

Characterization of risk for general population exposure to perfluorooctanoate

John L. Butenhoff,^{a,*} David W. Gaylor,^b John A. Moore,^c Geary W. Olsen,^a Joseph Rodricks,^d Jeffrey H. Mandel,^e and Larry R. Zobel^a

^a Medical Department, 3M Company, Building 220-2E-02, St. Paul, MN 55144, USA

^b David W. Gaylor Associates, Little Rock, AR, USA

^c Hollyhouse, Inc., Wicomico Church, VA, USA

^d Environ Health Sciences Institute, Arlington, VA, USA

^e Exponent, Chicago, IL, USA

Received 10 November 2003

Available online 16 April 2004

Abstract

Perfluorooctanoate (PFOA), an environmentally and metabolically stable perfluorinated carboxylic acid, has been detected in the serum of children, adults and the elderly from the United States with the upper bound of the 95th percentile estimate in the range of 0.011–0.014 µg/mL (ppm). In this risk characterization, margins of exposure (MOE), which can provide a realistic perspective on potential for human risk, were determined by comparison of general population serum PFOA concentrations with serum concentrations from toxicological studies that are associated with the lower 95% confidence limit of a modeled 10 percent response or incidence level (LBMIC₁₀) using USEPA BMDS software. The LBMIC₁₀ was estimated using surrogate data from other studies or pharmacokinetic relationships if serum PFOA data were not available. Modeled dose–responses (with resulting LBMIC₁₀ values) included post-natal effects in rats (29 µg/mL), liver-weight increase (23 µg/mL), and body-weight change (60 µg/mL) in rats and monkeys, and incidence of Leydig cell adenoma (125 µg/mL) in rats. MOE values based on the upper bound 95th percentile population serum PFOA concentration were large, ranging from 1600 (liver-weight increase) to 8900 (Leydig cell adenoma). These MOE values represent substantial protection of children, adults, and the elderly.

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Keywords: Perfluorooctanoate; Perfluorooctanoic acid; C8; PFOA; APFO; Risk characterization; Biomonitoring; Benchmark dose; Benchmark internal concentration; Toxicokinetics

1. Introduction

Perfluorooctanoic acid is a fully fluorinated carboxylic acid that, due to the strength of the carbon–fluorine bond, is exceptionally stable to metabolic and environmental degradation. The presence of fluorine in the carbon chain imparts a high electron-withdrawing capacity, rendering the carboxyl function highly acidic relative to other organic acids. Salts of perfluorooctanoic acid have been used as surfactants and processing aids in the production of fluoropolymers, and these salts

are considered critical to the production of certain fluoropolymers and fluoroelastomers.

Perfluorooctanoate (PFOA), the dissociated anion of the acid, has been found in the serum of children, adults, and the elderly as part of a broad biomonitoring study of the United States (US) population (Olsen et al., 2003c, 2004a,b). Serum concentrations of PFOA follow a log-normal distribution with geometric mean concentrations of 0.004–0.005 µg/mL. PFOA was quantifiable in over 90% of the serum samples. Upper 95% confidence limits of the 95th percentile estimated serum PFOA concentrations ranged from 0.011 to 0.014 µg/mL (Table 1), and the highest measured individual values in these general populations were 0.056, 0.052, and 0.017 µg/mL for children (ages 2–12), adults (ages 20–69), and the elderly

* Corresponding author. Fax: 1-651-733-1773.

E-mail address: jlbutenhoff@mmm.com (J.L. Butenhoff).

Table 1
Serum PFOA concentrations measured in three non-occupationally exposed populations from the United States

General population monitoring study	Location	Sample size ^c	Upper bound of the 95th percentile estimate of the population ($\mu\text{g/mL}$)
Children ^a	23 states	645	0.011
Adult ^b	6 cities ^d	598	0.014
Elderly ^c	Seattle (WA)	238	0.011

^a Olsen et al. (2004a).

^b Olsen et al. (2003c).

^c Olsen et al. (2004b).

^d Portland (OR), Los Angeles (CA), Minneapolis-St. Paul (MN), Charlotte (NC), Hagerstown (MD), and Boston (MA).

^e Sample size in each study was equally represented by sex. Geometric mean serum PFOA concentrations were similar by sex.

(ages 65–96), respectively. Although possible sources and pathways of exposure have been suggested (environmental releases and some consumer products), the source(s) and pathway(s) responsible for human exposure are not known. The United States Environmental Protection Agency (USEPA) and current and former manufacturers and users of PFOA and its salts have joined in a process to better understand sources and pathways of PFOA exposure (USEPA, 2003a).

Based on interim results from a study of nine retired workers, PFOA appears to be poorly excreted in humans, as the current estimate of serum elimination half-life from an on-going study of retired workers is 4.4 ± 3.5 years (Burris et al., 2002). The finding of PFOA in most human sera samples tested and the extended retention time in the body have prompted consideration of the potential health risk of low-level continuous exposure to PFOA.

The potential toxicity of PFOA has been studied extensively, and recent reviews of PFOA toxicity are available (Kennedy et al., 2004; USEPA, 2002). While many of these studies have been published or are in various stages of preparation for publication, the USEPA administrative record 226 (AR-226) contains copies of many original studies not yet available in the peer-reviewed literature, as well as source data for several published studies. Most studies were conducted with the ammonium salt of PFOA, which readily dissociates in aqueous media at physiological pH. The designation, APFO, will be used when referring specifically to this salt. Numerous acute, shorter-term, and longer-term toxicity studies have been conducted in multiple species by different routes of exposure. In addition, two chronic dietary studies in rats have been reported. The toxicological database includes developmental toxicity, reproductive toxicity, immunotoxicity, genotoxicity, carcinogenicity, pharmacokinetics, and various mode-of-action studies. In addition to toxicological studies, medical surveillance and epidemiological studies of PFOA-exposed workers at the 3M Company have been ongoing since the late 1970s (3M Company, 2003a,b; Alexander, 2001; Gilliland, 1992; Gilliland and Mandel, 1996; NIOSH, 2001; Olsen et al., 1998, 2000, 2003a; Ubel et al., 1980).

PFOA should not be confused with perfluorooctanesulfonate (PFOS, $\text{C}_8\text{F}_{17}\text{SO}_3$), another eight-carbon perfluorinated acid that has also been found to be eliminated slowly in humans (Burris et al., 2002), is widely distributed in humans (Olsen et al., 2003c, 2004a,b), and has been found in wildlife samples from many areas of the world (3M Company, 2003c). There are key differences in the production, use, environmental distribution, potential toxicity, and pharmacokinetics between PFOA and PFOS; therefore, these two perfluorinated acids should be evaluated separately.

There have been two recently documented preliminary risk assessments focused on PFOA. In the first, the State of West Virginia Department of Environmental Protection released a report on the establishment of preliminary risk screening levels based on RfD and RfC values derived by a team of experts for PFOA in drinking water, soil, and air in proximity to a manufacturing facility that uses APFO in fluoropolymer production (West Virginia Department of Environmental Protection, 2002). All water samples collected in the vicinity of this facility were below the risk screening level of $150 \mu\text{g/L}$ derived for drinking water in this process. Water samples from 50 private well and cisterns used for drinking water and the nine public water supplies were below $3 \mu\text{g/L}$. No measured air or soil concentrations were available.

Another preliminary risk assessment was released by USEPA (USEPA, 2003b), which presented a range of margin-of-exposure (MOE) values based on comparisons of human serum concentrations of PFOA (Olsen et al., 2003c, 2004a,b) and the serum concentrations in samples taken from rats involved in a two-generation reproduction study. However, the USEPA cautioned that these MOE values should not be considered to represent the range of possible MOE values for general populations because of uncertainties resulting from the lack of appropriate toxicokinetic data in weanling rats and their relationship to human serum levels of PFOA. The USEPA continues to develop a more comprehensive risk characterization that is intended to better define potential human MOE values (Butenhoff et al., 2004; USEPA, 2004).

This document presents a characterization of potential health risk for the general population using measured serum PFOA concentrations in the biomonitoring studies conducted by Olsen et al. (2003c, 2004a,b). Dose–response data from toxicological studies have been used to estimate concentrations of PFOA in serum associated with a 10% benchmark response (BMR) for several key endpoints. Further, the lower 95% confidence limits of these benchmark internal concentrations (LBMIC₁₀, Gaylor et al., 2003) have been used as a basis for comparison with general population serum PFOA concentrations. This method takes advantage of the facts that:

- (1) PFOA is chemically stable and not readily subject to environmental and metabolic degradation (Goecke et al., 1992; Kuslikis et al., 1992; Vanden Heuvel et al., 1991).
- (2) Extensive toxicological (including non-human primates) and worker-health studies are available (Kennedy et al., 2004; USEPA, 2002) that allow for examination of most toxicological endpoints (e.g., developmental toxicity, reproductive toxicity, immunotoxicity, genotoxicity, carcinogenicity, pharmacokinetics, and mode-of-action).
- (3) Serum PFOA measurements have either been made in connection with studies or can be estimated based

on new information on the toxicokinetics of PFOA in the rat (Han, 2003; Kemper, 2003; Mylchreest, 2003) and monkey (Kerstner-Wood et al., 2003; Noker, 2003) to facilitate cross-species extrapolation.

- (4) Population exposure to PFOA has been well characterized through serum PFOA concentration measurements in biomonitoring studies that include children (Olsen et al., 2004a), adults (Olsen et al., 2003c), and the elderly (Olsen et al., 2004b).

The use of these factors can be instrumental in reducing uncertainty in the risk characterization of PFOA.

2. Methods

2.1. Selection of studies and endpoints

A review of the toxicological database for PFOA was conducted in order to select studies that covered a variety of endpoints, were sufficiently robust, and provided good dose–response data. The endpoints and associated studies chosen are presented in Table 2. Sensitive indicators of response that were chosen for the determination or estimation of benchmark internal concentration values (LBMIC₁₀, as described in Section 2.3) were post-natal developmental effects (rats), liver-weight increase

Table 2
Endpoints and source studies used in evaluating dose–response

Endpoint	Source study	Source data table
Post-natal development in rats ^a	Two-generation reproduction study (Butenhoff et al., 2004)	Table 3
Liver-to-brain-weight ratio in rats ^b	Two-generation reproduction study (Butenhoff et al., 2004)	Table 3
Body-weight change in rats ^c	Two-generation reproduction study (Butenhoff et al., 2004)	Table 3
Liver-to-brain-weight ratio in rats ^d	13-week dietary study (Palazzolo, 1993)	Table 4
Body-weight change	13-week dietary study (Palazzolo, 1993)	Table 4
Liver-to-brain-weight ratio in monkeys ^e	6-month oral toxicity study (Butenhoff et al., 2002b)	Table 5
Body-weight change in monkeys ^f	6-month oral toxicity study (Butenhoff et al., 2002b)	Table 5
Leydig cell tumors in rats ^g	Two-year cancer bioassay (Sibinski et al., 1983)	Table 6

^a The following endpoints were evaluated separately: (1) pre-weaning mortality (combined sexes); (2) pup body-weight at weaning (combined sexes); (3) post-weaning mortality in males and females (separately); (4) days to preputial separation in males; and (5) days to vaginal patency in females.

^b Male liver-weight-to-brain-weight ratio was selected because male rats respond to a greater extent than females to the liver-enlarging effects of PFOA. PFOA affects body weight; therefore, use of liver-weight-to-brain-weight ratio normalizes for body-weight changes, since brain is not responsive to body-weight change from dietary restriction (Feron et al., 1973). F₀ and F₁ data were evaluated separately. The two-generation reproduction study involved oral dosing of male rats in both the F₀ and F₁ generations for more than 90 days, the typical term of a subchronic study, and, therefore, has the advantage of following a subchronic dosing response over two generations and group sizes of approximately 30.

^c Body-weight change was evaluated as reduced body-weight gain compared to controls only in male rats, which were more sensitive than female rats to PFOA-induced reductions in weight gain. F₀ and F₁ data were evaluated separately.

^d Liver-weight-to-brain-weight ratio was used to minimize effects of body-weight reduction and reduced feed consumption. The 13-week (90-day) subchronic dietary study in male rats (Palazzolo, 1993) is useful in that serum PFOA concentrations were made at all dose levels.

^e Since the male monkeys from this study varied in age and weight at the beginning of the study, and dosing with APFO caused significant weight loss among the high-dose-group monkeys, only data from male monkeys dosed until terminal sacrifice were used, which excludes data from three high-dose-group monkeys for whom dosing was suspended.

^f For male cynomolgus monkeys, body-weight change was represented by the actual percentage change in individual body weight from pre-study baseline weight through weight at or near termination (scheduled or unscheduled) of dosing. Because these were adult monkeys of various ages and weights, and due to the fact that only two of six monkeys were dosed continuously for six months at the high dose, percent change in body weight from baseline was considered more meaningful than comparison of body-weight change or terminal body weight between treated and control groups.

^g Human epidemiological studies have not shown statistically significant associations of exposure to PFOA with increased cancer mortality risk (Alexander, 2001). Leydig cell adenoma incidence from the two-year cancer bioassay in rats was used.

(rats and monkeys), body-weight change (rats and monkeys), and incidence of Leydig cell adenoma (rats). Source data are presented in Tables 3–6.

A comment regarding the choice of Leydig cell adenoma incidence from a two-year cancer bioassay in rats (Sibinski et al., 1983) is in order. Human epidemiological

studies have not shown associations of exposure to PFOA with increased cancer mortality risk (Alexander, 2001). The two-year cancer bioassay in rats by Sibinski et al. (1983) produced significant increases in Leydig cell adenoma (males) and mammary fibroadenoma (females). The mammary fibroadenoma incidence

Table 3

Dose–response data for post-natal developmental endpoints in a two-generation reproduction study with ammonium perfluorooctanoate (APFO) in rats (Butenhoff et al., 2004)

Post-natal effect	Oral gavage dose level (mg/kg/day)				
	0	1	3	10	30
Post-weaning mortality, males, %	5.0 (3/60) ^a	5.0 (3/60)	5.0 (3/60)	3.3 (2/60)	12 (7/60)
Post-weaning mortality, females, %	0.0 (0/60) ^a	3.3 (2/60)	1.7 (1/60)	1.7 (1/60)	10 (6/60)*
Mean days to preputial separation	48.5	49.5	49.4	49.7	52.2*
Mean days to vaginal patency	34.9	35.5	34.1	34.8	36.6*
Pre-weaning mortality, combined sexes, %	2.6 (10/385) ^a	3.0 (11/372)	4.4 (17/388)*	2.5 (10/400)	6.7 (26/388)*
Mean weight at weaning, both sexes, g	37.4	36.7	39.7	38.8	35.7
F ₀ male liver-to-brain-weight ratio	9.0 ± 1.2 (30) ^b	10.7 ± 1.5 (30)*	12.3 ± 1.2 (30)*	12.8 ± 1.8 (30)*	12.5 ± 1.4 (29)*
F ₁ male liver-to-brain-weight ratio	9.3 ± 1.4 (30) ^b	10.8 ± 1.5 (29)*	12.2 ± 1.8 (30)*	12.9 ± 1.6 (30)*	13.6 ± 1.7 (29)*
F ₀ male body-weight change, g	400 ± 37 (30) ^b	395 ± 46 (30)	362 ± 48 (30)*	335 ± 53 (30)*	253 ± 63 (30)*
F ₁ male body-weight change, g	512 ± 55 (30) ^b	473 ± 52 (29)*	468 ± 50 (30)*	445 ± 58 (30)*	388 ± 37 (30)*

^a Incidence is given in parentheses.

^b Sample size (*n*) is given in parentheses.

* Statistically significant compared to controls (*p* < 0.05).

Table 4

Dose–response data from a 13-week subchronic dietary study with ammonium perfluorooctanoate in rats (Palazzolo, 1993)

Estimated dose (mg/kg/day)	Serum [PFOA] (µg/ml)	Liver-weight-to-brain-weight ratio	Body-weight change (g)
0 (ad libitum)	<1 (10) ^a	9.03 ± 1.20 (15)	339 ± 34.1 (25)
0 (pair fed) ^b	<1 (10)	7.64 ± 0.774 (15)	296 ± 17.8 (25)
0.06	7 ± 1 (10)	8.19 ± 1.56 (14)	343 ± 33.1 (25)
0.64	41 ± 13 (10)	9.41 ± 1.33 (15)	348 ± 37.8 (25)
1.94	70 ± 16 (10)	10.8 ± 1.96 (15)	327 ± 42.2 (25)
6.5	138 ± 34(10)	12.6 ± 2.88 (14)*.#	290 ± 57.4 (25)*

^a Sample size (*n*) is given in parentheses.

^b Control group pair-fed to high-dose group.

* Statistically significant when compared to ad libitum control.

Statistically significant when compared to pair-fed control.

Table 5

Dose–response data from a six-month oral toxicity study of ammonium perfluorooctanoate in male cynomolgus monkeys (Butenhoff et al., 2002b)

Dose (mg/kg)	[PFOA] serum ^a (µg/mL)	Liver-weight-to-brain-weight ratio	[PFOA] serum ^b (µg/mL)	Body-weight change (%)
0	0.16 ± 0.15 (4) ^c	0.934 ± 0.074 (4)	0.21 ± 0.14 (6)	17 ± 6 (6)
3 ^d	72 ± 47 (4)	1.34 ± 0.23 (4)*	72.1 ± 44.4 (4)	13 ± 8 (4)
10	85 ± 20 (4)	1.30 ± 0.23 (4)*	81.3 ± 25.2 (6)	15 ± 5 (6)
30/20	155 ± 102 (2) ^e	1.22 ± 1.2 (2) ^c	284 ± 212 (6)	−5 ± 9 (6)*

^a Mean serum values ± SDs from samples taken during weeks 20, 22, 24, and 26.

^b Mean serum values ± SDs from single samples taken at termination of dosing or mean of multiple samples taken at termination of dosing and/or within two weeks prior to termination of dosing.

^c Sample size (*n*) is given in parentheses.

^d Includes data derived from monkey I05721M, which was sacrificed in week 20 on day 137.

^e Includes data derived from the two monkeys that were dosed until scheduled end of dosing at 26 weeks. Does not include data from monkey I05724 that was humanely sacrificed in week 5 on day 29. Monkey I05724 had a body weight of 3505 g, a liver weight of 83 g, a liver weight % of body weight of 2.37, a liver weight % of brain weight of 1.48, and a serum concentration of 822 µg/mL in week 4.

* Statistically significant when compared to controls (*p* < 0.05).

Table 6

Poly-3 procedure adjusted Leydig cell adenoma incidence rates for rats given ammonium perfluorooctanoate in diet for a lifetime

Dietary dose level (ppm)	Overall mean APFO intake (mg/kg/day) ^a	Leydig cell adenoma ^b
0	0	0/44
30	1.3	2/44 (4.6%)
300	14	7/48 (15%)
<i>Estimated LBMD</i> ₁₀ ^c LBMD ₁₀ = 100 ppm	LBMD ₁₀ = 4.8 mg/kg/day	(10%)
<i>Estimated LBMIC</i> ₁₀ ^d	LBMIC ₁₀ = 125 µg PFOA/mL	(10%)

^a Estimated from feed consumption and analysis of diet for ammonium perfluorooctanoate.^b The values in the denominator represent the effective lifetime number of rats at risk of cancer based on the Poly-3 adjustment procedure (Bailer and Portier, 1988) and value shown is the corresponding age-adjusted tumor incidence rate.^c The lower 95% CI of the benchmark dose based on the multistage model and a 10% incidence.^d Estimated using the equation of the trendline in Fig. 2 ($y = 46.1 \times x^{0.6347}$) to yield a LBMIC₁₀ of 125 µg PFOA/mL serum corresponding to 4.8 mg/kg/day.

was reported to be within the range of reported spontaneous incidences for this tumor type. Recent evaluation of historical control data from studies conducted at DuPont Haskell Laboratory and those offered on the animal supplier's (Charles River Laboratories) web site (http://www.criver.com/techdocs/tech_pdf/2001TOXDATA.pdf) indicate that this assertion is correct, and these benign mammary adenomas are not likely to be related to treatment (Butenhoff et al., 2002a). A mechanistic two-year dietary study in male rats reported by Biegel et al. (2001), in addition to finding an increase in Leydig cell adenoma (8/76 (11%) versus 0/80 (ad libitum control) and 2/78 (2.5%, pair-fed control)), found an increase in pancreatic acinar cell adenoma/carcinoma (7/76 (9.2%) versus 0/80 (ad libitum control) and 1/79 (1.3%, pair-fed control)), and hepatocellular adenoma (10/79 (13%) versus 2/80 (2.5%, ad libitum control) and 1/79 (1.3%, pair-fed control)) at the single study dose of 300 ppm in diet. The single-treatment-level data available from the Biegel et al. (2001) study do not allow insight into the characteristics of the dose–response relationships for tumor incidence. With respect to the liver tumors, the proposed mechanism of peroxisome-proliferator-activated-receptor- α (PPAR- α) activation suggests that these tumors have questionable or no relevance to humans (Ashby et al., 1994; Bentley et al., 1993; Cattley et al., 1998). Therefore, because the Leydig cell adenoma incidence in the Sibinski et al. (1983) study (Table 6) was higher than or comparable to tumor incidences in Biegel et al. (2001) and provided a means to model dose–response, it was used to develop LBMIC₁₀ and MOE values for nonlinear cancer risk. The human relevance of Leydig cell tumors observed by Sibinski et al. (1983) as well as Leydig cell, and pancreatic acinar cell tumors observed by Biegel et al. (2001) remains uncertain (Ashby et al., 1994; Bentley et al., 1993; Biegel et al., 1995; Cattley et al., 1998; Clegg et al., 1997; Cook et al., 1992, 1999; Liu et al., 1996a,b).

2.2. Choice of general population serum PFOA value for comparison to LBMIC₁₀ values from toxicological studies

Four studies that survey PFOA concentrations in serum samples from non-occupationally exposed populations in the US have been conducted. Separate studies of serum samples from children enrolled in a Group A *Streptococcal* clinical trial ($N = 598$), adult American Red Cross blood donors ($N = 645$), and dementia-free elderly ($N = 238$) from a prospective study of cognitive function have shown serum concentrations of PFOA averaging approximately 0.005 µg PFOA/mL with an upper bound of the 95th percentile estimate approximating 0.011–0.014 µg PFOA/mL, as shown in Table 1 (Olsen et al., 2003c, 2004a,b). In another study, Olsen et al. (2003b) examined a total of 30 human donor livers for the presence of PFOA. All donor livers were below the lower limit of quantitation for at least one of two analyses per sample except for one liver that had an average of 0.047 µg/g. The value from Table 1 that will be used to represent the upper bound of general population exposure is the highest upper bound 95th percentile estimate, 0.014 µg/mL, from the adult blood donor biomonitoring study (Olsen et al., 2003c).

2.3. Derivation of benchmark internal concentration (LBMIC₁₀) values

2.3.1. Dose metrics and the use of serum PFOA concentration data

This risk characterization uses either: (1) measured serum PFOA concentration at presumed steady state; (2) pharmacokinetic estimates of steady state; or (3) 24-h mean serum PFOA concentration, as a measure that is associated with biological responses to PFOA and compared to representative general population serum PFOA concentrations. This method requires that PFOA be accurately measured in serum, and that the measured

serum PFOA concentration be related to response in toxicological studies. It also requires an understanding of the pharmacokinetics related to dosing that affect bioavailability and serum concentration. The assumption is made that the dose of PFOA is related to serum PFOA concentration, which, in turn, is associated with response. Factors affecting the measurement of serum PFOA concentration include dosage form and frequency, absorption rate constant, elimination rate constant, time of sampling relative to dose administration, and the precision and variability in the analytical method (measurements of serum PFOA used in this characterization had a coefficient of variation of approximately $\pm 30\%$). If a non-metabolized compound is absorbed readily and poorly excreted, these factors have less influence on the value of measured serum concentration than if it is readily absorbed and quickly excreted. In the latter case, time of sampling relative to dose administration becomes critical, since the serum concentration of the compound changes rapidly. The striking differences in PFOA elimination rates between species, or sexes within species, are demonstrated in Table 7. These differences in PFOA elimination rate are largely overcome in estimating margins of exposure based on direct comparisons of serum concentrations at steady state or by using area-under-the-serum-concentration-versus-time curve (AUC, $\mu\text{g h/mL}$) to estimate steady-state or mean 24-h serum concentrations. For example, humans, who have a long elimination half-life (Burris et al., 2002), would exhibit *de minimis* change in their serum concentration over 24 h; thus, the 24-h mean serum concentration (AUC/24 h) represents steady state and is essentially the measured serum PFOA concentration. In the case of adult female rats, which would have a rapidly changing profile of PFOA serum

concentration due to essentially complete excretion of PFOA in a 24-h period (Table 7), AUC/24 h provides a time-weighted average serum concentration over the course of a dosing interval that can be used for comparison to human values. AUC_∞ has been measured for male and female rats given single oral doses of PFOA (Kemper, 2003). The strong linear relationship between AUC_∞ and oral dose at the doses employed (0.1, 1, 5, and 25 mg/kg) allows interpolation of the value of AUC_∞ for any dose within the range 0.1–25 mg/kg (Fig. 1). AUC can be used to estimate steady state, because, in the theory of linear pharmacokinetics, the AUC from time zero to infinity for a single dose (AUC_∞ , $\mu\text{g h/mL}$) will equal the AUC for a dosage interval at steady state, assuming that absorption and elimination rates remain constant from dose to dose (Wagner, 1975). Therefore, when dosing intervals are shorter than the elimination half-life, steady-state serum concentration may be estimated by AUC/24 h. The use of these concepts in estimating serum PFOA values for use in risk characterization is described below.

Serum PFOA concentrations corresponding to a 10% change from normal or control were determined using USEPA National Center for Exposure Assessment software (BMDS version 1.3.1) and in general accordance with a draft guidance document by USEPA (USEPA, 2000) on use of the benchmark dose. For most categorical data from toxicology studies, a 10% response level is fairly representative of the limits in which a change can be accurately determined. For continuous, normally distributed data, a shift in the distribution of 1.0 standard deviation represents approximately an extra 10% of the individual values being greater than approximately the 99th percentile or about an extra 10% less than approximately the 1st percentile of the

Table 7
Reported values for elimination $T_{1/2}$ of perfluorooctanoate in various species

Species	Sex	Dose form	Observed $T_{1/2}$ (days)	<i>N</i>	Reference
Rat	Male	Oral	5	3	Gibson and Johnson (1979)
Rat	Male	Inhalation	5–7	4–5/group	Kennedy et al. (1986)
Rat	Male	Dermal	5–7	5/group	Kennedy (1985)
Rat	Male	Oral	9 (liver)	6	Ylinen et al. (1990)
Rat	Male	Oral	6–8	4	Kemper (2003)
Rat	Male	i.p.	15	4	Vanden Heuvel et al. (1991)
Rat	Male	i.v.	5.6	3	Ohmori et al. (2003)
Rat	Pregnant female	Oral (GD 8–9)	<0.5	4	Gibson and Johnson (1983)
Rat	Female	Oral	<0.5	2	Gibson and Johnson (1983)
Rat	Female	Oral	0.13–0.67	4	Kemper (2003)
Rat	Female	Oral	2.5 (liver)	6	Ylinen et al. (1990)
Rat	Female	i.p.	<1	4	Vanden Heuvel et al. (1991)
Rat	Female	i.v.	0.08	3	Ohmori et al. (2003)
Dog	Male	i.v.	20 and 23	2	Hanhijärvi et al. (1988)
Dog	Female	i.v.	8–13	2	Hanhijärvi et al. (1988)
Monkey	Male	i.v.	21	3	Noker (2003)
Monkey	Female	i.v.	33	3	Noker (2003)
Retired workers	Male	Occupational exposure	1600 \pm 1300	9	Burris et al. (2002)

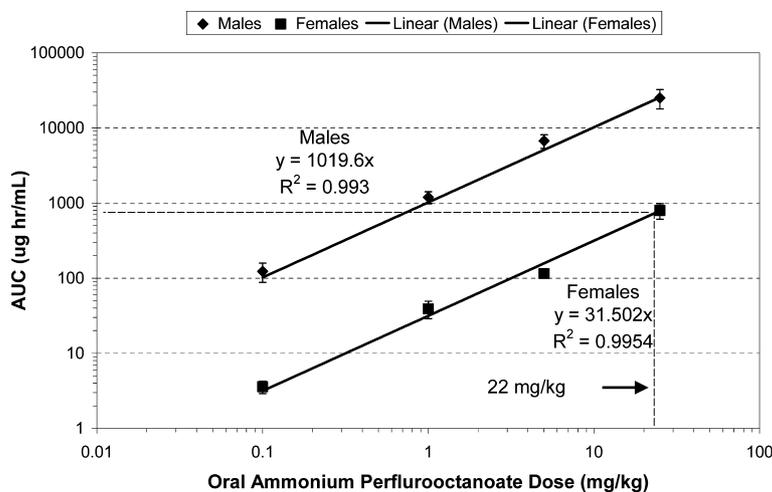


Fig. 1. Relationship of single oral dose of ammonium perfluorooctanoate (APFO) to serum perfluorooctanoate (PFOA) area-under-the-curve in male and female rats over a range of oral doses. These relationships are used in estimating mean serum concentrations of PFOA at the LBMD₁₀. The AUC corresponding to the LBMD₁₀ for a response in male or female rats is divided by 24 h to obtain a time-weighted average serum concentration over the daily dosing interval. For example, the dose corresponding to the LBMD₁₀ for post-natal effects (22 mg/kg/day) is marked with an arrow. The corresponding AUC ($\approx 700 \mu\text{g h/mL}$) was divided by 24 h to obtain the estimated LBMIC of 29 $\mu\text{g/mL}$. Data are based on Kemper (2003).

distribution in controls. For continuous data, linear, polynomial, Hill, and power models were attempted, and those providing adequate goodness-of-fit, as determined by $p > 0.05$ and reasonable fit of modeled dose-response curves in the range of the benchmark response on visual inspection, were used. In certain cases, elimination of the high-dose group was necessary to obtain adequate fit in the curve region in which the benchmark response level occurred. The resulting values from the modeling are referred to in this analysis as the Benchmark Internal Concentration for a 10% response (BMIC₁₀), and the lower 95% CL of the BMIC₁₀ (LBMIC₁₀) was used as the point of departure (POD) for estimating MOE values.

Serum concentrations representing the LBMIC₁₀ were determined by either of three methods. For the 13-week dietary study of APFO toxicity in male Sprague–Dawley rats (Palazzolo, 1993) and the six-month oral dosing study of APFO in cynomolgus monkeys (Butenhoff et al., 2002b), in which appropriate serum PFOA concentration data were available, the serum PFOA concentration was entered into the BMDS modelling program as the “dose,” and this will be referred to as the “calculated” LBMIC₁₀. For the cynomolgus monkey study, in order to derive LBMD₁₀ and LBMIC₁₀ values for body-weight change, serum PFOA concentrations at scheduled or unscheduled termination of dosing were used, along with any valid serum PFOA determinations within a two-week period prior to cessation of dosing. A second method was used for the two-generation reproduction study in Sprague–Dawley rats (Butenhoff et al., 2004), in which representative serum PFOA concentration data were not available for all groups. In this case, the lower 95% CL of the benchmark administered dose for a 10% change (LBMD₁₀) was determined, and the equations for AUC

versus dose for male and female rats from Fig. 1 were used to calculate AUC from the LBMD₁₀. The resulting AUC was divided by 24 h to provide an average serum concentration over a 24-h dosing interval, and the result of this method will be referred to as the “estimated” LBMIC₁₀. For example, a LBMD₁₀ value of 22 mg/kg was used to calculate a corresponding estimated LBMIC₁₀ of 29 $\mu\text{g PFOA/mL}$ serum by calculating the AUC from the relationship in Fig. 1 ($\text{AUC} = 31.5 \times 22 \text{ mg/kg} = 704 \mu\text{g h/mL}$) and dividing by 24 h ($704 \mu\text{g h/mL} / 24 \text{ h} = 29 \mu\text{g/mL}$). The latter method provides an estimated mean serum PFOA concentration over the course of a daily gavage-dosing interval that should approximate steady-state serum PFOA concentrations in adult male rats (see Section 2.1 above). In the case of adult female rats, which would not reach a steady-state serum PFOA concentration on daily gavage dosing due to rapid elimination of PFOA (Table 7), the latter method provides a mean 24-h serum PFOA concentration that is more meaningful than a value taken at a single time point on a rapid serum PFOA elimination curve that effectively reaches baseline within 24 h. For weanling rats, with the exception of one report in the literature (Kojo et al., 1986) that is lacking in detail, the elimination kinetics have not been described previously. However, recent work investigating the time course of development of sex differences in elimination of PFOA indicates that PFOA elimination in weanling rats is intermediate between adult male and female rats until four to five weeks of age, at which time the sex differences observed in adult rats become apparent (Han, 2003). Therefore, use of the adult female rat AUC-versus-oral-dose relationship shown in Fig. 1 to estimate 24-h mean serum PFOA concentrations in F₁ male and female weanling rats between three and four weeks of age is warranted, if not conservative. Finally, serum PFOA

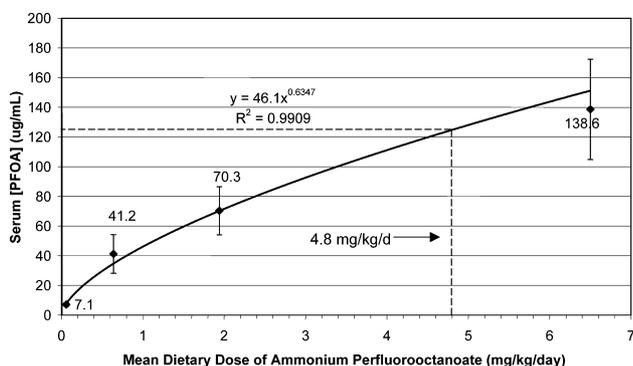


Fig. 2. The relationship of serum perfluorooctanoate concentration to dose in male rats after dietary treatment with ammonium perfluorooctanoate (APFO) for 13 weeks. These data were used in calculating LBMIC₁₀ for liver-to-brain-weight ratio and body-weight change in the 13-week dietary study (Palazzolo, 1993) and in estimating the LBMIC₁₀ from the LBMD₁₀ for Leydig cell tumors (4.8 mg/kg/day, arrow) in the two-year dietary cancer bioassay dietary toxicology studies of ammonium perfluorooctanoate (APFO) in male rats (Sibinski et al., 1983). Data are derived from Palazzolo (1993).

data were also not available for Leydig cell adenoma incidence in rats from the two-year dietary cancer bioassay (Sibinski et al., 1983). In this case, the LBMD₁₀ was determined (USEPA, 1996, 1999, 2003c) after obtaining tumor incidences shown in Table 6 by the Poly-3 procedure (Bailer and Portier, 1988) for calculating the effective lifetime number of rats at risk for cancer. The LBMIC₁₀ was estimated using the serum PFOA concentration data from the subchronic study in rats (Palazzolo, 1993). Serum PFOA concentration versus mean mg/kg/day intake of APFO from Table 4 were fit to a power curve (Fig. 2) to provide a means of interpolating the serum value corresponding to the LBMD₁₀ for Leydig cell tumors, and, thereby, estimating the LBMIC₁₀.

2.4. Estimation of margins of exposure

In this risk characterization, MOE values are calculated based on comparisons of serum PFOA

concentrations. The MOE was estimated by dividing the lowest LBMIC₁₀ values (or, points of departure) for the chosen toxicological endpoints in Table 2 by human serum PFOA concentrations considered to represent upper bound of the 95th percentile estimate of the general population serum PFOA concentration (0.014 µg/mL).

3. Results of calculated or estimated of LBMIC₁₀ values

3.1. Post-natal developmental effects

Benchmark doses (LBMD₁₀) calculated for post-natal developmental endpoints of F₁ pups in the two-generation reproduction study (Butenhoff et al., 2004) are shown in Table 8. Because data on serum PFOA concentration in pups were not available from the study, the LBMIC₁₀ correlating to the lowest LBMD₁₀ for post-natal developmental effects (22 mg/kg/day based on F₁ female post-weaning mortality, plot for LBMD₁₀ shown in Fig. 3) was calculated based on the relationship of adult female rat AUC to administered dose, as explained in Section 2.3. Using this relationship, the value of AUC/24 h at a dose of 22 mg/kg/day was calculated to be 29 µg/ml. The fact that adult F₀ males had

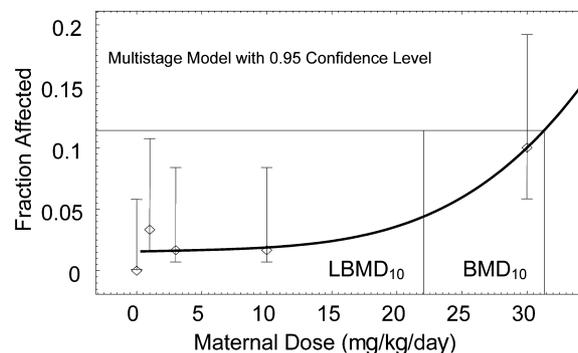


Fig. 3. Plot of BMDS-modeled dose (mg/kg/day)–response curve for post-weaning mortality in F₁ female rats (dichotomous multi-stage model, 10% response level).

Table 8

Estimates of the BMD₁₀, LBMD₁₀, and LBMIC₁₀ for post-natal effects in F₁ rat pups in a two-generation reproduction study with ammonium perfluorooctanoate (Butenhoff et al., 2004)

Effect	Model	<i>p</i> value ^a	BMD ₁₀ (mg/kg/day)	LBMD ₁₀ (mg/kg/day)	LBMIC ₁₀ ^b (µg/mL)
Days to preputial separation	Linear	0.33	27	22	29
Post-lactational mortality in females	Multistage	0.34	31	22	29
Post-lactational mortality in males	Multistage	0.96	33	24	32
Days to vaginal patency ^c	Linear	0.001	41	30	40
Pre-weaning mortality (both sexes)	Multistage	0.39	39	34	45
Day 22 pup weight (both sexes)	Linear	0.21	97	44	59

^a Goodness-of-fit *p* values greater than 0.05 indicate an adequate fit.

^b Estimated based on relationship of AUC to dose; e.g., the LBMD₁₀ value of 22 mg/kg was used to calculate a corresponding estimated LBMIC₁₀ of 29 µg PFOA/mL serum by calculating the AUC from the relationship in Fig. 1 (AUC = 32 × 22 mg/kg = 704 µg h/mL) and dividing by 24 h (704 µg h/mL/24 h = 29 µg/mL).

^c None of the available models provided a good fit to the data.

Table 9

LBMD₁₀ and LBMIC₁₀ values for liver-weight-to-brain-weight ratio and for body-weight change in male rats and monkeys dosed with ammonium perfluorooctanoate

Species/study	LBMD ₁₀ values			LBMIC ₁₀ values		
	Model	<i>p</i> value ^a	LBMD ₁₀ (mg/kg)	Model	<i>p</i> value	LBMIC ₁₀ (µg/mL)
Liver-weight-to-brain-weight ratio						
F ₀ rats/2-Gen ^b	Hill	0.30	0.60	NA ^c	NA	25 ^d
F ₀ rats/2-Gen	Linear	0.07	1.0	NA	NA	42
F ₁ rats/2-Gen	Hill	0.25	0.60	NA	NA	25
F ₁ rats/2-Gen	Linear	0.17	1.3	NA	NA	54
Rats/13-week ^e	Linear	0.18	1.4	Linear	0.23	34
				NA	NA	58
Monkeys/6-mo. ^f	Linear	0.01 ^g	3.9	Linear	0.39	23
Body-weight change ^h						
F ₀ rats/2-Gen	Linear	0.12	9.2	NA	NA	380
F ₀ rats/2-Gen	Polynomial	0.25	5.2	NA	NA	220
F ₁ rats/2-Gen	Power ⁱ	0.20	1.5	NA	NA	63
Rats/13-week	Power	0.46	3.0	Power	0.42	88
				NA	NA	130
Monkey/6-mo.	Power	0.34	10	Power	0.55	64
Monkeys/6-mo.				Linear	0.45	60

^a Goodness-of-fit *p* values greater than 0.05 indicate an adequate fit.

^b Two-generation reproduction study (Butenhoff et al., 2004).

^c Not applicable. The value of the LBMIC was estimated based on the LBMD (see footnote d).

^d Values in italics are estimated based on relationship of AUC to dose; e.g., the LBMD₁₀ value of 0.6 mg/kg was used to calculate a corresponding estimated LBMIC₁₀ of 25 µg PFOA/mL serum by calculating the AUC from the relationship in Fig. 1 (AUC = 1000 × 0.6 mg/kg = 600 µg h/mL) and dividing by 24 h (600 µg h/mL/24 h = 25 µg/mL).

^e Thirteen-week dietary study (Palazzolo, 1993).

^f Six-month oral dosing study in male cynomolgus monkeys (Butenhoff et al., 2002b).

^g Even though *p* < 0.05, this provides some reasonable level of fit and is shown for comparative purposes.

^h Body-weight change from initiation of dosing through termination of dosing.

ⁱ Unrestricted power model (power not restricted to ≥1).

mean serum concentrations at termination of 51 ± 9.3 and 45 ± 13 µg/mL at 10 and 30 mg/kg/day, respectively (Butenhoff et al., 2004), suggests that a LBMIC₁₀ based on measured adult male values would be 45–50 µg/mL. Therefore, it is conservative and appropriate to use the adult female-based LBMIC₁₀ (29 µg/ml) for estimation of MOE values based on post-natal developmental effects.

3.2. Liver-weight increase

3.2.1. Rats

The LBMD₁₀ values and calculated or estimated (AUC/24 h) LBMIC₁₀ for male rat liver-weight-to-brain-weight ratio increases are shown in Table 9. As can be seen, the Hill and linear models provided adequate fits for LBMD₁₀, and all resulting values (0.60–1.4) are tightly clustered within a factor of 2.4. There is also little difference between F₀ and F₁ males. The linear model provided an adequate fit for the LBMIC₁₀ based on the serum PFOA concentration data from the Palazzolo (1993) study. Although not used for estimation of MOE values, LBMD₁₀ and LBMIC₁₀ values for absolute liver-weight increase and liver-weight-to-body-weight ratio were in the range of those derived from liver-weight-to-brain-weight ratios (data not shown).

3.2.2. Monkeys

In the six-month oral-dosing study in male cynomolgus monkeys (Butenhoff et al., 2002b), liver-weight-to-brain-weight ratios were elevated at all APFO treatment levels relative to controls (Table 5); however, this parameter did not show an increase in response with increasing dose. Using values for dose, serum concentration as related to dose, and liver-weight-to-brain-weight ratio from Table 5, the calculated values of LBMD₁₀ and LBMIC₁₀ for liver-weight-to-brain-weight ratio are shown in Table 9. Fig. 4 shows the resulting plot that provided the LBMIC₁₀ of 23 µg/mL used in MOE calculations.

3.3. Body-weight change

3.3.1. Rats

LBMD₁₀ and LBMIC₁₀ values (calculated or estimated) for body-weight change from the two-generation study (Butenhoff et al., 2004) F₀ and F₁ males, and the 13-week dietary study (Palazzolo, 1993) males are presented in Table 9.

3.3.2. Monkeys

Body-weight change (decrease), a prominent effect noted in male cynomolgus monkeys of the 30/20 mg/kg/day

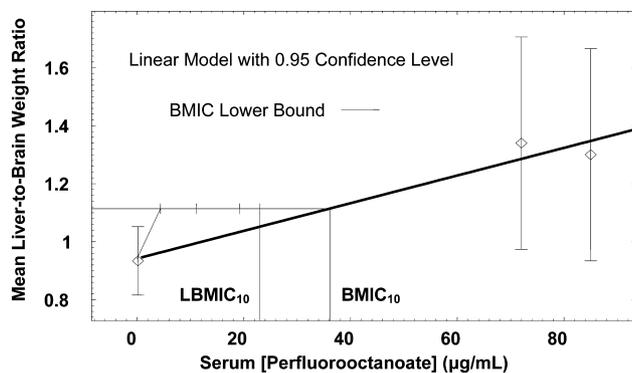


Fig. 4. Plot of BMDS-modeled serum PFOA concentration (axis label “dose,” $\mu\text{g/mL}$) versus liver-weight-to-brain-weight ratio for male cynomolgus monkeys (linear continuous model, top dose-group data eliminated, one standard deviation from mean benchmark response, constant variance).

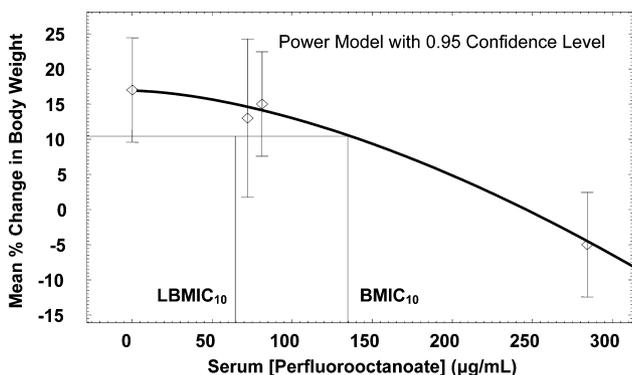


Fig. 5. Plot of BMDS-modeled serum PFOA concentration (axis label “dose,” $\mu\text{g/mL}$) versus body-weight change for male cynomolgus monkeys (linear continuous model, one standard deviation from mean benchmark response, constant variance).

dose group in the six-month oral dosing study (Butenhoff et al., 2002b), produced calculated values of LBMD_{10} and LBMIC_{10} presented in Table 9. The plot providing the LBMIC_{10} of $60 \mu\text{g/mL}$ that was used in estimating the MOE for body-weight change is shown in Fig. 5.

3.4. Leydig cell adenoma in rats

Poly-3 (Bailer and Portier, 1988) adjusted incidence of Leydig cell adenoma in rats from the Sibinski et al. (1983) study are presented in Table 6. Fitting the multistage model to these age-adjusted incidence rates gave an estimated LBMD_{10} of 100 ppm in diet (4.8 mg APFO/kg/day) for a lifetime for Leydig cell tumors in male rats (Table 6 and Fig. 6). The latter mg/kg/day value is interpolated from the average estimated mg/kg/day dose of APFO over the two-year study in male Sprague–Dawley rats (1.3 mg/kg/day for the 30 ppm dose group and 14 mg/kg/day for the 300 ppm dose group, Table 6). Using the equation of

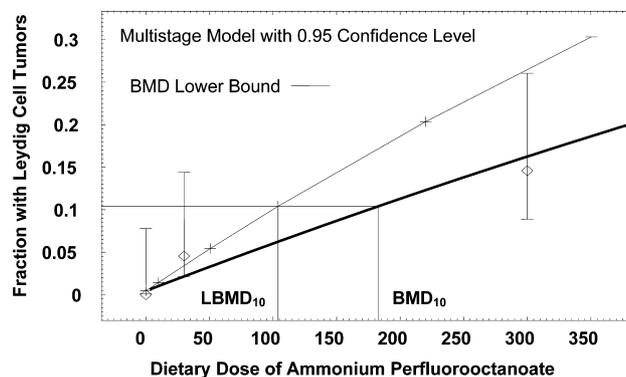


Fig. 6. Plot of BMDS-modeled dose (ppm APFO in diet)–response curve for Leydig cell adenoma in male rats (dichotomous multi-stage model, 10% response).

the trendline in Fig. 2 ($y = 46.1 \times x^{0.6347}$) yielded a LBMIC_{10} of $125 \mu\text{g PFOA/mL}$ serum at 4.8 mg/kg/day (Table 6). The serum PFOA concentration data used in this analysis were obtained after dietary dosing for 14 weeks, and male rats in the 100 ppm dose group in the subchronic dietary study (Palazzolo, 1993) had received a mean of 6.5 mg/kg/day over the 14-week period. Since the LBMD_{10} for Leydig cell tumors of 100 ppm APFO in diet is near the geometric mean of the two dietary APFO dose groups in the cancer study (30 and 300 ppm), the geometric mean of the two conversion factors for ppm APFO to mg/kg/day APFO at 14 weeks in the cancer study could be used to estimate the mg/kg/day dose at 100 ppm after 14 weeks. Mean mg/kg/day doses at 30 and 300 ppm were 1.75 and 21.5 mg/kg/day , respectively, after 14 weeks. Therefore, at the LBMD_{10} of 100 ppm, the estimated conversion factor would be $1 \text{ ppm} = (0.0717 \times 0.0650)^{0.5} = 0.068 \text{ mg/kg/day}$. Hence, over the first 14 weeks in the cancer study, the average dose at the LBMD_{10} for Leydig cell tumors is estimated to be $(100 \times 0.068) = 6.8 \text{ mg/kg/day}$, which is quite comparable to the mg/kg/day dose from the subchronic study and corresponds to $156 \mu\text{g PFOA/mL}$ serum based on Fig. 2. In another approach, use of the relationship in Fig. 1 to estimate LBMIC_{10} at 4.8 mg/kg/day from AUC yields $204 \mu\text{g/mL}$ for the value of $\text{AUC}/24 \text{ h}$. Thus, the use of the LBMIC_{10} value of $125 \mu\text{g/mL}$ based on 4.8 mg/kg/day is most conservative and will be used in estimating the MOE.

4. Characterization of risk

4.1. Points of departure

Table 10 presents the values of the LBMIC_{10} for post-natal developmental effects in rats, liver-weight-to-brain-weight ratio increase in monkeys, body-weight change in monkeys, and tumorigenesis (Leydig cell) in rats that were used as POD to estimate MOE values

Table 10

Margin of exposure values based on various LBMIC₁₀ points of departure and highest upper bound 95th percentile estimate of general population serum PFOA concentrations

Response (species)	Source table	Point of departure LBMIC ₁₀ (µg/mL)	Margin of exposure ^a
Post-natal effects (rats)	Table 8	29 ^b	2100
Liver-weight-to-brain-weight ratio ^c (monkeys)	Table 9	23	1600
Body-weight change (monkeys)	Table 9	60	4300
Leydig cell tumors (rats)	Table 6	125	8900

^a The margin of exposure is calculated by dividing the LBMIC₁₀ (µg/mL) by the general population serum [PFOA] representing the upper 95% confidence limit of the estimate of the 95th percentile general population serum [PFOA] (0.014 µg/mL). Margins of exposure based on the upper bound of the geometric mean general population serum [PFOA] (0.005 µg/mL) are approximately three times higher.

^b The serum [PFOA] in post-weaning rat pups was estimated conservatively based on adult-female rat AUC at the LBMD₁₀ value of 22 mg/kg/day for post-natal effects using the relationship of AUC to administered oral dose from Fig. 1. Results from studies currently in progress support the premise that this is a conservative estimate for weanling rat pups (Han, 2003; Mylchreest, 2003). Availability of data in the future may require adjustment of this estimate of the MOE.

^c Liver-weight increase is not necessarily reflective of an adverse effect, as this is a normal adaptive response. This endpoint was used as a sensitive indication of biological response.

based on serum PFOA concentration. The POD LBMIC₁₀ values ranged from 23 µg/mL (liver-weight-to-brain-weight ratio increase in monkeys) to 125 µg/mL (Leydig cell adenoma in rats).

4.2. Margins of exposure

The results of dividing POD LBMIC₁₀ values by the upper-bound estimated 95th percentile general population serum concentration (0.014 µg/mL) to estimate MOE values are presented in Table 10. These MOE values varied from 1600 for increased liver-weight-to-brain-weight ratio in monkeys to 8900 for Leydig cell adenoma in rats.

4.3. Analysis of uncertainty

This subsection discusses factors that tend to reduce uncertainty as well as residual uncertainty in this risk characterization. Current methods for qualitatively assigning uncertainty factors in a deterministic approach to assessing risk have been recently reviewed by Kalberlah et al. (2003). Most methods currently used to assign uncertainty include factors for intraspecies and interspecies extrapolation. Subdividing the latter two uncertainty factors into subfactors for toxicokinetics and toxicodynamics has gained acceptance. A third area of uncertainty comes from differences in length of exposure (chronicity).

4.3.1. Toxicokinetic factors

4.3.1.1. Interspecies toxicokinetic comparisons. A number of factors reduce uncertainty with respect to interspecies differences in toxicokinetics. A primary factor is that PFOA is not metabolized; therefore, species differences in metabolic handling do not need to be accounted for. A second factor is the availability of sound PFOA toxicokinetic data from multiple studies and species,

including pregnant rats, weanling rats, rodents, dogs, monkeys, and humans (Table 7; Goecke et al., 1992; Kuslikis et al., 1992; Kerstner-Wood et al., 2003). These studies provide a reasonable understanding of absorption, distribution, and elimination of PFOA across species. With respect to residual uncertainty in toxicokinetics between species, the notable sex and species differences presented in Table 7 can be overcome, in part, through the use of direct serum PFOA comparisons or comparisons with estimated serum PFOA concentrations based on pharmacokinetic factors such as AUC. The volume of distribution in male rats and male and female cynomolgus monkeys is quite similar, and suggests that PFOA is distributed primarily in extracellular spaces (Kemper, 2003; Noker, 2003). In addition, PFOA has been shown to have similar plasma binding characteristics in rats, monkeys, and humans (Kerstner-Wood et al., 2003). A third factor that is of considerable value in reducing uncertainty, when estimating MOE values based on comparisons of serum PFOA concentrations associated with a level of response in toxicological studies with general population serum PFOA concentration, is the availability of human serum PFOA concentration data from large cohorts of the general population that allow for analysis of differences by age and gender and include children, adults, and the elderly. Use of the upper bound of the highest 95th percentile estimated general population serum concentration for comparison with POD LBMIC₁₀ values from toxicological studies narrows the MOE, thus accounting for any uncertainty relative to the variability in the distribution of human serum PFOA concentrations.

The MOE estimates are based on the assumption of steady-state serum PFOA concentrations, with the exception of the female rat, for which 24-h average serum PFOA concentrations based on the relationship of dose to AUC were used. A near steady state is likely in the general population because of the prolonged elimination half-life (Burriss et al., 2002) and minimal exposure (plus

the tight distribution of PFOA serum concentrations from individual sampling of children, adults, and the elderly). During repeated daily dosing or dietary intake in toxicology studies, male rats and monkeys appear to reach steady state within approximately one month (Butenhoff et al., 2002b; Kemper, 2003); therefore, uncertainty around the use of serum level is further minimized. Female cynomolgus monkeys have a similar, perhaps slightly longer (approximately 30 versus 20 days), elimination half-life as compared to males (Noker, 2003). In the case of the female rat, a steady-state condition cannot be reached on daily dosing due to the rapid elimination of PFOA (see discussion in text). As a result, the authors believe that mean 24-h serum PFOA concentration (AUC divided by 24 h) is an appropriate comparative measure.

4.3.1.2. Intraspecies toxicokinetic comparisons. Individual differences in metabolism do not exist. The general population monitoring data available for PFOA also decrease uncertainty related to the variability in exposure, since serum PFOA concentrations have been characterized in a group of children, in adult American Red Cross blood donors, and in an elderly population (Table 1). Group sizes were large and covered a reasonably representative geographical distribution and age distribution. The fact that these serum measurements are tightly distributed and do not show major differences between age or gender groups (Table 1) reduces the chance that large portions of the population may not be adequately represented in a risk characterization. The tight distribution of general population serum PFOA concentrations (Table 1), particularly across age groups and between sexes, is not consistent with initial expectations. If the elimination half-life in humans is indeed in the range of that estimated by Burris et al. (2002), then steady-state serum PFOA concentrations would not be expected until after 5–25 years of exposure or longer. However, PFOA concentrations in general population serum do not increase with age. Therefore, the estimated serum elimination rate provided by Burris et al. (2002) for retired workers may not be representative of the elimination rate for the general population with much lower serum PFOA concentrations. The external exposure and pharmacokinetic factors that influence the observations from the general population biomonitoring studies of Olsen et al. (2003c, 2004a,b) remain to be discovered. Once again, these matters point to the utility of risk characterization based on direct comparison of serum concentration, which has the effect of reducing overall uncertainty.

Another fact that aids in reducing uncertainty in intraspecies toxicokinetic factors is that medical monitoring of exposed workers has included measurements of serum PFOA concentration for a quarter century (3M Company, 2003a,b; Gilliland, 1992; Gilliland and

Mandel, 1996; Olsen et al., 1998, 2000; Ubel et al., 1980). Ubel et al. (1980) originally reported that serum total organic fluorine concentrations (assumed to be due predominantly to the presence of PFOA) among 3M Cottage Grove, Minnesota fluorochemical workers ranged from 1 to 71 $\mu\text{g}/\text{mL}$. Serum PFOA concentrations during the 1990s have remained comparable to those initially estimated by Ubel et al. (1980) with means (range in parentheses) of 5.0 $\mu\text{g}/\text{mL}$ (0–80), 6.8 $\mu\text{g}/\text{mL}$ (0.0–114), and 6.4 $\mu\text{g}/\text{mL}$ (0.1–81) in 1993, 1995, and 1997, respectively. Serum PFOA concentrations of other 3M fluorochemical production workers in Decatur, Alabama, and Antwerp, Belgium have averaged 1–2 $\mu\text{g}/\text{mL}$ with highest concentrations reported to be 13 $\mu\text{g}/\text{mL}$ (Olsen et al., 2003a).

In estimating weanling rat serum PFOA concentrations, the relationship between dose and AUC (Fig. 1) for adult female rats was exploited. Based on the study by Kemper (2003), the latter relationship is linear over a broad range of doses. The adult female serum concentration associated with the LBMD_{10} for post-natal effects can be calculated from this relationship. This was accomplished by taking the value of the AUC at the LBMD_{10} ($\mu\text{g h}/\text{mL}$) and dividing this by 24 h to provide an average serum concentration. Based on results from a recent study sponsored by 3M and DuPont (Han, 2003) the 24-h average adult female serum concentration may underestimate the actual concentration in weanling rats. The study by Han (2003) demonstrates that the elimination of PFOA in weanling rats is intermediate between the elimination in adult males and females until sometime between four and five weeks of age, when the hormonally regulated sex differences in PFOA elimination became apparent. In the latter study, 24 h after a single oral dose (10 mg/kg), female serum PFOA concentrations were 2.4-fold lower at five weeks of age than at four weeks of age and were not further affected through study termination at eight weeks of age. Serum PFOA concentrations in males were 2.7-fold higher than females at four weeks of age, and five-week-old males were 5.4-fold higher than four-week-old males. This recent finding is consistent with the suggestion by Kudo et al. (2002) that sexual hormone regulation of the expression of certain organic anion transporters in kidney (OAT2, OAT3, and *oatp1*) may account for sex differences in PFOA elimination in rats. They found OAT2 to be more highly expressed in female rat kidney and subject to up-regulation by estradiol. Buist et al. (2002) examined differences in expression of organic anion transporter proteins during post-natal development in the rat. These researchers confirmed the much greater expression of OAT2 in female rat kidney as compared to male rats and showed that OAT2 does not increase in expression during development through post-natal day 45 in the male but does increase between post-natal days 35 and 40 in females. Based on these recent studies, it is

likely that male and female weanling rat pups in the first days after weaning (i.e., between three and four weeks of age), where a statistically significant increase in mortality was observed, have lower PFOA excretion rates when compared to adult female rats. Based on another recent study co-sponsored by 3M and DuPont (Mylchreest, 2003), body burdens of PFOA from gestational and lactational exposure existed prior to the initiation of dosing at weaning in the two-generation reproduction study reported by Butenhoff et al. (2004). Therefore, the estimated adult female 24-h mean serum PFOA concentration for the LBMIC₁₀ for post-natal developmental effects is believed to provide a realistic and conservative estimation of serum PFOA concentrations for the weanling rats.

In male rats, dietary dosing with APFO produced higher serum PFOA concentrations at a calculated mg/kg/day dose level than did gavage dosing, as is evidenced on comparison of steady-state serum PFOA concentration data from the two-generation study by gavage (Butenhoff et al., 2004) with the 13-week subchronic dietary study (Palazzolo, 1993). This may be due to potentially lower absorption with a bolus dose as compared to lower level continuous dietary intake. Another possible explanation for this difference may relate to the observed elevation of estradiol in male rats on treatment with APFO (Biegel et al., 2001; Liu et al., 1996b), which may be more pronounced in gavage dosing due to potentially higher C_{\max} values. This could lead to a higher rate of increased urinary excretion in gavage dosing due to greater up-regulation of urinary transport systems by elevated estradiol in male rats, as observed by Kudo et al. (2002).

Considering potential male/female differences in toxicokinetics within primate species, male and female rhesus monkeys had similar serum PFOA concentrations after dosing for 90 days at 3 and 10 mg/kg/day (Griffith and Long, 1980). In cynomolgus monkeys, a sex difference in elimination rate of less than twofold has been noted after a single i.v. dose of potassium PFOA, with females having the lower mean elimination rate (Noker, 2003).

4.3.2. Toxicodynamic factors

The toxicodynamic response in experimental studies can be related to serum PFOA concentration, and, in some cases, target tissue dose. Therefore, it is possible to gain insight into the variability of some toxicodynamic responses across species and within a species. This is true not only for the species used in experimental studies, but also for humans.

4.3.2.1. Interspecies toxicodynamic comparisons. This risk characterization benefits from the availability of a large number of studies covering most toxicological endpoints of interest. The fact that non-human primate

toxicology studies are available as well as epidemiological and medical monitoring studies of PFOA-exposed workers is a significant factor in reducing uncertainty with regard to extrapolation of responses from studies with test species to humans. A number of the toxicological studies as well as the worker-health studies have measured serum PFOA concentrations that can be associated with observations from the studies. For studies where serum PFOA concentration data are not available, estimates of serum PFOA concentration can be made using established pharmacokinetic factors. Therefore, it was possible in this risk characterization to relate serum PFOA concentration to selected responses across species. In inspecting the results of modeled dose-response and serum-PFOA-concentration-response relationships for liver-weight increase and body-weight change from Table 9, the LBMIC₁₀ values for male rats and monkeys are reasonably comparable, a fact that supports the comparison of serum PFOA concentration associated with response across species.

With respect to liver responses, mitochondrial proliferation has been observed in rats and monkeys (Berthiaume and Wallace, 2002; Butenhoff et al., 2002b); however, only rats have shown increased PPAR- α agonism (peroxisome proliferation) after treatment with PFOA (Berthiaume and Wallace, 2002; Biegel et al., 2001; Palazzolo, 1993), a fact that is consistent with primates being generally non-responsive to PPAR- α agonists (Ashby et al., 1994; Bentley et al., 1993; Cattley et al., 1998). Medical monitoring of human workers exposed to PFOA has not shown associations with liver function abnormalities (liver enzymes in serum) or other measured endpoints at serum PFOA concentrations within an order of magnitude of the LBMIC₁₀ for body-weight and liver-weight effects in male monkeys (Olsen et al., 2000).

The relevance to humans of the observed tumors in the two chronic dietary studies (Biegel et al., 2001; Sibinski et al., 1983) is uncertain. The hepatocellular tumors observed by Biegel et al. (2001) are likely to be related to PPAR- α agonism; therefore, they likely are not relevant to humans (Ashby et al., 1994; Bentley et al., 1993; Cattley et al., 1998). The Leydig cell tumors may result from hormonal changes brought about by induction of aromatase (Biegel et al., 1995; Liu et al., 1996a,b), and this proposed mechanism, in addition to being nonlinear, would be of questionable relevance to humans (Clegg et al., 1997; Cook et al., 1999). The incidence of pancreatic acinar cell adenomas in the Biegel et al. (2001) study was 0/80, 1/79 (1.3%), and 7/76 (9.2%) in control, control pair-fed, and 300 ppm PFOA groups, respectively. A higher incidence of pancreatic acinar cell adenoma was also observed with the potent PPAR- α agonist, WY-14,643, in the same study (Biegel et al., 2001). The mechanism of PFOA-induced pancreatic acinar tumors in rats remains to be elucidated. Most

human pancreatic cancers are of ductal origin, and current understanding of the mechanisms underlying human pancreatic cancers suggest that mutations in oncogenes (predominantly K-ras) and genes coding for certain tumor suppressor factors are involved in most human pancreatic cancers (Anderson et al., 1996; Fernandez-Zapico et al., 2003; Li and Jiao, 2003; Moore et al., 2003; Schneider and Schmid, 2003; Urrutia, 2002). PFOA has not shown mutagenic or clastogenic activity in a variety of standard assay systems, and is unlikely to be a complete carcinogen. A statistically significant increase in acinar cell adenoma was observed in one of two rat two-year studies at a dose of 300 ppm in diet, although, acinar cell hyperplasia was evident in both studies (Frame and McConnell, 2003).

If the assumption is made that the PFOA-induced acinar cell tumors in rats in the Biegel et al. (2001) study are likely to have been the result of mechanisms that involve epigenetic or proliferative mechanisms as opposed to direct mutations, it is likely that the dose–response curve is nonlinear; therefore, it would be appropriate to consider benchmark-dose methodology in risk characterization. When compared to the age-adjusted Leydig cell adenoma incidence in the Sibinski et al. (1983) study (0/48, 2/48, and 7/48 for the control, 30 ppm, and 300 ppm dose groups, respectively) for which an LBMIC₁₀ has been estimated, it becomes evident that the LBMIC₁₀ for Leydig cell tumor incidence could be expected to be lower than that for pancreatic acinar cell tumors in the study by Biegel et al. (2001). Therefore, Leydig cell tumors were used in this risk characterization to represent nonlinear cancer risk.

4.3.2.2. Intraspecies toxicodynamic comparisons. Medical monitoring and epidemiological studies among 3M Company fluorochemical workers in Cottage Grove, Minnesota, that were engaged in PFOA production and processing have not found associations of PFOA exposure with altered health status, including clinical chemistry and hormonal abnormalities, and no statistically significant increases in standardized mortality ratios (SMR) were found for total cancer (SMR = 0.9, 95% CI 0.7–1.1), liver cancer (SMR = 0.6, 95% CI 0.1–3.3), or pancreatic cancer (SMR = 1.4, 95% CI 0.5–3.1) (3M Company, 2003a,b; Alexander, 2001; Gilliland, 1992; Gilliland and Mandel, 1996; NIOSH, 2001; Olsen et al., 1998, 2000, 2003a). There was one death attributed to testicular cancer (approximately 0.5 expected) among these fluorochemical production workers (Alexander, 2001). The low case-fatality rate for testicular cancer does not allow for a straight-forward interpretation of results from an occupational cohort mortality study. These epidemiological data provide a level of comfort in characterizing health risk of the population. The lack of observed effect at the higher serum PFOA concentrations experienced by workers reduces uncertainty in

considering the toxicodynamic response in non-occupational populations with serum PFOA concentration levels two-to-three orders of magnitude lower.

In regard to male/female differences, differences in response may be due, in part, to toxicokinetic differences. For example, in the rhesus monkey study reported by Griffith and Long (1980), there were no obvious differences between the response of males and females; although, the numbers per dose group (two per sex) limit interpretation. This is consistent with serum PFOA concentrations being similar. In contrast to monkeys, adult male rats are notably more responsive to body-weight and liver-weight effects of PFOA than females at similar administered doses. The striking differences in elimination rate between male and female rats (Table 7) may partially explain this apparent toxicodynamic difference.

The differences in tumor outcome between the two-year dietary studies with APFO in rats reported by Sibinski et al. (1983) and Biegel et al. (2001) is not understood. Both studies included a 300 ppm APFO dietary dose, and compound consumption over two years of dosing averaged 13.9 and 13.6 mg/kg/day in the Sibinski et al. (1983) and Biegel et al. (2001) studies, respectively. Biegel et al. (2001) report purity of the sample to be 98–100%, and mixed the sample with Certified Rodent Diet #5002 (PMI Feeds, Inc.) in a Hobart mixer at high speed for 6 min. Sibinski et al. (1983) report sample purity to be 97.6–98.4% mixed C₈ isomers, and the sample was determined to be 79% linear, with 9% terminal branching, and 12% backbone branching. The latter sample was mixed with Certified Purina Laboratory Chow (Ralston Purina, St. Louis, Missouri). Three possibilities arise that may explain the toxicodynamic differences seen in these two studies. These are: (1) possible differences in amount of branched vs. linear APFO; (2) possible influences of base diet; and (3) genetic drift in Sprague–Dawley rats over time. Other possibilities may also be raised, but it is not possible at this time to explain why hepatocellular adenoma and pancreatic acinar cell adenoma were seen by Biegel et al. (2001) but not by Sibinski et al. (1983).

4.3.3. Factors reducing uncertainty related to chronicity of exposure

The finding of PFOA in the serum of children, adults, and a group of elderly combined with the observed long elimination half-life suggests lifetime internal presence of PFOA. Several factors mitigate the degree of uncertainty that is related in applying experimental study results to humans. First, experimental studies have been conducted that cover all periods of development and lifetime exposure in rats. These include the two cancer studies (Biegel et al., 2001; Sibinski et al., 1983), developmental toxicity studies in rats and rabbits (Gortner, 1981; Gortner, 1982; Staples et al., 1984), and the two-

generation reproduction study in rats (Butenhoff et al., 2004). A second factor that addresses uncertainty related to chronic exposure is the availability of epidemiological and medical monitoring studies in exposed workers over several decades, where exposures on a serum PFOA concentration basis have been significantly higher than those of the general population (3M Company, 2003a,b; Alexander, 2001; Gilliland, 1992; Gilliland and Mandel, 1996; NIOSH, 2001; Olsen et al., 1998; Olsen et al., 2000; Olsen et al., 2003a; Ubel et al., 1980). These worker studies have not found consistent health effect associations related to PFOA exposure.

5. Discussion

5.1. Approach

The approach used in this risk characterization has the advantage of deriving MOE values using multiple biological responses in rodents and monkeys by comparison of concentrations of PFOA in serum. In addition, the use of LBMIC₁₀ values as PODs has an advantage over the use of a NOAEL or LOAEL in that the benchmarked values represent a defined level of excess response (risk). This provides a better view of species differences in response by normalizing response at a specified level. Therefore, unlike the NOAEL or LOAEL from a study, the benchmark value is directly related to a given level of response, or risk, and can be used in probabilistic risk assessment (Gaylor and Kodell, 2002). The benchmark response level (BMR) that is used to calculate the benchmark dose may vary depending on the endpoint that is being benchmarked. For most categorical data from toxicology studies, a 10% response level (BMD₁₀) is fairly representative of the limits in which a change can be accurately determined. For continuous, normally distributed data, a shift in the distribution of 1.0 standard deviation represents approximately an extra 10% of the individual values being greater than near the 99th percentile or about an extra 10% less than near the 1st percentile of the distribution in controls. To be conservative, the lower 95% CL of the BMIC (LBMIC₁₀) has been used.

Uncertainty related to variations in response within and between species is further reduced by the fact that PFOA is not metabolized, and the pharmacokinetics of PFOA have been investigated in rodents and primates. The use of serum PFOA concentration also has advantages in that internal dose is more directly related to biological response (toxicodynamics), and the influence of rates of absorption and elimination (toxicokinetics) on response when using external (administered) dose are minimized. When all of the above advantages are combined, the result is reduction in uncertainty.

Serum PFOA concentrations measured in children (Olsen et al., 2004a), adults (Olsen et al., 2003c), and the elderly (Olsen et al., 2004b) represent exposure from all sources. Age and sex of the individual human subjects was known; however, intensity and duration of exposure cannot be known. That said, the distribution of serum PFOA concentrations is comprised of data from several hundred individuals for adults and children, and over 200 for the elderly. In the case of adults and children, the samples are from a representative geographical cross-section of the United States. Because these samples represent a “snap-shot” of serum PFOA concentration at a point in time for these individuals, the distribution will account for the variation in intensity and duration of exposure among individuals. As the data show, the distribution is remarkably tight, adding confidence to the suitability of our methodology for this risk characterization.

It could be argued that comparisons of PFOA concentrations in liver tissue, a primary target of PFOA toxicity, would be more meaningful for risk characterization. As stated in the introduction, Olsen et al. (2003b) examined a total of 30 human donor livers for the presence of PFOA. All donor livers were below the lower limit of quantitation for at least one of two analyses per sample except for one liver that had an average of 0.047 µg/g. Although many serum PFOA analyses in paired samples from this donor population were also below the limit of quantitation, there were more serum values that were quantifiable than liver values. Concentrations of PFOA in liver measured in toxicology studies in rats (Griffith and Long, 1980) and monkeys (Butenhoff et al., 2002b) have been comparable to serum concentrations. Also, analysis of pharmacokinetic data obtained during the six-month oral toxicity study in male cynomolgus monkeys (Butenhoff et al., 2002b) does not suggest that PFOA is eliminated from liver to a lesser extent than it is eliminated from serum (Butenhoff et al., manuscript in preparation). Therefore, the authors believe that serum PFOA concentration can be correlated with effects that involve liver tissue.

5.2. Points of departure

A brief discussion on the choice of points of departure is in order. Post-natal developmental effects were used, specifically, the LBMD₁₀ and LBMIC₁₀ values associated with post-weaning mortality. This POD represents an adverse outcome in rats that has the greatest meaning for risk characterization. Liver-weight increase measures were also used as POD because this is a sensitive response in male rats and monkeys. It must be emphasized that liver-weight increase does not necessarily represent an adverse effect, as it is typically an adaptive response to dosing; thus, use of liver-weight increase as a point of departure is believed to be

conservative. Liver injury does occur under higher dosing conditions, and liver is the primary target organ. The same thoughts can be expressed for body-weight change, a response that is not necessarily adverse. With PFOA, the threshold for body-weight change is higher than that for liver-weight increase, and body-weight change is closer on a continuum to the occurrence of adverse effects. As such, it is also a conservative endpoint for a point of departure, but has more relevance to potential adverse effects because it may represent effects on appetite or metabolism. Male rats and monkeys respond with both liver-weight increase and body-weight change, with benchmark doses and internal concentrations that are relatively similar. Finally, a benchmark dose was developed for Leydig cell tumors in the chronic dietary study of Sibinski et al. (1983). Leydig cell tumors are rare in humans (Anderson et al., 1996), and Biegel et al. (2001) suggest that the mode of action for APFO-induced Leydig cell adenoma may be a sustained increase in estradiol after induction of aromatase. Due to the fact that APFO was administered at a single treatment level (300 ppm in diet), dose–response data over a range of doses were not available for the liver and pancreatic acinar cell tumors observed in the study by Biegel et al. (2001). The weight of evidence pertaining to PPAR α agonists would indicate that it is unlikely that the liver tumors are relevant to humans (Ashby et al., 1994; Bentley et al., 1993; Cattley et al., 1998). The pancreatic tumors observed in the Biegel et al. (2001) study were likely the result of epigenetic and/or proliferative mechanisms, because pancreatic acinar cell proliferation was elevated in 300-ppm-treated rats when measured at 15, 18, and 21 months. Furthermore, PFOA is not known to cause mutations or chromosomal aberrations. Therefore, PFOA is not likely to be a complete pancreatic carcinogen in humans. It would follow that a nonlinear model would be appropriate in assessing cancer risk from PFOA.

In calculating benchmark dose and benchmark internal concentration, all models were explored, and, if the study data fit more than one model adequately, the results of calculations for multiple models were displayed. In choosing points of departure, the lowest LBMIC₁₀ value for the endpoint was employed. The small number of animals in the monkey study (Butenhoff et al., 2002b) should be taken into consideration, and, given the somewhat similar responses to liver-weight increase and body-weight change, use of the lower value regardless of species seems appropriate.

5.3. Context on the margins of exposure

Based on the discussion above, there are justifiable reasons to reduce the default uncertainty factors of 10 for intraspecies and interspecies uncertainty as well as chronicity of exposure in the traditional approach to

uncertainty analysis. Among the most significant factors discussed above are the lack of metabolism of PFOA, the extensive experimental database, human biomonitoring data, occupational epidemiological studies, medical monitoring of workers, and the ability to relate serum PFOA concentration to effect. Because there are reasons to suggest reductions in the default uncertainty factors, it is reasonable to conclude that the lowest MOE determined in this risk characterization (1600) represents a substantial level of protection based on the current norms (e.g., European Commission, 2002). MOEs based on the geometric means of population serum PFOA determinations would be approximately 2–3 times higher than those based on the upper bound of the 95th percentile estimated serum PFOA concentration. If the highest measured individual serum PFOA concentration of 56 $\mu\text{g}/\text{mL}$ from the biomonitoring studies of Olsen et al. (2003c, 2004a,b) is considered, MOE values would still indicate substantial protection.

6. Conclusion

This risk characterization used responses that included postnatal developmental effects in rats, liver-weight-to-brain-weight ratio increase in rats and monkeys, body-weight change in rats and monkeys, and increased incidence of Leydig cell adenoma. The upper bound of the highest 95th percentile estimated general population serum PFOA concentration (0.014 $\mu\text{g}/\text{mL}$) that occurred in three biomonitoring studies of United States general populations was used to represent human exposure. The use of serum PFOA concentration metrics in calculating MOE values reduced uncertainty in the risk characterization. Using approaches that relate serum PFOA concentration to response, MOE values based on the upper bound 95th percentile estimated population serum PFOA concentration were large, ranging from 1600 (liver-weight increase) to 8900 (Leydig cell adenoma). These MOE values represent substantial protection of children, adults, and the elderly in the general population.

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