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Review

The developmental toxicity of perfluoroalkyl acids and their derivatives☆

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Abstract

Perfluoroalkyl acids such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have applications in numerous industrial and consumer products. Although the toxicology of some of these compounds has been investigated in the past, the widespread prevalence of PFOS and PFOA in humans, as demonstrated in recent bio-monitoring studies, has drawn considerable interest from the public and regulatory agencies as well as renewed efforts to better understand the hazards that may be inherent in these compounds. This review provides a brief overview of the perfluoroalkyl chemicals and a summary of the available information on the developmental toxicity of the eight-carbon compounds, PFOS and PFOA. Although the teratological potentials of some of these chemicals had been studied in the past and the findings were generally unremarkable, results from recent postnatal studies on developmental and reproductive indices have prompted consideration of their relevance to human health risk. Based on current understanding of the developmental effects of PFOS and PFOA in rodents, several avenues of research are suggested that would further support the risk assessment of these perfluorinated organic chemicals.

Keywords: PFOS; PFOA; PFBS; PFDA; Developmental toxicity; Rodents; Review

Environmental exposures of perfluoroalkyl acids

In recent years, perfluoroalkyl acids have been identified at low parts per billion (ppb) concentrations in samples of human serum taken from the general population as well as certain wildlife samples (Giesy and Kannan, 2001, 2002; Hansen et al., 2001; Hoff et al., 2003a, 2003b; Kannan et al., 2001a, 2001b, 2002a, 2002b, 2002c, 2002d; Martin et al., 2004; Olsen et al., 2002, 2003a, 2004; Sottani and Minoia, 2002). The presence of organic fluoride in humans was actually first reported by Taves (1968) and Shen and Taves (1974) over 30 years ago and the organic form was originally suggested to be perfluorooctanoate (PFOA) or, possibly, perfluorooctane sulfonate (PFOS) (Taves et al., 1976). It is only in the past several years that significant advances in analytical chemistry have enabled routine detection of individual perfluoroalkyl acids in various matrices in the ppb or lower range by high-performance liquid or gas chromatography followed by electrospray tandem mass spectrometry (ES/MS/MS) (Hansen et al., 2001; Hebert et al., 2002; Martin et al., 2002; Sottani and Minoia, 2002). Equipped with such improvement in detection sensitivity, Olsen et al. (1999, 2001, 2003b, 2003c) reported mean serum PFOS levels of 1–2 ppm (range 0.1–13 ppm) and mean PFOA serum concentrations (Olsen et al., 1998, 2000, 2003b) of approximately 5 ppm (range <0.1–114 ppm) in 3M fluorochemical production workers. A broad survey of individual blood samples from adult Red Cross blood donors (Olsen et al., 2003a), children from a clinical trial (Olsen et al., 2002), and elderly subjects enrolled in a longitudinal study (Olsen et al., 2004)

Abbreviations: POSF, perfluorooctane sulfonyl fluoride; PFOS, perfluorooctane sulfonate; PFBS, perfluorobutane sulfonate; PFOA, perfluorooctanoic acid; PFDA, perfluorodecanoic acid.

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indicated geometric mean serum concentrations of PFOA, perfluorohexanesulfonate (PFHS), and PFOS in these populations of approximately 4–5, 2–5, and 30–40 ppb, respectively. More recently, Olsen et al. (2003d) reported liver concentrations of PFOS ranging from <4.5 to 57 ppb in human organ donors from the general population. The hepatic levels of PFOA in most of the organ donor subjects were below current detection limits.

Several recent studies by Giesy and Kannan (2001, 2002), Kannan et al. (2001a, 2001b, 2002a, 2002b, 2002c, 2002d), and others (Hoff et al., 2003a, 2003b; Martin et al., 2004) have reported detection of PFOS in a variety of wildlife species including fresh water and marine mammals, fish, birds, and shellfish. Although distribution of the chemical appears to be widespread in the northern hemisphere, including remote locations in the Arctic and North Pacific Oceans, concentrations of PFOS in these animals are generally greater in the more populated and industrial regions. These investigators and others (Martin et al., 2003b) also suggested that PFOS can be bio-magnified in the top levels of the food chain. Although PFOA was also found occasionally in the serum and liver of various wildlife species at different geographic locations, concentrations are generally lower than those of PFOS (Kannan et al., 2002a, 2002b, 2002d).

The prevalence of these perfluoroalkyl acids in humans and wildlife populations has thus drawn considerable attention from regulatory agencies (US EPA, 2003a, 2003b) and the public (Renner, 2001). Largely because of the observed broad distribution and persistence of PFOS and PFOA in humans and the environment, 3M Company (one of the chief manufacturers) phased out production of these chemicals by the end of 2002; although other perfluoroalkyl acid products are poised to take up the void left by PFOS in the commercial markets. In addition, the Center of Disease Control and Prevention (CDC) has recently nominated PFOS and PFOA to be included in their National Health and Nutrition Examination Survey (NHANES) for future exposure reports, in order to provide a better assessment of the distribution of these chemicals among human populations. It is therefore timely to review the toxicology of these perfluorochemicals, and this paper will focus on the current understanding of developmental toxicity of these compounds.

A search of the extant literature revealed only limited peer-reviewed publications on this topic. Because the issues related to developmental toxicity of these chemicals arise from recently completed studies, manuscripts are still in preparation and substantial toxicity data are available only in the form of reports submitted to the U.S. EPA and other regulatory agencies. Information from the latter source is included in this review and details of these studies are generally accessible to the public upon request from these agencies.

As a class, these perfluorochemicals share unique physical and chemical properties that are related to their commercial applications as well as potentials for toxicity. A clear understanding of these properties will be useful for toxicologists and risk assessors in evaluating the toxic potentials of these compounds. Thus, although the focus of this review is directed toward the developmental toxicity of perfluoroalkyl acids, a brief background on their chemistry, environmental distribution, and pharmacokinetic profiles is also provided for the readers' purview.

Chemistry, production, and uses of perfluoroalkyl acids

Organic fluorochemicals are compounds in which one or more carbon–hydrogen (C-H) bonds are replaced by carbon–fluorine (C-F) bonds. Fluorine is one of the most reactive elements in ionic form and one of the most stable in bound form. Hence, the fully fluorinated hydrocarbons, or perfluorocarbons, are stable in air even at high temperatures (in excess of 150 °C), nonflammable, not readily degraded by strong acids, alkalis or oxidizing agents, and not subject to photolysis. The unique stability of these chemicals thus renders them practically nonbiodegradable and thus, very persistent in the environment (Key et al., 1997, 1998; Prescher et al., 1985).

Naturally occurring fluorinated organic chemicals are rare in biological systems due to the high energy required to form or break the C-F bonds, and all known biologically produced fluorinated organics contain only one fluorine atom (review: Key et al., 1997). However, partially or fully fluorinated (perfluorinated) organic molecules can be synthesized in the laboratory and in full-scale manufacturing operations.

One method for production employs the Simons Electrochemical Fluorination (ECF) process that was discovered in 1937. The ECF process is based on the reaction between organic feedstocks such as 1-octanesulfonyl fluoride ($C_8H_{17}SO_2F$) and anhydrous hydrogen fluoride (HF), which is fueled by an electrical current, causing the hydrogen atoms on the carbon backbone to be replaced by fluorine atoms, with resultant products such as perfluorooctane sulfonyl fluoride (POSF, $C_8F_{17}SO_2F$). However, during this process, fragmentation and rearrangement of the carbon skeleton can occur, producing fluorinated organic molecules of various carbon chain lengths (less than eight) and a mixture of linear, branched, and cyclic isomers of the chemical. Typically 70–85% of the mixture was linear and 15–30% was a mixture of branched isomers. 3M has produced fluorochemicals by the ECF process for industrial and consumer use since the 1950s, although this manufacturer has now phased out essentially all production of perfluorooctanyl (PFOA and POSF-based) chemistries.

An alternative production method for the functionalized perfluoroalkyl acids involves telomerization of tetrafluoroethylene units, and many manufacturers employ this technology to produce materials that, in some cases, have similar properties to the ECF-based materials. In this pro-

cess, a telogen such as pentafluoroethyl iodide ($F(CF_2CF_2)I$) polymerizes with terafluoroethylene (CF_2CF_2) to form telomer intermediates of desired carbon chain length (typically up to 12); the resultant telomer iodides are then reacted with ethylene via free radical addition. The telomerization process thus yields straight-chain alcohols ($F(CF_2CF_2)_nCH_2CH_2OH$) that can be converted into final products for commercial application. In general, these telomer-derived products may consist of a distribution of various carbon chain lengths. Hence, the fluorinated organic chemicals found in the environment represent a family of compounds of various sizes and isomeric forms.

The high ionization potential and low polarizability of fluorine lead to weak inter- and intramolecular interactions that are reflected by the extremely low surface tension of the perfluoroalkyl acids. The partitioning behavior of perfluoroalkyl acids is also unique; when they are mixed with water and hydrocarbons, three immiscible phases are formed, indicating the hydrophobic and oleophobic nature of these compounds. By attaching a charged moiety such as carboxylic acid, sulfonic acid, or phosphate to the perfluorinated chain, the molecule becomes more water soluble due to the hydrophilic nature of the added functional group. Together, the physical properties of the functionalized perfluoroalkyl acids render these compounds ideal surfactants (Kissa, 2001). Although all amphoteric perfluoroalkyl acids share some surfactant properties, the eight-carbon chemicals such as POSF-based materials, PFOS and PFOA have been most effective in commercial uses. Indeed, POSF- and telomer-related products are found in over 200 known industrial and consumer applications ranging from water-, soil-, and stain-resistant coatings for clothing fabrics, leather, upholstery, and carpets, to oil-resistant coatings for paper products approved for food contact, electroplating, and electronic etching bath surfactants, photographic emulsifier, aviation hydraulic fluids, fire-fighting foams, floor polishes, and insecticide formulations (Renner, 2001; Seacat et al., 2002). According to 3M, the global production of POSF for 2000 (the last year of active production) was estimated at about 3,545 metric tons, and in 2001, the worldwide production was 175 metric tons (3M, 2003). Information on PFOA production by various manufacturers is not readily available, but can be estimated in excess of 500 metric tons per year.

In commercial applications, POSF was converted into other intermediates such as acids, alcohols, and sulfonamides for further polymerization and esterification, but some of these compounds and their intermediates have the potential to break down metabolically or in the environment to PFOS as an end-stage metabolite and product. PFOA (primarily ammonium salt) can be used as a surfactant and an emulsifier in the production of polytetrafluoroethylene as well as other fluoropolymers and fluoroelastomers. Telomer-based molecules may have the potential to degrade metabolically to perfluorinated acids (Hagen et al., 1981). Most of the polymers based on fluorochemistry tend to be relatively stable and resistant to degradation. Current understanding of fluorochemistry suggests that PFOS and PFOA are substantially different compounds, and that toxicological evaluation of these two chemicals should be considered separately.

Distribution of perfluoroalkyl acids in the environment

POSF is a volatile liquid at normal temperature and pressure; however, PFOS and PFOA have very low volatility and vapor pressure. Certain sulfonamide intermediary compounds derived from POSF, such as alkyl amides, alkyl alcohols, and alkyl acids, may sublime (3M, 2003; Schal, 1992). However, little is known about the transport and fate of these fluorochemicals in the environment. Recent studies reported <0.017 to 2,260 μg/l for perfluoroalkane sulfonate (C-6–C-8) and <0.009 to 11.3 μg/l for PFOA in the surface water of a Canadian tributary after an accidental release of fire-fighting foams (Moody et al., 2002). Both PFOS and PFOA were detected in water samples from the Tennessee River near a 3M fluorochemical manufacturing site (Hansen et al., 2002). Concentrations ranging from a low of approximately 17 ppt (below quantitation limit) upstream of the facility to a high of 144 ppt downstream of the site for PFOS, and a low of 25 ppt to a high of approximately 600 ppt for PFOA were reported. Monitoring of drinking water sources near a production plant in West Virginia indicated the presence of PFOA at below 3 μg/l, substantially below the interim risk screening of 150 μg/l established by the State and US EPA region (West Virginia Department of Environmental Protection, 2002). Martin et al. (2002) also reported airborne levels of certain perfluorooctanesulfonamide-based chemistries and telomer alcohols ranging from approximately 10 to 400 pg/m^3 in Ontario (Toronto and Long Point), Canada.

Pharmacokinetics of PFOS and PFOA

PFOS and PFOA have been observed in retired fluorochemical workers to have mean elimination half-lives of several years (Burris et al., 2002). PFOS is readily absorbed and distributed primarily in the serum and liver (most likely through enterohepatic circulation), but poorly eliminated (Burris et al., 2002; Johnson and Ober, 1979; Johnson et al., 1979, 1984; Noker and Gorman, 2003a; Seacat et al., 2002). The elimination half-life of PFOS in rats and monkeys has been estimated at >90 days for male rats (Johnson et al., 1979) and approximately 100–200 days for male and female cynomolgus monkeys (Noker and Gorman, 2003a; Seacat et al., 2002). PFOA is also readily absorbed (Kemper and Jepson, 2003); however, notable differences in elimination between species have been reported (Kennedy et al., 2004; Kojo et al., 1986; Kudo and Kawashima, 2003; US EPA, 2003a). In the rat, a major

gender difference exists in the elimination of PFOA, with half-lives estimated at 1.9–24 h for females and 4.4–9 days for males (Hanhijarvi et al., 1982; Kemper and Jepson, 2003; Kudo et al., 2002; Ophaug and Singer, 1980; 1987; Vanden Heuvel et al., 1991a; Ylinen et al., 1990). The gender difference in rats appears to be under steroid hormonal control (Kawashima et al., 1991; Kudo et al., 2002; Hanhijarvi et al., 1982; Vanden Heuvel et al., 1991a, 1992; Ylinen et al., 1989). Gibson and Johnson (1983) reported that hormonal changes during pregnancy did not appear to alter the rate of PFOA elimination. A smaller sex-related difference in PFOA elimination was found in beagle dogs (Hanhijarvi et al., 1988), with plasma half-lives of 202 and 473 h estimated for females and males, respectively. However, it is noteworthy that striking gender differences in PFOA elimination have not been observed in primates and humans (Burris et al., 2002; Noker and Gorman, 2003b), and appear to be unlikely in the mouse (Sohlenius et al., 1992; Uy-Yu et al., 1990; US EPA, 2003a).

Toxicity of perfluorooctane sulfonate

The potential toxicities of PFOS in monkeys (Seacat et al., 2002), rats (Austin et al., 2003; Seacat et al., 2003; Thibodeaux et al., 2003), fish (Hoff et al., 2003b; Martin et al., 2003a, 2003b), and humans (Olsen et al., 1999, 2003b, 2003c) have been characterized and summarized in recent reviews (3M, 2003; US EPA, 2003b; link to OECD: http://www.oecd.org/dataoecd/23/18/2382880.pdf). In brief, subchronic exposure to PFOS led to significant weight loss accompanied by hepatotoxicity and reductions of serum cholesterol and thyroid hormones. Teratological studies have been conducted in rat, rabbit, and mouse with PFOS (potassium and lithium salts) (Case et al., 2001b; Christian et al., 1999a; Gortner, 1980; Henwood et al., 1994; Thibodeaux et al., 2003; Wetzel, 1983). The findings are in agreement between laboratories and across species examined, and are generally unremarkable when maternal effects are taken into consideration. Observed developmental effects include reduction of fetal weight, cleft palate, anasarca (edema), delayed ossification of bones (sternebrae and phalanges), and cardiac abnormalities (ventricular septal defects and enlargement of the right atrium). Nonetheless, it should be noted that a preponderance of these structural abnormalities was found in the highest PFOS dose groups, where significant reductions of weight gain and food consumption were also observed in the pregnant dams. On the other hand, maternal malnutrition is not likely the sole factor accounting for the induction of birth defects. Indeed, Thibodeaux et al. (2003) have shown that equivalent or higher incidence of malformations was seen in the mouse, compared to the rat; yet, the deficits of maternal weight gain and food consumption in the mouse were much less extensive than those in the rat.

When rodent dams exposed to PFOS throughout pregnancy were allowed to give birth, dose-dependent deleterious effects were seen in the newborns (Lau et al., 2003; Fig. 1). As a reference to body burdens of the chemical, serum levels of PFOS in the pregnant dams at term and in the newborns are summarized in Table 1. All rat pups were born alive, pink, and active. However, in the highest dose (10 mg/kg) group evaluated, the neonates became pale, inactive, and moribund within 30–60 min, and all died soon afterward. In the next highest dose group (5 mg/kg), the neonates also became moribund but survived for a longer period of time (8–12 h). Over 95% of these animals did not survive the first day of postnatal life, and only a few pups reached puberty. Survival improved with lower PFOS exposure, and was about 50% at 3 mg/kg. The morbidity and mortality of

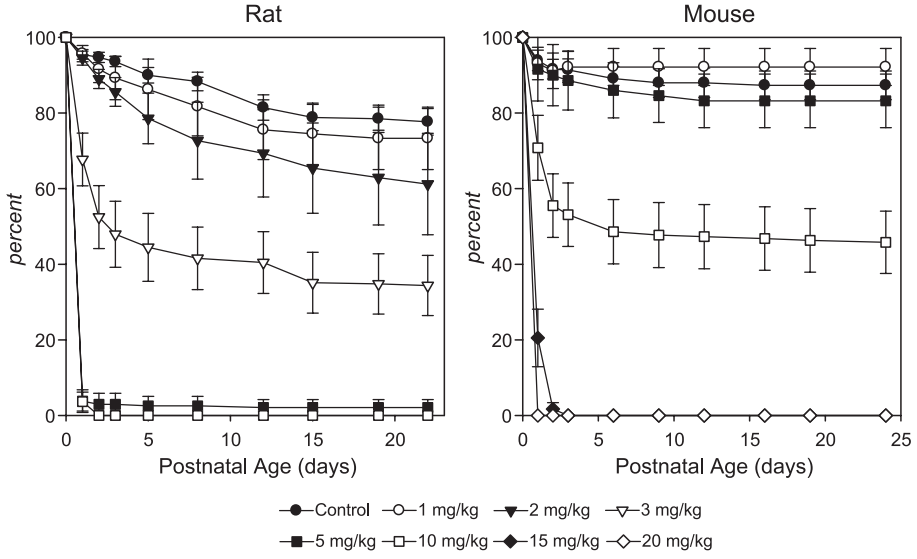

Fig. 1. Effects of prenatal exposure to PFOS on postnatal survival in rats and mice. Each data point represent means ± SE of 9–18 litters for rats and 7–18 litters for mice. Results are excerpted and slightly modified from the primary study that contained the experimental details (Lau et al., 2003).

Table 1
Concentrations of PFOS in rat maternal serum at near-term (GD 21) and in newborn pups

Treatment dose (mg/kg)	Maternal serum (ppm)	Newborn serum (ppm)
0	0.244 ± 0.044	0.188 ± 0.070
1	19.6 ± 1.8	35.9 ± 0.6
2	45.0 ± 1.6	71.9 ± 4.1
3	71.9 ± 6.6	86.5 ± 10.0
5	80.6 ± 3.0	108.2 ± 4.6
10	189.9 ± 6.6	–

Data represent means ± SE of 9–14 dams and 3–5 newborns, and are excerpted from primary studies that contained the experimental details (Lau et al., 2003; Thibodeaux et al., 2003).

the newborn rats appeared to be correlated to their body burden of the fluorochemical. After the first week of postnatal life, the mortality rate was not different between control and treated groups. Cross-fostering the PFOS-exposed rat pups (5 mg/kg) to control nursing dams immediately after birth failed to improve survival of the neonates, thus ruling out a role for abnormal maternal behaviors associated with the fluorochemical treatment. The maternal dose corresponding to the $BMDL_5$ (lower limit of the 95% confidence interval on the Benchmark Dose that would predict a 5% increase in response above background incidence) for survival of rat pups at postnatal day 8 was estimated at 0.58 mg/kg.

Postnatal growth of surviving rat pups was somewhat stunted by in utero exposure to PFOS. Body weights of pups in the 2 mg/kg and higher dose groups significantly lagged behind those in controls, and this effect persisted past weaning. Development of the PFOS-exposed pups was also significantly hindered as the average age at eye opening was delayed by a day, although no significant changes in the onset of puberty were noted. Hypothyroxinemia was observed in PFOS-exposed neonates. Because thyroid hormones are known to regulate brain development, the ontogeny of neurochemical and neurobehavioral markers was evaluated. Prenatal exposure to PFOS produced only marginal, but statistically significant deficits in the developmental patterns of choline acetyltransferase activity (an enzyme marker sensitive to thyroid hormone status), and did not affect learning and memory behaviors determined by T-maze delayed alternation. This lack of notable changes in the CNS in these studies may have been related to the extent of thyroid hormone imbalance (reductions of thyroxine but not triiodothyronine or TSH) induced by the prenatal PFOS treatment.

A similar profile of postnatal mortality was observed in the mouse (Fig. 1), although the maternal dosage required for the effect was higher than that in the rat. Most mouse offspring of dams exposed to 15 or 20 mg/kg PFOS did not survive for 24 h after birth. The LD_{50} was estimated at 10 mg/kg (compared to approximately 3 mg/kg in the rat), whereas survival of the lower dose groups (1 and 5 mg/kg) was not different from that of controls. The maternal dose corresponding to the $BMDL_5$ for survival of mouse pups at postnatal day 6 was estimated at 3.88 mg/kg, approximately six times higher than that of the rat. Among survivors, a trend toward growth deficit was evident. Compared to the rat, a more pronounced increase of liver weight was noted in PFOS-exposed neonatal mice. Hepatic enlargement persisted through the duration of the study (to PD 35). Serum thyroxine levels in the developing mice exposed to PFOS prenatally tended to be lower, but less consistently so than in PFOS-exposed rat pups. A significant delay in eye opening was also detected in treated mouse neonates.

Similar effects of PFOS on newborn rodents can be achieved by different treatment paradigms. Butenhoff et al. (2002b) treated female rats with PFOS for 42 days before mating and throughout pregnancy at lower doses (0.4–2 mg/kg/day) and reported marked reduction of viability in pups in the two highest dose groups (49% and 17%, respectively, for the 1.6 and 2 mg/kg groups), as well as significant retardation in postnatal growth among the survivors, even in the lower dose groups. These results confirmed those from a two-generation reproductive study with the same treatment period for parental rats at doses of 0.1, 0.4, 1.6, and 3.2 mg/kg/day PFOS. In this study, the 1.6 and 3.2 mg/kg doses produced 34% and 100% F_1 pup mortality within the first days postpartum, respectively, transient reductions in weight gain in F_2 pups during the lactation period at maternal doses of 0.4 mg/kg/day, and no effects at 0.1 mg/kg/day (Christian et al., 1999a). In a cross-foster study with the same treatment paradigm, postpartum transfer of the PFOS-exposed pups (1.6 mg/kg) to control nursing dams failed to reduce mortality (Case et al., 2001a). Grasty et al. (2003b) examined critical windows of PFOS toxicity and reported a similar increase of neonatal mortality in the rat with exposure of pregnant dams to high doses of PFOS (25 mg/kg/day) for a 4-day period during various stages of pregnancy. Neonatal mortality occurred after dosing in all time periods, but the incidence of neonatal death increased as the exposure period fell later during gestation, reaching 100% in the treatment group of GD 17–20. In another experiment, dosing with 50 mg/kg/day of PFOS on GD 19 and 20 only was sufficient to produce almost 100% mortality. Hence, the neonatal mortality resulting from PFOS administration to pregnant rats does not require PFOS exposure before day 19 of gestation, suggesting that the critical period is late-gestational or perinatal. Interestingly, although the incidence of neonatal mortality was correspondingly lower in the early exposure groups, body weights of the newborns in these groups (4-day treatment on GD2–5, 6–9, or 10–14) were significantly lower than those of controls or late exposure groups Grasty et al. (2003b).

The pathophysiology underlying PFOS-induced neonatal mortality is largely unknown at present. Results from Grasty et al. (2003b) suggest that organ systems developing late in gestation may be targets for PFOS insult. This hypothesis is also consistent with the relatively unremarkable teratological findings. Considering that PFOS-induced organ toxicity

is incompatible with postnatal survival, maturation of the lung and pulmonary function is a plausible target.

Numerous chemicals have previously been shown to interfere with fetal lung development (Lau and Kavlock, 1994). The profile of neonatal mortality induced by PFOS is in fact reminiscent of the developmental toxicity of nitrofen, a herbicide that perturbs fetal lung maturation, ultimately leading to compromised cardiopulmonary function and death in newborn rats (Lau et al., 1986, 1988; Stone and Manson, 1981). Indeed, the most recent findings reported by Grasty et al. (2003a, 2003b) are promising in support of this contention. These investigators described significant differences in histological and morphometric evaluations of the lung between control and PFOS-treated newborns that suggest PFOS-induced inhibition of or delayed perinatal lung maturation.

Nevertheless, the involvement of other possible mechanisms of PFOS toxicity such as interference with mitochondrial bioenergetics (Berthiaume and Wallace, 2002; Starkov and Wallace, 2002), impedance of cell–cell communication through gap junctions (Hu et al., 2002), interactions with fatty-acid binding proteins (Luebker et al., 2002a), hepatotoxicity (peroxisome proliferation) (Ikeda et al., 1987; Sohlenius et al., 1993), and alterations of maternal and fetal thyroid hormone economy (Thibodeaux et al., 2003) warrants consideration. On the other hand, a recent report did not provide evidence to support PFOS-induced interference with cholesterol synthesis (through inhibition of HMG CoA reductase activity, Haughom and Spydevold, 1992) as a potential mechanism for developmental toxicity of the chemical because cholesterol or mevalonic acid supplementation failed to ameliorate the adverse effects of PFOS (Luebker et al., 2002b).

Toxicity of *N-ethyl-N-(2-hydroxyethyl)-perfluorooctanesulfonamide*

N-ethyl-*N*-(2-hydroxyethyl)-perfluorooctanesulfonamide (*N*-EtFOSE) represents a building block for POSF-related products for commercial applications and can be degraded metabolically and in the environment to PFOS. Case et al. (2001b) evaluated its potential developmental effects in rats (with doses of 1, 5, 10, and 20 mg/kg) and rabbits (with doses of 0.1, 1, 2.5, and 3.75 mg/kg). In the rat, maternal body weight gains were reduced in the high dose groups, where reductions of fetal weight were also evident; but teratological findings were unremarkable. In the rabbit, dose-dependent reductions of maternal weight gain were seen and late-term resorptions were increased at the highest dose. No structural aberrations were noted in fetal examinations.

A two-generation reproductive study in rats was conducted with *N*-EtFOSE (Christian et al., 1999b) at doses of 1, 5, 10, and 15 mg/kg/day, and the profile of developmental toxicity was quite similar to that described with PFOS (Case et al., 2001a, 2001b; Christian et al., 1999a; Lau et al., 2003; Thibodeaux et al., 2003). Doses of the fluorochemical as high as 15 mg/kg/day had no effect on estrous cycling, mating, or fertility of the F_0 generation rats, although a decrease in numbers of implantations and viable embryos was noted. Postnatal survival of the F_1 generation pups was significantly reduced in the 10 and 15 mg/kg dose groups, characterized by stillbirth and mortality during the first 72 h of life; in addition, weight gain deficits were evident among the survivors whose reflex and physical development were slightly delayed. These adverse effects were less pronounced in the lower dose groups, and their reproductive performances were not adversely affected. However, the number of F_1 dams in the 5 mg/kg dose group that had stillborns or neonatal mortality (F_2) was slightly, but significantly increased, and the weight gain of F_2 pups was also reduced. There were no significant adverse effects at 1 mg/kg/day.

Toxicity of perfluorooctanoic acid

The general toxicity profile of PFOA has been characterized in monkeys (Butenhoff et al., 2002a; Griffith and Long, 1980), rats (Abdellatif et al., 1991; Biegel et al., 1995; Cook et al., 1992; Diaz et al., 1994; Griffith and Long, 1980; Kennedy, 1985; Kennedy et al., 1986; Kudo et al., 1999; Liu et al., 1996; Ohmori et al., 2003; Olson and Andersen, 1983; Pastoor et al., 1987; Reo et al., 1994), mice (Yang et al., 2000, 2001), rabbits (Griffith and Long, 1980; Kennedy, 1985), fish (Martin et al., 2003a, 2003b), and humans (Gilliland and Mandel, 1993, 1996, Olsen et al., 1999, 2000, 2003b), and summarized in a recent review (Kennedy et al., 2004; Kudo and Kawashima, 2003). A preliminary risk assessment of the developmental toxicity of PFOA was recently conducted by the Office of Pollution Prevention and Toxic Substances of the U.S. Environmental Protection Agency (US EPA, 2003a). Hence, only an abbreviated summary of the animal studies mentioned in the risk assessment document will be provided here.

Because of the peculiar gender difference in the clearance and excretion of PFOA in some animal species (*vide supra*), the choice of animal models is critical when evaluating the reproductive and developmental toxicity of this chemical. Thus far, only information pertaining to the rat and the rabbit is available. Teratological studies of PFOA have been conducted by Gortner (1981) with rats and rabbits (Gortner, 1982), and Staples et al. (1984) with rats, but neither laboratory reported any significant findings with administered doses up to 100–150 mg/kg/day for rats and 50 mg/kg/day for rabbits.

More recently, Butenhoff et al. (2004) undertook a two-generation study of ammonium PFOA where rats were given 1, 3, 10, or 30 mg/kg daily. In the F_1 generation, observations during the lactational period were unremarkable, with the exception of a significant lag of weight gain in the offspring of the 30 mg/kg PFOA-treated group noted during the first postnatal week and a statistically significant

decrease in the lactation index. A statistically significant increase of mortality was seen in both male and female pups at 30 mg/kg, especially in the first week after initiating gavage dosing at weaning. Nine of the thirteen neonatal deaths in the postweaning period involved the lightest pups and occurred in the first few days postweaning, suggesting that low body weight or immaturity at weaning was a likely factor in the mortality of these pups. Among the survivors, significantly reduced weight gain was observed in males, reaching statistical significance in all treated groups by termination of the study at 113 days postweaning. F_1 females in the 30 mg/kg dose group had significant reductions in body weight after weaning and these deficits persisted through the remainder of the study. Both F_1-generation males and females, at the highest dose (30 mg/kg/day) evaluated, experienced statistically significant delays (by 2 and 4 days for females and males, respectively) in reaching sexual maturity. However, no effects on mating or fertility parameters were apparent, and findings in the F_2 generation were unremarkable. Histological examination of tissues by light microscopy did not reveal any abnormalities in sex organs. However, hypertrophy and vacuolation of the zona glomerulosa of the adrenals of P-generation (10 and 30 mg/kg) and F_1-generation (30 mg/kg) males were seen, and hepatocellular hypertrophy was present in F_1 males at doses of 3 mg/kg and higher.

Interpretation of these data and their extrapolation for human health risk assessment are quite challenging because sexually mature female rats possess an innate ability to excrete PFOA rapidly, with an elimination half-life of 2–3 h, as opposed to days or weeks in male rats, dogs, and monkeys, or years in humans. Preliminary results from a pharmacokinetic study (Kemper and Jepson, 2003) further complicate the issue. These investigators reported that plasma kinetics in adult female rats appeared to be biphasic at the two highest administered single oral doses of 5 and 25 mg/kg (unlike the monophasic profile in male rats), and terminal elimination half-lives were dependent on the dose level. Thus, the toxic manifestation of PFOA in female pregnant rats, where steady state of the chemical is unlikely to be reached on a daily gavage dosing regimen and significant variations in the serum concentration of PFOA occur between the treatment intervals, may be quite different from those species (including humans) where the steady-state kinetics is reached gradually.

The postweaning mortality and delays in pubertal onset in the F_1 generation at 30 mg/kg (Butenhoff et al., 2004) reflect potential developmental effects of PFOA that may be partially explained by reduced body weight at weaning or weight gain thereafter. Interestingly, the fact that these effects occurred in both genders suggests a lack of the sex hormone-mediated differences in excretion. This contention would be consistent with the observation that the organic anion transporter protein (OAT2) that has been proposed to be responsible for the high PFOA excretion rate in adult female rats (Kudo et al., 2002) does not begin to be expressed until sometime between postnatal days 35 and 40 (Buist et al., 2002). These data suggest that pharmacokinetic profiles in sexually immature rats may be different from those in the adults.

Toxicity of other perfluoroalkyl acids

Compared to the C-8 perfluorochemicals, less is known about perfluoroalkyl acids of other carbon chain lengths. Perfluorohexanesulfonate (PFHS) has been found in serum samples from the general population including children, adult Red Cross blood donors, and elderly subjects enrolled in a prospective study of cognitive disorders (Olsen et al., 2002, 2003a, 2004), with geometric means of 0.002–0.005 μg/ml. In the same studies, geometric mean serum concentrations for PFOA were approximately 0.005 μg/ml, and, for PFOS, they ranged from approximately 0.03–0.04 μg/ml. Perfluorobutane sulfonate (PFBS) is known to have a much shorter half-life than PFOS (Paul Lieder, 3M, personal communication); in contrast, perfluorodecanoate (PFDA) appears to have a low rate of elimination in the rat (Vanden Heuval et al., 1991b). In this regard, it will be of interest in future pharmacokinetic and structure-activity studies to determine to what extent the rate of elimination for these fluorochemicals is directly related to their carbon chain length.

Like PFOA, both perfluorononanoate (PFNA) and PFDA are peroxisome proliferators (Harrison et al., 1988; Ikeda et al., 1985; Kinney et al., 1989). The toxic potency of PFDA is quite high, with an LD_{50} 4.6-fold lower than that estimated for PFOA (Olson and Andersen, 1983). PFDA has also been reported to produce hepatotoxicity, anorexia, alteration of fatty acid metabolism, reduction of circulating thyroid hormones and androgen, bradycardia, and hypothermia in the rat (Bookstaff et al., 1990; George and Andersen, 1986; Gutshall et al., 1988, 1989; Langley and Pilcher, 1985; Pilcher and Langley, 1986; Pilcher et al., 1987; Singer et al., 1990).

Both potassium PFBS and potassium PFHS have recently been assessed for developmental and reproductive effects. Maternal exposure to PFBS did not produce any adverse effects on embryo/fetal development, and no significant alterations were noted in a two-generation study in rats at doses as high as 1 g/kg (Paul Lieder, 3M, personal communication). PFHS did not produce developmental or reproductive effects in rats at doses up to 10 mg/kg in a one-generation reproductive and developmental toxicity screening test (Hoberman and York, 2003). In addition, Harris and Birnbaum (1989) reported a lack of teratogenic effects of PFDA in the mouse.

Future research needs and directions

The industrial and consumer uses of perfluoroalkyl acids and their derivatives have increased tremendously over the

past half century, and the unique physical and chemical properties of these materials makes substitution by other organic materials difficult for some of these products and applications. On the other hand, several of these fluorochemicals have become ubiquitous in the environment and the presence of PFOS and PFOA in humans in particular has drawn considerable attention in the public arena. Although the toxicology of some of these compounds has been investigated in the past, the widespread exposure demonstrated in recent bio-monitoring studies has renewed efforts to understand the hazards that may be associated with these chemicals, and new information has come to light in recent studies. This review has summarized recent findings from several laboratories on the developmental toxicity of perfluoroalkyl acids. Although the teratological potentials of some of these chemicals had been studied in the past and the findings were generally unremarkable, information from postnatal studies on developmental and reproductive indices have been published or submitted to the U.S. EPA public docket recently, and these findings have prompted further consideration of their relevance to human health risk. Confirmation and elucidation of the pharmacokinetic and mechanistic bases of these findings by different experimental approaches and study designs will be important to establish the context of these findings for human health risk assessment. This goal should be attainable by active investigation within the scientific community in the next few years.

Other research avenues can be useful in defining the potential health risks of fluorochemicals. At present, little is known about the environmental exposure, transport, and fate of these compounds, particularly PFOA. In June 2003, the U.S. Environmental Protection Agency began an Enforceable Consent Agreement process with industry to explore the environmental fate and transport of the PFOA, telomer-based materials, and related compounds, and to enhance understanding of the sources of PFOA in the environment, as well as the pathways by which human exposure to this chemical can occur. Information gathered under this agreement will complement the data collected in bio-monitoring programs and may lead to predictive models of exposure. Because of their physical properties (being hydrophobic, lipophobic and protein-bound; Han et al., 2003), perfluoroalkyl chemicals may have unique profiles of distribution in the body. Thus, pharmacokinetic characterization of these compounds, especially during pregnancy and early postnatal ages (before sexual maturity), will greatly facilitate our understanding of chemical disposition and potential cellular targets of PFOS and PFOA. In addition, as information pertaining to body burden of these fluorochemicals comes to light in the next few years (from bio-monitoring surveys by the CDC, for instance), this "internal dosimetric" can be used readily for cross-species (from laboratory animal models, such as rodents and monkeys to humans) as well as low-dose (from experimental doses to exposure levels) extrapolations for risk analysis. More in-depth descriptive information will undoubtedly provide additional clues to the modes and mechanisms of PFOS and PFOA developmental toxicity in laboratory animal models. Cellular and molecular mechanistic findings will be instrumental in extrapolating the health risk potential of these compounds for humans. For PFOA, potential disparities between genders and species (i.e. pharmacokinetic parameters between rats and humans) have to be reconciled to aid interspecies extrapolation. As other perfluoroalkyl acids are slowly coming onto the commercial market to replace PFOS, the potential developmental toxicity of these compounds should be investigated thoroughly.

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