger et al. 2004 Kallenborn et al. 2004

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Mammals (bottlenose dolphin) plasma	Plasma was placed in a centrifuge tube; internal standard (PFHpA or ^{13}C-PFOA) Na_2CO_3 and ion-pairing agent (TBAS-tetrabutylammonium hydrogensulfate pH adjusted to 10) were added; the mixture was extracted using MTBE; after centrifugation the organic layer was separated; extracts were blown to dryness with nitrogen; after addition of methanol and vortexing, the extract was filtered	HPLC/MS/MS	LOQ: 0.5 ng/g// 93%	Houde et al., 2005
Marine fish/mammals, and birds: liver and blood	Sample preparation acc. to Hansen et al. (2001)	HPLC/MS/MS	LOQ: 1–72 ng/g ww (liver) LOQ: 2.5 ng/ml (blood)// <40–90 % (recovery depended on animals investigated)	Kannan et al. 2002b
Marine mammals: blood, liver, and tissue	Ion-pair extraction procedure acc. to Hansen et al. (2001); internal standard (1H,1H,2H,2H-perfluorooctanesulfonate and/or perfluorobutane-sulfonate) was added before extraction	HPLC/MS/MS	LOQ: 3–20 ng/ml// 75–120%	Corsolini and Kannan, 2004
Marine mammals: liver	Acc. to Hansen et al. (2001) and Hoff et al. (2003): Sample homogenized on ice with water; homogenate was mixed with internal standard (1H,1H,2H,2H-perfluorooctane sulfonic acid, tetrabutylammonium hydrogen sulfate,, sodium carbonate buffer; methyl tert-butyl ether was added and the mixture shaken; organic and aqueous layer separation via centrifugation; after concentrating, the residue was dissolved in methanol and filtered	LC/MS/MS	LOQ: 10–110 ng/g ww (for all PFCAs)// NR	Van de Vijver et al. 2003 Van de Vijver et al. 2004

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Marine food web	analysis was conducted according to the method of Hansen et al. (2001) with few modifications	HPLC/MS/MS	LOD: 0.2 ng/g// Recovery: NR	Tomy et al. 2004
Fish, mussels, amphipods, and algae: tissue	Tissue was cut into small pieces; water was added and the samples homogenized; after addition of acetonitrile the sample was shaken; the organic and aqueous layers were separated via centrifugation; water was added to the acetonitrile phase; extraction was carried out using SPE cartridges; cartridges were eluted with methanol	HPLC/MS/MS	LOQ: 1–10 ng/g ww// <50%	Kannan et al. 2005
Polar bear	analysis was conducted according to the method of Hansen et al. (2001): samples were homogenized with sodium carbonate buffer, tetrabutylammonium solution (pH 10) and internal standard; extraction with methyl tert-butyl ether; the organic and aqueous phase were separated by centrifugation; MTBE fraction was evaporated to dryness; the residue was dissolved in methanol/water; the solution was vortexed and filtered	LC/MS/MS	LOD: 2 ng/g ww// 100%	Smithwick et al. 2005b
Polar bear: liver	Liver samples were extracted using an ion pairing agent; after drying of the extracts distilled water was added; derivatization with 2,4-difluoroaniline	GC/MS	NR	Ellis et al. 2004

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Polar bear: liver	Liver tissue homogenized in centrifuge tubes containing Na_2CO_3, water, ion-pairing agent TBAS (tetrabutyl-ammonium hydrogensulfate, pH 10), and internal standard; extraction with methyl tert-butyl ether; the extraction procedure was repeated several times and the supernatants were collected after centrifugation; solvent was removed by nitrogen stream; addition of water/methanol and vortexing; filtration	LC/MS/MS	LOD: 2 ng/g ww// >80%	Martin et al. 2004a
Polar bear: liver	Homogenized tissue was extracted using an ion-pairing agent and MTBE; extracts combined and evaporated to dryness; residues dissolved in distilled water and acidified to pH 1.0; NaCl, ethyl acetate, 1,3-dicyclohexylcarbodiimide as catalyst and 2,4-difluoroaniline (derivatizing agent) were added; after stirring of the mixture for one hour NaCl was added; mixture separated via a separatory funnel; organic phase was washed with HCl, $NaHCO_3$ and NaCl solution; organic phase was filtered through Na_2SO_4 and evaporated to dryness; residues were dissolved in hexane-diethyl ether (95%/5%); further cleanup was performed using a silica gel column and elution with hexane-diethyl ether; eluents were evaporated to 200 µL	GC-MS	LOD: 0.02 pg// Recovery: NR	De Silva and Mabury 2004
Beef cattle: blood plasma	Plasma was mixed with tetrabutylammonium hydrogen sulfate and buffer (pH 10); the mixture was extracted 3 times with MTBE; after shaking and centrifugation the extract was concentrated under nitrogen; finally, methanol was added and the solution filtered	HPLC/MS/MS	NR (solvent blank: 90 pg/ml)// 92%	Guruge et al. 2004

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Guillemot: eggs	Extraction acc. to Hansen et al. (2001): egg samples were homogenized in water; homogenate was mixed with sodium carbonate buffer and tetrabutylammonium solution (pH 10); mixture was vortexed and methyl tert-butyl ether added; the organic and aqueous phase were separated by centrifugation; MTBE fraction was evaporated to dryness; the residue was dissolved in methanol and filtered	HPLC/MS/MS	LOD: 3 ng/g// NR	Holmström et al. 2005
Wood mice: liver	Analysis was performed according to Giesy and Kannan (2001)	HPLC/MS/MS	LOD: 0.11 µg/g ww// NR	Hoff et al., 2004
Turtle: plasma	Plasma samples were spiked with PFBS; plasma, tetrabutylammonium hydrogen sulfate (pH 10) and sodium carbonate were thoroughly mixed and then shaken with MTBE; after centrifugation, the MBTE extract was transferred to a polypropylene tube; extract was evaporated under nitrogen; methanol was added and the mixture vortexed and filtered.	HPLC/MS/MS	LOD: 0.05 ng/ml// 70-120%	Keller et al. 2004; 2005
Mink and river otters: liver	Liver homogenized in water; homogenate was mixed with tetrabutyl-ammonium hydrogen sulfate solution (pH 10) and sodium carbonate buffer; mixture was vortexed and methyl tert-butyl ether added; the organic and aqueous phase were separated by centrifugation; MTBE fraction was removed from the solution; aqueous solution was rinsed with MTBE and separated twice; rinses were combined; solvent was allowed to evaporate; the residue was dissolved in methanol, vortexed and filtered	HPLC/MS/MS	LOQ: 4.5–75 ng/g ww// 53–140%	Kannan et al. 2002c

Table 53 Analysis of biota samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
Bald eagle: liver, kidney, muscle, testes, ovaries, and gallbladder Fish: liver, eggs, muscle Frog, mink: liver Snapping turtle: plasma	Homogenates were prepared in water; tetrabutylammonium hydrogen sulfate and sodium carbonate buffer (pH 10) were added; mixture was thoroughly shaken; extraction was carried out using methyl tert-butyl ether; after shaking and centrifugation the MTBE phase was separated; solvent was removed; the residue was redissolved in methanol and filtered	HPLC/MS/MS	LOQ: 7.5–75 ng/g ww (tissue) LOQ: <2.5 ng/ml (plasma)// <50%	Kannan et al. 2005

Table 54 Analysis of other samples

Sample	Sample preparation	Analysis	Limit of detection and/ or quantification// Recovery	Reference
All-weather jackets for children	10 x 10 cm textile were extracted with ethyl acetate and methanol	HPLC/MS	LOD: <0.80 µg/m²; LOD: <6.1 ng/g// NR	SNF 2006
Textile (apparel), home furnishings and carpet samples	Samples cut into small pieces; extraction using the corresponding solvent; centrifugation of tubes;	LC/MS/MS.	LOQ: 1 ng PFOA/g sample (for samples extracted in water and sweat simulant)// 100% LOQ: 2.5–3 ng/g (for samples extracted in saliva simulant and methanol)//	Mawn et al. 2005
PTFE polymer (commercial lot and intermediate of commercial lot)	a. Reflux extraction procedure: Five reflux extraction systems run concurrently; solvent was added to each flask; recovery standard was added to one flask; approx. 0.75 g of each polymer was added to three of the flasks; the fifth flask served as solvent blank; flasks were refluxed for two hours, cooled and filtered; solvents were removed b. Pressurized solvent extraction (PSE) procedure: Five pressurized solvent extractions performed concurrently. Cells filled with sand to approximately 3 mm from the top; cells were closed and loaded onto the instrument; cells were preconditioned; new vessels were set in place; approx. 5/6 of the sand was removed and a weighed quantity of polymer added; cells were refilled to 3 mm from the top with the sand; one vessel for recovery check and one as solvent blank; extraction was now carried out; solvents were removed	LC/MS/MS	LOQ: 0.5 ng/g// 89 – 103 % (water, methanol, acetonitrile, ethanol as solvent and reflux conditions) 95 – 99 % (water, methanol, ethanol as solvent and PSE conditions)	Larsen et al., 2005