TOXICITY AND TOXICOKINETICS OF PERFLUOROOCTANOIC ACID IN HUMANS AND ANIMALS

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ABSTRACT — Perfluorooctanoic acid (PFOA) is an octanoic acid derivative to which all aliphatic hydrocarbons are substituted by fluorine. PFOA and its salts are commercially used in various industrial processes. The chemical is persistent in the environment and does not undergo biotransformation. It was reported that PFOA is found not only in the serum of occupationally exposed workers but also general populations. Recent studies have suggested that the biological half-life of PFOA in humans is 4.37 years based on study of occupationally exposed workers. It is increasingly suspect that PFOA accumulates and affects human health, although the toxicokinetics of PFOA in humans remain unclear. In experimental animals, PFOA seems low in toxicity. PFOA is well-absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. Once absorbed in the body, it distributes predominantly to the liver and plasma, and to a lesser extent the kidney and lungs. PFOA is excreted in both urine and feces. Biological half-life of PFOA is quite different between species and sexes and the difference is due mainly to the difference in renal clearance. In rats, renal clearance of PFOA is regulated by sex hormones, especially testosterone. PFOA is excreted into urine by active tubular secretion, and certain organic anion transporters are thought to be responsible for the secretion. Fecal excretion is also important in the elimination of PFOA. There is evidence that PFOA undergoes enterohepatic circulation resulting in reduced amounts of fecal excretion. Elucidation of the mechanisms of transport in biological systems leads to elimination and detoxification of this chemical in the human body.

KEY WORDS: Perfluorooctanoic acid, Ammonium perfluorooctanoate, Toxicokinetics, Toxicity

INTRODUCTION

Organic fluorochemical compounds have been manufactured for over 50 years. These chemicals are used in a wide variety of industrial and commercial applications and processes as refrigerants, surfactants and polymers, and as components of pharmaceuticals, fire retardants, lubricants, adhesives, cosmetics, paper coatings, and insecticides because of their unique properties. Perfluorooctanoic acid (PFOA) is used mainly as a chemical intermediate in the industrial synthesis of fluoroacrylic esters, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant use (3M Company, 2000).

PFOA is stable in the environment because of the strength of the carbon-fluorine bond. There is no evidence that PFOA undergoes direct or indirect photolysis (Hatfield, 2001). In a study at 3M Industrial Laboratory, the hydrolytic half-life of PFOA was estimated to be greater than 97 years (3M Company, 2000). In several studies, biodegradation of PFOA ammonium salt was investigated using an activated sludge inoculum or COD and BOD methods, but biodegradation was not observed (Reiner, 1978; Pace Analytical, 1997; Pace Analytical 2001, 3M Company, 1977, 1980, 1985). Also, PFOA does not undergo defluorination and phase II metabolism (Vanden Heuvel et al., 1991; Ophaug and Singer, 1980; Ylinen et al., 1989).

In 1968, it was reported that organic fluorine occurred in human sera (Taves). Over the next several decades, further reports indicated a relatively widespread distribution of organic fluorochemical compounds, although compound-specific information was
Recent development of analysis utilizing LC-MS and LC-MS/MS permitted compound-specific surveys. PFOA and perfluorooctanesulfonic acid were detected in the serum of occupationally exposed humans, and also detected in the serum of general populations; the levels in the latter case were far lower than those in workers exposed to PFOA (Olsen et al., 2002a, 2002b, 2002c). On May 16, 2000, the 3M company, the dominant global producer of PFOA-related chemicals, announced that it is discontinuing production of perfluoroctanoyl chemistry and related products including PFOA and its salts because of suspected toxicity and persistence in the environment. Toxicokinetics of PFOA in humans remain unclear despite its importance to assess the health effects of this chemical. In this review, we describe human biomonitoring and epidemiology and, in experimental animals, toxicity and toxicokinetics (absorption, distribution and elimination).

**HUMAN BIOMONITORING**

**Serum levels of PFOA**

The 3M Company has offered voluntary medical surveillance to plant workers exposed to perfluorinated compounds since 1976. This includes three plants: Cottage Grove, MN; Decatur, AL and Antwerp, Belgium. Workers at the Cottage Grove plant have the highest PFOA serum level (Olsen et al., 1998a). The mean serum PFOA level in 1997 was 6.4 ppm (ranging from 0.1-81.3 ppm). At the Decatur plant, the mean level was 1.78 ppm in 2000 (Olsen et al., 2001a). Mean level in males (n=215) was 1.90 ppm, which was higher than that in females (1.23 ppm, n=48). The mean serum PFOA at the Antwerp plant was 0.84 ppm in 2000 (Olsen et al., 2001b), which was lower than at the other two plants. The mean level of 3M’s employees who perform fluorochemical research was 0.106 ppm (Olsen et al., 2001c). Data on PFOA levels in the general population are very limited, but the mean level was lower than in workers exposed to PFOA. PFOA levels in pooled blood samples from US blood banks ranged from 3 to 17 ppb (3M Company, 1999). Individual blood samples from three different age populations, namely adults, elderly population (ages 65-96) and children (ages 2-12) were 4.6, 4.2 and 4.9 ppb, respectively.

**Half-life**

There are very limited data on the half-life of PFOA in humans. Ubel et al. (1980) reported that the organic fluorine is very slowly eliminated from the body, based on the data for one worker (1980). A half-life study on 27 retirees from 3M plants was undertaken, in which serum samples were drawn every 6 months over a 5-year period. For 9 of 27 original subjects, more accurate analysis was performed (Burris et al., 2002). There were 7 males and 2 females. The average age was 61 years and average body mass index was 27.9. The mean number of years worked at the plant was 27.7 years, and the average number of months retired was 18.9. The mean PFOA level in serum at the initiation of the study was 0.72 ppm (range, 0.06-1.84 ppm, SD=0.64) and the mean half-life was 4.37 years (range 1.50-13.49 years, SD=3.53). Age, body mass index, number of years worked or years since retirement were not significant predictors of serum half-lives in multivariable regression analysis.

**Epidemiology studies**

All epidemiology studies were reported by 3M Company. Multivariable regression analyses on the data from the 2000 medical surveillance program at the Decatur and Antwerp plants revealed that a positive significant association between PFOA and cholesterol (p=0.05), PFOA and triglyceride (p=0.002) and PFOA and triiodothyronine (p=0.01, Olsen et al., 2001d). HDL was negatively associated with PFOA (p=0.04). In another study based on data from the 1997 medical surveillance program at the Cottage Grove plant (n=74), employees’ serum PFOA levels were stratified into 3 categories (<1, 1-<10, and >10 ppm). A statistically significant (p=0.03) negative correlation was observed between three PFOA categories and cholecystokinin-33 (Olsen et al., 1998b).

A retrospective cohort mortality study was performed on employees at the Cottage Grove Plant where ammonium perfluorooctanoate (APFO) production was limited to the Chemical Division (Gilliland and Mandel, 1993). The cohort consisted of 3537 employees. Standardized Mortality Ratios (SMRs), adjusted for age, sex, and race were calculated and compared to US and Minnesota white death rates for men. When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer in the workers in the Chemical Division was 2.03. There was also a statistically significant association with length of employment in the Chemical Division and prostate cancer mortality. An update of this study was conducted to include the death experience of employees through 1997 (Alexander, 2001). The
cohort consisted of 3992 workers who were placed into three exposure groups based on job history information: definite PFOA exposure, probable PFOA exposure and not exposed to fluorochemicals. None of the SMRs were statistically significant at \( p=0.05 \).

In the medical surveillance program at the Cottage Grove plant in 1993 (\( n=111 \)) and 1995 (\( n=80 \)), eleven hormones were assayed to estimate endocrine effects: cortisol, dehydroepiandrosterone sulfate, estradiol, FSH, 17 gamma-hydroxyprogesterone, free testosterone, total testosterone, LH, prolactin, thyroid-stimulating hormone and sex hormone-binding globulin (Olsen et al., 1998a). PFOA was not highly correlated with any of the hormones or with the following covariates: age, alcohol consumption, body mass index (BMI) or cigarettes.

**TOXICITY IN ANIMALS**

**Acute toxicity**

Acute oral toxicity was tested in male and female rats by Glaza (1997) and 3M Company (1976). The former provided LD\(_{50}\) value of \( >500 \) mg/kg and 250-500 mg/kg for male and female Crl:CD(SD)BR rats, respectively, and the latter estimated LD\(_{50}\) value <1000 mg/kg for male and female Sherman-Wistar rats. Abnormal findings upon necropsy were observed at 500 mg/kg (Glaza, 1997). Clinical signs were observed as follows: red-stained face, stained urogenital area, wet orogenical area, hypoactivity, hunched posture, staggered gait, excessive salivation, ptosis, piloerection, ataxia, and corneal opacity.

The acute inhalation toxicity of APFO was tested in male and female Sprague-Dawley rats at a dose of 18.6 mg/L for 1-hr exposure (Bio/dynamics, Inc. 1979). The exposure was not fatal to rats.

The acute dermal toxicity of APFO was tested in male and female Hra(NZW)SPF rabbits, and dermal LD\(_{50}\) value was determined to be \( >2000 \) mg/kg (Glaza, 1995).

Olson and Andersen (1983) evaluated LD\(_{50}\)/30 day of PFOA of single i.p injection to be 189 (208-175 mg/kg).

**Subchronic toxicity**

Subchronic toxicity has been studied in rats, mice, rhesus monkeys and cynomolgus monkeys. In a 28-day study of ChR-CD albino rats, male and female rats received similar feed containing 0, 30, 100, 300, 1000, 3000, 10000 or 30000 ppm APFO for 28 days (Metrick and Marias, 1977). All animals in the 10000 and 30000-ppm groups died before the end of the first week. Body weight gains were reduced in the groups receiving 1000 or more ppm. In a study by Goldenthal (1978a), CD rats were administered PFOA at dietary levels of 0, 10, 30, 100, 300 and 1000 ppm. Reduction of mean body weight was observed in males in the 1000-ppm group. Males in the 30, 100, 300 and 1000-ppm groups had a significantly reduced number of erythrocytes and had increased glucose levels, while similar changes were not observed in female rats under the same conditions. In a study by Palazzolo (1993), male Sprague-Dawley rats were fed \textit{ad libitum} diet containing 1, 10, 30, or 100 ppm for 13 weeks followed by 8 weeks of non-treatment. At 100 ppm, mean body weight gains were significantly higher than pair-fed control group during week 1 and significantly lower than the non pair-fed control group during weeks 1-13. There were no significant differences among the groups for serum hormone levels. Hepatic palmitoyl-CoA oxidase activity was significantly higher at 30 and 100 ppm. The increased activity was returned to control levels by the end of the recovery period. In this study, a NOAEL of 1.0 ppm and a LOAEL of 10 ppm were indicated based on reductions in body weight and body weight gain, and on increases in liver weights with hepatocellular hypertrophy.

In a 28-day study of Charles-RiverCD albino mice, male and female mice received similar feed containing 0, 30, 100, 300, 1000, 3000, 10000 and 30000 ppm of APFO for 28 days (Christopher and Marias, 1977). All animals in the 1000-ppm and higher groups died before the end of day 9. The entire 300-ppm group died within 26 days except for 1 male. Clinical signs were observed in mice exposed to 100 ppm and higher doses of APFO. All mice fed APFO lost weight.

In a study by Goldenthal (1978b), rhesus monkeys (2 males and females in each group) were administered PFOA at doses of 0, 3, 10, 30 or 100 mg/kg/day in 0.5% Methocel 7 by gavage for 90 days. All monkeys in the 100 mg/kg/day group died during the study. One male and two female monkeys in the 30 mg/kg/day group died during the study. Monkeys from the 30 and 100 mg/kg/day groups lost body weight after week 1. At 30 mg/kg/day the surviving male had a decreased number of erythrocytes, decreased hemoglobin, decreased hematocrit and increased platelet. In this monkey, serum cholesterol was elevated and total protein and albumin were reduced. Recently, a 6-month toxicity study in cynomolgus monkeys was performed by Thomford (2001a, b). Male cynomolgus monkeys were administered APFO by oral capsule at doses of 0,
3, 10 or 30/20 mg/kg/day for 26 weeks. Monkeys given 30 mg/kg/day from days 1-11 had clinical signs of few feces and low food consumption. Mean body weight changes of this group were lower. Thyroid hormones were decreased beginning on day 35 in animals in the 10 or 30/20 mg/kg/day groups. There were no consistent or clearly dose-related effects on estrone, estradiol, thyroid-stimulating hormone or testosterone. At 26 weeks, absolute liver weights and relative liver weights were significantly increased in all dose groups. There were no macroscopic or microscopic changes in any organs at 26 weeks, including liver, adrenal, spleen, pancreas and testis. In this study the LOAEL was 3 mg/kg/day (liver toxicity and possibly mortality) and a NOAEL was not established.

Developmental toxicity

Oral developmental toxicity of PFOA was studied by Gortner (1981). Sprague-Dawley rats were administered 0-150 mg/kg/day APFO in distilled water by gavage on gestation days 6-15. Mean maternal body weight was significantly reduced on gestation days 9, 12 and 15 at the dose of 150 mg/kg/day. At this dose, other signs of maternal toxicity were observed such as ataxia and death in rat dams. No significant differences between control groups and treated groups were observed for the developmental parameters including the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora leuta and mean fetus weight. In this study, a NOAEL of 5 mg/kg/day and a LOAEL of 150 mg/kg/day for maternal toxicity, and a NOAEL for developmental toxicity of 150 mg/kg/day were indicated. A similar study was conducted in rabbits where rabbits were administered 0-50 mg/kg/day APFO on gestation days 6-18. Transient reduction in maternal body weight gain was observed on gestation days 6-9; body weight gain returned to control levels on gestation days 12-29. No clinical or other treatment-related signs were reported. Significant differences between control groups and treated groups were not observed for the developmental parameters, including the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora leuta and mean fetus weight. A NOAEL of 50 mg/kg/day for maternal toxicity and a LOAEL for developmental toxicity of 50 mg/kg/day were indicated.

Carcinogenicity

The carcinogenic potential of APFO was investigated in a two-year feeding study in rats (3M Company, 1987). Male and female Sprague-Dawley rats were fed diets containing 0, 30, or 300 ppm APFO for two years. At the termination of the study, a slight increase in the incidence of various neoplasms was seen in the treated rats. Increases in the incidence of Leydig cell adenomas in high-dose male rats and of mammary fibroadenoma in both groups of female rats were statistically significant. Two other studies showed that PFOA promotes liver carcinogenesis in rodents (Abdellatif et al., 1991; Nilsson et al., 1991). The induction of tumors by APFO appears to be due to a non-genotoxic mechanism involving activation of receptors and perturbations of the endocrine system, although the mechanism is not fully understood. IARC (1995) concluded that the liver tumors induced in rodents by peroxisome proliferator-activated receptor α (PPARα)-agonists are unlikely to be operative in humans. This conclusion is based on the scientific knowledge on PPARα-induced liver tumors in rodents and relevance to humans.

Immunotoxicity

In the study of Yang et al. (2000), male C57Bl/6 mice were fed a diet containing 0.02% PFOA for up to 10 days. Significant decreases in thymus and spleen weight were detected and the number of thymocytes and splenocytes also decreased. The above effects were observed at doses higher that 0.01% (Yang et al., 2001). By comparing the effects between PPAR-α null mice and wild-type mice, these effects were almost PPARα-dependent processes (Yang et al., 2002).

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Absorption

APFO is well-absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. In the study by Ophaug and Singer (1980), non-ionic fluorine was found in urine within 96 hr after stomach intubation of an aqueous solution of 2 mg PFOA. After a single oral dose of 14C-PFOA (11.0 mg/kg) to male rats, at least 93% of the total 14C was absorbed at 24 hr (Gibson and Johnson, 1979). Concentration of organic fluorine was 108 ppm immediately after the tenth period after head-only inhalation exposure of male rats to AFPO (6 hr/day, 5 days/week for 2 week, 84 mg/m3, Kennedy et al., 1986). According to another study by Kennedy (1985), subchronic dermal APFO treatment in rats and rabbits resulted in the elevation of blood organofluorine levels in a dose-
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dependent manner. In a study by O’Malley and Ebbens (1981), mortality of rabbits was 100%, 75% and 0% when PFOA was applied to 40% of the shaved trunk of the rabbits for 5 days/week over 14 days.

**Distribution**

Several studies have indicated that PFOA distributes primarily to the liver, plasma and kidney, and to a lesser extent, other tissues of the body. Vanden Heuvel et al. (1991) determined tissue distribution of 14C-PFOA-derived radioactivity after an intraperitoneal administration (9.4 mmol/kg). In male rats, the liver had the highest concentration of PFOA, followed by plasma and kidney. Far lower concentrations were observed in the heart, testis, fat and gastrocnemius muscle. In female rats at 2 hr post-dose, the highest concentration of PFOA was found in serum, followed by kidney, liver and ovaries in that order. Ylinen et al. (1990) studied the distribution and accumulation of PFOA in male and female Wistar rats after a single and subchronic administration. In the single-dose study (50 mg/kg, i.p.), PFOA was found in serum, liver, kidney, spleen and brain but not in adipose tissue. After subchronic administration (3, 10, and 30 mg/kg/day by gavage), PFOA was mainly distributed in the serum. High concentrations of PFOA were also found in liver, kidney and lung of male and female rats.

**Half-life**

Biological half-life of PFOA was determined in rats, mice, rabbits and monkeys. There is a significant species-related difference in half-life of PFOA. Sex-related difference is also significant in rats, but not in other species such as rabbits, mice and dogs.

In a study by Gibson and Johnson (1979), the half-life for elimination of 14C-PFOA was calculated as 4.8 days after a single oral dose of PFOA (11 mg/kg). Vanden Heuvel et al. (1991) determined half-lives of PFOA in blood and tissues. In male and female rats administered 14C-PFOA (9.4 mmol/kg, i.p.), blood half-life of PFOA in male and female rats was 9 days and 4 hr, respectively. The half-life for elimination of PFOA from the liver in male and female rats was 11 days and 3 hr, respectively. In a study by Ylinen et al. (1990), male and female Wistar rats were intraperitoneally administered a dose of 50 mg/kg. The half-life in the serum was 105 and 24 hr in male and female rats, respectively. Ohmori et al. (2003) reported that the half-life of PFOA in the serum was 5.63 and 0.08 days in male and female Wistar rats, respectively. In rabbits intravenously administered PFOA, half-life of PFOA in the serum was on the order of 4 hr (Johnson, 1995). In male and female NZW rabbits intraperitoneally administered PFOA at the dose of 20 mg/kg, half life in the plasma was calculated to be 5.5 and 7.0 hr, respectively (Kudo et al., unpublished data). Serum half-life of PFOA in male and female ddY mice was 12 and 20 days, respectively (Kudo et al., unpublished data). Hanhijarvi et al. (1988) determined half-life of PFOA in dogs. Male and female beagle dogs were given an intravenous dose of 30 mg/kg PFOA. The plasma half-lives of two male dogs were 473 hr and 541 hr, values that were longer than those in two female dogs (202 hr and 305 hr).

**Metabolism**

Vanden Heuvel (1991) investigated the metabolism of PFOA in rats. Bile extract and urine samples were prepared from the rats intraperitoneally administered with 14C-PFOA. Only parent compound was found in urine samples, bile extracts and tissue extracts. PFOA did not undergo defluorination because fluoride concentrations in plasma and urine after PFOA treatment were not different from those before PFOA treatment. Ophaug and Singer (1980) also found that the level of ionic fluoride was not altered after an oral administration of PFOA. There was no evidence showing that PFOA undergoes phase II metabolism following a single intraperitoneal administration of PFOA in both male and female rats (Ylinen et al., 1989).

**Elimination**

PFOA is excreted in both urine and feces without biotransformation. There are significant sex-related differences in the elimination of PFOA in rats. The difference is primarily due to the difference in renal clearance. In contrast to rats, sex-related difference in the elimination is not significant in other species.

Vanden Heuvel et al. (1991) demonstrated that urine was the major route of PFOA excretion in male rats, while the urine and feces were both the major routes in female rats. Male and female rats were intraperitoneally administered 14C-PFOA (9.4 µmol/kg). In female rats, 91% of dosed PFOA was excreted in the urine, while male rats excreted only 6% of the dose in the first 24 hr. During 28 days after dosing, male rats excreted PFOA in urine and feces 36.4% and 35.1%, respectively. In a study by Gibson and Johnson (1980), female rats excreted essentially all of the dose in urine in 24 hr after an intravenous injection of 14C-PFOA, while male rats excreted 20% of the dose. Metabolic
fate of PFOA after an oral intubation was studied in female rats by Ophaug and Singer (1980). Nonionic fluorine recovered in urine and feces was 89% and 14.3% of the dosed PFOA at 96 hr. Hanhijarvi et al. (1982) also demonstrated that female rats excreted 76% of the dose in urine in female rats, while male rats excreted only 7.8% of the dose over a 7-hr period after an oral administration of APFO. Kudo et al. (2002) estimated renal clearance of PFOA in male and female rats. Rats were intravenously administered PFOA (48.6 μmol/kg) and then infused 10% mannitol/0.9% NaCl. Renal clearance of PFOA in male and female rats was 0.032 and 0.732 mL/min/kg, respectively. In rats, PFOA seems to undergo active tubular secretion because renal clearance of PFOA was reduced by probenecid, an inhibitor of active tubular secretion (Hanhijarvi et al., 1988). Renal clearance of PFOA in beagle dogs, both male and female, was reduced by probenecid. According to unpublished data by Kudo, et al., renal clearance of male and female mice was approximately 10 and 16 mL/day/kg, respectively; and that in male and female rabbits was 640 and 670 mL/min/kg, respectively.

PFOA appears to undergo enterohepatic circulation. Feeding of cholestyramine to rats enhanced the fecal elimination of APFO (Johnson et al., 1984). PFOA eliminated in feces during 14 days after an intravenous injection of 14C-APFO (13 mg/kg) was 43% of the dose in the rats given 4% cholestyramine in feed, a percentage 9.8-fold that of cholestyramine-untreated rats.

Toxicokinetics of PFOA-related compounds

Ohmori et al. (2003) determined renal clearance of perfluorocarboxylic acids with 7, 9 and 10 carbon atoms (C7, C9 and C10) as structurally related compounds of PFOA. Sex-related difference in renal clearance was observed in C9 but not in C7 and C10. Renal clearance of these perfluorinated fatty acids was C7>C8>C9=C10 in male rats and C7=C8>C9>C10 in female rats.

CONCLUSIONS

PFOA and other fluorinated compounds are thought to be very useful chemicals with low toxicity. Estimation of PFOA toxicity in experimental animals revealed that PFOA is toxic when animals are exposed to relatively high amounts of PFOA. However, it is increasingly suspect that PFOA accumulates in human, animals and the environment because of their persistence. In addition, several biomonitoring studies on humans show that PFOA accumulates not only in occupationally exposed workers but also in general populations. It is obvious that these chemicals should be carefully handled, although, to date, epidemiological studies did not show evidence that PFOA affects human health. Biological half-lives of PFOA vary between animal species. Half-life of PFOA in humans determined from occupationally exposed workers is far longer than these experimental animals. Accordingly, toxicokinetics of PFOA in humans cannot be predicted based on animal data. The reason for this difference remains to be resolved. It may be due to the difference...
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in experimental conditions, because occupationally exposed workers were chronically exposed to PFOA at very low levels. Animal data suggest that PFOA is recognized in certain organic anion transporters or at least in renal transport. Further studies on the mechanism of PFOA transport in biological systems may lead to better understanding of the toxicokinetics of PFOA in humans.

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