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PRELIMINARY RISK ASSESSMENT OF THE DEVELOPMENTAL TOXICITY ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID AND ITS SALTS

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Executive Summary

As part of the effort by the Office of Pollution Prevention and Toxics (OPPT) to understand health and environmental issues presented by fluorochemicals in the wake of unexpected toxicological and bioaccumulation discoveries with respect to perfluorooctane sulfonates (PFOS), OPPT has been investigating perfluorooctanoic acid (PFOA) and its salts. PFOA and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses. PFOA and its salts are persistent in the environment.

Human Health Effects and Biomonitoring

Little information is available concerning the pharmacokinetics of PFOA and its salts in humans. An ongoing 5-year, half-life study in 7 male and 2 female retired workers has suggested a mean serum PFOA half-life of 4.37 years (range, 1.50 – 13.49 years). Animal studies have shown that the ammonium salt of PFOA (APFO) is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. PFOA distributes primarily to the liver and plasma. 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 rats. In female rats, estimates of the serum half life range from 1.9 to 24 hours, while in male rats estimates of the serum half life range from 4.4 to 9 days. In female rats elimination of PFOA appears to be biphasic; a fast phase occurs with a half life of approximately 2- 4 hours while a slow phase occurs with a half life of approximately 24 hours. 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. This gender difference has not been observed in humans based on the limited data available in the half life study in retired workers.

While the environmental concentrations and pathways of human exposure to PFOA and its salts are unknown, there are limited data on PFOA serum levels in workers and the general population. Occupational data from certain plants in the U.S. and Belgium that manufacture or use PFOA indicate that mean serum levels in U.S. workers in 2000 range from 0.84 to 6.4 ppm. At another U.S. plant where the most recently reported data are from 1997, the highest level reported in a worker was 81.3 ppm. In non-occupational populations, serum PFOA levels were much lower. In both pooled blood bank samples and in individual samples, mean serum PFOA levels ranged from 3 to 17 ppb. The highest serum PFOA levels of the general public were reported in a sample of children from different geographic regions in the U.S. (mean, 5.6 ppb; range, 1.9 – 56.1 ppb).

Epidemiological studies on the effects of PFOA in humans have been conducted on workers. However, these studies have not examined developmental outcomes. The majority of production workers at facilities that produce or use PFOA are male. Two mortality studies, a morbidity study, and studies examining effects on the liver, pancreas, endocrine system, and lipid metabolism, have been conducted to date. In addition, a longitudinal study of the worker surveillance data recently became available.

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 a recent update to this study in which more specific exposure measures were used, a significant association for prostate cancer was not observed. In an "episodes of care"study, workers with the highest PFOA exposures for the longest durations sought care more often for prostate cancer treatment than workers with lower exposures. However, this finding was not statistically significant and the 95% confidence interval was very wide.

Another study reported an increase in estradiol levels in workers with the highest PFOA serum levels; however, none of the other hormone levels analyzed indicated any adverse effects. Some of the same employees who participated in the hormone study also were included in a study of cholecystokinin (CCK) levels in employees. No positive association was noted between CCK values and PFOA. The other available study examined cholesterol and other serum components in workers. There did not appear to be any significant differences among workers of different exposure levels. At plants where the serum PFOA levels were lower, cross-sectional and longitudinal studies found positive significant associations between PFOA and cholesterol and triglyceride levels. In addition, a positive, significant association was reported between PFOA and T3 hormone and a negative association with HDL in the cross-sectional study. There are many limitations to the studies conducted to date, and therefore, all of these results must be interpreted carefully.

Prenatal developmental toxicity studies in rats resulted in death and reduced body weight in dams exposed to oral doses of 100 mg/kg/day or by inhalation to 25 mg/m^3 of the ammonium salt of PFOA (APFO). There was no evidence of developmental toxicity after oral exposure to doses as high as 150 mg/kg/day, while inhalation exposure to 25 mg/m^3 resulted in reduced fetal body weights. In a rabbit oral 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 two-generation reproductive toxicity study in rats exposed to 0, 1, 3, 10, and 30 mg/kg/day APFO, significant increases in absolute and relative liver and kidney weights were observed in F0 males at 1 mg/kg/day, while significant reductions in absolute and relative kidney weights were observed in F0 females at 30 mg/kg/day. Reproductive indices were not affected in the F0 animals. Serum levels of the 10 and 30 mg/kg/day groups were measured for F0 males after mating and F0 females at weaning of the F1 pups. In F0 males, the serum levels were (average $\pm$SD) 51.1$\pm$9.30 and 45.3$\pm$12.6 ug/l, respectively for the 10 and 30 mg/kg/day groups, and in F0 females, the serum levels were 0.37$\pm$0.0805 and 1.02$\pm$0.425 ug/l, respectively for the 10 and 30

mg/kg/day groups. In F1 animals, there was a significant reduction in mean body weight (sexes combined) during lactation in the 30 mg/kg/day group. In F1 females, there was a significant increase in post weaning mortality, a significant decrease in mean body weight, and a significant delay in sexual maturation at 30 mg/kg/day. In F1 males, significant decreases in body weights and body weight gains, and significant changes in absolute liver and spleen weights and in the ratios of liver, kidney, and spleen weights-to-brain weights were observed in all treated groups. The increase in post weaning mortality and the delay in sexual maturation were also noted in F1 males at 30 mg/kg/day. Reproductive indices were not affected in the F1 animals. The LOAEL for the F1 females was 30 mg/kg/day, and the NOAEL was 10 mg/kg/day; the LOAEL for F1 males was 1 mg/kg/day and a NOAEL was not determined. It should be noted that these effect levels reflect effects seen throughout the study (i.e. developmental and adult exposures), and should not be confused with the effect levels that are used in the preliminary risk assessment for strictly developmental exposures and effects. The difference in sensitivity is presumed to be related to the gender difference in elimination of APFO. No treatment-related effects were observed in the F2 generation. However, the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain if the post-weaning effects that were noted in the F1 generation occurred in the F2 animals.

Preliminary Risk Assessment

This preliminary risk assessment focused on the potential risks for developmental toxicity associated with exposure to PFOA and its salts. A margin of exposure (MOE) approach was used; the MOE is calculated as the ratio of the NOAEL, LOAEL, or BMDL for a specific endpoint to the estimated human exposure level. The MOE does not provide an estimate of population risk, but simply describes the relative "distance" between the exposure level and the NOAEL, LOAEL, or BMDL. For many risk assessments, the MOE is calculated as the ratio of the administered dose from the animal toxicology study to the estimated human exposure level. The human exposure is estimated from a variety of potential exposure scenarios, each of which requires a variety of assumptions. A more accurate estimate of the MOE can be derived if measures of internal dose are available for humans and the animal model. In this preliminary risk assessment, serum levels of PFOA, which are a measure of internal dose, were available for the rat two-generation reproductive toxicology study and from human biomonitoring studies. Thus, internal dose was used for the calculation of MOEs in this assessment.

For this preliminary risk assessment, the endpoints from the two-generation reproductive toxicity study that were considered relevant for assessing developmental toxicity included the significant reduction in F1 mean body weight during lactation (sexes combined). In addition, for F1 females, postweaning mortality and delayed sexual maturation were noted at 30 mg/kg/day APFO; the NOAEL for developmental effects for F1 females was 10 mg/kg/day. Postweaning mortality, delayed sexual maturation and a significant reduction in postweaning body weights were noted in F1 males at 30 mg/kg/day, and a significant reduction in postweaning body weight was noted at 10 mg/kg/day. For F1 males, the LOAEL for developmental effects was 10 mg/kg/day and the NOAEL was 3 mg/kg/day. Thus, the LOAEL for developmental effects from the study was 10 mg/kg/day and the NOAEL was 3 mg/kg/day.

In the rat two-generation reproductive toxicity study, serum levels of PFOA were only measured in the F0 animals. In order to use these serum levels as surrogates for the serum levels in the F1 animals, several areas of uncertainty had to be considered. It is not known whether the effects on postweaning mortality, body weight, or age of sexual maturation were due to prenatal exposures, lactational exposures, postweaning exposures, or a combination of one or more of these exposure periods. In most risk assessments of developmental toxicity, no attempt is made to determine which of these exposure periods is important. A major strength of this preliminary assessment is that each of these exposure periods was considered in order to determine the appropriateness and uncertainties associated with the use of the serum levels from the F0 animals.

It was reasoned that if prenatal and/or lactational exposures were important then the serum levels in the F0 females would be the most appropriate estimate for the F1 animals. If postweaning exposures were important then the serum levels for the F0 males would be the most appropriate estimate for the F1 males, and similarly the serum levels in the F0 females would be the most appropriate estimate for the F1 females. It was not possible to make a "direct" estimate of F1 serum levels from the serum levels in the F0 females for several reasons. First, there is a gender difference in the elimination of PFOA in rats. In female rats, estimates of the serum half life range from 1.9 to 24 hours, while in male rats estimates of the serum half life range from 4.4 to 9 days. In female rats elimination of PFOA appears to be biphasic; a fast phase occurs with a half life of approximately 2- 4 hours while a slow phase occurs with a half life of approximately 24 hours. In the two generation reproductive toxicity study, the animals were dosed by gavage once daily. The serum levels were measured 24 hours after dosing. Thus, the values obtained for the F0 females represent the low end of exposure. With no knowledge of the peak exposures, it was reasoned that it was unlikely that the peak exposure would be higher than the serum level in the F0 males in the same dose group since they would tend to accumulate PFOA with a daily dosing regime. Therefore, the strategy that was employed in this assessment was to use the MOEs that were calculated from the serum levels in the F0 males and females as a range or as a means to bracket the low and high ends of exposure.

For calculation of the MOEs, the human populations that were considered included women of child bearing age and children. Estimates of general human population exposure were available from recent analyses of individual serum samples from a group of children (2-12 years) and adults (20-69 years). For the populations of interest, calculations using human adult serum levels and children serum levels in combination with rat serum values from the parental (F0) females and males produced a range of overlapping MOE values that extends from less than 100 to greater than 9000. There are a number of important uncertainties discussed in this document that provide a context for considering these MOEs as a range of potential values.

It is important to note that MOEs that were calculated from the serum levels in the F0 female and male rats provide a means to bracket the low and high ends of experimental animal exposures. This is an unusual situation in that MOE estimates, which typically represent point estimates, are described here as a range of potential values due to uncertainties in the rat serum data. This situation arises from the fact that the available data do not allow selection of a particular departure point for the MOE calculations. It is likely that MOEs calculated using the

F0 female rat serum level are lower than what would be anticipated in the human population, and it is likely that MOEs calculated using the F0 male rat serum level are higher than what would be anticipated in the human population. As uncertainty around the rat serum values decreases the end brackets are likely to shift towards the middle of the current range. Therefore, MOE values presented in this document should not be interpreted as representing the range of possible MOEs in the US population. It is likely that when more extensive rat kinetic data are available, the resultant, refined estimated range of MOEs will constitute a narrower subset of the range presented here. Interpretation of the significance of the MOEs for ascertaining potential levels of concern will necessitate a better understanding of the appropriate dose metric in rats, and the relationship of the dose metric to the human serum levels.

1.0 Scope of the Assessment

As part of the effort by the Office of Pollution Prevention and Toxics (OPPT) to understand health and environmental issues presented by fluorochemicals in the wake of unexpected toxicological and bioaccumulation discoveries with respect to perfluorooctane sulfonates (PFOS), OPPT has been investigating perfluorooctanoic acid and its salts (PFOA). PFOA and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses.

OPPT released a preliminary *Draft Hazard Assessment of Perfluorooctanoic Acid and Its Salts*, dated February 20, 2002, on March 28, 2002, and issued a minor correction to that document on April 15, 2002. That draft assessment indicated that PFOA and its salts are persistent in the environment and in humans with a half life of years. The assessment noted the potential systemic toxicity and carcinogenicity associated with the ammonium salt of PFOA (APFO), which has been the focus of the animal toxicology studies, and observed that blood monitoring data suggested widespread exposure to the general population, albeit at low levels. The Agency has since received considerable additional animal toxicology data on APFO that suggest a potential for developmental/reproductive toxicity and immunotoxicity, and additional human biomonitoring data that indicate low level exposures to the general population that cannot be explained at this time.

On September 27, 2002, the Director of OPPT issued a memorandum announcing that OPPT would initiate a priority review to determine whether PFOA and its salts meets the criteria for action under section 4(f) of the Toxic Substances Control Act. As part of the priority review, the hazard assessment was revised and released on September 30, 2002. Another revision was then released November 4, 2002. In addition, OPPT conducted a preliminary risk assessment of PFOA and its salts. OPPT recognizes that there is a wide range of toxicological endpoints associated with exposure to APFO, but at this time only the endpoints that are included in section 4(f) were considered; these include cancer, mutations, and birth defects. OPPT did not include gene mutations in the preliminary risk assessment since APFO is not known to be mutagenic. In addition, APFO is a peroxisome proliferation activating receptor-α-agonist and through this mode of action could lead to the formation of liver tumors in rodents. The relevance of this mode of action for humans is currently under scientific debate, and the Agency is engaged in activities to resolve this issue. Therefore, at this time, OPPT has narrowly restricted the analysis to examine only the potential risks of developmental toxicity.

The relevant information pertaining to chemical properties, pharmacokinetics and metabolism, epidemiology, prenatal developmental toxicity, reproductive toxicity, and human exposure have been included in this preliminary risk assessment. Other information pertaining to systemic toxicity, carcinogenicity, ecotoxicity, production and uses, fate and transport, and environmental monitoring can be found in the *Draft Hazard Assessment of Perfluorooctanoic Acid and Its Salts*, dated November 4, 2002.

2.0 Chemical Identity

Chemical Name: Perfluorooctanoic Acid
Molecular formula: C8 H F15 O2

Structural formula: F-CF2-CF2-CF2-CF2-CF2-CF2-CF2-C(=O)-X,

The free acid and some common derivatives have the following CAS numbers:
The perfluorooctanoate anion does not have a specific CAS number.

Free Acid	(X = OM+; M = H)	[335-67-1]
Ammonium Salt	(X = OM+; M = NH4)	[3825-26-1]
Sodium Salt	(X = OM+; M = Na)	[335-95-5]
Potassium Salt	(X = OM+; M = K)	[2395-00-8]
Silver Salt	(X = OM+; M = Ag)	[335-93-3]
Acid Fluoride	(X = F)	[335-66-0]
Methyl Ester	(X = CH3)	[376-27-2]
Ethyl Ester	(X = CH2-CH3)	[3108-24-5]

Synonyms: 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-PFOA

2.1 Physicochemical Properties

PFOA is a completely fluorinated organic acid. The typical structure has a linear chain of eight carbon atoms. The physical chemical properties noted below are for the free acid, unless otherwise stated. The data for the free acid, pentadecafluorooctanoic acid [335-67-1], is the most complete. The reported vapor pressure of 10 mm Hg appears high for a low melting solid when compared to other low melting solids (chloroacetic acid: solid; MP = 61 to 63 °C; BP = 189 °C; VP = 0.1 kPa (0.75 mm Hg) @ 20 °C; NIOSH), but is consistent with other perfluorinated compounds with similar boiling points (perfluorobutanoic acid BP = 120 °C, VP 10 mm Hg @ 20 °C; Beilstein, 1975). Another explanation may be that the 10 mm vapor pressure was measured at an elevated temperature (but the temperature inadvertently omitted), as perfluorooctanoic acid is typically handled as a liquid at 65 °C (3M data sheet for FC-26). The free acid is expected to completely dissociate in water, leaving the anionic carboxylate in the water and the perfluoroalkyl chain on the surface. In aqueous solutions, individual molecules of PFOA anion loosely associate on the water surface and partition between the air / water interface. Several reports note that PFOA salts self-associate at the surface, but with agitation they disperse and micelles form at higher concentrations. (Simister et al., 1992; Calfours, 1985; Edwards, 1997). Water solubility has been reported for PFOA, but it is unclear whether these values are for a microdispersion of micelles, rather than true solubility. Due to these same surface-active properties of PFOA, and the test protocol for the OECD shake flask method, PFOA is anticipated to form multiple layers in octanol/water, much like those observed for PFOS. Therefore, an n-octanol/water partition coefficient cannot be determined.

The available physicochemical properties for the PFOA free acid are:

Molecular weight: 414 (Beilstein, 1975)
Melting point: 45 – 50 °C (Beilstein, 1975)
Boiling point: 189 – 192 °C / 736 mm Hg (Beilstein, 1975)
Vapor pressure: 10 mm Hg @ 25 °C (approx.) (Exfluor MSDS)
Water solubility: 3.4 g/L (telomeric [MP = 34 °C ref. 0.01 - 0.02 mol/L ~4 - 8 g/L) (MSDS from Merck, Fischer, and Chinameilan Internet sites)
pKa: 2.5 (USEPA AR226- 0473)
pH (1g/L): 2.6 (MSDS Merck)

The PFOA derivative of greatest concern and most wide spread use is the ammonium salt (APFO; CAS No. 3825-26-1). The water solubility of APFO has been inconsistently reported. One 3M study reported the water solubility of APFO to be > 10%. It was noted in an earlier study that at concentrations of 20 g/L, the solution "gelled" (3M, 1979). These numbers seem surprising low for a salt in light of Apollo Scientific selling a 31% aqueous solution of APFO. One author reported the APFO partition coefficient log Pow = 5. Another author reported an estimated APFO log Pow = -0.9. This value might not be accurate due to the estimation method used (Hansch and Leo 1979). Again, the anticipated formation of an emulsified layer between the octanol and water surface interface would make determination of log Kow impossible.

Determination of the vapor pressure of APFO is complicated. A vapor pressure of 7 x 10-5 mm Hg at 20 °C has been reported for APFO; however, this appears to be too low for a material that sublimates as the ammonium salt (3M Environmental Laboratory, 1993). The ammonium salt begins to sublimate at 130 °C. As the temperature increases from when APFO begins to sublimate, 20% of the sample weight is lost by 169 °C. Other salts (Cs, K, Ag, Pb, Li) do not demonstrate similar weight loss until 237 °C or higher. (Lines, 1984). Decomposition of different salts produces perfluoroheptene (loss of metal fluoride and carbon dioxide). This occurs at 320 °C for the sodium salt and at 250-290 °C for the silver salt (Beilstein 1975).

The physicochemical properties of PFOA and its common derivatives are summarized in Table 1.

Table 1. Reported Physicochemical Properties

Compound	CAS REG #	MP	BP	VP	Sol.-H2O	Log P*
R-C(=O)Cl	335-64-8		131 °C			
R-CO2H	335-67-1	55 °C	189 °C	10 mm Hg	3.4 g/L	
R-CO2-NH4+	3825-26-1	130 °C (sub)	sublimes	1 x 10E-5 mm Hg	20 g/L gels	
R-C(=O)OMe	376-27-2		159 °C			
pH (1 g free acid /L Water) = 1.5 – 2.5			Free acid pKa is approximately 0.6			
Sodium or Silver salts of PFOA decompose above 250 °C to generate perfluoroolefins.						

- Surfactants traditionally emulsify octanol and water

3.0 Hazard Characterization

3.1 Metabolism and Pharmacokinetics in Humans

3.1.1 Half-life Studies in Humans

There are very limited data on the half-life of PFOA. With the exception of a 1980 study in which total organic fluorine in blood serum was measured in one worker, no other data were available until June 2000 (Ubel et al., 1980). A half-life study on 27 retirees from the Decatur and Cottage Grove 3M plants was undertaken, in which serum samples were drawn every 6 months over a 5-year period. Two interim reports describing the results thus far have been submitted (Burris et al., 2000; Burris et al., 2002). The first interim report suggested a median serum half-life of PFOA of 344 days, with a range of 109 to 1308 days. The two highest half-life calculations were for the 2 female retirees who participated in this study (654 and 1308 days).

There were several limitations to this first analysis including: 1) the limited data available and the range of serum PFOA levels measured; 2) serum was analyzed after each collection period with only one measurement per time period on different days using slightly different analytical techniques; and 3) the reference material purity was not determined until after the first 3 samples had been analyzed.

An effort was made to minimize experimental error, including systematic and random error in the analytical method. Serum samples were collected from 9 of the original 27 subjects over 4 time periods spanning 180 days, measured in triplicate with all time points from each subject analyzed in the same analytical run. This would allow for statistical evaluation of the precision of the measurement and assure that all systematic error inherent in the assay equally affected each sample used for half-life determination. Single serum measurements were made on samples of the remaining 18 retirees, but were not included because triplicate analyses of all time points were not conducted.

Of the 9 retirees included in this analysis, there were 7 males and 2 females, all from the Decatur plant. The average age of the retirees was 61 years, the mean number of years worked at Decatur was 27.7 years, and the average number of months retired from the plant at study initiation was 18.9. The average body mass index (BMI) of this group was 27.9 (range 22.5-33, SD = 3.6). The mean PFOA value at study initiation was 0.72 ppm (range 0.06 – 1.84 ppm, SD = 0.64).

The mean serum half-life for PFOA was 4.37 years (range 1.50 – 13.49 years, SD = 3.53). Only 1 employee had a half-life value that exceeded 4.3 years. The 2 females had values of 3.1 and 3.9 years. Age, BMI, number of years worked or years since retirement were not significant predictors of serum half-lives in multivariable regression analyses.

This analysis has attempted to reduce experimental error in the determination of a half-life for PFOA. However, two issues should be noted. First, the effect of continual non-occupational, low-level exposure on the half-life is unknown. Second, systematic error of the analytical method could be as high as +/- 20% and still satisfy the data quality criteria.

3.2 Metabolism and Pharmacokinetic Studies in Animals

The metabolism and pharmacokinetics of APFO have been fairly extensively studied in animals. Animal studies have shown that APFO is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. The compound distributes primarily to the liver and plasma. 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 major routes of excretion in male rats. There are major gender differences in the elimination of PFOA in rats. In female rats, estimates of the serum half life range from 1.9 to 24 hours, while in male rats estimates of the serum half life range from 4.4 to 9 days. In female rats elimination of PFOA appears to be biphasic; a fast phase occurs with a half life of approximately 2- 4 hours while a slow phase occurs with a half life of approximately 24 hours. 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. This gender difference has not been observed in primates and humans. The relevant studies are summarized below.

3.2.1 Absorption Studies in Animals

PFOA and its salts are well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. The serum levels of PFOA were measured in a two-generation reproductive toxicity study in rats. These results are presented with the summary of that study in section 3.5. Other studies are summarized here.

Gibson and Johnson (1979) administered a single oral dose of 11.0 mg/kg ^{14}C-PFOA to groups of 3 male CD rats. Twenty-four hr 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. Seven hundred forty-nine ug or 37% of the fluorine in the administered dose was recovered in the urine within 4.5 hr after administration of PFOA. The quantity of nonionic fluorine recovered in the urine increased to 61%, 76% and 89% at 8, 24 and 96 hr, respectively, after administration. Ionic fluoride and total fluorine was also measured. Four and half hours after the administration of PFOA, serum from treated rats had a nonionic fluorine level of 13.6 ppm, virtually all of which was bound to components in the serum and was not ultrafilterable. 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 increase 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 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.

O'Malley and Ebbins (1981) conducted a range finding study that indicates significant dermal absorption of PFOA in male and female New Zealand White rabbits. PFOA at concentrations of

100, 1,000 and 2,000 mg/kg in a saline slurry was applied to approximately 40% of the shaved trunk of the animals (2/sex/group). Animals were then fitted with a plastic collar, and the trunk was wrapped with impervious plastic sheeting. The exposure period was 24 hr/day 5 days/week 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.

Kennedy (1985) treated rats and rabbits dermally with a total of 10 applications of APFO at doses of 0, 20, 200 or 2,000 mg/kg. Doses were applied on a split schedule of 5 days dosing, 2 days of rest, and 5 days of dosing. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner.

Kennedy et al. (1986) exposed male rats by head-only inhalation to doses of 0, 1, 8 or 84 mg/m3 APFO for 6/hr/day 5day/wk for 2 weeks. Immediately after the tenth exposure, mean organofluoride concentrations in the blood were 13, 47 and 108 ppm, respectively in the 1, 8 and 84 mg/m3 dose groups.

3.2.2 Distribution Studies in Animals

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, but does bind to macromolecules in the tissues. There is evidence of enterohepatic circulation of the compound.

Griffith and Long (1980) determined the serum and liver concentrations of PFOA in rhesus monkeys (2 per sex/group) in a 90 day oral toxicity study. In monkeys that received a dose of 3 mg/kg/day, mean serum PFOA levels were 50 ppm in males and 58 ppm in females. At the same dose, males had 3 ppm and females had 7 ppm in liver samples. At a dose of 10 mg/kg/day, male monkeys had mean serum PFOA levels of 58 ppm and females had mean levels 75 ppm. Liver levels of PFOA, measured as organic fluoride, were 9 and 10 ppm for males and females, respectively.

Johnson et al. (1984) investigated the effect of feeding cholestyramine to rats on the fecal 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 and weighing 300-324 g, were given a single iv 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. Carbon-14 in the liver equaled 4% or 12.1±2.1 ug eq/g in cholestyramine treated rats and 8 % or 22.3±6.2 ug eq/g rats that did not receive cholestryamine. In plasma, the levels were 5.1±1.7 ug eq/ml in cholestyramine treated rats and 14.7±6.8 ug eq/ml in rats that did not receive cholestyramine. In red blood cells, the levels were 1.8±0.7 ug eq/ml in cholestryamine treated rats and 4.2±2.4 ug 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 carbon-14 nearly 10-fold suggests that there is enterohepatic circulation of PFOA.

Hanhijarvi et al. (1987) compared the disposition of PFOA in male and female Wistar rats during subchronic administration. PFOA was administered by gavage to 48 newly-weaned animals at 0, 3, 10, and 30 mg/kg/day for 28 consecutive days. Urine was collected on the 7th and 28th day of the study. At the end of the study, blood was collected via cardiac puncture. 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 ppm, 83.1±24.7 ppm, and 53.4 ± 11.2 ppm, respectively for the 3, 10 and 30 mg/kg/day dose levels. The corresponding figures for female rats were 2.43±5.96 ppm, 11.3±8.59 ppm, and 9.06±8.80 ppm respectively, for the 3, 10 and 30 mg/kg/day dose levels. Although the plasma PFOA concentrations were significantly higher in the male rats, no significant histopathological differences between the sexes were observed at necropsy.

Ylinen et al. (1990) studied the difference between male and female Wistar rats in the distribution and accumulation of PFOA after single and subchronic administration. For the single dose study, 50 mg/kg of PFOA was administered by ip injection to groups of 20 male and 20 female 10 week old rats. For the subchronic study, PFOA was administered by gavage at doses of 3, 10, and 30 mg/kg/day to groups of 18 male and 18 female newly weaned rats.
For both studies, samples were collected for determination of PFOA levels 12 hr after treatment, at 24-168 hr at 24 hr intervals, at 244 hr and at 336 hrs after treatment. For the subchronic study, samples were also taken on Day 28. Serum was collected by cardiac puncture; the brain was collected afer decapitation and at necropsy samples from the liver, kidney, lung, spleen, ovary, testis, and adipose tissue were collected and frozen. The biological half-life of PFOA in the serum and tissues was determined from the linear relationship between time and PFOA concentration in the semilogarithmic plot.

In the single-dose study, concentrations of PFOA in the serum and tissues were higher in males than females at all time periods. Twelve hours after the administration of PFOA about 10% of the administered dose was found in the serum of females, whereas about 40% of the administered dose was in the serum of males. In females, the concentration of PFOA in the serum, liver, and kidney occurred in a discontinuous fashion, indicating distinct phases. The half-life in the serum was 24 hr in females and 105 hr in the males. In the females, a half-life of 60 hr was estimated in the liver during the first week. In the males, the half-life in liver was 210 hr. Although PFOA was retained by the liver, it was not found in the lipid fraction. In the kidney, the half-life was 130 hr and 145 hr in females and males, respectively. In the spleen, the half-life was 73 hr in the females and 170 hr in males. PFOA was also found in brain tissue. PFOA was not detectable in adipose tissue.

Samples taken on the 28th day indicated significantly higher PFOA concentrations in the serum and tissues of males versus females at all three dose levels. After subchronic, as well as single-dose administration, PFOA was mainly distributed in the serum of rats. High concentrations of PFOA were also found in the liver, kidney, and lung of males and females. At the 30 mg/kg/day dose level, females and males exhibited, respectively, serum concentrations of 13.92 and 51.65

ppm, liver concentrations of 6.64 ug/g and 49.77 ug/g, kidney concentrations of 12.54 ug/g and 39.81 ug/g, spleen concentrations of 1.59 ug/g and 4.10 ug/g, lung concentrations of 0.75 ug/g and 23.71 ug/g, and brain concentrations of 0.044 ug/g and 0.710 ug/g. The ovary contained 1.16 ug/g and the testis contained 7.22 ug/g. At the 10 mg/kg/day dose level, females and males exhibited, respectively, serum concentrations of 12.47 and 87.27 ppm, liver concentrations of 3.45 ug/g and 51.71 ug/g, kidney concentrations of 7.36 ug/g and 40.56 ug/g, spleen concentrations of 0.38 ug/g and 7.59 ug/g, lung concentrations of 0.22 ug/g and 22.58 ug/g, and brain concentrations of 0.029 ug/g and 1.464 ug/g. The ovary contained 0.41 ug/g and the testis contained 9.35 ug/g. At the 3 mg/kg/day dose level, females and males exhibited, respectively, serum concentrations of 2.40 and 48.60 ppm, liver concentrations of 1.81 ug/g and 39.90 ug/g, kidney concentrations of 0.06 ug/g and 1.55 ug/g, spleen concentrations of 0.15 ug/g and 4.75 ug/g, lung concentrations of 0.24 ug/g and 2.95 ug/g, and brain concentrations of < limits of quantitation and 0.398 ug/g. The ovary contained less than the limits of quantitation and the testis contained 6.24 ug/g. A significant positive correlation existed between the administered dose and the concentration of PFOA in the liver, kidney, spleen, and lung of females. On the contrary, no significant correlation between the administered dose and the concentration of PFOA was observed in the males, as 10 mg/kg/day produced higher PFOA concentrations in the serum and organs than 30 mg/kg/day. However, in males, the concentration in the spleen, testis, and brain correlated positively with the concentration in the serum.

Vanden Heuvel et al. (1991b) administered 9.4 umol/kg ^{14}C-PFOA by ip injection, to male and female Harlan Sprague-Dawley rats. The concentration of ^{14}C-PFOA-derived radioactivity in the blood was higher and eliminated more slowly in males than in females. In males, the t½ was 9 days while the t½ was 4 hr in females.. In the male rats, 21% of the administered dose was present in the liver at 2 hr after treatment followed by levels in the plasma and kidney. By 28 days post-treatment, levels in the liver had fallen to 2% of the administered dose. Far lower PFOA concentrations were found in the heart, testis, fat, and gastrocnemius muscle. In females at 2 hr post dose, the highest concentrations of PFOA were found in the plasma followed by the kidney, liver and ovaries in that order. The average t½ for elimination of PFOA from the liver in male rats was 11 days compared to an average of 9 days for extrahepatic tissues. In females, the average t½ for tissue elimination was approximately 3 hr.

Vanden Heuvel et al. (1991a) investigated the disposition of PFOA in perfused male rat liver. Liver was infused with 0.08 umol ^{14}C-PFOA/min over a 48 min period for a total of 3.84 umol ^{14}C. Approximately 11% of the cumulative dose of ^{14}C-PFOA infused was extracted by the liver during 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. Pooled daily urine samples taken 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 previously with ^{14}C-PFOA 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. Female rats eliminated PFOA-derived radioactivity rapidly in the urine with 91% of the dose being excreted

in the first 24 hr, while male rats excreted only 6% of the dose in the same time period. Negligible radioactivity was recovered in the feces of female rats. In male rats during the 28-day collection period, the cumulative excretion of PFOA-derived ^{14}C in urine and feces was 36.4% in urine and 35.1% in feces. The female rat retained less than 10% of the administered dose after 24 hr, while the male rats retained 30% of the administered dose after 28 days. The whole-body elimination half-life in females was less than one day, and in males it was 15 days. In renal-ligated rats injected ip with ^{14}C-PFOA, approximately 0.3% of the PFOA-derived radioactivity was excreted in the bile after 6 hours. No sex-related difference in the biliary excretion of PFOA was observed when the kidneys were ligated.

Vanden Heuvel et al. (1992) demonstrated that PFOA covalently binds to proteins in the liver, plasma, and testes of rats. Carbon-14-labeled PFOA at a dose of 9.4 umol/kg was administered by ip injection to six-week old male Harlan Sprague-Dawley rats. No time-dependent changes in either absolute or relative concentrations of covalently bound PFOA-derived ^{14}C were found at 2 hours, 1 and 4 days post-treatment. Covalently bound PFOA was represented by 0.1% to 0.3% of the tissue ^{14}C content. The absolute concentration of covalently bound PFOA was significantly higher in the plasma than in the liver. The testes had the highest relative concentration of covalently bound PFOA-derived radioactivity.

Johnson (1995a) reported on the disposition of the tetrabutyl ammonium salt of perfluorooctanoic acid in female rabbits. Individual rabbits were given intravenous doses of 0, 4, 16, 24 and 40 mg/kg. The animal give 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 ppm, 4.09 ppm, 14.9 ppm, and 41.0 ppm, respectively. There was a rapid decrease of total organic fluorine in the serum with time; it 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 ug, 43 ug, 66 ug, and 54 ug, respectively.

3.2.3 Metabolism Studies in Animals

Vanden Heuvel et al. (1991b) investigated the metabolism of PFOA in Harlan Sprague-Dawley rats administered ^{14}C-PFOA (9.4 umol/kg, ip). 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 previously with ^{14}C-PFOA 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.

Ophaug and Singer (1980) also 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.

3.2.4 Elimination Studies in Animals

Gibson and Johnson (1980) observed a sex difference in extent and rate of excretion of total carbon-14 between male and female CD rats after a single iv dose of ^{14}C-PFOA. 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.

The urinary excretion of APFO in rats was investigated by Hanhijarvi et al. (1982). Four male and six female Holtzman rats were administered 2 mg APFO in 2 ml aqueous solution by stomach intubation. Seven female rats were administered 2 ml distilled water as controls. The animals were then placed in metabolism cages with rat chow and tap water. Urine was collected until 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. For clearance studies of APFO and inulin, the rats were anesthetized with Inactin 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 ip injection and additional clearance tests were performed. In the cumulative excretion study, rats were dosed iv with a mixture of 10%-20% radiolabeled-APFO and 80-90% unlabeled APFO. Five percent mannitol was infused and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65-68 mg/kg by ip injection at least 30 min prior to the administration of APFO. Twenty-four hours after oral administration of APFO, 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 ppm, 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 ppm. 97.5±0.25% of the APFO was bound in the plasma of both male and female rats. The clearance studies demonstrated major differences between the sexes in rats. The APFO clearance in female rats was several times greater than the inulin clearance. Administration of probenecid, which strongly inhibits active renal secretion of organic acids, reduced the APFO/inulin clearance ratio in females from 14.5 to 0.46. APFO clearance was reduced from 5.8 to 0.11 ml/min/100g. Net APFO excretion was reduced from 4.6 ug/min to 0.13 ug/min/100g. In male rats, however, the APFO/inulin clearance ratio and the net excretion of APFO were virtually unaffected by probenecid. In the males, APFO clearance was 0.17 ml/min/100g, the APFO/inulin clearance ratio was 0.22, and net APFO excretion was 0.17 ug/min/mg. In the cumulative excretion studies, female rats excreted 76% of the administered dose of APFO, 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 APFO elimination to 11.8%. The authors concluded that the female rat possesses an active secretory mechanism which rapidly eliminates

APFO from the body. This secretory mechanism is lacking or is relatively inactive in male rats and accounts for the greater toxicity of APFO in males.

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 2 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.

Hanhijarvi et al. (1988) investigated the excretion of PFOA in the beagle dog. Six laboratory bred beagle dogs, 3 males and 3 females were given an iv dose of 30 mg/kg of PFOA followed by continuous infusion with 5% mannitol. Urine was collected at 10 minute intervals for 60 min. A 5 ml blood sample was collected in the middle of each urine sampling period. Thirty mg/kg probenecid was then administered by iv 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 males and 2 females, were given 30 mg/kg of PFOA by iv 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.

Ylinen et al (1989) studied the urinary excretion of PFOA in male Wistar rats after castration and estradiol administration. They also studied urinary excretion in intact males and females. 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 ug/kg estradiol valerate by sc injection every second day for 14 days before administration of PFOA. PFOA was administered as a single ip 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 was no statistically significant difference 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 umol/kg, ^{14}C-PFOA by ip injection. Castration increased the elimination of PFOA in the urine by >1-fold (36% of the dose was eliminated in 4 days versus 16% in controls), demonstrating 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) demonstrated in male and female Wistar rats that renal clearance (CL_R) of PFOA and the renal mRNA levels of specific organic anion transporters are markedly affected by sex hormones. The biological half-life of PFOA in male rats was found to be 70 times longer (5.7 days versus 1.9 hours) than in female rats and this difference is due primarily to low CL_R in male rats. 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. 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 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 organic anion transporter 2 (OAT2) and OAT3 are responsible for urinary elimination of PFOA in the rat.

3.3 Epidemiology Studies

3M has conducted several epidemiology and medical surveillance studies of the workers at its Decatur, Antwerp, and Cottage Grove plants. However, these studies have not provided information regarding the potential for developmental toxicity since the majority of production workers at facilities that produce or use PFOA are male, and reproductive outcomes have not been examined. The results of these studies are summarized below for the readers' information.

3.3.1 Mortality Studies in Humans

A retrospective cohort mortality study was performed on employees at the 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 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 die from cerebrovascular disease (5 deaths observed, 1.94 expected; 95% CI = 0.84 – 6.03).

In the probable exposure group, 3 SMRs should be noted: 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 employees 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.

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.

It is difficult to compare the results of the first and second mortality studies at the Cottage Grove plant since the exposure categories were modified. 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 (and Decatur) 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.

3.3.2 Hormone Study in Humans

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 (although 80 ppm was the limit of detection that year, so it could have been higher) 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, FSH, 17 gamma-hydroxyprogesterone (17-HP), free testosterone, total testosterone, 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, 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.

Estradiol was 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. 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. 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.

Free testosterone was highly correlated with age in both 1993 and 1995. The authors did not report a negative association between PFOA serum levels and testosterone. There were no statistically significant trends 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). There were no significant associations between PFOA and cortisol, DHEAS, FSH, LH, and SHBG.

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.

3.3.3 Study on Episodes of Care (Morbidity)

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). An "episode of care" is a series of health care services provided from the start of a particular 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.

3.3.4 Medical Surveillance Studies from the Antwerp and Decatur Plants

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, body mass index (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. 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. 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.

3.3.5 Medical Surveillance Studies from the Cottage Grove Plant

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, Olsen et al., 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. Mean serum PFOA levels and ranges are provided in Table 2 of the Biomonitoring Section of this report. It should be noted that 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 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.

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), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (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).

3.4 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, 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.

A second 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 iv 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.

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 males and females, dead or live fetuses, and 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 13th rib, with statistical significance at the high-dose group (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). A statistically significant increase in 13th ribs-spurred occurred in the mid-dose group of 5 mg/kg/day; however, the biological significance of this effect is uncertain since in both the high- and low-dose groups, this effect occurred at the same rate and was not statistically significantly different from controls. Therefore, under the conditions of the study, a LOAEL for developmental toxicity of 50 mg/kg/day (highest dose group) was indicated.

Staples et al. (1984) also conducted a developmental toxicity study of APFO. 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 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 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 and in the control group pair-fed 25 mg/m^3. However, interpretation of the decreased fetal body weight is difficult given the high incidence of mortality in the dams. Under EPA guidance, data at doses exceeding 10% mortality are generally discounted. 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.

3.5 Reproductive Toxicity Studies in Animals

York (2002) conducted an oral two-generation reproductive toxicity study of APFO, which is summarized below. Although this preliminary risk assessment focuses on developmental toxicity, the summary below of the two generation reproductive toxicity study includes all endpoints.

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 and assigned to individual housing. 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 and frozen for future analysis. The methods section cites that liver samples were also collected, but no other details were provided and the results did not appear to be available at the time of the report.

The F1 generation pups in each litter were counted once daily. The litter sizes were not standardized on day 4. 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.

Parental Males (F0)

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 at 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. 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 significantly reduced at 30 mg/kg/day. 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 significantly increased in all dose-groups. Kidney weights were significantly increased in the 1, 3, and 10 mg/kg/day dose groups, but significantly decreased in the 30 mg/kg/day group. All organ weight-to-terminal body weight and ratios were significantly increased in all treated 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 the 10 and 30 mg/kg/day dose groups.

Serum analysis for the F0 generation males 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$\pm$ 0.0148 ppm PFOA. Levels of PFOA found in male sera remained the same between the two dose groups; treated males had 51.1$\pm$9.30 and 45.3$\pm$12.6 ppm, respectively for the 10 and 30 mg/kg/day dose groups.

Parental Females (F0)

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 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 ratio of liver weights-to-terminal body weight was also significantly reduced at 3 and 10 mg/kg/day.

Results of the serum analysis in F0 generation females sampled on LD 22 showed that PFOA was present in all samples tested, except in controls where the level was below the limits of quantitation (0.00528 ppm). 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 ppm, respectively for the 10 and 30 mg/kg/day dose groups.

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.

Pup body weight on a per litter basis (sexes combined) was reduced throughout lactation in the 30 mg/kg/day group, and statistical significance was achieved on days 1, 5, and 8. 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.

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 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.

Body weights and body weight gains were statistically significantly reduced prior to and during cohabitation and during the entire dosing period in all treated groups. Statistically significant reductions in body weights 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. Statistically significant, dose-related reductions in body weight gains were observed for the entire dosage period (days 1-113 postweaning). 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 of parental F1 males were observed in the 1,3,10, and 30 mg/kg/day dose groups. The absolute weights of the liver were significantly increased and the absolute weights of the spleen were significantly decreased at all treated groups. The absolute weights of the left and/or right kidneys were significantly increased in the 1 and 3 mg/kg/day dose groups and significantly decreased in the 30 mg/kg/day dose group. The absolute weight of the thymus was also significantly decreased in the 10 and 30 mg/kg/day dose groups. The absolute weight of the prostate, brain and left adrenal gland were significantly decreased in the 30 mg/kg/day dosage group. The ratios of the weights of the seminal vesicles, with and without fluid, liver and left and right kidneys to the terminal body weights were significantly increased in all treated groups. The ratios of the weights of the left testis, with and without the tunica albuginea and the right testis to the terminal body weight, were significantly increased at 3 mg/kg/day and higher. The ratios of the weights of the left epididymis, left cauda epididymis, right epididymis and brain to the terminal body weight were

significantly increased at 10 mg/kg/day and higher. The ratios of the weight of the seminal vesicles with fluid to the brain weight were increased at 1 mg/kg/day and higher, with statistical significance at 1 and 10 mg/kg/day. The ratios of the liver weight-to-brain weight were significantly increased in the 1 mg/kg/day and higher dosage groups, and the ratios of the left and right kidney weights-to-brain weight were significantly increased in all treated groups. The ratios of the spleen weight-to-brain weight were significantly decreased at 1 mg/kg/day and higher, and the ratios of the thymus weight-to-brain weight were significantly decreased at 10 and 30 mg/kg/day. The ratios of the left and right testes weight-to-brain weight were increased in the 3 mg/kg/day and higher dosage groups. These ratios were significantly increased at 10 mg/kg/day (right testis only) and 30 mg/kg/day.

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.

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 and body weight gains 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. Statistically significant decreases in absolute food consumption were observed during days 1-8, 8-15 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 Company (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.

No treatment-related effects were observed on terminal body weights. The absolute weight of the pituitary and the ratios of the pituitary weight-to-terminal body weight and to the brain weight were significantly decreased at 3 mg/kg/day and higher, but did not show a dose-response. No other differences were reported for the absolute weights or ratios for other organs evaluated. No treatment-related effects were reported following necroscopic and histopathologic examinations.

F2 Generation

No treatment-related adverse clinical signs were observed at any dose level. Likewise, no treatment-related effects were reported following necroscopic examination, with the exception of no milk in the stomach of the pups that were found dead. The total number of pups found either dead or stillborn did not show a dose-response (3/28, 6/28, 10/28, 10/28, and 6/28 in 0, 1, 3, 10, and 30 mg/kg/day dose groups, respectively; ratios refer to total pups/total number of litters) and therefore were unlikely related to treatment.

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.

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.

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.

Conclusions

Dosing with APFO at 30 mg/kg/day appeared to delay 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 the $p \leq 0.05$. 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.

Therefore, under the conditions of the study, the LOAEL for F0 parental males is considered to be 1 mg/kg/day, the lowest dose tested, based on significant increases in the liver and kidney weights-to-terminal body weight and to brain weight ratios. A NOAEL for the F0 parental males could not be determined since treatment-related effects were seen at all doses tested.

The NOAEL and LOAEL for F0 parental females are considered to be 10 and 30 mg/kg/day, respectively, based on significant reductions in kidney weight and kidney weight-to-terminal body weight and to brain weight ratios observed at the highest dose.

The LOAEL for F1 generation males is considered to be 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 liver and spleen weights and in the ratios of liver, kidney, and spleen weights-to-brain weights. A NOAEL for the F1 males could not be determined since treatment-related effects were seen at all doses tested.

The NOAEL and LOAEL for F1 generation females are considered to be 10 and 30 mg/kg/day, respectively, based on statistically significant increases in postweaning mortality, delays in sexual maturation (time to vaginal patency), decreases in body weight and body weight gains, and decreases in absolute food consumption, all observed at the highest dose tested.

The NOAEL for the F2 generation offspring was considered to be 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.

It is important to note that the LOAELs and NOAELs summarized above are for the entire duration of the study, and therefore represent effects resulting from developmental and/or adult exposures. These effect levels differ from those described in section 5.1. The LOAELs and NOAELs described in section 5.1 only refer to effects resulting from developmental exposures.

4.0 Exposure Characterization

PFOA has been detected in human serum of workers occupationally exposed to APFO, and it has also been measured in the general population. In general the levels in the general population are much lower than in the workers. However, it should be noted that the highest levels reported to date in the general population are similar to some of the lowest levels in workers exposed to PFOA occupationally. It is not known what the environmental concentrations of APFO are or the pathways of exposure to the general population.

4.1 Occupational exposures

3M has been offering voluntary medical surveillance to workers at plants that produce or use perfluorinated compounds since 1976. Serum PFOA levels have been measured and reported since 1993. Prior to this time, only total organic fluorine was measured. The results of biomonitoring for PFOA have been reported for 3 plants: Cottage Grove, MN; Decatur, AL and Antwerp, Belgium. Surveillance years include 1993, 1995, 1997, 1998, and 2000, although not all of the plants offered surveillance in all of these years. The 1998 data reported for the Decatur plant consist of a random sample of employees; however, volunteers participated in all of the

other sampling periods for all of the plants. The results of these studies are summarized in Table 2.

Table 2. Summary of Occupational Exposures (ppm)

Plant	Arithmetic Mean	Range	Geometric Mean	95% CI
Cottage Grove				
1997 (n =7 4)	6.4	0.1 - 81.3	NA	NA
1995 (n = 80)	6.8	0 - 114.1	NA	NA
1993 (n = 111)	5.0	0 - 80.0	NA	NA
Decatur				
2000 (n = 263)	1.78	0.04 - 12.7	1.13	0.99 - 1.3
1998 (n = 126)	1.54	0.02 - 6.76	0.9	0.72 - 1.12
1997 (n = 126)	1.57	NA	NA	NA
1995 (n = 90)	1.46	NA	NA	NA
Antwerp				
2000 (n = 258)	0.84	0.01 - 7.04	0.33	0.27 - 0.4
1995 (n = 93)	1.13	0 - 13.2	NA	NA
Building 236				
2000 (n = 45)	0.106	0.008 - 0.668	0.053	0.037 - 0.076

Mean serum PFOA levels have increased slightly at both the Cottage Grove and Decatur plants since 1993. Workers at the Cottage Grove plant, where PFOA exposures are highest, have the highest PFOA serum levels. The latest sample was in 1997 (Olsen, et al., 1998b). The mean serum PFOA level was 6.4 ppm (range = 0.1 – 81.3 ppm). Only 74 employees participated in the 1997 surveillance. The eligible voluntary participation rates ranged from approximately 50% in 1997 to 70% in 1993.

At the Decatur plant, 263 of 500 employees participated in 2000 (Olsen, et al., 2001a). The mean serum PFOA level was 1.78 ppm. It was higher in males (n = 215) than females (n = 48), 1.90 and 1.23 ppm, respectively. In addition, male production employees had higher mean serum levels (2.34 ppm). Five employees had serum levels greater than 5 ppm, the Biological Limit Value established by the 3M Exposure Guideline Committee. Cell operators had the largest increase in serum PFOA between 1998 and 2000. The highest level was in a chemical operator on the Scotchgard team (12.70 ppm). The mean level for the rest of the members of the team was 5.06 ppm (range 5 - 9 ppm). Other job categories did not exhibit such a large increase. 3M reports that this is due to increased PFOA production at the Decatur plant beginning in 1999. Serum PFOA levels for the Antwerp plant are lower than at Decatur or Cottage Grove, and have decreased slightly since 1995 (Olsen, et al., 2001b). Participation in medical surveillance at the

Antwerp plant was the highest it had ever been in 2000 (258 volunteers out of 340 workers). The mean serum PFOA level was 0.84 ppm, and the highest serum level reported was 7.04 ppm. As in the Decatur plant, males (n = 209) had higher mean serum PFOA levels (1.03 ppm) than females (n = 49, 0.07 ppm). Three employees had levels greater than 5 ppm.

3M's Specialty Materials Manufacturing Division Laboratories, where employees perform fluorochemical research (Building 236), conducted voluntary biomonitoring of 45 employees in 2000 (Olsen, et al., 2001c). The mean PFOA serum level was 0.106 ppm (range 0.008 – 0.668 ppm).

4.2 Non-occupational Exposures

Serum PFOA levels in corporate staff and managers at a 3M plant in St. Paul, MN, where occupational exposure to PFOA should not have occurred, were reported (3M Report, 1999). Four of 31 employees had serum PFOA levels greater than the detection limit of 10 ppb. The mean for these employees was 12.5 ppb.

4.3 General Population Exposures

Data on PFOA levels in the general population are very limited. They are very recent so that trends over time cannot be established. The mean serum PFOA levels are lower in the general population than in workers exposed to PFOA. The available data are summarized in Table 3.

Table 3. Summary of General Population Exposures (ppb)

Sample	Arithmetic Mean	Range	Geometric Mean	95% CI
Pooled Samples				
Commerical sources of blood, 1999 (n = 35 lots)	3	1 - 13	NA	NA
Blood banks, 1998 (n = 18 lots, 340-680 donors)	17*	12 - 22	NA	NA
Individual Samples				
American Red Cross blood banks, 2000 (n = 645)	5.6	1.9 - 52.3	4.6	4.3 - 4.8
Elderly (65 - 96 years), 2000 (n = 238)	NA	1.4 - 16.7	4.2	3.9 - 4.5
Children (2 - 12 years), 1995 (n = 598)	5.6	1.9 - 56.1	4.9	4.7 - 5.1

* PFOA detected in about 1/3 of the pooled samples but quantifiable in only 2.

Pooled blood samples from U.S. blood banks indicate mean PFOA levels of 3 to 17 ppb (3M Company, Feb. 5, 1999; 3M Company, May 26, 1999). The highest pooled sample reported was 22 ppb. Samples were collected in 1998 and 1999. However, it cannot be assumed that these levels are generalizable to the U.S. population for several reasons: 1) blood donors are a unique group that does not necessarily reflect the U.S. population as a whole, 2) many of the blood banks originally contacted for possible inclusion in the study declined to participate, 3) only a small number of samples have actually been analyzed for PFOA, and 4) no other data such as age, sex, or other demographic information are available on the donors.

Individual blood samples from 3 different age populations were recently analyzed for PFOA and other fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) (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 ppb, 4.2 ppb, and 4.9 ppb, respectively). The geometric means and 95% tolerance limits (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. However, the data appeared to have a log normal distribution and therefore geometric means were calculated. 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 ppb. The range was <lower limit of quantitation (1.9 ppb) to 52.3 ppb. Only 2 samples were less than the LLOQ. Males had significantly higher ($p < .05$) geometric mean PFOA levels than females (4.9 ppb vs. 4.2 ppb). 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 ppb, range: 2.1 – 29.0) and the lowest from Portland (3.6 ppb, range: 2.1 – 16.7). The highest individual value was reported in Hagerstown (52.3 ppb).

Serum PFOA levels were reported for 238 (118 males and 120 females) elderly volunteers in Seattle participating in a study designed to examine cognitive function in adults aged 65-96 (Olsen, et al., 2002b). 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 ppb (95% CI, 3.9 – 4.5). The range was 1.4 – 16.7 ppb. Only 5 samples were less than the LLOQ of 1.4 ppb. 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. In bootstrap analyses, the mean of the 95% tolerance limit for PFOA was 9.7 ppb with an upper 95% confidence limit of 11.3 ppb. 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). 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 ppb. The geometric mean of PFOA for all of the participants was 4.9 ppb (95% CI, 4.7 – 5.1). The range was 1.9 to 56.1 ppb. Male children had significantly ($p<.01$) higher geometric mean serum PFOA levels than females: 5.2 ppb and 4.7 ppb, 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 ppb) and lowest at age 12 (3.5 ppb). 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 the data and given the limited sample size for each gender/location subgroup. In bootstrap analyses, the mean of the 95% tolerance limit for PFOA was 10.1 ppb with an upper 95% confidence limit of 11.0 ppb. PFOS and PFOA were highly correlated (r = .70) in this study. PFOA and PFHS (perfluorohexanesulfonate) were also correlated (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. 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 (Olsen et al., 2001d). Thirty-one donor samples were obtained from 16 males and 15 females over an 18-month period from the International Institute for the Advancement of Medicine (IIAM). 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. Serum samples were obtained from 5 ml of blood; liver samples consisted of 10 g of tissue. Samples were frozen at IIAM and shipped frozen to 3M for analysis. Samples were extracted using an ion-pairing extraction procedure and were quantitatively assayed using HPLC-ESMSMS and evaluated versus an unextracted curve. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the extraction procedure. 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 3.1 ng/mL with a geometric mean of 2.5 ng/mL. No liver to serum ratios were provided because more than 90% of the individual liver samples were <LOQ.

5.0 Preliminary Risk Assessment

For this preliminary risk assessment, a margin of exposure (MOE) approach was used to describe the potential for developmental toxicity associated with exposure to PFOA and its salts. The MOE is calculated as the ratio of the NOAEL, LOAEL, or BMDL for a specific endpoint to the estimated human exposure level. The MOE does not provide an estimate of population risk, but simply describes the relative "distance" between the exposure level and the NOAEL, LOAEL, or BMDL.

For many risk assessments, the MOE is calculated as the ratio of the administered dose from the animal toxicology study to the estimated human exposure level. The human exposure is estimated from a variety of potential exposure scenarios, each of which requires a variety of

assumptions. A more accurate estimate of the MOE can be derived if measures of internal dose are available for humans and the animal model. In this preliminary risk assessment, serum levels of PFOA, which is a measure of internal dose, were available for the animal toxicology studies and from human biomonitoring studies. Thus, internal dose was used for the calculation of MOEs in this assessment.

5.1 Selection of Developmental Endpoints

As stated in the section entitled "Scope of the Assessment", the purpose of this preliminary assessment was to determine the potential of developmental toxicity associated with exposure to PFOA and its salts. It was therefore necessary to determine which endpoints from the animal toxicology studies would be relevant for this assessment. The selection of developmental endpoints for this assessment was based on the Agency's Developmental Toxicity Risk Assessment Guidelines (EPA, 1991). According to the guidelines, the period of exposure for developmental toxicity is prior to conception to 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.

Several oral prenatal developmental toxicity studies of APFO have been conducted in rats and rabbits. A summary of the exposure duration and the LOAELs and NOAELs are presented in Table 4.

Table 4. Summary of Oral Prenatal Developmental Toxicity Studies

Species	Exposure Duration	Time Endpoints Assessed	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)	Reference
Sprague-Dawley rat (n=22/group)	GD 6-15	GD 20	none	150	Gortner, 1981
Sprague-Dawley rat (n=25/group)	GD 6-15	GD 20	none	100	Staples, 1984
Sprague-Dawley rat (n=12/group)	GD 6-15	PND 35	none	100	Staples, 1984
New Zealand white rabbit (n=18/group)	GD 6-18	GD 29	50*	5	Gortner, 1982

* - there was a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at the high-dose group (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).

In addition, developmental effects were observed in the oral two generation reproductive toxicity study that was conducted in Sprague-Dawley rats (York, 2002). For selection of the developmental endpoints from this 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 this preliminary risk characterization. In the high dose group administered 30 mg/kg/day APFO, there was a reduction in F1 mean body weight on a litter basis during lactation (sexes combined). For F1 females, 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 in the 30 mg/kg/day group. For F1 females, the LOAEL for developmental toxicity was considered to be 30 mg/kg/day, and the NOAEL was 10 mg/kg/day. For F1 males in the 30 mg/kg/day group, there was an increase in mortality mainly during the first few days after weaning, a significant delay in the timing of sexual maturation, and a significant reduction in mean postweaning body weight that began on day 8 and continued through the duration of the study. At 10 mg/kg/day, mean body weights were significantly reduced beginning on day 36 postweaning and continuing through the duration of the study. For F1 males, the LOAEL for developmental toxicity was considered to be 10 mg//kg/day, and the NOAEL was 3 mg/kg/day. The NOAEL for the F2 generation offspring was considered to be 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.

The database that is available to examine the potential developmental toxicity associated with oral exposure to APFO thus consists of two prenatal studies in Sprague-Dawley rats, a prenatal study in New Zealand white rabbits, and a two generation reproductive toxicity study in Sprague-Dawley rats. Since no developmental toxicity was noted in the prenatal studies in Sprague-Dawley rats these studies were not considered for the calculation of the MOEs. The effects noted in the prenatal study in New Zealand white rabbits and in the two generation reproductive toxicity study in Sprague-Dawley rats were considered important for the calculation of the MOEs.

5.2 Use of Serum Levels as a Measure of Internal Dose for Humans

Serum levels of PFOA were available from human biomonitoring studies. These provide a measure of total human exposure and a measure of internal dose. The populations that were considered relevant for assessing the potential for developmental toxicity included children and women of child bearing age. Estimates of general human population exposure were available from recent analyses of individual serum samples from a group of children (2-12 years) and adults (20-69 years). Individual serum data were also available from a recent analysis of a group of elderly adults (65-96 years). However, these data were not included in this analysis given that the concerns are for women of child bearing age. The data obtained from the pooled blood samples from blood banks and commercial sources were not used in the calculation of the MOEs given the limitations of these data.

A summary of the human serum levels of PFOA that were considered in the calculation of MOEs is provided in Table 5. The arithmetic means and ranges are presented in order to display

the higher end of the range of PFOA serum levels in a small segment of these populations. The geometric means and 95% confidence intervals are presented because the serum levels represent what appears to be a log normal distribution. Gender specific data were available for the geometric mean and range, but not for the arithmetic mean. Since the geometric means for the males and females were very similar (4.2 ppb for females and 4.9 ppb for males), the value used in the MOE calculation was the mean for sexes combined which was 4.6 ppb. The value for sexes combined was used so that MOEs could be calculated using both the arithmetic and geometric means. The use of the value of 4.6 ppb versus the gender specific levels of 4.2 and 4.9 ppb has minimal impact on the resulting MOE in this preliminary risk assessment.

Table 5. Summary of Levels of PFOA in the Serum of Human Populations

Population	Arithmetic Mean	Range	Geometric Mean	95% CI
Adults (20 - 69 years, American Red Cross blood banks, 2000, n=645)	5.6 ppb	1.9 - 52.3 ppb	4.6 ppb	4.3 - 4.8 ppb
Children (2-12 years, 1995, n=598)	5.6 ppb	1.9 - 56.1 ppb	4.9 ppb	4.7 - 5.1 ppb

5.3 Use of Serum Levels as a Measure of Internal Dose for Animal Studies

Serum levels of PFOA were available as a measure of the internal dose of humans. Thus, only those animal studies with serum levels of PFOA were considered for the calculation of the MOEs. Since no information on serum levels was available for the prenatal developmental toxicity study in New Zealand white rabbits, MOEs were not calculated for the endpoints from this study. Serum levels were available for the two generation reproductive study in Sprague-Dawley rats. Therefore, this study was used for the calculation of the MOEs.

In the two generation reproductive toxicity study in Sprague-Dawley rats, the LOAEL for developmental toxicity was considered to be 30 mg/kg/day, and the NOAEL was 10 mg/kg/day for the F1 females. For the F1 males, the LOAEL for developmental toxicity was considered to be 10 mg/kg/day, and the NOAEL was 3 mg/kg/day. Serum levels of PFOA were not measured in the F1 animals. Serum levels were available for F0 animals in the control, 10 and 30 mg/kg/day groups. Serum levels were not measured in the 1 and 3 mg/kg/day groups. The serum levels were measured in the F0 males at the end of the cohabitation period, while serum levels were measured on lactation day 22 in the F0 females. For both sexes, the serum levels were measured 24 hours after the administration of the last dose.

For this preliminary risk assessment, the serum levels for the F0 animals were used to provide an estimated range of potential serum levels in the F1 animals. The following approach was employed. It was reasoned that if prenatal and/or lactational exposures were important in

eliciting the developmental effects, then the serum levels in the dam (i.e. F0 females who were being administered APFO) would be the most representative of the serum levels in the F1 pups. The serum levels in the F0 males would not be informative. It was further reasoned that if postweaning exposures were important then the serum levels for the F0 males would be the most appropriate estimate for the F1 males, and similarly the serum levels in the F0 females would be the most appropriate estimate for the F1 females.

If prenatal and/or lactational exposures were important, several other factors had to be considered. First, serum levels were measured in the F0 females 24 hours after dosing. Given that the serum half-life in female rats is less than 24 hours, this value would represent the low end of exposure. Since the peak exposure of the F0 females is not known, it was reasoned that it was unlikely that the peak exposure to the F0 females was higher than the serum level in the F0 males in the same dose group since the serum half life of PFOA in male rats is 4.4 - 9 days, and therefore with a daily dosing regime they would tend to accumulate PFOA. Therefore, the serum levels in the F0 males and females in the 10 mg/kg/day were used to provide a range of values for the calculation of the MOEs; the serum levels were 51.1 and 0.37 ppm in the F0 males and females, respectively. The serum levels of the F0 females probably represent a low internal dose for the F1 animals and the serum levels of the F0 males probably represent a high internal dose for the F1 animals.

For the scenario where postweaning exposures are important, the serum levels in F0 females would be the most appropriate estimate of the serum levels in F1 females. For F1 females, the same rationale was applied for this scenario as was applied to the previous scenario for prenatal and/or lactational exposures. Therefore, the serum levels in the F0 males and females in the 10 mg/kg/day were used to provide a range of values for the calculation of the MOEs.

Similarly, for the scenario where postweaning exposures are important, the serum levels in the F0 males would be the most appropriate estimate of the serum levels in F1 males. For this assessment, the LOAEL for developmental effects in the F1 males was considered to be10 mg/kg/day and the NOAEL was considered to be 3 mg/kg/day. However, serum levels for the F0 males were only measured in the 10 and 30 mg/kg/day groups; there was no information available for the 3 mg/kg/day group. In addition, it was not possible to extrapolate the serum levels to the lower administered doses as the values appear to have reached a plateau at 10 and 30 mg/kg/day (51.1 and 45.3 ppm, respectively) and are not linear. Therefore, for this preliminary assessment the serum levels from the F0 males in the 10 mg/kg/day group were used in the calculation of the MOEs.

5.4 Calculation of MOEs

The human populations of concern for this preliminary assessment are women of child bearing age and children. As stated in section 5.2, the serum data from the American Red Cross study included both men and women, ages 20-69. As explained in section 5.2, since the data were not consistently reported separately for each gender and since the geometric means were very similar for males and females, the means for the sexes combined are used as a surrogate for women of child bearing age. The MOEs were calculated by dividing the serum values for the F0 female

and male rats in the 10 mg/kg/day group (0.37 ppm and 51.1 ppm, respectively) in the two generation reproductive toxicity study by the American Red Cross blood samples and children's samples presented in Table 5. The MOEs for potentially exposed populations are presented in Table 6.

It is important to note that MOEs that were calculated from the serum levels in the F0 female and male rats provide a means to bracket the low and high ends of reported experimental exposures. This is an unusual situation in that MOE estimates, which typically represent point estimates, are described here as a range of potential values due to uncertainties in the rat serum data. This situation arises from the fact that the available data do not allow selection of a particular departure point for the MOE calculations. It is likely that the MOEs calculated using the F0 female rat serum level are lower than what would be anticipated in the human population, and it is likely that MOEs calculated using the F0 male rat serum level are higher than what would be anticipated in the human population. As uncertainty around the rat serum values decreases the end brackets are likely to shift towards the middle of the current range. Therefore, MOE values presented in Table 6 should not be interpreted as representing the range of possible MOEs in the US population. It is likely that when more extensive rat kinetic data are available, the resultant, refined estimated range of MOEs will constitute a narrower subset of the range presented here.

Table 6. MOE Calculations for Potentially Exposed Populations Using F0 Rat Serum Values and Human Serum Values

Human Population	**MOE values calculated using rat serum values from the 2-generation reproductive study[1]**
Women of Childbearing Age[2]	
Arithmetic mean	66 ----- 9125
Geometric mean	80 -----11,109
Children[3]	
Arithmetic mean	66 ---- 9125
Geometric mean	75 ---- 10,429

[1]Estimated MOE values are bracketed by the serum level concentrations in the F0 females (0.31 ppm) and the F0 males (51.1 ppm).
[2]The American Red Cross serum samples were used as an estimate of the arithmetic and geometric means in women of child bearing age.
[3]The samples from the Children's study were used as an estimate of the arithmetic and geometric means.

The MOE that was calculated at the low end of the range using the rat female F0 serum levels and the arithmetic mean for the adults is 66, while the MOE that was calculated using the geometric mean for the adults is 80. The MOEs that were calculated at the high end of the range using the serum levels of the rat F0 males and the arithmetic or geometric means for the adults range from approximately 9,000-11,000. If the upper and lower values of the human serum levels are used in the MOE calculation with the rat F0 females, the resulting MOEs are 195 and 7. If the upper and lower values of the human serum levels are used in the MOE calculation with the rat F0 males, the resulting MOEs are 26,895 and 911.

The MOEs for children were calculated by dividing the serum values for the rat F0 females and males in the 10 mg/kg/day group (0.37 ppm and 51.1 ppm, respectively) by the biomonitoring values presented in Table 5. The MOE that was calculated at the low end of the range using the rat female F0 serum levels and the arithmetic mean for the children is 66, while the MOE that was calculated using the geometric mean for the children is 75. The MOEs that were calculated at the high end of the range using the serum levels of the rat F0 males and the arithmetic or geometric means for the children range from approximately 9,000-10,400. If the upper and lower values of the childrens' serum levels are used in the MOE calculation with the F0 females, the resulting MOEs are 195 and 6.6. If the upper and lower values of the childrens' serum levels are used in the MOE calculation with the F0 males, the MOEs are 26,895 and 911.

5.5 Uncertainties in the Preliminary Risk Characterization

Some of the uncertainties encountered in this preliminary risk assessment are common for many risk assessments. One such example pertains to the choice of the animal model. In this preliminary assessment, serum levels were not available for the oral rabbit prenatal developmental toxicity study and therefore this study was not used in the calculation of the MOEs. It is not known whether rabbits are a more appropriate animal model than rats.

Other uncertainties that are common for many assessments have been avoided in this preliminary assessment through the use of a measure of internal dose of PFOA for both humans and the rat animal model. This approach has avoided many of the pitfalls encountered in trying to estimate human exposure levels through the application of various models and assumptions. Unlike many environmental chemicals where it is only hypothesized that humans are exposed, serum data from humans gives direct evidence that exposure to PFOA has occurred in the general public, and provides a measure of internal dose. Although, it is not known when or how exposure has occurred, this is less of an issue for PFOA given its long half life in humans. Therefore, the MOEs calculated in this preliminary risk assessment can be considered to be a more accurate comparison of the relative "distance" between the exposure level and the NOAEL than if administered dose had been used.

Although the use of serum levels as a measure of internal dose introduces uncertainties that are unique to this assessment, many of these have been accounted for through the use of a range of MOEs. For example, one area of uncertainty pertains to the use of serum data for the F0 animals as estimates of serum levels in the F1 animals. As noted in the previous section, it is not known whether the effects on postweaning mortality, body weight, or age of sexual maturation were due

to prenatal exposures, lactational exposures, postweaning exposures, or a combination of one or more of these exposure periods. In most risk assessments, no attempt is made to determine which of these exposure periods is important. A major strength of this preliminary assessment is that each of these exposure periods was considered in order to determine the appropriateness and uncertainties associated with the use of the serum levels from the F0 animals.

It was reasoned that if prenatal and/or lactational exposures were important then the serum levels in the F0 females would be the most appropriate estimate for the F1 animals. If postweaning exposures were important then the serum levels for the F0 males would be the most appropriate estimate for the F1 males, and similarly the serum levels in the F0 females would be the most appropriate estimate for the F1 females. It was not possible to make a "direct" estimate of F1 serum levels from the serum levels in the F0 females for the prenatal and/or lactational exposure scenario for several reasons. First, as noted in the previous sections, there is a gender difference in the elimination of PFOA in rats. In female rats, estimates of the serum half life range from 1.9 to 24 hours, while in male rats estimates of the serum half life range from 4.4 to 9 days. In female rats elimination of PFOA appears to be biphasic; a fast phase occurs with a half life of approximately 2- 4 hours while a slow phase occurs with a half life of approximately 24 hours. In the two generation reproductive toxicity study, the animals were dosed by gavage once daily. The serum levels were measured 24 hours after dosing. Thus, the values obtained for the F0 females represent the low end of exposure. With no knowledge of the peak exposures, it was reasoned that it was unlikely that the peak exposure would be higher than the serum level in the F0 males in the same dose group since they would tend to accumulate PFOA with a daily dosing regime. Therefore, the strategy that was employed in this assessment was to use the MOEs that were calculated from the serum levels in the F0 females and males as a range or as a means to bracket the low and high ends of exposure. Thus, it is likely that the MOEs that were calculated using the F0 female serum levels are probably too low, while the values calculated from the F0 male serum levels are probably too high.

As noted above, the serum levels from the F0 females would be the most appropriate estimate for the F1 females if postweaning exposures were important. Given the issues associated with the gender difference in elimination of PFOA in rats and the timing of serum collection in the F0 females, the same logic which was applied for the prenatal and lactational exposures was also used for this scenario. Again, the MOEs that were calculated from the serum levels in the F0 females and males were viewed as a range or as a means to bracket the low and high ends of potential exposure.

Similarly, the serum levels from the F0 males would be the most appropriate estimate for the F1 males if postweaning exposures were important. As stated above, the LOAEL for developmental effects in the F1 males was considered to be10 mg/kg/day and the NOAEL was considered to be 3 mg/kg/day. However, serum levels for the F0 males were only measured in the 10 and 30 mg/kg/day groups; there was no information available for the 3 mg/kg/day group. In addition, it was not possible to extrapolate the serum levels to the lower administered doses as the values appear to have reached a plateau at 10 and 30 mg/kg/day (51.1 and 45.3 ppm, respectively) and are not linear. Thus, if postweaning exposures were important, the MOEs based on the serum levels in the F0 males would be too high and underestimate potential risks. Again, this

uncertainty has been accounted for in this preliminary assessment through the calculation of a range of MOEs.

In the scenarios presented above, the serum levels of the F0 males were viewed as an overestimate given that male rats tend to accumulate PFOA given the long half life and the fact that saturation had apparently occurred. One line of reasoning that would support the statement that the serum levels from the F0 males are probably too high an estimate of peak exposure in the F0 females is provided by consideration of the serum half life of PFOA in female rats. Four studies have examined serum half life in a variety of rat strains; these studies have employed different routes of exposure and different methods for quantitating serum PFOA levels. Two of these studies were most relevant to the rat strain and route employed in the two generation reproductive toxicity study. Ophaug and Singer (1980) estimated a serum half life of 10 hours after administering female Holtzman rats a gavage dose of 8 mg/kg, while Vanden Heuvel et al. (1991b) estimated a serum half life of 4 hours after administering female Harlan Sprague-Dawley rats an intraperitoneal dose of 4 mg/kg. Using a serum half life value of 10 hours would yield a peak internal dose of approximately 1.85 ppm, while a serum half life of 4 hours would yield a peak internal dose of approximately 24.7 ppm. While neither of these values may be absolutely correct given that serum half life has not been reported for the rat strain and dosing conditions used in the two generation reproductive toxicity study, both values do support the statement that the serum levels in the F0 males are probably too high (1.85 and 24.7 ppm versus 51.1 ppm). Thus the resultant MOEs using the F0 male serum values probably underestimate the potential risks.

It is possible that lactational and postweaning exposures to the F1 pups may have been higher than the exposures to the F0 females due to the time required for maturation of the clearance mechanism. First, the clearance is under hormonal control and these hormones do not reach adult levels until puberty. Second, recent studies have examined the role of three organic anion transporters (OAT), OAT1, OAT2, and OAT3, in the urinary elimination of PFOA in the rat. Kudo et al. (2002) has provided evidence that OAT2 and OAT3 may be involved. A study of developmental and gender-specific influences on the expression of rat OATs in the kidney has shown that at birth all OAT mRNA levels are low (Buist et al., 2002). Renal OAT1 expression approaches adult level at 30 days, where at day 40 and 45 OAT1 levels were greater in males than females. OAT2 expression was minimal through day 30 but increased dramatically only in females at day 35. OAT3 expression matured the earliest and reached adult levels at 10 days. If these OATs are important, then this developmental profile would suggest that the PFOA clearance in juvenile female rats is less than in adult female rats. Therefore, the serum levels in the females prior to sexual maturation may be higher than in the adult, and the resultant MOEs would also be higher. However, it is important to note that the MOEs in this situation would still fall within the range of MOEs that were calculated in this preliminary assessment.

There are several other uncertainties unique to this assessment. The first pertains to the choice of developmental endpoints. This preliminary assessment utilized endpoints from the two generation reproductive toxicity study that could be directly attributed to developmental exposures. Another way to ascertain potential developmental effects would be to compare the magnitude and dose levels of the systemic toxicity that was observed in the F1 animals

(developmental and adult exposures) at the end of the study period with those systemic effects noted in the F0 animals (adult exposures only). For example, if organ weight changes were greater in F1 than F0 animals or if changes occurred at lower doses in the F1 animals, this may be indicative of the importance of developmental exposures. In the F0 males, there were significant reductions in the 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 at 30 mg/kg/day. In the F1 males, the effects on many of these organ weights occurred at lower doses. This may indicate that the developmental exposures were important, and that the LOAEL and NOAEL are lower than the values used in this preliminary assessment. If true, this preliminary assessment would have underestimated potential risks.

Another area of uncertainty pertains to the use of the serum levels as a measure of internal dose. PFOA behaves differently from many other persistent environmental contaminants in that it is not stored in adipose tissue. It is not clear whether it binds to proteins or other macromolecules, but it is clear, based on animal studies, that PFOA enters enterohepatic circulation. Animal studies indicate that PFOA mainly partitions to blood serum and the liver. In this preliminary assessment, serum levels were used primarily because this was the only information available for humans. However, it is possible that area under the curve, liver levels, the ratio of serum to liver levels, or some other measure may be more appropriate dose metrics.

In addition, the apparent biphasic elimination of PFOA in female rats raises an important issue, whether the observed effects are due to the kinetics associated with the fast or slow elimination phase in the females. The data currently available do not allow development of this potentially important issue. In this preliminary assessment, the serum values for the F0 males were used to provide an estimate of the peak exposure of the F0 females. The MOEs that were then calculated from the serum levels in the F0 females and males provide a means to bracket the high and low ends of exposure. If the effects are associated with the slow elimination phase, then the serum levels in the F0 females and the resultant MOEs would be more realistic.

Another area of uncertainty pertains to the differences in the serum half life in humans and rats. In humans, the serum half life is years, while in the rat it is less than 24 hours in females and 105 hours in males. To date, there is no evidence of a gender difference in the elimination of PFOA in humans. Thus, humans appear to be more similar to male rats in that they will tend to accumulate PFOA and will have a more continuous internal exposure. It is not known how this would impact potential risks of developmental toxicity in humans.

Finally, there is some uncertainty regarding the use of the human biomonitoring data. Although the available data include a range of populations with various demographics, there may be some populations that may not be represented. Since it is unknown how the human exposures are occurring, proximity to a manufacturing plant may be a factor in exposure. However, populations living near the plants were not sampled. Therefore, it is possible that PFOA serum levels may be underestimated for certain portions of the U.S. population. The children's sample was derived from blood collected in 1994/1995; therefore, it may not reflect the current status of PFOA in children's blood. It is not clear how PFOA may affect more sensitive subpopulations or if their exposures would vary.

6.0 Overall Conclusions

This preliminary risk assessment focused on the potential risks for developmental toxicity associated with exposure to PFOA and its salts. Concerns for developmental toxicity were raised from the results of a rat two-generation reproductive toxicity study of APFO . In this study, there was a reduction in F1 mean body weight on a litter basis during lactation (sexes combined). Postweaning mortality and delayed sexual maturation were noted in F1 females administered 30 mg/kg/day APFO; the NOAEL for developmental effects for F1 females was 10 mg/kg/day. Postweaning mortality, delayed sexual maturation and a significant reduction in postweaning body weights were noted in F1 males at 30 mg/kg/day, and a significant reduction in postweaning body weight was noted at 10 mg/kg/day. For F1 males, the LOAEL for developmental effects was 10 mg/kg/day and the NOAEL was 3 mg/kg/day.

For calculation of the MOEs, the human populations that were considered included women of child bearing age and children. Estimates of general human population exposure were available from recent analyses of individual serum samples from a group of children (2-12 years) and adults (20-69 years). For the populations of interest, calculations using human adult serum levels and children serum levels in combination with rat serum values from the parental (F0) females and males produced a range of overlapping MOE values that extends from less than 100 to greater than 9000. There are a number of important uncertainties discussed in this document that provide a context for considering these MOEs as a range of potential values. Interpretation of the significance of the MOEs for ascertaining potential levels of concern in exposed populations will necessitate a better understanding of the appropriate measure of exposure in rats, and the relationship of the latter to human serum levels.

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