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DRAFT RISK ASSESSMENT OF THE POTENTIAL HUMAN HEALTH EFFECTS ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID AND ITS SALTS

Executive Summary

U.S. Environmental Protection Agency
Office of Pollution Prevention and Toxics
Risk Assessment Division

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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. Most of the toxicology studies have been conducted with the ammonium salt of perfluorooctanoic acid, which is referred to as APFO in this report.

Human Health Effects

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

Little information is available concerning the pharmacokinetics of PFOA and its salts in humans. 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). Gender differences in elimination of PFOA have not been observed in humans based on the limited data available in the half-life study in retired workers. Metabolism and pharmacokinetic studies in non-human primates are limited to a study of 3 male and 3 female cynomolgus monkeys administered a single i.v. dose of 10 mg/kg potassium PFOA. In male monkeys, the average serum half-life was 20.9 days. In female monkeys, the average serum half-life was 32.6 days.

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

There are gender differences in the elimination of PFOA in adult rats following administration by gavage and by i.v. and i.p.injection. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8 to 16 hours,

while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138 to 202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

Several recent studies have been conducted to examine the kinetics of PFOA in the developing Sprague-Dawley rat. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. During lactation and for the first several weeks after weaning, the elimination of PFOA is similar in males and females pups. Between 4-5 weeks of age, the elimination in male rats assumes the adult pattern and the gender difference becomes readily apparent. Distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

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

APFO is not mutagenic. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation. APFO did not induce gene mutation when tested with or without metabolic activation in the K-1 line of Chinese hamster ovary (CHO) cells in culture. APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in C_3H $10T_{½}$ mouse embryo fibroblasts and in the mouse micronucleus assay.

Repeat-dose studies have been conducted in non-human primates. In a 13-week study with Rhesus monkeys, exposure to doses of 30 mg/kg-day or higher resulted in death. Clinical signs of toxicity were noted at doses as low as 3 mg/kg-day. Unlike rodent studies, analyses of the serum and liver levels did not reveal a gender difference in monkeys, but the sample size was very small. In a 6-month study of male cynomolgus monkeys, there was a steep dose response curve for mortality. Increases in liver weight were noted at doses as low as 3 mg/kg-day, but there was no evidence of peroxisome proliferator-activated receptor alpha activity (PPARα).

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

and reductions in body weight and hematological effects in females.

The carcinogenic potential of PFOA has been investigated in two dietary carcinogenicity studies in rats. Under the conditions of these studies, there is some evidence that PFOA is carcinogenic, inducing liver tumors, Leydig cell tumors (LCT), and pancreatic acinar cell tumors (PACT) in male Sprague-Dawley rats. The evidence for mammary fibroadenomas in the female rats is equivocal since the incidences were comparable to some historical background incidences. There is sufficient evidence to indicate that PFOA is a PPARα-agonist and that the liver carcinogenicity (and toxicity) of PFOA is mediated by binding to the PPARα in the liver. A mode of action analysis has demonstrated that the hepatic effects are due to PPARα-agonism, and that this mode of action is unlikely to occur in humans. The mode(s) of action for the Leydig cell and pancreatic acinar cell tumors have been investigated, but there is insufficient evidence to link these modes of action to PPARα. The LCT and PACT induced in the rat by PFOA probably do not represent a significant cancer hazard for humans because of quantitative differences in the expressions of LH and CCK_A receptors and of other toxicodynamic differences between the rat and the human. Based on no adequate human studies and uncertain relevance of the tumors from the rat studies, PFOA may be best described as "suggestive evidence of carcinogenicty, but not sufficient to assess human carcinogenic potential" under the draft 1999 EPA Guidelines for Carcinogen Risk Assessment.

PFOA appears to be immunotoxic in mice. Feeding C57Bl/6 mice a diet containing 0.02% PFOA resulted in adverse effects to both the thymus and spleen. In addition, this feeding regimen resulted in suppression of the specific humoral immune response to horse red blood cells, and suppression of splenic lymphocyte proliferation. The suppressed mice recovered their ability to generate a humoral immune response when they were fed a diet devoid of PFOA. Studies using transgenic mice showed that the PPARα was involved in causing the adverse effects to the immune system.

There was no evidence of prenatal developmental toxicity in rats after oral exposure to doses as high as 150 mg/kg-day. Maternal toxicity was seen at 100 mg/kg-day. In a rabbit oral prenatal developmental toxicity study there was a significant increase in skeletal variations after exposure to 50 mg/kg-day APFO. There was no evidence of maternal toxicity at 50 mg/kg-day, the highest dose tested.

A variety of endpoints were evaluated throughout different lifestages in a two-generation reproductive toxicity study in rats exposed to 0, 1, 3, 10, and 30 mg/kg/day APFO. In that study, a reduction in F1 pup mean body weight on a litter basis was observed during lactation (sexes combined) in the 30 mg/kg-day group. F1 male pups in the 10 and 30 mg/kg-day groups exhibited a significant reduction in body weight gain during days 8-50 postweaning, and body weights were significantly reduced in the 10 mg/kg-day group beginning on postweaning day 36, and in the 30 mg/kg-day group beginning on postweaning day 8. F1 female pups in the 30 mg/kg-day group exhibited a significant reduction in body weight gain on days 1-15 postweaning, and in body weights beginning on day 8 postweaning. Reproductive indices were not affected in the F1 animals. There was a significant increase in mortality mainly during the first few days after weaning, and a significant delay in the timing of sexual maturation for F1 male and female pups in the 30 mg/kg-day group. No effects were observed in the F2 pups. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation. Adult systemic toxicity consisted of reductions in body weight in both the F0 and F1 animals.

Human Biomonitoring

While the environmental concentrations and pathways of human exposure to PFOA and its salts are unknown, there are data on PFOA serum levels in workers and the general population. PFOA has been measured in the serum of workers occupationally exposed to perfluorinated chemicals for many years. PFOA has also been detected recently in the serum of the general U.S. population, but at much lower levels than those reported in occupational biomonitoring studies. Individual blood serum samples from 3 separate non-occupational cohorts have been analyzed for PFOA. Cohorts of adults (n= 645) and children (n=598) from various geographic areas of the U.S. and an elderly cohort from Seattle (n = 238) have indicated consistent mean serum levels of PFOA (approximately 5 ng/ml or 5 ppb). The reports indicate that serum levels for most of the individuals in these samples are below 10 ng/ml; however, some of the levels are as high as 56 ng/ml, indicating that a small number of individuals are being exposed at higher concentrations than the rest of the general population.

Risk Assessment and Uncertainties

A margin of exposure (MOE) approach can be used to describe the potential for human health effects associated with exposure to a chemical. The MOE is calculated as the ratio of the NOAEL or LOAEL for a specific endpoint to the estimated human exposure level. The specific endpoint may be from an epidemiology study or an animal toxicology study. The MOE does not provide an estimate of population risk, but simply describes the relative "distance" between the exposure level and the NOAEL or LOAEL. In this risk assessment there is no information on the sources or pathways of human exposure. However, serum levels of PFOA, which are a measure of cumulative exposure, were available from human biomonitoring studies. In addition, serum levels of PFOA were available for many of the animal toxicology studies or there was sufficient pharmacokinetic information to estimate serum levels. Thus, in this assessment internal doses from animal and human studies were compared; this is somewhat analogous to a MOE approach which uses external exposure estimates.

The results of existing epidemiology studies are not adequate for use in quantitative risk assessment, and therefore the analysis was restricted to endpoints in the animal toxicology studies. MOEs were calculated for the general U.S. population. Although some serum level data were available for workers, the data were not adequate to calculate MOEs for occupational exposures. In general, the mean serum levels following occupational exposures appear to be orders of magnitude higher than observed in the general population. Thus, MOEs for workers would be expected to be much less than for the general population.

A variety of endpoints from the animal toxicology studies were used to calculate MOEs for this draft risk assessment. The endpoints encompassed different species, gender and life stages. For this draft assessment, specific recommendations on the most appropriate endpoint/lifestage/species/gender have not been made; rather, all have been presented to provide transparency.

For adults, two sets of MOEs were calculated based on the toxicology studies in non-human primates and rats. First, MOEs were calculated from the cynomolgus monkey study and are based on increased liver weight and possible mortality. The MOE using the geometric mean for the human serum level is 16,739 (8,191 for the 90th percentile). Second, MOEs were calculated from the adult rat studies and are based on reductions in body weight. MOEs were calculated separately for the female and male rat due to the gender differences in pharmacokinetics in this

species. MOEs were calculated by dividing the AUC in the adult female rat by the AUC for the adult humans which is 398 (195 for the 90th percentile) and by dividing the AUC for the adult male rat by the AUC for the adult humans which is 9158 (4481 for the 90th percentile).

MOEs were calculated for the developmental effects in the two-generation reproductive toxicity study in rats. These effects were observed at various times during the maturation of the F1 pups. For both F1 males and females there were reductions in body weight during lactation; significant increases in mortality during the first few days after weaning; and significant delays in the timing of sexual maturation. Mean body weights were also significantly reduced in the time period prior to sexual maturation in both the F1 males and females. The critical period of exposure for each of the effects is not known. For example, it is not known whether prenatal and/or lactational exposure is important for the reduced body weight that was observed during lactation. Similarly, it is not known whether the reduced body weight, mortality, or delayed sexual maturation that occurred during the postweaning period are due to prenatal, lactational, and/or postweaning exposures. Ideally, MOEs would be calculated for each of these exposure periods; however MOEs were not calculated for the lactation period due to uncertainties in pharmacokinetics.

For the prenatal period, MOEs were calculated for the pregnant human female. MOEs were not calculated for the fetus since there is no information on human serum levels in fetuses. MOEs were calculated using both C_{max} and AUC; the MOE based on C_{max} is 3,095 (1548 for the 90th percentile) and the MOE based on the AUC is 823 (412 for the 90th percentile).

For the postweaning period, MOEs were calculated for several endpoints including reductions in body weight, mortality and delayed sexual maturation. These MOEs were based on the geometric mean for children and range from 10,484 - 78,546 (the range using the 90th is 6,044 - 45,279).

This assessment has provided a range of MOEs for several life stages. Several uncertainties have been discussed in a qualitative fashion in the assessment, which highlight the need to interpret the MOEs with caution. For example, MOEs were not calculated for the lactation period due to insufficient data, although this may represent an important exposure period. Similarly, the biomonitoring data for the children are from samples collected in 1994 and may not be representative of current children's serum levels. Finally, there is some uncertainty associated with the determination of the adequacy of a specific MOE in protecting human health in the present context. Traditionally, MOEs are calculated from administered dose levels and estimates of human exposure. In this assessment, the MOEs were calculated from internal dose metrics in animals and humans. While use of internal dose metrics reduces many uncertainties pertaining to exposure, there is little experience or guidance on the factors that should be considered in making judgements about the level of concern associated with a given MOE. Approaches that are used for conventional MOEs, if applied unchanged, indicate that among the populations of interest some individuals are highly exposed, for reasons not understood at this time. However, if conventional approaches for determining levels of concern are not appropriate for MOEs based on internal dose metrics, then this conclusion would have to be re-evaluated as the understanding of this question evolves.