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Exposure to Perfluorooctane Sulfonate during Pregnancy in Rat and Mouse. I: Maternal and Prenatal Evaluations

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The maternal and developmental toxicities of perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) were evaluated in the rat and mouse. PFOS is an environmentally persistent compound used as a surfactant and occurs as a degradation product of both perfluorooctane sulfonyl fluoride and substituted perfluorooctane sulfonamido components found in many commercial and consumer applications. Pregnant Sprague-Dawley rats were given 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from gestational day (GD) 2 to GD 20; CD-1 mice were similarly treated with 1, 5, 10, 15, and 20 mg/kg PFOS from GD 1 to GD 17. Controls received 0.5% Tween-20 vehicle (1 ml/kg for rats and 10 ml/kg for mice). Maternal weight gain, food and water consumption, and serum chemistry were monitored. Rats were euthanized on GD 21 and mice on GD 18. PFOS levels in maternal serum and in maternal and fetal livers were determined. Maternal weight gains in both species were suppressed by PFOS in a dose-dependent manner, likely attributed to reduced food and water intake. Serum PFOS levels increased with dosage, and liver levels were approximately fourfold higher than serum. Serum thyroxine (T_4) and triiodothyronine (T_3) in the PFOS-treated rat dams were significantly reduced as early as one week after chemical exposure, although no feedback response of thyroid-stimulating hormone (TSH) was observed. A similar pattern of reduction in T_4 was also seen in the pregnant mice. Maternal serum triglycerides were significantly reduced, particularly in the high-dose groups, although cholesterol levels were not affected. In the mouse dams, PFOS produced a marked enlargement of the liver at 10 mg/kg and higher dosages. In the rat fetuses, PFOS was detected in the liver but at levels nearly half of those in the maternal counterparts, regardless of administered doses. In both rodent species, PFOS did not alter the numbers of implantations or live fetuses at term, although small deficits in fetal weight were noted in the rat. A host of birth defects, including cleft palate, anasarca, ventricular septal defect, and enlargement of the right atrium, were seen in both rats and mice, primarily in the 10 and 20 mg/kg dosage groups, respectively. Our results demonstrate both maternal and developmental toxicity of PFOS in the rat and mouse.

Key Words: **perfluorooctane sulfonate; maternal; prenatal; toxicity; rodent.**

Organic fluorochemicals are compounds in which one or more carbon-hydrogen (C-H) bond is replaced by a carbon-fluorine (C-F) bond. These C-F bonds are one of the strongest in nature and contribute to the unique stability of fluorochemicals in the environment, even at high temperatures. In perfluorinated compounds, all of the C-H bonds are replaced by C-F bonds (Kissa, 1994). When these compounds are mixed with hydrocarbons and water, three immiscible phases are formed, indicating that the perfluoroalkanes are both oleophobic and hydrophobic. By adding a charged moiety (such as a sulfonic acid) to a perfluorinated carbon chain, the chemical molecule becomes more water soluble, resulting from the hydrophilic nature of the added functional group. These amphoteric perfluorinated organic chemicals are used in commerce principally for their surfactant properties.

Perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) is a perfluorinated alkane with a sulfonyl group. The intermediate precursor, perfluorooctane sulfonyl fluoride, provides a link to products with other functional groups, such as free acids, metal salts, sulfonyl halides, and sulfonamides. Since the 1950s, with the commercial scale-up of electrochemical fluorination, PFOS and other perfluorinated organic compounds that metabolize into PFOS (as an end-stage metabolite and breakdown product) have been used in a wide variety of industrial and consumer applications that include stain-resistant coatings for fabrics and carpets, oil-resistant coatings for paper products approved for food contact, fire-fighting foams, mining and oil well surfactants, floor polishes, and insecticide formulations (Renner,

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2001; Seacat *et al*., 2002). In all, PFOS or products degrading to PFOS are used in over 200 products and applications. However, 3M Company, the primary manufacturer of these compounds and products, discontinued production at the end of 2002.

The widespread use of PFOS and its related products, as well as the environmental stability of the perfluorinated organic chemical, have led to documentation of its presence in both human and wildlife populations worldwide (Giesy and Kannan, 2001; Hansen *et al.*, 2001; Kannan *et al*., 2001a,b; 2002a,b,c; Olsen *et al*., 2001a,b,c). Olsen *et al.* (1999) reported an average serum PFOS level of 2 ppm in 3M production workers, with 5% of them having levels $\geq$ 6 ppm. Current information from a broad survey of individual blood samples from adult Red Cross blood donors, children (ages 2–12) from a streptococcal A clinical trial, and a group of elderly subjects enrolled in a longitudinal study of cognitive function indicates that the upper bound of the 95th percentile serum concentration is approximately 100 ppb, with a mean of approximately 30–40 ppb (Olsen *et al*., 2001a,b,c). Recent studies by Giesy and coworkers (Giesy and Kannan, 2001; Kannan *et al*., 2001a,b; 2002a,b,c) reported detection of PFOS in a variety of wildlife species, including fresh-water and marine mammals, fishes, birds, and shellfish. Although distribution of the chemical appears to be global, including remote locations in the Arctic and North Pacific Oceans, concentrations of PFOS in these animals are relatively greater in the more populated and industrial regions. These investigators also suggested that PFOS can be biomagnified in the top levels of the food chain.

Pharmacokinetic studies have shown that PFOS is readily absorbed, distributed, and accumulated in the serum and liver but poorly eliminated (urinary and fecal excretion half-life estimated at >90 days in the rat; Johnson and Ober, 1979; Johnson *et al*., 1979, 1984; Seacat *et al*., 2003). In the rat, a serum elimination half-life of 7.5 days was reported after an oral treatment of PFOS (Johnson *et al*., 1979); in Cynomolgus monkeys, a half-life of 200 days was described (Seacat *et al*., 2002); and in humans, a mean half-life of approximately 8.7 years was recently estimated from retired production workers (Geary Olsen, 3M, 2002, personal communication). The potential mammalian toxicity of PFOS has been investigated. In the rat, reduction of body weight, liver hypertrophy, and decreased serum cholesterol and triglycerides have been reported after subchronic exposure to PFOS (Seacat *et al*., 2003). PFOS has been suggested to interfere with mitochondrial bioenergetics, gap junctional intercellular communication, and fatty acid-protein binding in the liver (Berthiaume and Wallace, 2002; Hu *et al*., 2002; Luebker *et al*., 2002a; Starkov and Wallace, 2002). In addition, PFOS-induced hepatic peroxisome proliferation has been indicated in both rat and mouse (Berthiaume and Wallace, 2002; Haughom and Spydevold, 1992; Sohlenius *et al*., 1993). Seacat and coworkers (2002) have evaluated PFOS toxicity in Cynomolgus monkeys and reported weight loss, hepatocellular hypertrophy, and lipid vacuolation, as well as reductions in serum cholesterol, triiodothyronine, and estradiol. The potential reproductive and developmental toxicities of PFOS have not been fully elaborated. Case *et al*. (2001) examined the effects of PFOS in rabbits and noted reductions of maternal weight gain and fetal weight at 3.75 mg/kg and higher dosage but no significant incidence of malformation. More recently, 3M completed a multigenerational reproduction study with PFOS in rats (Butenhoff *et al*., 2002), which did not indicate any adverse effects on mating and fertility; however, significant reductions of body weight and perinatal viability were noted.

In light of the prevalence and persistence of PFOS in both humans and wildlife, this study was undertaken to provide a comprehensive evaluation of the developmental toxicity of this fluorochemical. This article summarizes the observations from the pregnant dams and term fetuses, and a companion article (Lau *et al*., 2003) will address the postnatal findings in rats and mice.

MATERIALS AND METHODS

Chemicals

Perfluorooctane sulfonate (PFOS, potassium salt; 91% pure) was purchased from Fluka Chemical (Steinheim, Switzerland). Our analysis indicated that approximately 71% of the chemical was straight-chain, and the remaining 29% was branched. Additional chromatographic analysis indicated that the chemical obtained from Fluka appeared to be the same material produced by the 3M Company (St. Paul, MN) and tested in the earlier developmental and reproductive studies by 3M.

Animal Treatment

Timed-pregnant Sprague-Dawley rats and CD-1 mice obtained from Charles River Laboratories (Raleigh, NC) were bred within a 4-h period and overnight, respectively. Those animals with spermatozoa in a vaginal smear and/or with a copulatory plug were considered to be at gestational day (GD) 0. In a separate study, mature female rats weighing 200 g were obtained from the same supplier. Animals were housed individually in polypropylene cages with heat-treated pine shavings for bedding and provided pellet chow (LabDiet 5001, PMI Nutrition International, Brentwood, MO) and tap water *ad libitum*. Animal facilities were controlled for temperature (20–24°C) and relative humidity (40–60%), and operated under a 12-h light-dark cycle.

Rats. PFOS was freshly prepared daily at 1, 2, 3, 5, and 10 mg/ml of 0.5% Tween-20 vehicle and administered to the pregnant dams by gavage at a volume of 1 ml/kg/day from GD 2 through GD 20. Controls received vehicle alone. Throughout gestation and treatment, maternal body weights as well as food and water consumption were recorded. Blood from each dam was collected between 9–11 A.M. on GDs 7 and 14 from the lateral tail vein and on GD 21 after decapitation. Aliquots of serum from these blood samples were stored at –20°C for subsequent analysis of PFOS concentration, thyroid hormones, corticosterone, prolactin, cholesterol, and lipid content. On GD 21, both maternal and some fetal livers were removed, weighed, and immediately frozen on dry ice and stored at –80°C for PFOS analysis. For other animals, the gravid uterus was removed and examined, and individual live fetuses were weighed and prepared for teratological evaluation.

In a separate study, adult female rats were given either 3 or 5 mg/kg PFOS daily for 20 days; controls received the Tween-20 vehicle. Blood samples were withdrawn from tail vein at 3, 7, and 14 days after the initiation of PFOS exposure, and trunk blood was obtained from decapitation after 20 days of

chemical treatment. Serum samples were prepared and stored at –20°C for T_3, T_4, and thyroid-stimulating hormone (TSH) analyses.

Mice. PFOS (0.1, 0.5, 1.0, 1.5, and 2.0 mg/ml vehicle) was similarly prepared and administered by gavage at a volume of 10 ml/kg/day from GD 1 through GD 17. Maternal weight as well as food and water consumption were monitored throughout gestation. Some mice were sacrificed on GDs 6 and 12 by CO_2 asphyxiation. The remaining dams were sacrificed on GD 18. Blood was collected from the descending aorta, and serum samples were prepared and analyzed for PFOS concentration as well as lipid content. On GD 18, maternal livers from representative animals were dissected, weighed, and immediately frozen on dry ice and stored at -80°C for PFOS analysis. For other animals, the gravid uterus was removed and examined, and individual live fetuses were weighed and prepared for teratological evaluation.

Teratological Evaluation

The gravid uterus of the pregnant rat or mouse was removed and weighed; the numbers and positions of the live or dead fetuses, as well as resorptions, were recorded. Live fetuses were weighed individually, gender-determined, and examined for external abnormalities. Half of the fetuses were prepared for skeletal examination, and the other half were prepared for visceral evaluation.

Skeletal evaluation. Fetuses were killed with an overdose of pentobarbital, eviscerated, and fixed in 95% ethanol. Specimens were subsequently stained with Alizarin red and Alcian blue to visualize bone and cartilage, respectively. Skeletal morphology was evaluated as described previously (Narotsky and Rogers, 2000).

Visceral evaluation. Fetuses were fixed in Bodian's solution (2% formaldehyde, 5% acetic acid, 72% ethanol, 21% water). Examination of the head, thoracic, and abdominal viscera were carried out using a freehand razor dissection.

Serum Chemistry

Serum samples were analyzed for total cholesterol, triglycerides, sorbitol dehydrogenase, glucose, bile acids, and total bilirubin levels using a Cobas Fara II chemistry analyzer (Roche Diagnostics, Basel, Switzerland).

Radioimmunoassays

Concentrations of serum hormones were derived from a standard curve encompassing a range of reference standards specific for each assay. If the value of unknown fell above or below this range, it was arbitrarily assigned the values of the highest or lowest reference standard. Internal standards from rat sera were routinely used to monitor interassay differences.

T_4 and T_3. Serum samples were thawed and levels of total thyroxine (T_4), free T_4, and triiodothyronine (T_3) were measured in duplicate using specific radioimmunoassay (RIA) kits (Diagnostics Products Corporation, Los Angeles, CA). Sensitivity of the total T_4 assay was 5–240 ng/ml; that of the free T_4 assay was 1–100 pg/ml; and that of the T_3 assay was 0.1–6 ng/ml. Because of the surfactant properties of PFOS, a preliminary experiment was conducted to determine whether the chemical might alter performance of the RIA directly. PFOS (at a final concentration of 5 or 10 mg/ml) was added directly to the assay tubes containing the T_4 standards and serum samples from untreated control rats. Under these conditions, PFOS did not interfere with the RIA performance.

TSH and prolactin. Serum samples were thawed, and the levels of TSH and prolactin were quantified by specific RIA. The TSH assay was performed using the following materials supplied by the National Hormone and Pituitary Program (Torrance, CA): iodination preparation NIDDK-rTSH-I-9, reference preparation NIDDK-rTSH-RP-3, and antiserum NIDDK-antirat TSH-RIA-6. Similarly, the prolactin assay was performed with iodination preparation NIDDK-rPRL-I-6, reference preparation NIDDK-rPRL-RP-3, and antiserum NIDDK-antirat PRL-RIA-9. Iodination materials were radiolabeled with ^{125}I (Perkin Elmer/New England Nuclear, Boston, MA) by a modification of the chloramine-T method of Greenwood *et al*. (1963). Labeled TSH or prolactin was separated from the unreacted iodide by gel filtration chromatography, as described previously (Goldman *et al*., 1986).

Serum was pipetted with the appropriate dilutions to a final assay volume of 500 μl with 100 mM phosphate buffer containing 1% BSA. Reference TSH standards ranging from 0.195 to 200 ng/ml and prolactin standards ranging from 0.39 to 100 ng/ml were prepared by serial dilution. Primary antiserum (200 μl) at a dilution of 1:437,500 prepared in a mixture of 100 mM potassium phosphate, 76.8 mM EDTA, 1% BSA, and 3% normal rabbit serum was then pipetted to each assay tube, vortexed, and incubated at 4°C for 24 h; 100 μl of either ^{125}I-TSH or ^{125}I-prolactin was then added to each tube, vortexed, and incubated for another 24 h. Second antibody (goat antirabbit gamma globulin [Calbiochem, San Diego, CA] at a dilution of 1 U/100 μl) was then added, vortexed, and incubated for a third 24 h. The samples were then centrifuged at 1,260 × *g* for 30 min; the supernatant was aspirated, and the sample tube with pellet was counted in a gamma counter.

Corticosterone. Serum samples were thawed and levels of corticosterone were measured in duplicate using an RIA kit (ICN Biomedical Inc., Costa Mesa, CA). Sensitivity of the assay was 25–1000 ng/ml.

Determination of PFOS Concentrations

Serum samples were diluted and liver samples were homogenized in five volumes of reagent-grade water. An aliquot of each dilution was spiked with the appropriate internal standards. Acetonitrile (5 ml) was added as an extraction solvent, which also served to precipitate the proteins. The samples were shaken at 300 rpm for 20 min and centrifuged at 850 × *g* for 10 min. The supernatant was transferred to a clean tube, diluted with 40 ml of water, and passed through a preconditioned C18 SPE cartridge. PFOS was eluted from the SPE cartridge with 2 ml methanol and analyzed by high-performance liquid

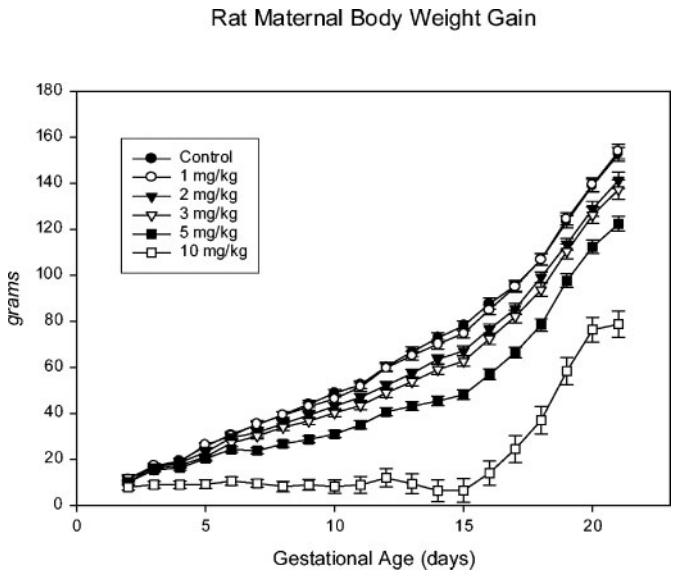

FIG. 1. Effects of PFOS on weight gain in pregnant rats. Each data point represents mean ± SE of determination from 25–50 rats. Two-way ANOVA indicates a significant treatment effect ($p < 0.0001$) and a time × treatment interaction ($p < 0.0001$). Duncan's multiple-range test indicates that, with the exception of the 1 mg/kg group, all dose groups are significantly different from controls. When individual PFOS dose groups are compared with controls, ANOVA indicates a significant treatment effect ($p < 0.0001$) for dose groups at 2 mg/kg and higher. Dunnett's *t*-test indicates significant variations from controls for the 10 mg/kg dose group beginning at GD 4, the 5 mg/kg dose group at GD 5, the 3 mg/kg dose group at GD 7, and the 2 mg/kg dose group from GDs 12 to 17.

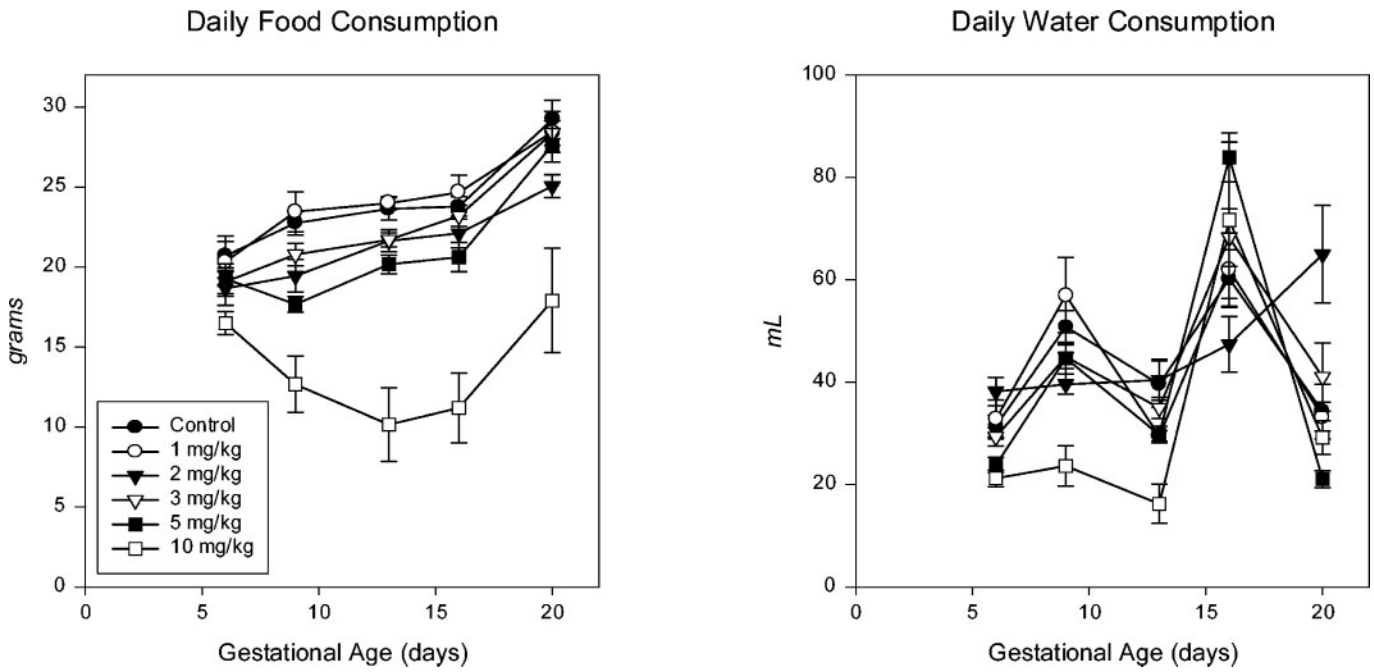

FIG. 2. Effects of PFOS on food and water consumption in pregnant rats. Each data point represents mean ± S.E. of determination from 9–20 rats, with the exception of the 1 and 10 mg/kg dose groups, where $n = 5$. Two-way ANOVA indicates a significant treatment effect ($p < 0.0001$) and a time × treatment interaction ($p < 0.05$) for food consumption. Duncan's multiple-range test indicates that only the 5 and 10 mg/kg dosage groups are significantly different from controls. When individual treatment groups are compared with controls, ANOVA indicates a significant dose effect ($p < 0.0001$) for the 5 and 10 mg/kg dose groups. Dunnett's *t*-test indicates significant variations from controls for the 5 mg/kg dose group from GDs 9 through 16 and for the 10 mg/kg dose group from GD 9 to term. Two-way ANOVA indicates a significant treatment effect ($p < 0.05$) and a time × treatment interaction ($p < 0.0001$) for water consumption. Duncan's multiple-range test indicates that only the 10 mg/kg dose group is significantly different from control values. When individual treatment groups are compared with controls, ANOVA indicates a significant main effect ($p < 0.05$) for the 5 and 10 mg/kg dose groups. Dunnett's *t*-test indicates significant variations at the 0.05 level from control values for the 5 mg/kg dose group on GD 6 and for the 10 mg/kg dose group from GDs 6 to 13.

chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS) according to the method described by Hansen *et al*. (2001).

Data Analysis

Data are presented as means and standard errors. Statistical significance was determined by ANOVA, using individual litter as the statistical unit. Maternal weight gains and food and water consumption were analyzed by ANOVA with repeated measure. When a significant treatment effect or interaction was detected, Duncan's multiple-range test or Dunnett's *t*-test were performed post hoc. Statistically significant differences were determined at $p \leq 0.05$.

The U.S. Environmental Protection Agency (EPA) now uses the benchmark dose (BMD) approach (Barnes *et al*., 1995; Crump, 1984) for noncancer risk assessment (EPA, 1995). This approach is designed to provide a more quantitative alternative to dose-response assessment than the no-observed-adverse-effect-level (NOAEL) process by constructing mathematical models to fit all data points in the dose-response study and to take data variance into consideration. In this study, BMD_5 and $BMDL_5$ values were calculated for maternal and developmental toxicity after PFOS exposure. BMD_5 refers to the central estimate of the administered dose predicted to cause a 5% increase in response above background, and $BMDL_5$ is defined as the corresponding lower limit of the 95% confidence interval on the BMD (Allen *et al*., 1994). Benchmark Dose Software (EPA, 2000) was used to calculate the BMD_5 values. Selection of a specific curve-fitting model for the BMD determination was based on the Akaike's Information Criterion (AIC) value. The AIC is equal to $-2L + 2p$, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and p is the number of model parameters estimated. The model that demonstrates "goodness of fit" with the lowest AIC value is presumed to be the most appropriate.

RESULTS

Rat

PFOS reduced maternal weight gain in a dose-dependent manner, significantly in the 2 mg/kg and higher dosage groups (Fig. 1). Dams exposed to 3 mg/kg PFOS showed significant weight deficits ($p < 0.0001$) by GD 7, whereas those exposed to 5 and 10 mg/kg PFOS revealed significant lags ($p < 0.0001$) by GDs 5 and 3, respectively. Effects on maternal weight at the two highest dosage groups were particularly profound. Dams in the 10 mg/kg dosage group failed to gain any weight until the last week of pregnancy. These weight gain deficits corresponded to significant reductions in food and water consumption throughout gestation (Fig. 2).

With the 20-day exposure scheme, PFOS did not affect maternal liver weight in rats (Table 1), but liver/body weight ratio was increased in the 10 mg/kg dosage group, most likely reflecting the marked body weight deficit in these animals. Negligible levels of PFOS were detected in the sera and livers

TABLE 1
Rat Maternal Liver Weight, Serum Chemistry, and Hormones at Term (A); Rat Reproductive Outcome and Fetal Teratology, Examined at Term (B)

	PFOS Exposure level					
	Control	1 mg/kg	2 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg
A. Maternal examinations (*N*)	14	14	9	9	14	16
Liver wt (g)	14.7 ± 0.6[a]	16.1 ± 0.5[a]	15.0 ± 0.4[a]	14.7 ± 0.5[a]	15.1 ± 0.5[a]	14.8 ± 0.5[a]
Relative liver wt (%)	5.0 ± 0.1[a]	5.2 ± 0.1[a]	5.1 ± 0.1[a]	5.0 ± 0.1[a]	5.3 ± 0.2[a]	6.0 ± 0.1[b]
Serum chemistry (*N*)	12	12	9	9	14	12
Cholesterol (mg/dl)	90.6 ± 5.2[a,b]	98.7 ± 6.3[a]	92.7 ± 4.6[a]	98.0 ± 5.1[a]	88.5 ± 3.1[a,b]	77.5 ± 4.0[b]
Triglycerides (mg/dl)	510 ± 44[a]	522 ± 54[a]	443 ± 38[a,b]	421 ± 64[a,b]	477 ± 34[a,b]	337 ± 44[b]
Sorbitol dehydrogenase (units/l)	15.9 ± 2.1[a]	13.1 ± 2.4[a]	16.1 ± 1.2[a]	16.5 ± 2.0[a]	14.3 ± 2.6[a]	14.5 ± 3.4[a]
Serum hormones (*N*)	5	5	—	—	5	5
Corticosterone (ng/ml)	141 ± 22[a]	147 ± 37[a]	—	—	102 ± 6[a]	188 ± 31[a]
Prolactin (ng/ml)	1.54 ± 0.06[a]	1.27 ± 0.36[a]	—	—	0.95 ± 0.15[a]	1.71 ± 0.39[a]
B. Fetal examinations (*N*)	13	14	9	8	14	15
Implantation sites (# per dam)	14.7 ± 0.5[a]	15.7 ± 0.8[a]	14.3 ± 0.6[a]	13.8 ± 1.0[a]	14.8 ± 0.5[a]	15.2 ± 0.4[a]
Live fetuses (%)	96.9 ± 1.3[a]	92.7 ± 2.1[a]	97.8 ± 1.5[a]	88.6 ± 8.5[a]	96.3 ± 1.6[a]	86.6 ± 6.0[a]
Body wt (g)	3.87 ± 0.07[a]	3.85 ± 0.05[a]	3.74 ± 0.06[a]	3.86 ± 0.05[a]	3.73 ± 0.08[a]	3.38 ± 0.09[b]
Liver wt (g)	0.29 ± 0.01[a]	0.32 ± 0.01[a]	0.31 ± 0.01[a]	0.30 ± 0.01[a]	0.28 ± 0.02[a]	0.28 ± 0.02[a]
Relative liver wt (%)	7.56 ± 0.24[a]	8.14 ± 0.24[a]	8.32 ± 0.16[a]	7.94 ± 0.34[a]	7.55 ± 0.28[a]	7.97 ± 0.22[a]
Notable skeletal defects (*N*)	13	11	9	7	14	15
Cleft palate (%)	0[a]	9 ± 9[a]	14 ± 14[a]	10 ± 10[a]	0[a]	60 ± 13[b]
Sternal defects (# per fetus)*	1.2 ± 0.3[a]	1.7 ± 0.3[a,b]	2.1 ± 0.3[b,c]	2.6 ± 0.2[a,b]	2.1 ± 0.2[a,b]	3.4 ± 0.4[c]
Ossified proximal phalanges						
(# per forelimb)	1.8 ± 0.4[a,b,c]	1.9 ± 0.4[a,b,c]	2.5 ± 0.6[a,b]	2.0 ± 0.3[a]	0.8 ± 0.2[c]	1.0 ± 0.4[b,c]
(# per hindlimb)	2.1 ± 0.5[a,b]	2.5 ± 0.5[a,b,c]	3.9 ± 0.1[b,c]	3.2 ± 0.3[c]	1.9 ± 0.5[a,b]	1.6 ± 0.4[a]
Notable visceral defects (*N*)	13	6	9	8	14	15
Anasarca (%)	0[a]	0[a]	0[a]	18.1 ± 9.1[a]	16.6 ± 7.9[a]	44.3 ± 11.7[b]
Enlarged right atrium (%)	0[a]	2.3 ± 2.3[a]	8.3 ± 8.3[a,b]	0[a]	22.9 ± 7.4[b]	8.7 ± 3.5[a,b]
Ventricular septal defects (%)	0[a]	0[a]	0[a]	0[a]	12.9 ± 6.1[a]	14.6 ± 5.2[a]

Note. (A) Data represent means ± SE of dams examined as indicated. (B) Data represent means ± S.E. of numbers of litters examined as indicated. Significant differences ($p < 0.05$) were determined by Duncan's multiple-range test and are depicted by different letters ([a], [b], and [c]); thus, groups sharing the same letter are not significantly different from each other.

*Sternal defects induced by PFOS were primarily bilobed and bipartite.

of the controls (Fig. 3); the source of this slight contamination may have been derived from fish meal in the chow (Seacat *et al*., 2003). With daily chemical treatment, the serum concentrations of PFOS increased monotonically in proportion to dosage; however, the level of all dosage groups fell toward the end of pregnancy. At term, PFOS concentration as well as the total hepatic burden also increased linearly with PFOS dosage. When these data were expressed as ppm, the liver samples were found to contain approximately four times higher concentrations of PFOS, compared with the corresponding serum samples. Fetal liver weight was not influenced by PFOS exposure (Table 1). An accumulation of PFOS that was proportional to the treatment dosage was also detected in the fetal liver (Fig. 3); based on concentration, fetal livers appeared to contain approximately half as much PFOS as their maternal counterparts.

Analysis of the serum chemistry of pregnant rats at term revealed that PFOS caused a significant reduction in circulating cholesterol and triglycerides only in the 10 mg/kg dosage group (Table 1). Sorbitol dehydrogenase, glucose, bile acid, and bilirubin levels were not altered by PFOS treatment (data not shown). In contrast, PFOS produced a marked reduction in both total and free serum T_4 in all dosage groups as early as GD 7 (Fig. 4) and in serum T_3 to a lesser extent, as well. However, no difference in serum TSH was observed among the treatment groups. An additional study was conducted with adult female (nonpregnant) rats in which the animals were exposed to PFOS (3 or 5 mg/kg) for 20 days. Similar to findings in pregnant rats, serum T_4 (both total and free) and T_3 levels in the nonpregnant rats were markedly reduced by the chemical treatment (as early as 3 days after the initiation of exposure) (Fig. 5). The pattern of TSH response is somewhat confounding and appears to be dose dependent. For the 3 mg/kg dosage group, a significant elevation (47%) of serum TSH was detected after 7 days of PFOS treatment. This hormonal increase was maintained for another week, although it was no longer statistically different from controls. After 20 days of chemical treatment, the alteration of TSH was completely attenuated. In contrast, the serum TSH levels in the 5 mg/kg dosage group were

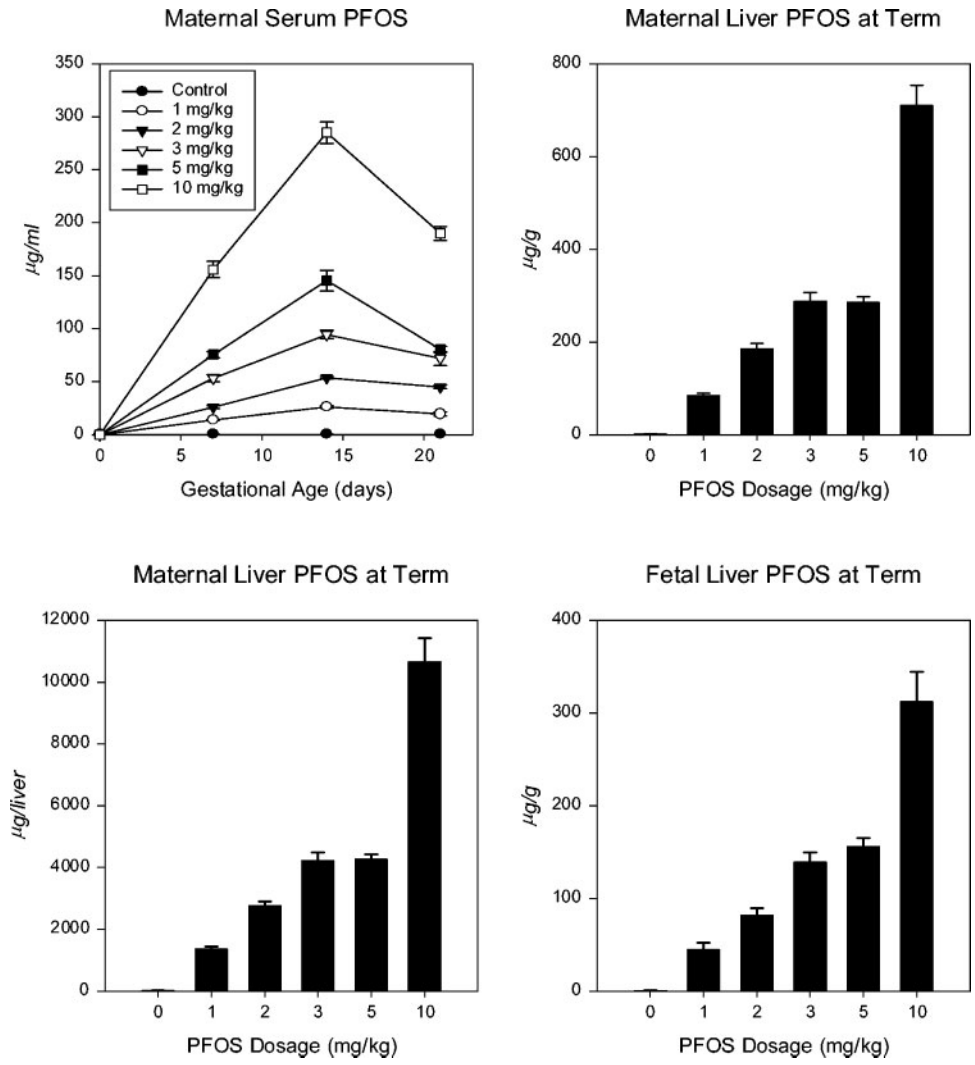

FIG. 3. Concentrations of PFOS in rat maternal serum throughout gestation and maternal and fetal liver at term. Each data point or bar represents mean ± SE of determination from 9–14 rats.

slightly lower than controls at the initial stages of PFOS exposure (by 26% and 21%, respectively, after 3 and 7 days); these changes were also abolished after 20 days of treatment. By comparison, PFOS did not alter serum corticosterone or prolactin levels appreciably in the pregnant rats at term (Table 1).

In utero exposure to PFOS throughout gestation did not produce adverse effects on the number of live fetuses or postimplantation loss in the treated dams (Table 1). However, a significant reduction of fetal weight was apparent in the 10 mg/kg group. Gross and skeletal examinations revealed a significant increase in the incidence of cleft palate, defective sternebrae, anasarca, enlarged right atrium, and ventricular septal defects, primarily in the fetuses exposed to the highest level of PFOS (Table 1).

Mouse

PFOS-induced deficits in maternal weight gain were not as pronounced in the mouse as in the rat. Statistically significant differences in body weight gain were observed only in the 20 mg/kg dosage group at late gestation (Fig. 6). Likewise, food and water consumption were less affected by the chemical exposure (Fig. 7). In contrast, PFOS treatment increased maternal liver weight in a dose-dependent fashion; indeed, in the highest dosage group (20 mg/kg), the livers almost doubled their weight, compared with those in controls (Table 2). Serum PFOS concentrations in the mouse were comparable with those found in the rat (Fig. 8); for the 10 mg/kg dosage group, the mean (± SE) maternal rat serum at term was 190 ± 7 μg/ml, and the maternal mouse serum at term was 179 ± 35 μg/ml. Additionally, serum PFOS in the mouse appeared to reach a saturated concentration at 250 μg/ml. A similar pattern of PFOS accumulation and saturation was seen in the maternal mouse liver. Indeed, in the 10 mg/kg dosage group, the rat maternal liver PFOS concentration was 710 ± 44 μg/g, and that for the mouse liver was 560 ± 52 μg/g.

As observed in the rat, maternal serum triglycerides were

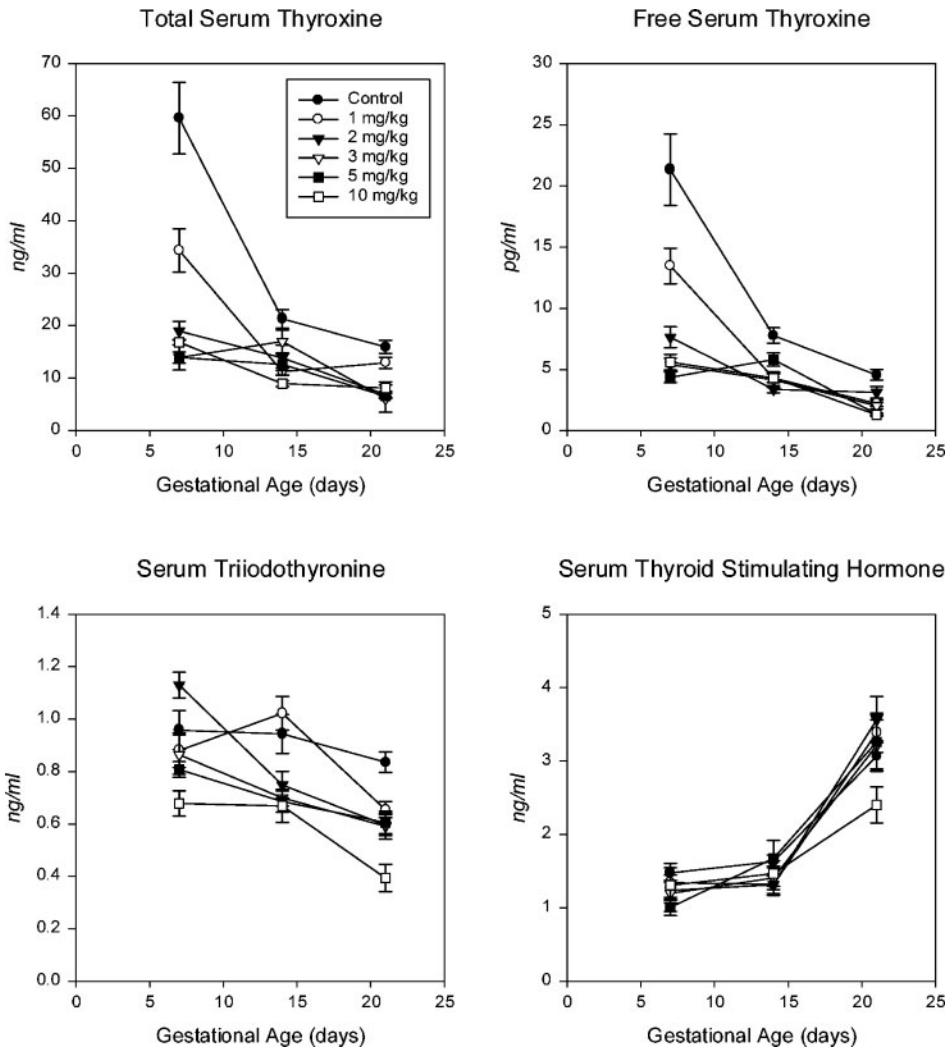

FIG. 4. Effects of PFOS on circulating thyroid hormones in pregnant rats. Each data point represents mean ± SE of duplicate determination from 9–14 rats. Two-way ANOVA indicates significant effects on time and treatment, and time × treatment interaction for serum total and free T_4 levels ($p < 0.0001$) and for serum T_3 levels ($p < 0.002$). Duncan's multiple-range test indicates that all doses are significantly different from control values at all time points evaluated for T_4; whereas significant differences from controls are detected in 10 mg/kg group on GD 7, in 3, 5, and 10 mg/kg groups on GD14, and all doses groups on GD 21 for T_3. For serum TSH, two-way ANOVA indicates a significant effect of time ($p < 0.0001$) but not of treatment, and no interaction.

also significantly lowered by PFOS in the mouse in a dose-dependent manner (Table 2), although neither serum cholesterol nor sorbitol dehydrogenase was significantly altered. As seen in the rat, a rapid decline of serum thyroxine was noted in the mouse during pregnancy (Fig. 9). However, the adverse effect of PFOS on thyroid hormones was less pronounced in the mouse than in the rat. Serum T_4 levels were reduced by the chemical treatment in a dose-dependent manner by GD 6, but hormone levels in the PFOS-exposed mice were no longer different from controls during the last week of pregnancy (Fig. 9).

Exposure of pregnant mice to PFOS throughout gestation did not alter the number of implantation sites; however, a significant increase in postimplantation loss was seen in the 20 mg/kg dosage group (Table 2). Small but significant reductions of fetal weight were detectable in the 10 and 15 mg/kg dosage groups. In addition, fetal liver weights (absolute and relative) were significantly elevated at 20 mg/kg. Fetal examination revealed cleft palate, defective sternebrae, enlargement of the right atrium, and ventricular septal defects, but primarily in the higher dosage groups (15 and 20 mg/kg) (Table 2).

DISCUSSION

Consistent with recent findings with Cynomolgus monkeys (Seacat *et al.*, 2002), accumulated body burdens of PFOS in pregnant rodents were found to be directly proportional to exposure levels in this study. At term, the correlation coefficients (r^2) between administered dosage and PFOS levels in the rat were 0.980 for serum and 0.964 for liver. In the mouse, saturation kinetic was apparent between 15 and 20 mg/kg; hence, for dosages below 15 mg/kg, the r^2 between treatment dosages and PFOS levels was 0.993 for serum and 0.989 for liver. The existence of such linear relationships across these three species and a wide dose range (0.03 mg/kg/day for monkey to 15 mg/kg/day for mouse) lends support to similar crude extrapolations for other species and exposure levels. PFOS was preferentially accumulated in the liver; the ratio of

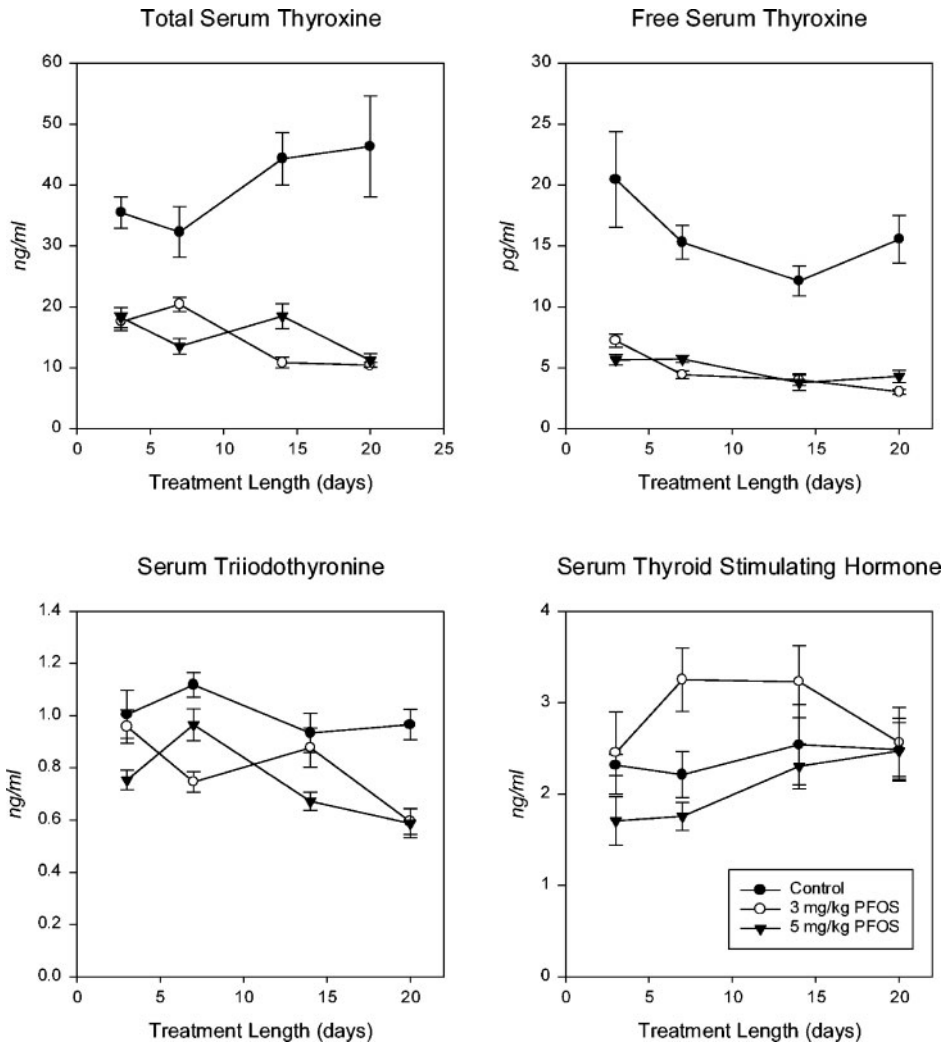

FIG. 5. Effects of PFOS on circulating thyroid hormones in adult female nonpregnant rats. Each data point represents mean ± SE of duplicate determination from 6–8 rats. Two-way ANOVA indicates a significant treatment effect ($p < 0.001$) and a time × treatment interaction ($p < 0.02$) for total T_4; a significant treatment effect ($p < 0.001$) but no interaction for free T_4; and a significant treatment effect ($p < 0.005$) and a time × treatment interaction ($p < 0.005$) for T_3. When individual PFOS dose groups are compared with controls, ANOVA indicates a significant treatment effect ($p < 0.0001$) for both 3 and 5 mg/kg dose groups. For TSH, two-way ANOVA indicates a significant treatment effect ($p < 0.004$) but no interaction; Duncan's multiple-range test indicates a significant difference between the 3 mg/kg dose group and controls and between the 3 mg/kg and 5 mg/kg dose groups but not between the 5 mg/kg dose group and controls.

serum to liver concentration was approximately 1:4 in both rodent species, regardless of the administered dosages and comparable with the reported values for rat (at approximately 1:5, Seacat *et al*., 2003) and monkey (at approximately 1:2, Seacat *et al*., 2002). These data are consistent with the previous observation of enterohepatic circulation of PFOS (Johnson *et al*., 1984). PFOS levels in the fetal liver were nearly half of those in the maternal counterparts, regardless of administered dose. Although the PFOS levels in fetal circulation were not measured in this study, data from a postnatal evaluation of PFOS toxicity described in the companion article (Lau *et al*., 2003) indicate that serum concentrations of the fluorochemical in the newborns were comparable to those in maternal circulation. Thus, the lower accumulation of hepatic PFOS in the fetuses would likely suggest a reduced capacity of chemical uptake/storage in the liver or immaturity of the enterohepatic circulation. However, it must be cautioned that the pharmacokinetic properties of PFOS, particularly during pregnancy, are complex and have not yet been characterized. For instance, the decline of serum PFOS levels in the rat at term (Fig. 3) most likely reflects a marked expansion of maternal blood volume that is characteristic of the late term of pregnancy (Barron, 1987; Tam and Chan, 1977). Hence, an accurate profile of PFOS disposition, particularly during pregnancy, must await the construction of a detailed pharmacokinetic model for the fluorochemical.

Maternal toxicity of PFOS, indicated by deficits in weight gain during pregnancy, was observed in both rat and mouse. In both rodents, the severity of the adverse effects was dose-dependent; at term, a BMD_5 for maternal weight reduction is estimated at 0.22 mg/kg and a $BMDL_5$ at 0.15 mg/kg for the rat, and a BMD_5 of 15.2 mg/kg and a $BMDL_5$ of 3.1 mg/kg are determined for the mouse (polynomial model). In the rat, the lag in weight gain during pregnancy was particularly pronounced in the two highest dosage groups (5 and 10 mg/kg), which exhibited marked reductions of food and water intake. The PFOS-induced reductions of maternal weight gain in the rat and mouse seen here are comparable to similar alterations produced by the fluorochemical in the rabbit or by N-alkyl perfluorooctanesulfonamido ethyl alcohol in the rat and rabbit

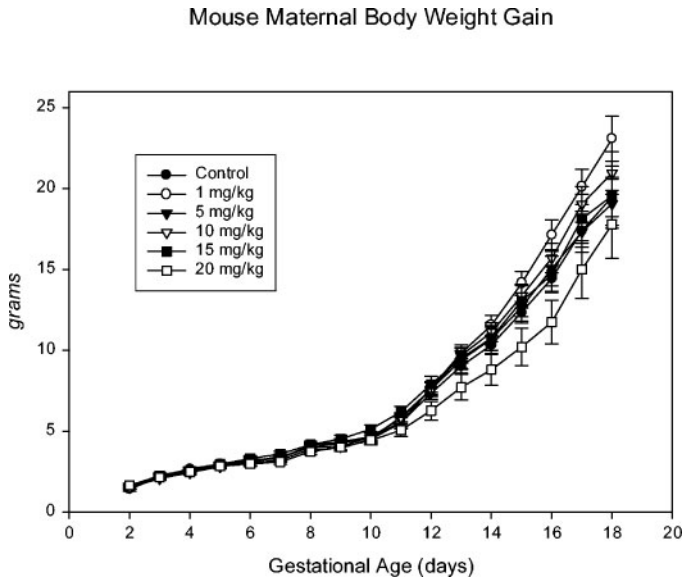

FIG. 6. Effects of PFOS on body weight gain in pregnant mice. Each data point represents mean ± SE of determination from 60–80 mice. Two-way ANOVA indicates a significant treatment effect ($p < 0.0001$) and a time × treatment interaction ($p < 0.02$). Duncan's multiple-range test indicates that the 10 and 20 mg/kg dose groups vary significantly from control values and from each other.

(Case *et al*., 2001), indicating that the adverse effect on maternal weight gain may be a common feature of toxicity for the perfluorochemicals. Liver enlargement with associated histological abnormalities is another feature often seen after exposure to PFOS and related compounds (Case *et al*., 2001; Haughom and Spydevold, 1992; Ikeda *et al*., 1987; Seacat *et al*., 2002; 2003; Sohlenius *et al*., 1993). A somewhat similar finding was obtained in our studies. An increase of liver weight is generally observed in rodents during pregnancy (by about 24% in rat, Buelke-Sam *et al*., 1982; and 56% in mouse, observation in our laboratory, data not shown). Above and beyond this physiological change, significant elevations of hepatic weight were found in the PFOS-exposed mice, and increases in the high-dose groups were as much as twofold over the corresponding controls. Serum triglycerides in these mice were significantly reduced. Interestingly, a comparable PFOS-induced liver enlargement was absent in the pregnant rat; the small increase in the relative liver weight in the 10 mg/kg dosage group largely reflected the reduction of body weight, rather than a net increase of liver weight. Serum cholesterol and triglycerides in the rat were also not altered appreciably by PFOS exposure. An explanation for these disparate findings (compared to the results reported by Seacat *et al*., 2003, for instance) is not readily available and may be attributed to the relatively short duration of PFOS exposure (20 days) in our study (compared to 14 weeks in the Seacat study). Nonetheless, the high sensitivity to PFOS-induced liver toxicity in the mouse should be noted, with a BMD_5 and a $BMDL_5$ of liver weight increase estimated at 2.61 mg/kg and 1.31 mg/kg (Hill model), respectively.

Seacat and co-workers (2002) reported reductions of serum T_3 and elevations of TSH in monkeys after exposure to PFOS for 182 days. In this study, PFOS produced a much more marked reduction of both T_3 and T_4 in pregnant rats and at a much earlier onset. For the T_4 effects at GD 7, a BMD_5 at 0.23 mg/kg and a $BMDL_5$ at 0.05 mg/kg (Hill model) are estimated. Interestingly, the accumulated serum PFOS level where thyroid imbalance was detected in the monkey (171 ppm) is comparable to that in the pregnant rat (53–155 ppm). Yet in rats, despite these deficits in circulating hormones, a feedback elevation of TSH through activation of the hypothalamic-pituitary-thyroid (HPT) axis was not apparent. Because the level of serum T_4 (and to a lesser extent, T_3) falls and that of TSH rises during pregnancy (Versloot *et al*., 1994; Figure 4 in this study), these physiological changes might have masked the true effects of PFOS. However, the T_3 and T_4 results from the study with nonpregnant female rats by and large substantiated the findings in pregnant dams, discounting potential confounding effects of pregnancy. The dose-dependent, paradoxical

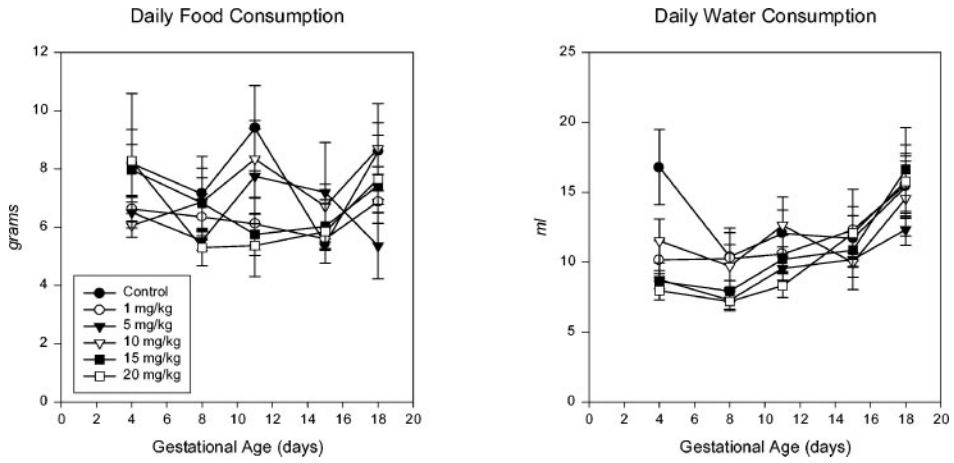

FIG. 7. Effects of various doses of PFOS on food and water consumption in pregnant mice. Each data point represents mean ± SE of determination from 14–19 mice. Two-way ANOVA indicates no significant treatment effect or interaction associated with food consumption but a significant treatment effect ($p < 0.05$) for water consumption.

TABLE 2
Mouse Maternal Liver Weight and Serum Chemistry at Term (A), and Mouse Reproductive Outcome and Fetal Teratology, Examined at Term (B)

	PFOS Exposure level					
	Control	1 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg	20 mg/kg
A. Maternal examinations *(N)*	20	27	29	24	26	25
Liver wt (g)	2.19 ± 0.08^{a}	2.46 ± 0.10^{a,b}	2.78 ± 0.08^{b}	3.86 ± 0.10^{c}	4.61 ± 0.17^{d}	4.25 ± 0.21^{e}
Relative liver wt (%)	7.42 ± 0.24^{a}	7.36 ± 0.20^{a}	9.05 ± 0.28^{b}	12.25 ± 0.19^{c}	14.45 ± 0.34^{d}	15.51 ± 0.26^{e}
Serum chemistry *(N)*	19	19	20	21	9	5
Cholesterol (mg/dl)	68.9 ± 4.9^{a,b}	69.7 ± 6.2^{a,b}	83.3 ± 6.0^{a}	83.1 ± 5.4^{a}	78.0 ± 5.8^{a,b}	58.4 ± 10.2^{b}
Triglycerides (mg/dl)	284 ± 18^{a}	220 ± 21^{a,b}	196 ± 18^{b}	162 ± 11^{b,c}	191 ± 19^{b,c}	119 ± 18^{c}
Sorbitol dehydrogenase (units/l)	20.3 ± 3.1^{a}	23.5 ± 3.6^{a}	22.3 ± 4.8^{a}	23.5 ± 3.3^{a}	20.7 ± 3.1^{a}	23.3 ± 6.1^{a}
B. Fetal examinations *(N)*	19	26	12	24	18	11
Implantation sites (# per dam)	12.3 ± 0.9^{a}	13.7 ± 1.1^{a}	11.1 ± 1.1^{a}	12.7 ± 0.8^{a}	12.4 ± 1.4^{a}	12.9 ± 0.6^{a}
Live fetuses (%)	97.9 ± 1.0^{a}	96.6 ± 1.1^{a}	91.9 ± 3.1^{a,b}	96.0 ± 0.9^{a}	95.3 ± 1.0^{a,b}	89.1 ± 5.5^{b}
Body wt (g)	1.05 ± 0.03^{a}	1.05 ± 0.01^{a}	1.05 ± 0.04^{a}	0.97 ± 0.01^{b,c}	0.94 ± 0.02^{b}	1.03 ± 0.02^{a,c}
Liver wt (mg)	77 ± 2^{a,b,c}	67 ± 4^{a}	88 ± 5^{c,d}	76 ± 2^{a,b}	79 ± 3^{b,c}	91 ± 3^{d}
Relative liver wt (%)	7.13 ± 0.20^{a}	6.26 ± 0.39^{b}	7.93 ± 0.20^{a}	7.99 ± 0.18^{a}	7.95 ± 0.16^{a}	9.07 ± 0.17^{c}
Notable skeletal defects *(N)*	8	3	4	9	11	6
Cleft palate (%)	0^{a}	0^{a}	0^{a}	1.8 ± 1.8^{a,b}	21.1 ± 8.3^{b}	73.2 ± 11.7^{c}
Sternal defects (# per fetus)*	0.5 ± 0.2^{a}	1.0 ± 0.3^{a,b}	2.1 ± 0.4^{b,c}	2.1 ± 0.2^{b,c}	2.6 ± 0.2^{b,c,d}	3.3 ± 0.7^{d}
Notable visceral defects (*N*)	9	5	3	8	12	8
Enlarged right atrium (%)	0^{a}	0^{a}	0^{a}	25.8 ± 7.8^{b}	22.5 ± 7.2^{b}	34.5 ± 9.2^{b}
Ventricular septal defects (%)	1.9 ± 1.9^{a}	0^{a}	0^{a}	5.4 ± 2.7^{a}	4.9 ± 3.3^{a}	30.0 ± 15.6^{b}

Note. (A) Data represent means ± SE of dams examined as indicated. (B) Data represent means ± SE of litters examined as indicated. Significant differences ($p < 0.05$) were determined by Duncan's multiple-range test and are depicted by different letters (^{a}, ^{b}, ^{c}, ^{d}, and ^{e}); thus, groups sharing the same letter are not significantly different from each other.

*Sternal defects induced by PFOS were primarily bilobed and bipartite.

responses of serum TSH in the nonpregnant rats are intriguing. The near-identical response patterns between total and free T_4 rule out the potential involvement of the hormone binding proteins. Although feedback increases of the pituitary hormone in the 3 mg/kg dosage group were relatively small (27–50%), compared with the two- to threefold increase induced by propylthiouracil (Cooper *et al*., 1983), these changes nonetheless indicate the integrity of the HPT axis. The recovery of TSH after a transient response, despite the persistent reductions of serum T_3 and T_4, suggests that the homeostatic balance of thyroid hormone economy may have been reset. These findings resemble those previously reported with long-term chemical

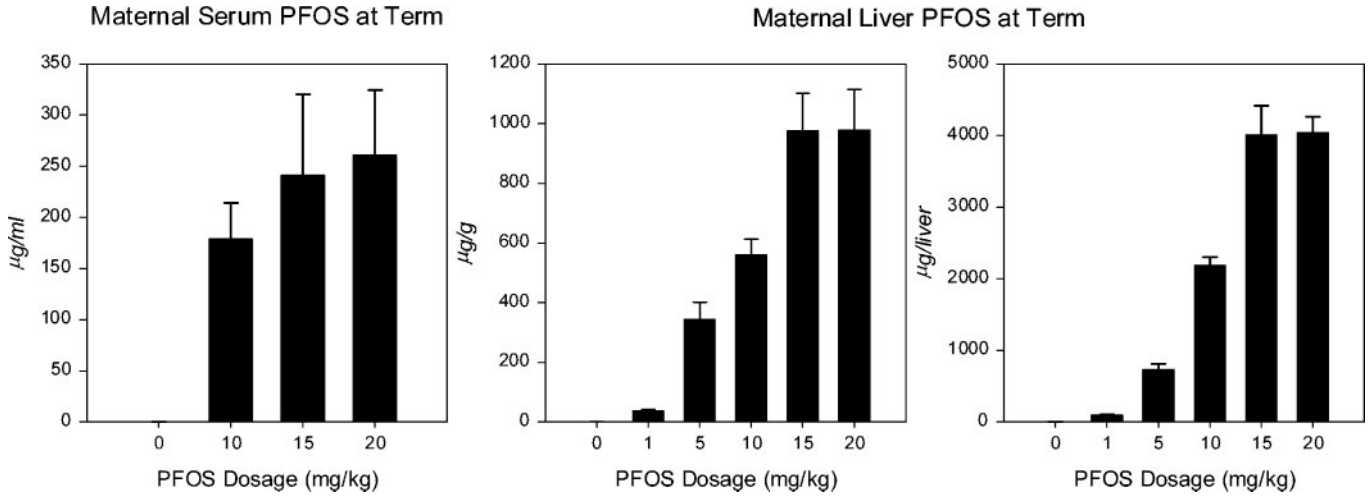

FIG. 8. Concentrations of PFOS in mouse maternal serum and liver at term. Each data point or bar represents mean ± SE of determination from six mice.

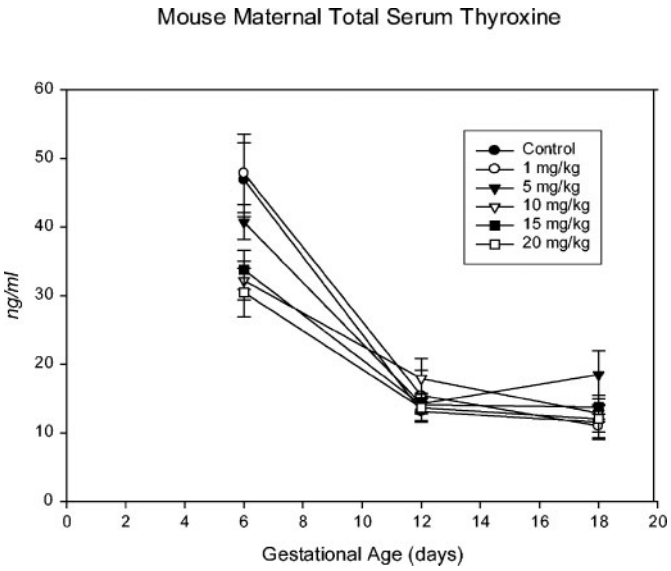

FIG. 9. Effects of PFOS on circulating total T_4 levels in pregnant mice. Two-way ANOVA indicates a significant effect of time ($p < 0.0001$) and a time × treatment interaction ($p < 0.005$). Dunnett's *t*-test demonstrates that the T_4 level associated with the 20 mg/kg dose group is significantly different at the 0.05 level from the control value on GD 6.

disruption of the thyroid axis (Biegel *et al*., 1995). The absence of serum TSH elevation (through the HPT feedback mechanism) in the 5 mg/kg dosage group is perplexing. In fact, the TSH levels of these rats were slightly depressed in the initial stage of PFOS exposure. These results point toward a more complex, dose-dependent effect of the fluorochemical that is not yet fully understood.

Pregnant mice exhibited a pattern of PFOS-induced T_4 reductions similar to that seen in the pregnant rat, with a BMD_5 of 0.51 mg/kg and a $BMDL_5$ of 0.35 mg/kg estimated at GD 6 (linear model). This profile of thyroid hormone imbalance (reductions of T_3 and T_4 without a compensatory elevation of TSH) produced by PFOS, though puzzling, is not unique. Chemically induced decreases of serum T_4 and T_3 without significant feedback increase of TSH have been reported with polychlorinated biphenyls (PCBs) (Goldey *et al*., 1995; Liu *et al*., 1995). The mechanism(s) underlying the thyroidal effects of PFOS remains to be elaborated. Similar patterns of alterations between total and free T_4 rule out involvement of hormone binding proteins. Alternatively, the hepatotoxicity of PFOS presents a prime possibility. Altered thyroid hormone metabolism through induction of hepatic enzymes has been described with chemicals such as phenobarbital, pregnenolone-16α-carbonitrile, 3-methylcholanthrane, PCB, and brominated diphenyl ethers (Byrne *et al*., 1987; Liu *et al*., 1995; Zhou *et al*., 2002). Regardless, the thyroid hormone deficits produced by PFOS during pregnancy are of potential concern, particularly if the feedback mechanism via the HPT axis is compromised. Thyroid hormones play a critical role in the normal development of the lung, inner ears, and nervous system (particularly CNS), and they regulate growth, metabolic rate, cardiac performance, and body temperature (Lucas *et al*., 1988; Glinoer, 2001). During *in utero* development, the embryo and fetus rely completely on maternal supplies of thyroid hormones through placental transfer until maturation of the fetal thyroid gland toward late gestation. Perinatal hypothyroidism has been shown to cause retardation of neurodevelopment and stunted growth (Porterfield, 1993), and recent epidemiological findings indicate that even subtle changes of the thyroid economy (subclinical hypothyroidism) during maturation may have long-lasting effects on the development of intellectual and motor skills (Haddow *et al*., 1999). Decreased availability of maternal T_4 to the developing brain poses an increased risk of poor neuropsychological development, and a direct relationship between the degree of neonatal hypothyroxinemia and subsequent neurodevelopment has been established (Morreale de Escobar *et al*., 2000).

In view of the profound deficits in maternal weight gain in PFOS-exposed rats, it was surprising to find little adverse effect on the viability of the fetuses at term. In fact, only small decrements of fetal weight were noted. Similar results were obtained with the mouse, even at higher exposures. On the other hand, anasarca, craniofacial malformation (cleft palate), cardiac defects (ventricular septal defects, enlargement of the right atrium), and delayed ossification (sternebrae, phalanges) were detected in the PFOS-exposed fetuses. A BMD_5 for the sternal defects is estimated at 0.31 mg/kg, with a $BMDL_5$ at 0.12 mg/kg (logistic model); and a BMD_5 for cleft palate at 8.85 mg/kg, with a $BMDL_5$ at 3.33 mg/kg (logistic model). The enlarged right atrium may be associated with complications of pulmonary function. The mouse essentially produced an identical teratological profile. For comparison, BMD_5 and $BMDL_5$ for the sternal defects in the mouse are estimated at 0.06 mg/kg and 0.02 mg/kg, respectively (logistic model); those for cleft palate are 7.03 mg/kg and 3.53 mg/kg, respectively (NCTR model). These results are in agreement with previous teratological findings with lithium perfluorooctane sulfonate and N-ethylperfluorooctanesulfonamido ethyl alcohol in the rat (Case *et al*., 2001; Henwood *et al*., 1994) and PFOS in the

TABLE 3
Species Comparison of the Benchmark Doses for Various Parameters of PFOS Maternal and Developmental Toxicity

	Rat		Mouse	
	BMD_5 (mg/kg)	$BMDL_5$ (mg/kg)	BMD_5 (mg/kg)	$BMDL_5$ (mg/kg)
Maternal body wt at term	0.224	0.150	15.15	3.14
Maternal serum total T_4 (Rat GD 7; mouse GD 6)	0.234	0.046	0.513	0.352
Maternal liver wt at term	—	—	2.61	1.31
Fetal sternal defects	0.313	0.122	0.055	0.016
Fetal cleft palate	.85	3.33	7.03	3.53

rabbit (Case *et al*., 2001). Nonetheless, it should be noted that a preponderance of these structural abnormalities was found in the highest PFOS dosage group (10 mg/kg for the rat and 20 mg/kg in the mouse). Although a significant reduction of weight gain and food consumption was noted in this group of pregnant rats, malnutrition is not likely the sole factor accounting for the induction of birth defects. Indeed, equivalent or higher incidence of malformations was seen in the mouse fetuses, yet the deficits of weight gain and food consumption in the mouse dams were much less extensive than those of the rat.

Previous studies have shown that PFOS can interfere with cholesterol synthesis through inhibition of HMG CoA reductase activity (Haughom and Spydevold, 1992). Because cholesterol is known to play a role in development through the molecular signaling of *sonic hedgehog* (Brewer *et al*., 1993), alterations of this metabolic precursor may be involved in the mechanism of dysmorphogenesis (Fitzky *et al*., 2001). Yet in this study, maternal serum cholesterol was not significantly lowered by PFOS treatment in either rodent species. Indeed, results from a preliminary study (Luebker *et al*., 2002b) indicated that cholesterol or mevalonic acid supplement failed to ameliorate PFOS-induced developmental toxicity in the rat. Alternatively, altered thyroid status in the dam may raise concerns regarding developmental toxicity. Thyroid hormone effects on cell proliferation and differentiation, as well as on organ growth and maturation, have been well documented. On the other hand, changes of these parameters are often subtle (for instance, at a functional rather than morphological level) and not easily discernible by standard teratological assessment. Hence, evaluations for potential developmental toxicity of PFOS have been extended to postnatal examination, and the results are described in a companion article (Lau *et al*., 2003).

In summary, exposure to PFOS during pregnancy led to significant physiological alterations in the rat and mouse that are indicative of maternal toxicity, as well as to anatomical defects observed in the fetuses at term at high dosages. These adverse outcomes are dose-dependent and can be correlated with body burden of the fluorochemical. Generally, the mouse appeared to be a less sensitive species than the rat in regard to the PFOS-induced toxicity. A species comparison of the benchmark doses for various parameters is provided in Table 3.

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REFERENCES

Allen, B. C., Kavlock, R. J., Kimmel, C. A., and Faustman, E. M. (1994). Dose-response assessment for developmental toxicity. II. Comparison of generic benchmark dose estimates with no observed adverse effect levels. *Fundam. Appl. Toxicol.* **23,** 487–495.

Barnes, D. G., Daston, G. P., Evans, J. S., Jarabek, A. M., Kavlock, R. J., Kimmel, C. A., Park, C., and Spitzer, H. L. (1995). Benchmark Dose Workshop: Criteria for use of a benchmark dose to estimate a reference dose. *Reg. Toxicol. Pharmacol*. **21,** 296–306.

Barron, W. M. (1987). Volume homeostasis during pregnancy in the rat. *Am. J. Kidney Dis.* **9,** 296–302.

Berthiaume, J., and Wallace, K. B. (2002). Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol: Peroxisome proliferation and mitochondrial biogenesis. *Toxicol. Lett*. **129,** 23–32.

Biegel, L. B, Cook, J. C., O'Connor, C., Aschiero, M., Arduengo, A. J., and Slone, T. W. (1995). Subchronic toxicity study in rats with 1-methyl-3-propylimidazole-2-thione (PTI): Effects on the thyroid. *Fundam. Appl. Toxicol.* **27,** 185–194.

Brewer, L. M., Sheardown, S. A., and Brown, N. A. (1993). HMG-CoA reductase mRNA in the post-implantation rat embryo by *in situ* hybridization. *Teratology* **47,** 137–146.

Buelke-Sam, J., Nelson, C. J., Byrd, R. A., and Holson, J. F. (1982). Blood flow during pregnancy in the rat: I. Flow patterns to maternal organs. *Teratology* **26,** 269–277.

Butenhoff, J. L., York, R., Seacat, A., and Luebker, D. (2002). Perfluorooctanesulfonate-induced perinatal mortality in rat pups is associated with a steep dose-response. *Toxicologist* **66,** 25.

Byrne, J. J., Carbone, J. P., and Hanson, E. A. (1987). Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. *Endocrinology* **121,** 520–527.

Case, M. T., York, R. G., and Christian, M. S. (2001). Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. *Int. J. Toxicol*. **20,** 101–109.

Cooper, D. S., Kieffer, D., Halpern, R., Saxe, V., Mover, H., Maloof, F., and Ridgway, E. C. (1983). Propylthiouracil (PTU) pharmacology in the rat. II. Effects of PTU on thyroid function. *Endocrinology* **113,** 921–928.

Crump, K. S. (1984). A new method for determining allowable daily intakes. *Fundam. Appl. Toxicol.* **4,** 854–871.

Environmental Protection Agency (EPA). (1995). The use of the benchmark dose approach in health risk assessment. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development.

Environmental Protection Agency (EPA). (2000). Benchmark dose software. Washington, DC: U.S. Environmental Protection Agency, National Center for Environmental Assessment.

Fitzky, B. U., Moebius, F. F., Asaoka, H., Waage-Baudet, H., Xu, L., Xu, G., Maeda, N., Kluckman, K., Hiller, S., Yu, H., *et al.* (2001). 7-Dehydrocholesterol-dependent proteolysis of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. *J. Clin. Invest.* **108,** 905–915.

Giesy, J. P., and Kannan, K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **35,** 1339–1342.

Glinoer, D. (2001). Potential consequences of maternal hypothyroidism on the offspring: Evidence and implications. *Horm. Res*. **55,** 109–114.

Goldey, E. S. , Kehn, L. S., Lau, C., Rehnberg, G. L., and Crofton, K. M. (1995). Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Toxicol. Appl. Pharmacol*. **135,** 77–88.

Goldman, J. M., Cooper, R. L., Rehnberg, G. L., Hein, J. F., McElroy, W. K., and Gray, L. E. (1986). Effects of low subchronic doses of methoxychlor on the rat hypothalamic-pituitary reproductive axis. *Toxicol. Appl. Pharmacol*. **86,** 474–483.

Greenwood, F. C., Hunter, W. M., and Glover, T. (1963). The preparation of

^{131}I-labeled human growth hormone of high specific activity. *Biochem. J.* **89,** 114–123.

Haddow, J. E., Palomaski, G. E., Allan, W. C., Williams, J. R., Knight, G. J., Gagnon, J., O'Heir, C. E., Mitchell, M. L., Hermos, R. J., Waisbren, S. E., *et al.* (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N. Engl. J. Med.* **341,** 549–555.

Hansen, K. J., Clemen, L. A., Ellefson, M. E., and Johnson, H. O. (2001). Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ. Sci. Technol.* **35,** 766–770.

Haughom, B., and Spydevold, O. (1992). The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. *Biochim. Biophys. Acta* **1128,** 65–72.

Henwood, S. M., McKee-Pesick, P., Costello, A. C., and Osmitz, T. G. (1994). Developmental toxicity study with lithium perfluorooctane sulfonate in rats. *Teratology* **49,** 398.

Hu, W., Jones, P. D., Upham, B. L., Trosko, J. E., Lau, C., and Giesy, J. P. (2002). Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines *in vitro* and Sprague-Dawley rats *in vivo*. *Toxicol. Sci.* **68,** 429–436.

Ikeda, T., Fukuda, K., Mori, I., Enomoto, M., Komai, T., and Suga, T. (1987). Induction of cytochrome P-450 and peroxisome proliferation in rat liver by perfluorinated octanesulfonic acid. In *Peroxisomes in Biology and Medicine* (H. D. Fahimi and H. Sies, Eds.), pp. 304–308. Springer-Verlag, New York.

Johnson, J. D., Gibson, S. J., and Ober, R. E. (1979). Extent and route of excretion and tissue distribution of total carbon-14 in rats after a single i.v. dose of FC-95–^{14}C. Project No. 8900310200, Riker Laboratories, Inc., St. Paul, MN. (EPA Docket No. 8(e)HQ-1180-00374).

Johnson, J. D., Gibson, S. J., and Ober, R. E. (1984). Cholestramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C]perfluorooctanoate or potassium [^{14}C]perfluorooctanesulfonate. *Fundam. Appl. Toxicol.* **4,** 972–976.

Johnson, J. D., and Ober, R. E. (1979). Absorption of FC-95–^{14}C in rats after a single oral dose. Project No. 8900310200, Riker Laboratories, Inc., St. Paul, MN. (EPA Docket No. 8(e)HQ-1180-00374).

Kannan, K., Choi, J. W., Iseki, N., Senthilkumar, K., Kim, D. H., and Giesy, J. P. (2002a). Concentrations of perfluorinated acids in livers of birds from Japan and Korea. *Chemosphere* **49,** 225–231.

Kannan, K., Corsolini, S., Falandysz, J., Oehme, G., Focardi, S., and Giesy, J. P. (2002b). Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. *Environ. Sci. Technol.* **36,** 3120–3126.

Kannan, K., Franson, J. C., Bowerman, W. W., Hansen, K. J., Jones, P. D., and Giesy, J. P. (2001a). Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. *Environ. Sci. Technol.* **35,** 3065–3070.

Kannan, K., Hansen, K. J., Wade, T. L., and Giesy, J. P. (2002c). Perfluorooctane sulfonate in oysters, *Crassostrea virginica*, from the Gulf of Mexico and the Chesapeake Bay, USA. *Arch. Environ. Contam. Toxicol.* **42,** 313–318.

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J. F., Hansen, K. J., Jones, P. D., Helle, E., Nyman, M., and Giesy, J. P. (2001b). Accumulation of perfluorooctane sulfonate in marine mammals. *Environ. Sci. Technol.* **35,** 1593–1598.

Kissa, E. (1994). *Fluorinated Surfactants*. Marcel Dekker, New York.

Lau, C., Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Stanton, M. E., Butenhoff, J. L., and Stevenson, L. A. (2003). Exposure to perfluorooctane sulfonate during pregnancy in laboratory rat and mouse. II. Postnatal evaluation. *Toxicol. Sci.* **74,** 382–392.

Liu, J., Liu, Y., Barter, R. A., and Klaassen, C. D. (1995). Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: A dose-response study. *J. Pharmacol. Exp. Ther.* **273,** 977–985.

Lucas, A., Rennie, J., Baker, B. A., and Morley, R. (1988). Low plasma triiodothyronine concentrations and outcome in preterm infants. *Arch. Dis. Child.* **63,** 1201–1206.

Luebker, D. J., Hansen, K. J., Bass, N. M., Butenhoff, J. L., and Seacat, A. M. (2002a). Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* **176,** 175–185.

Luebker, D., York, R., Seacat, A., and Butenhoff, J. (2002b). Perfluorooctanesulfonate-induced perinatal mortality in rat pups is not a result of reduced serum lipids. *Toxicologist* **66,** 26.

Morreale de Escobar, G., Obregon, M. J., and Del Rey, F. E. (2000). Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? *J. Clin. Endocr. Metab.* **85,** 3975–3987.

Narotsky, M. G., and Rogers, J. M. (2000). Examination of the axial skeleton of fetal rodents. In *Developmental Biology Protocols* Vol. I (R. S. Tuan and C. W. Lo, Eds.), pp. 139–150. Humana Press, New Jersey.

Olsen, G. W., Burlew, M. M., Hocking, B. B., Skratt, J. C., Burris, J. M., and Mandel, J. H. (2001a). An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993–1998. St. Paul, MN, 3M Company. EPA Docket AR-226.

Olsen, G. W., Burris, J. M., Mandel, J. H., and Zobel, L. R. (1999). Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. *J. Occup. Environ. Med.* **41,** 799–806.

Olsen, G. W., Logan, P. W., Simpson, C. A., Burris, J. M., Burlew, M. M., Lundberg, J. K., and Mandel, J. H. (2001b). Descriptive summary of serum fluorochemical levels among employee participants of the year 2000 Decatur fluorochemical medical surveillance program. St. Paul, MN, 3M Company. EPA Docket AR-226.

Olsen, G. W., Schmickler, M. N., Tierens, J. M., Logan, P. W., Burris, J. M., Burlew, M. M., Lundberg, J. K., and Mandel, J. H. (2001c). Descriptive summary of serum fluorochemical levels among employee participants of the year 2000 Antwerp fluorochemical medical surveillance program. St. Paul, MN, 3M Company. EPA Docket AR-226.

Porterfield, S. P. (1993). The role of thyroid hormones in prenatal and neonatal neurological development-current perspectives. *Endocr. Rev.* **14,** 94–106.

Renner, R. (2001). Growing concern over perfluorinated chemicals. *Environ. Sci. Technol.* **35,** 154A–160A.

Seacat, A. M., Thomford, P. J., Hansen, K. J., Clemen, L. A., Eldridge, S. R., Elcombe, C. R., and Butenhoff, J. L. (2003). Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. *Toxicology* **183,** 117–131.

Seacat, A. M., Thomford, P. J., Hansen, K. J., Olsen, G. W., Case, M. T., and Butenhoff, J. L. (2002). Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in Cynomolgus monkeys. *Toxicol. Sci.* **68,** 249–264.

Sohlenius, A. K., Eriksson, A. M., Hogstrom, C., Kimland, M., and DePierre, J. W. (1993). Perfluorooctane sulfonic acid is a potent inducer of peroxisomal fatty acid b-oxidation and other activities known to be affected by peroxisome proliferators in mouse liver. *Pharmacol. Toxicol.* **72,** 90–93.

Starkov, A. A., and Wallace, K. B. (2002). Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci.* **66,** 244–252.

Tam, P. P. L., and Chan, S. T. H. (1977). Changes in the composition of maternal plasma, fetal plasma and fetal extraembryonic fluid during gestation in the rat. *J. Reprod. Fertil.* **51,** 41–51.

Versloot, P. M., Gerritsen, J., Boogerd, L., Schroder-van der Elst, J. P., and van der Heide, D. (1994). Thyroxine and 3,5,3′-triiodothyronine production, metabolism, and distribution in pregnant rat near term. *Am. J. Physiol.* **267,** E860–E867.

Zhou, T., Taylor, M. M., DeVito, M. J., and Crofton, K. M. (2002). Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol. Sci.* **66,** 105–116.