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Subchronic Toxicity Studies on Perfluorooctanesulfonate Potassium Salt in Cynomolgus Monkeys

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This study was conducted to determine the earliest measurable response of primates to low-level perfluorooctanesulfonate (PFOS) exposure and to provide information to reduce uncertainty in human health risk assessment. Groups of male and female monkeys received 0, 0.03, 0.15, or 0.75 mg/kg/day potassium PFOS orally for 182 days. Recovery animals from each group, except the 0.03 mg/kg/day dose group, were monitored for one year after treatment. Significant adverse effects occurred only in the 0.75 mg/kg/day dose group and included compound-related mortality in 2 of 6 male monkeys, decreased body weights, increased liver weights, lowered serum total cholesterol, lowered triiodothyronine concentrations (without evidence of hypothyroidism), and lowered estradiol levels. Decreased serum total cholesterol occurred in the 0.75 mg/kg/day dose group at serum PFOS levels > 100 ppm. Hepatocellular hypertrophy and lipid vacuolation were present at term in the 0.75 mg/kg/day dose group. No peroxisomal (palmitoyl CoA oxidase) or cell proliferation (proliferating cell nuclear antigen immunohistochemistry) was detected. Complete reversal of clinical and hepatic effects and significant decreases in serum and liver PFOS occurred within 211 days posttreatment. Liver-to-serum PFOS ratios were comparable in all dose groups, with a range of 1:1 to 2:1. Serum concentrations associated with no adverse effects (0.15 mg/kg/day) were 82.6 ± 25.2 ppm for males and 66.8 ± 10.8 ppm for females. Comparison of serum PFOS concentrations associated with no adverse effect in this study to those reported in human blood samples (0.028 ± 0.014 ppm) indicated an adequate margin of safety.

Key Words: **perfluorooctanesulfonate; cholesterol; hypolipidemia; peroxisomes; hepatotoxicity; electron microscopy; primate.**

Perfluorooctanesulfonate (PFOS) and related N-alkyl perfluorooctanesulfonamido compounds have been manufactured by 3M Company from perfluorooctanesulfonyl fluoride since 1948. This class of compounds has been utilized in a wide variety of industrial and consumer products, such as protective coatings for carpets and apparel, paper coatings approved for food contact, insecticide formulations, and surfactants. These products contain chemistries that can degrade or metabolize to PFOS; however, no evidence has been found for metabolic or environmental degradation of PFOS. In May 2000, 3M announced that it would voluntarily cease producing PFOS due to concerns about its biopersistence and its widespread exposure to human populations and wildlife (Giesy and Kannan, 2001; Hansen *et al.,* 2001; Kannan *et al.,* 2001). Nonoccupational exposures to PFOS or precursors are not well understood at this time, but could include environmental sources, consumer products, or as indirect food additives. Following 3M's announcement to cease production of PFOS, the U.S. EPA proposed a Significant New Use Regulation (SNUR) that would regulate new uses of PFOS and related chemicals (EPA, 2000).

Analysis of serum samples from the general population showed PFOS concentrations in the tens of parts per billion concentration range (Hansen *et al.,* 2001). Serum PFOS concentrations in occupationally exposed production workers averaged 2.2 ppm (range = 0.0 to 12.8 ppm) in 1995 and 1.8 ppm (range = 0.1 to 9.9 ppm) in 1997 (Olsen *et al.,* 1999).

Perfluorooctanesulfonate is poorly eliminated from the body. In pharmacokinetic studies performed in male rats, the highest concentrations of PFOS were found in serum and liver, with liver concentrations several times higher than serum concentrations (Johnson *et al.,* 1979a,b). In an oral dosing study (Johnson *et al.,* 1979a) a serum elimination half-life of 7.5 days was reported. In an intravenous dosing study (Johnson *et al.,* 1979b) an elimination half-life was not reported; however, 89 days after a single intravenous dose, 30.2% of the dose was eliminated in the urine and 12.6% of the dose was eliminated in the feces. In the latter study at 89 days postdose, 25% of the dose was in the liver and 3% was in the plasma. In the rat, the administration of cholestyramine increased the elimination of radiolabeled PFOS 9.5-fold over 14 days, indicating that significant enterohepatic circulation occurs (Johnson *et al.,* 1984).

The properties of PFOS, good absorption, chemical stability toward degradation, and poor elimination, may explain the cumulative toxicity observed in previous subchronic rodent and primate toxicity studies (Butenhoff and Seacat, 2001; Goldenthal *et al.,* 1978a,b). The primary effects in rats were hepatocellular hypertrophy and vacuolation, decreased serum

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cholesterol, decreased triglycerides, increased liver-to-body-weight ratios, and decreased body weight, and death (Butenhoff and Seacat, 2001; Goldenthal *et al.*, 1978a). Serum cholesterol decreases were more pronounced in male rats than in female rats (Butenhoff and Seacat, 2001). In rhesus monkeys, reductions in body weight, reductions in serum lipids, and mortality appeared to be directly related to cumulative dose (Goldenthal *et al.*, 1978b).

In a two-generation rat reproduction study, toxic effects of PFOS included neonatal mortality, in the absence of significant maternal toxicity, that was attributed to exposure of the fetus *in utero* (Case *et al.*, 2001a). In developmental studies in rats and rabbits, there was no evidence of specific teratogenicity, and the embryotoxic effects observed were associated with maternal toxicity (Case *et al.*, 2001b; Henwood *et al.*, 1994a,b).

Medical surveillance of occupationally exposed 3M employees with serum PFOS concentrations less than 6 ppm revealed no associations with changes in mean values for serum hepatic enzymes, cholesterol, or lipoproteins (Olsen *et al.*, 1999). It was not possible to derive inferences from the few employees who had serum PFOS levels $\geq$ 6 ppm; although no differences were noted.

The purpose of the current study was to determine the earliest measurable responses in cynomolgus monkeys given PFOS and to define the serum PFOS concentrations at which these responses occur for use in human risk assessment. Cynomolgus monkeys were used as a representative primate species to test the hypothesis that primates are insensitive to the hepatic peroxisome proliferation observed in rodents. In addition, proliferating cell nuclear antigen immunohistochemistry (PCNA) was used to test the hypothesis that, if increased liver weight occurs as a result of PFOS exposure in primates, the increase is not due to hyperplasia. The results reported herein add significant new findings on the toxicity of PFOS in primates at lower dose levels than have been previously tested, and for the reversal of these effects in recovery. The prior study in Rhesus monkeys (Goldenthal *et al.*, 1978b) did not correlate effects with adequate measures of serum chemistry and serum PFOS concentration. Advances in the quantitation of PFOS in serum and liver tissues have made it possible to correlate the biological effects of PFOS to serum and tissue PFOS concentrations that approximate an internal dose, and to further define the toxicokinetics of PFOS in primates. The information obtained from this primate study is of value in human health risk assessment. Using serum concentration as a measure of exposure, the serum PFOS concentration associated with no adverse effects in the monkey can be compared to the results of serum concentration measurements in human populations.

MATERIALS AND METHODS

Materials, dose preparation, and analysis. Perfluorooctanesulfonate potassium salt (KPFOS, 86.9% purity, CAS 2795-39-3) was provided by 3M (Saint Paul, MN). The certificate of analysis (Center Analytical Laboratories COA reference # 023-018A), indicated 13.1% impurities. The LC/MS purity profile consisted of 8.4% lower chain length homologues of PFOS including 1.2% perfluorobutanesulfonic acid (C4), 1.3% perfluoropentanesulfonic acid (C5), 4.7% perfluorohexanesulfonic acid (C6), and 1.1% perfluoroheptanesulfonic acid (C7). The amounts of C4 and C6 homologues were calculated with authentic reference compounds, and the C5 and the C7 values were determined with the average response factors from the standard curves derived from the next lower and next higher homologues, respectively. Other impurities included 1.4% sodium, 0.6% inorganic fluoride, 0.3% perfluorooctanoate, 0.3% nonafluoropentanoic acid, and 0.1% heptafluorobutyric acid.

Lactose and acetone were purchased from Spectrum (New Brunswick, NJ, and Gardenia, CA, respectively). No. 2 gelatin capsules were purchased from Torpac Inc. (Fairfield, NJ). The dose mixtures were prepared once by mixing KPFOS dissolved in acetone with lactose, evaporating the acetone to dryness, then diluting with lactose to the desired concentration. The dose mixtures were stored at room temperature and analyzed by high-pressure liquid chromatography electrospray mass spectrometry/mass spectrometry (HPLC ESMS/MS) for perfluorooctanesulfonate anion content, homogeneity, and stability. Three samples (top, middle, and bottom) of the dose preparation for each dose group were extracted and analyzed for PFOS concentrations by HPLC-ESMS/MS methods that had been validated for serum analysis and were determined to be accurate to within $\pm$ 30%. The dose mixtures contained 103 $\pm$ 25% (average $\pm$ SD, $n = 3$) of the target concentration for the 0.15 and 0.75 mg/kg dose groups, which received different amounts of the same preparation, and 72 $\pm$ 35% for the 0.03 mg/kg dose. A combination of analytical uncertainty and lack of homogeneity of the 0.03 mg PFOS/kg/day dosing mixture may have contributed to the variation. The doses were considered to be the nominal dose for all subsequent calculations.

Animals and treatment. The study was carried out in an Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility and in compliance with the Animal Welfare Act regulations (9 CFR 1–4). Captive-bred, colony-raised, young-adult to adult male and female cynomolgus monkeys (*Macaca fasicularis*) were obtained from Covance Research Products, Inc. (Denver, PA) and acclimated for 57 days prior to treatment. Monkeys were housed individually in suspended stainless-steel cages. Room temperature was controlled to 23.5°C and humidity was controlled to 50%, with filtered air (10–15 air changes/h) and cycled lighting (12 h of light daily). Prior to the initiation of the study, all monkeys were found to be in good health, free of internal parasites and tuberculosis. Certified primate diet (#8726C, Harlan Teklad) was provided once or twice daily (unless otherwise specified), supplemented by fruit. Water was provided *ad libitum* and analysis for specific microorganisms and contaminants showed no significant findings.

At study initiation, monkeys weighing 2.4 to 4.4 kg were randomly assigned to one of the following treatment groups (6/sex/group, except where noted): vehicle control (lactose), 0.03 (4/sex), 0.15, or 0.75 mg/kg/day PFOS by intragastric intubation of a capsule dose for at least 26 weeks. Two monkeys/sex/group in the control, 0.15, and 0.75 mg/kg/day dose groups were monitored for one year after the end of the treatment period for delayed or reversible effects. Since PFOS is slowly eliminated, cumulative doses were calculated as the product of the nominal dose times the days dosed, and are expressed in units of mg/kg.

Animal observations. All monkeys were observed twice daily for mortality, morbidity, and clinical signs of adverse health effects and qualitative food consumption. Ophthalmic examinations were performed on each animal before initiation of treatment and before terminal sacrifice. Body weights were recorded predose and weekly thereafter.

PFOS level determinations in serum and liver tissues. Blood samples (~2 ml) were collected from the femoral vein of each animal 27 days before initiation of treatment, during treatment weeks 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, and 27 (day 183) and during recovery weeks 27 (days 184, 185, and 187), 28, 29, 30, 31, 35, 39, 43, 47, 51, and 53. The blood was allowed to clot, and serum was harvested within 1 h by centrifugation. Approximately 20 g of liver was collected from each animal at necropsy, and was flash-frozen in liquid nitrogen. Serum and liver samples were stored at –60 to –80°C and shipped on dry

ice to 3M Environmental Laboratory (Saint Paul, MN) for analysis of PFOS levels by HPLC ESMS/MS (Hansen *et al.*, 2001).

Hematology, serum chemistry, and hormone analysis. Blood was collected for hematology and serum chemistry on days –50, –40, and –27 before treatment, and on days 37, 62, 91, 153, and 182 of treatment from animals that had been fasted overnight. Hematologic measurements and calculations were performed using an Abbott Cell-Dyn 3500 analyzer (Abbott Laboratories, Abbott Park, IL). These measurements included: basophil count (BASO), eosinophil count (EOSIN), hematocrit (HCT), hemoglobin (HGB), lymphocyte count (LYMPH), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), monocyte count (MONO), platelet count (PLT), red blood cell count (RBC), segmented neutrophil count (N-SEG), white blood cell count (WBC), and blood cell morphology. Reticulocyte count (RETIC) was read manually from a slide.

Serum clinical chemistry parameters were assessed using a Hitachi® 704, 911 instrument (Roche Diagnostics). Measurements included: albumin (ALB), alkaline phosphatase (ALK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (BILI), blood urea nitrogen (BUN), calcium (CA), chloride (CL), cholesterol (CHOL), creatinine (CREAT), globulin (GLOB), glucose (GLU), inorganic phosphate (PHOS), potassium (K), serum bile acids (SBA), sorbitol dehydrogenase (SDH), sodium (NA), triglycerides (TRIG), and total protein (PROT). High-density lipoprotein cholesterol (HDL) was analyzed on treatment days 153 and 182 and in all samples collected after the cessation of treatment. Very low-density lipoprotein (VLDL) was analyzed from serum collected at necropsy.

For hormone analysis, serum samples were collected prior to treatment (days –50, –40, and –27), and on days 37, 62, 91, and 182 of treatment from animals that had been fasted overnight. Standard radioimmunoassay methods were used to measure serum levels of cortisol, testosterone, estradiol, estrone, estriol, total triiodothyronine (T_3), total thyroxin (T_4), free T_3 and free T_4 (AniLytics, Gaithersburg, MD). Free T_3 and free T_4 were only measured from samples taken at terminal sacrifice on day 184. Total T_3, total T_4, free T_4, and TSH values from samples taken at terminal sacrifice on day 184 were initially analyzed at AniLytics and then verified by Mayo Medical Laboratories, Rochester, MN. Thyroid hormones were analyzed using radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). Thyroid stimulating hormone (TSH) was measured by a double antibody radioimmunoassay (RIA) developed for determination of TSH in nonhuman primates by AniLytics, that used human TSH standards, polyclonal rabbit antihuman TSH antibodies (Sigma-Aldrich Co., Milwaukee, WI) and radiolabeled human TSH (Covance Laboratories Inc., Vienna, VA). Free T_3, free T_4, and TSH were measured in serum collected at necropsy by Anilytics using the methods above. Thyroid hormone values were confirmed from serum collected at necropsy by Mayo Medical Laboratories (Rochester, MN) using equilibrium dialysis followed by RIA for free T_4, and by standardized chemiluminometric immunoassays for the measurement of T_3, T_4, and TSH.

Urine and fecal analysis. Urine was collected on ice from each monkey during the overnight fasting period (16–18 h) on day –27 prior to treatment, and on days 37, 62, 91, 153, and 182 of treatment. Appearance (color and clarity), volume (graduated cylinder to the nearest ml), specific gravity (using an AO/TS refractometer), and osmolality (Osmette Precision Systems, Inc., Model 5004/Z430E) were determined. The pH, protein, bilirubin, blood, urobilinogen, glucose, and ketones were measured using Ames Multistix (Miles, Inc.). Sediment was examined by light microscopy at low and high power to observe crystals and casts.

Fecal samples, collected during the 23rd week of dosing from all animals in the control and high-dose groups, were analyzed for urobilinogen content (Mayo Medical Laboratories, Rochester, MN).

Necropsy. Four monkeys/sex from the control, 0.03, 0.15 mg/kg/day dose groups and 4 females and 2 males from the 0.75 mg/kg/day dose group were fasted overnight and sacrificed on days 184 and 185. Animals were anesthetized with ketamine and xylazine, weighed, bled for required tests, exsanguinated, necropsied, and examined for gross alterations. Unscheduled necropsies were performed on two 0.75 mg/kg/day dose group males prior to cessation of treatment, one that died on treatment day 155 and one that was sacrificed in a moribund condition on day 179. Organ weights were obtained for the adrenals, brain, epididymis, kidneys, liver, ovaries, pancreas, testes, and thyroid/parathyroid glands. Organ/body weight and organ/brain weight ratios were calculated. Sections of aorta, cecum, cervix, duodenum, esophagus, eyes (preserved in Davisons's fixative), femur, gall bladder, heart, ileum, jejunum, lesions, liver (left lateral lobe), lung, mammary gland, mesenteric lymph node, pituitary, prostate, rectum, mandibular salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, spleen, sternum with bone marrow, stomach, thymus, trachea, urinary bladder, uterus, and vagina were fixed in 10% formalin, stained with hematoxylin and eosin, and embedded in paraffin for subsequent histopathological evaluation. Sections of liver were analyzed by electron microscopy. Samples of liver, testes and pancreas were also examined for replicative DNA synthesis by immunohisotochemical detection, with PCNA. Bone marrow smears from the sternum were stained with Wright's stain and retained for possible future analysis.

Liver samples were obtained at necropsy from recovery 0.75 mg/kg/day dose group monkeys one year after the cessation of treatment (day 553). The mid-dose recovery animals were not sacrificed in this study.

Partial hepatectomy. Liver samples were obtained surgically under anesthesia by partial hepatectomy from the 0.75 mg/kg/day dose group recovery monkeys 6 months after cessation of treatment (day 393) and from the 0.15 mg/kg/day dose group recovery monkeys one year after the cessation of treatment (day 553).

Anatomic pathology. All tissues from the control and 0.75 mg/kg/day dose group monkeys were examined by light microscopy. Liver, thymus, and spinal cord were examined from animals in the 0.03 and 0.15 mg/kg/day dose groups. Liver tissue was also examined by electron microscopy (EM).

Determination of hepatic peroxisomal proliferation. Samples of the right lateral lobe of the liver were flash-frozen in liquid nitrogen at necropsy and stored at –70°C until analyzed for palmitoyl CoA oxidase activity using a Hitachi® 704,911 autoanalyzer (Abbott Laboratories, Abbott Park, IL) to determine peroxisome proliferation (Lazarow, 1981). Samples of liver tissue were fixed in gluteraldehyde and evaluated by transmission electron microscopy for the relative quantity and size of peroxisomes.

Immunohistochemistry for cell proliferation. PCNA was performed as previously described (Eldridge *et al.*, 1993) at Pathology Associates International (Frederick, MD). Briefly, tissue sections were incubated with a monoclonal antibody to PCNA. PCNA expression in cells was localized by the red chromagen, 3,3′-diaminobenzidine, within the nuclei compared to the hematoxylin-counterstained blue nuclei of nonlabeled cells. The labeling index was determined as the total number of labeled cells divided by the total number of cells counted (minimum $\geq$ 1000) multiplied by 100.

Regulatory compliance. All aspects of this study were conducted in accordance with the Environmental Protection Agency Good Laboratory Practice Regulations, 40 CFR 792, with the exception of the analysis performed at the Mayo Medical Laboratories.

Statistical analysis. Differences from control values were determined by one-way ANOVA, if applicable, and where significant, followed by a Dunnett's *t*-test used for control versus treated group comparisons (Dunnett, 1964; Levene, 1960; Winer, 1971). One-way analysis of covariance (ANCOVA) was used to analyze body weights, with initial body weight as the covariate, and where significant, the covariate adjusted mean (CAM) body weights were used for control versus treated group comparisons by a Dunnett's *t*-test. Differences from pretreatment values were determined by a paired Student's *t*-test for measurements taken at each time point during treatment (Daniel, 1987). *P*-values less than 0.05 were considered to be statistically significant. The term significant, when used in describing results, refers to statistically significant differences, unless stated otherwise. The highest biological significance was attributed when both the within-dose group and time-related pair-wise comparisons were statistically significant. Palmitoyl CoA oxidase activity less that 2-fold greater than control were not considered to be adverse effects (Doull *et al.*, 1999).

TABLE 1
Effects of PFOS on Body Weights, Absolute and Relative Liver Weights, and Serum and Liver PFOS Concentrations in Cynomolgus Monkeys after 183 Days of Treatment

Daily dose (cumulative dose)[a]	0 (0)		0.03 (4.6)		0.15 (22.9)		0.75 (114.7)	
	Male	Female	Male	Female	Male	Female	Male	Female
Body weights (kg)[b,c]	3.7 ± 0.7	3.0 ± 0.4	3.9 ± 0.6	3.2 ± 0.7	3.3 ± 0.3	3.1 ± 0.5	3.2 ± 0.8	2.8 ± 0.4
Body weight change (%)[c,d]	14 ± 11%	5 ± 5%	16 ± 8%	6 ± 7%	8 ± 7%	4 ± 5%	−8 ± 8%*	−4 ± 5%*
Mean liver weights (g)[e]	54.9 ± 8.1	51.1 ± 9.4	62.1 ± 5.3	56.8 ± 12.6	57.3 ± 5.5	57.0 ± 3.1	85.3 ± 38.4[c]	75.3 ± 13.3*
Mean relative liver weights (% of body weight)[e]	1.6 ± 0.2	1.8 ± 0.2	1.7 ± 0.3	1.9 ± 0.0	1.8 ± 0.1	2.1 ± 0.2	2.7 ± 0.3*	2.9 ± 0.3*
Mean relative liver weights (% of brain weight)[e]	0.87 ± 0.12	0.91 ± 0.22	1.01 ± 0.10	0.94 ± 0.21	0.91 ± 0.06	0.98 ± 0.06	1.26 ± 0.43	1.32 ± 0.23*
Mean serum PFOS (ppm)[c]	0.05 ± 0.01	0.05 ± 0.02	15.8 ± 1.4*	13.2 ± 1.4*	82.6 ± 25.2*	66.8 ± 10.8*	173 ± 37*	171 ± 22*
Mean liver PFOS (ppm)[e]	0.12 ± 0.03	0.11 ± 0.02	17.3 ± 4.7*	22.8 ± 2.1*	58.8 ± 19.5*	69.5 ± 14.9*	395 ± 24*	273 ± 14*
Liver PFOS:serum PFOS ratio (SD)[e, f]	2.2:1 (0.4)	2.7:1 (0.4)	2.2:1(0.4)	1.9:1 (0.2)	0.9:1 (0.3)	1.2:1 (0.3)	2.1:1 (0.3)	1.7:1 (0.4)
% PFOS in liver[g]	NA	NA	6.4 ± 1.4%	8.7 ± 1.0%	4.4 ± 1.6%	6.0 ± 1.5%	7.9 ± 1.6%	6.2 ± 1.0%

Note. Values are given as the mean ± SD, except where noted. Daily dose is given in mg/kg/day, cumulative dose in mg/kg.

[a]The cumulative dose of the perfluorooctanesulfonate anion was calculated as 80.6% of the nominal dose of KPFOS on a mole percent basis over the entire treatment period (183 days).

[b]Mean ± SD of the body weights on day 184.

[c]n = 6/sex for the 0, 0.15, and female 0.75 mg/kg/day dose groups; n = 4/sex for the 0.03 mg/kg/day dose group and the male 0.75 mg/kg/day dose group. The n includes values from the recovery group monkeys.

[d]Percent body weight change from day 0 to day 184 (mean ± SD).

[e]n = 4/sex/dose group, except that in the male control dose group n = 3, and in the male 0.75 mg/kg/day dose group n = 2 (values are: mean ± range).

[f]Values given for the Liver PFOS: Serum PFOS ratios are the mean L/S ratio followed by the SD in parenthesis.

[g]Percent of the cumulative PFOS administered during treatment that was present in liver at the end of treatment.

*Significantly different from control using Dunnett's t-test ($p \leq 0.05$).

RESULTS

Animal Observations

Two males in the 0.75 mg/kg/day dose group either died or were sacrificed *in extremis* during the study. A male that died on day 155 (cumulative dose = 116 mg/kg) had histological indications of pulmonary necrosis with a severe acute recurrence of pulmonary inflammation as a probable cause of death. This monkey also had an elevated serum creatinine phosphokinase (954 IU/l) from a serum sample taken on day 153. At death, this monkey weighed 2.8 kg and had lost 0.4 kg (13% of his initial body weight) since the beginning of treatment. Another 0.75 mg/kg/day male was found in a moribund condition on day 179 (cumulative dose = 134 mg/kg), and was sacrificed. This monkey had elevations in serum concentrations of creatine phosphokinase (4040 IU/l), creatinine (1.8 mg/dl), blood urea nitrogen (28 mg/dl), sorbitol dehydrogenase (10 mg/dl), alkaline phosphatase (876 IU/l), serum bile acids (58 μM) and potassium (7.5 mM). This male weighed 3.9 kg at sacrifice and had gained 0.5 kg (14% of his initial body weight). The specific cause of this monkey's moribund condition was not determined after thorough examination of all data available; however, the information available suggested hyperkalemia.

Body and Organ Weight

The mean percent change of initial body weight was significantly reduced in male and female monkeys given 0.75 mg/kg/day (Table 1). Two out of 6 females and 4 out of 6 males in this dose group lost weight beginning on treatment weeks 12 and 15, respectively.

Mean absolute liver weights were significantly increased in females, and liver-to-body weight ratios were significantly increased in both males and females in the 0.75 mg/kg/day dose group (Table 1). The liver-to-brain weight ratios were also significantly increased in the 0.75 mg/kg/day dose group females. The mean left adrenal-to-body weight percentages were significantly increased in males in the 0.75 mg/kg/day dose group (0.0120 ± 0.0003%, n = 2) compared to the controls (0.0083 ± 0.0012%, n = 4). No significant changes in absolute or relative organ weights (organ to body or organ to brain weight ratios), or anatomic pathology occurred in the 0.03 or 0.15 mg/kg/day dose groups.

Analysis of Serum and Liver PFOS Levels

The mean daily intake of PFOS was calculated on a mole percent basis as 80.6% of the nominal KPFOS dose for each dose group. Serum PFOS concentrations showed a linear increase in both the low- and mid-dose groups and a nonlinear

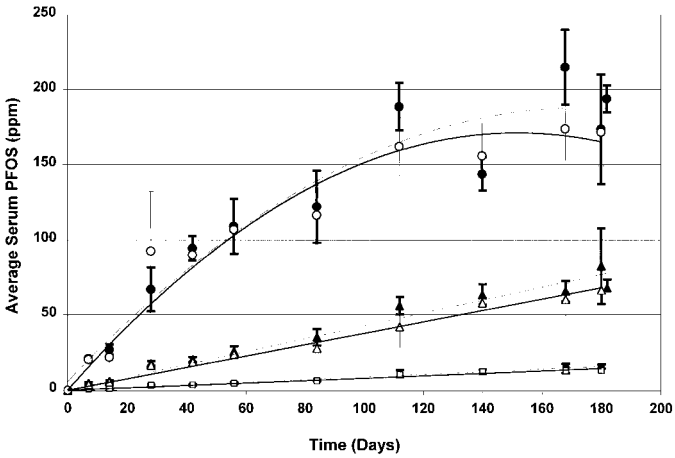

FIG. 1. Serum PFOS concentrations in monkeys given PFOS for 183 days. Male (filled symbols, dashed lines) and female (open symbols, solid lines) cynomolgus monkeys treated with 0.03 (squares), 0.15 (triangles), or 0.75 (circles) mg PFOS/kg/day.

response in the high-dose group, which appeared to plateau (Fig. 1). Serum PFOS concentrations were less than 0.05 ppm in the control males and females throughout the treatment period. The average liver-to-serum PFOS concentration ratios ranged from 0.9:1 to 2.7:1, without a dose-response relationship (Table 1). The average percent of the cumulative dose of PFOS found in the liver ranged from 4.4 ± 1.6% to 8.7 ± 1.0% without any apparent correlation to the dose group or gender (Table 1).

Hematology

Hematology results are indicated in Table 2. A reduction in hemoglobin was present in males of the 0.75 mg/kg/day dose group at termination of the study and was considered treatment-related. These males had a mean hemoglobin of 11.4 ± 0.8 g/dl as compared to a mean of 12.5 ± 0.5 in the control group. Although statistically significant, the reduction in hemaglobin observed in the male 0.75 mg/kg/day dose group are not considered biologically significant given that all values were within the published range and no stools were found to be discolored (black). There were no other consistent changes in hematological parameters for males that were considered treatment-related, and no changes in hematological parameters were observed in treated females.

Clinical Chemistry and Urinalysis

All significant results of the clinical chemistry determinations are represented in Table 3. Significant changes in total cholesterol values that were considered treatment-related were observed in males and females in the 0.75 mg/kg/day dose group on days 91, 153 and 182. Serum total cholesterol values in the 0.75 mg/kg/day dose group were significantly lower in males than in females at day 182 ($p = 0.013$). In this dose group, cholesterol decreased during the treatment period to 35 and 53% of their predose values in males and females, respectively. In the males, lower cholesterol occurred by day 91 at serum PFOS concentrations of approximately 150 ppm (cumulative dose = 68 mg/kg). In the females, decreased serum cholesterol occurred by day 62 at serum PFOS concentrations of approximately 110 ppm (cumulative dose = 46.5 mg/kg).

Serum total cholesterol values in the 0.03 mg/kg/day dose group males on days 62 and 182 and females on day 153 were significantly lower than control values, but not compared to within-group pretreatment values (Table 3). Since total serum cholesterol values were not reduced in the 0.15 mg/kg/day dose group males or females, the intermittent reductions as compared to control observed in the 0.03 mg/kg/day monkeys were not considered meaningful.

The HDL cholesterol was measured only on days 153 and 182, and pretreatment values were not obtained. In males, HDL concentrations were significantly lower than controls in the 0.03 and 0.75 mg/kg/day dose group at days 153 and 182 (Table 3). For females, HDL concentrations were significantly lower than controls in the 0.15 and 0.75 mg/kg/day dose groups at days 153 and 182 (Table 3).

Serum bilirubin in the 0.75 mg/kg/day dose group males were significantly lower than controls on days 91, 153, and 182. Elevated serum bile acid concentrations were observed in males given 0.75 mg/kg/day on day 182 only. This was partially the result of a value of 58 μM for a male sacrificed in moribund condition on day 179. No significant differences in serum bilirubin or serum bile acids were observed in females at any dose.

The creatine phosphokinase values were inconsistent over dose, time, and gender, and were exceptionally high in all pretreatment animals for unknown reasons. Therefore, subsequent mean values were primarily significantly lower than pretreatment values.

Considering the increase in absolute and/or relative liver weight observed at necropsy in the 0.75 mg/kg/day males and females, it is interesting to note that no treatment-related increases were observed in mean serum values for sorbitol dehydrogenase, transaminases, or alkaline phosphatase (Table 3).

No significant findings were observed for any of the other clinical chemistry measurements.

There were no significant changes in urinalyses except for a single observation, on day 62, when the average urine pH of females given 0.75 mg PFOS/kg/day dose group was significantly less than the control group (pH of 7.6 ± 0.4 vs. 8.2 ± 0.3, respectively).

Hormone Analysis

Thyroid hormone values for males and females are given in Table 4. On day 182, and based on confirmation in samples from terminal sacrifice on day 184, an increase in TSH (approximately twice control value) and a decrease in total T_3

TABLE 2
Effects of PFOS on Clinical Hematology Values in Cynomolgus Monkeys after 183 Days of Treatment

Dose group (mg/kg/day)	Day –27	Day 37	Day 62	Day 91	Day 153	Day 182
Males						
WBC ($\times 10^3/\mu l$)						
0	8.6 ± 1.8	7.6 ± 2.0	7.3 ± 0.8	6.7 ± 2.0	7.3 ± 2.6	9.7 ± 1.8
0.03	11.0 ± 1.0	10.8 ± 1.9	10.5 ± 1.8	8.5 ± 1.6	9.9 ± 1.0*	8.4 ± 0.7*
0.15	11.9 ± 2.0	10.0 ± 2.1*	11.8 ± 3.0**	10.3 ± 3.2	10.1 ± 2.4*	10.0 ± 2.4
0.75	9.4 ± 2.5	10.3 ± 4.2	9.6 ± 3.0	9.4 ± 2.6	9.0 ± 2.9	7.6 ± 2.1
N-SEG ($\times 10^3/\mu l$)						
0	3.9 ± 1.3	3.9 ± 2.0	3.0 ± 0.7	2.4 ± 0.6	2.7 ± 1.6	4.2 ± 1.7
0.03	5.2 ± 1.9	6.3 ± 2.4	6.1 ± 1.5**	4.4 ± 1.8	5.6 ± 1.7	4.8 ± 1.5
0.15	6.6 ± 2.4	6.0 ± 2.2	7.0 ± 3.2**	5.7 ± 1.9**	5.2 ± 2.8*	5.2 ± 2.1
0.75	3.7 ± 1.4	6.6 ± 3.7	5.3 ± 2.5	5.1 ± 2.1**	4.3 ± 3.0	2.9 ± 1.1
LYMPH ($\times 10^3/\mu l$)						
0	4.0 ± 0.9	3.1 ± 0.7	3.5 ± 0.9	3.7 ± 1.2	3.9 ± 0.9	4.4 ± 1.5
0.03	4.6 ± 1.1	3.7 ± 1.3	3.4 ± 0.8	3.3 ± 0.9	3.5 ± 1.1	2.8 ± 0.7
0.15	4.1 ± 1.1	3.2 ± 0.9	3.9 ± 1.2	3.7 ± 1.6	4.0 ± 1.2	4.1 ± 1.4
0.75	4.3 ± 1.25	2.9 ± 0.5	3.4 ± 0.5	3.5 ± 0.7	3.6 ± 1.1	4.0 ± 1.2
N-SEG %						
0	44 ± 9	50 ± 14	42 ± 11	37 ± 4	35 ± 9	45 ± 16
0.03	47 ± 15	58 ± 16	58 ± 10	50 ± 14	56 ± 15	57 ± 15
0.15	54 ± 13	58 ± 3	56 ± 16	56 ± 9**	49 ± 18	50 ± 15
0.75	39 ± 11	62 ± 10*	52 ± 10*	52 ± 10*·**	45 ± 16	38 ± 7
LYMPH %						
0	47 ± 9	43 ± 13	48 ± 8	55 ± 3	54 ± 8	46 ± 12
0.03	43 ± 9	36 ± 14	32 ± 7*	40 ± 13**	36 ± 14	34 ± 11
0.15	36 ± 11	33 ± 10	35 ± 15	36 ± 8**	42 ± 16	42 ± 14
0.75	46 ± 10	30 ± 8*	38 ± 11	38 ± 8*·**	44 ± 16	53 ± 9*
HCT (%)						
0	41.2 ± 1.30	40.6 ± 1.4	41.1 ± 1.8	40.3 ± 2.2	40.9 ± 2.3	41.1 ± 2.1
0.03	42.9 ± 3.0	42.4 ± 3.9	41.4 ± 1.4	42.5 ± 1.8	41.7 ± 1.9	42.1 ± 1.8
0.15	42.9 ± 1.9	41.2 ± 0.8	40.7 ± 1.4*	40.4 ± 0.9*	41.3 ± 1.5	41.0 ± 1.15
0.75	43.1 ± 2.1	40.2 ± 1.7*	41.2 ± 2.3*	41.0 ± 2.2*	40.0 ± 3.3*	38.8 ± 2.5*
RBC ($\times 10^6/\mu l$)						
0	6.45 ± 0.28	6.45 ± 0.22	6.49 ± 0.29	6.43 ± 0.32	6.68 ± 0.42	6.60 ± 035
0.03	6.99 ± 0.69	6.86 ± 0.50	6.84 ± 0.44	7.00 ± 0.63	7.08 ± 0.51	6.69 ± 0.63
0.15	6.76 ± 0.32	6.66 ± 0.39	6.60 ± 0.32	6.50 ± 0.30*	6.78 ± 0.48	6.65 ± 0.37
0.75	6.88 ± 0.56	6.53 ± 0.43*	6.73 ± 0.52*	6.66 ± 0.49	6.65 ± 0.64	6.32 ± 0.39
HGB (g/dl)						
0	12.2 ± 0.4	12.4 ± 0.6	12.5 ± 0.5	12.4 ± 0.5	12.5 ± 0.5	12.5 ± 0.5
0.03	12.4 ± 0.5	12.5 ± 0.4	12.4 ± 0.3	12.7 ± 0.5	12.6 ± 0.5	12.5 ± 0.5
0.15	12.6 ± 0.4	12.5 ± 0.3	12.5 ± 0.5	12.3 ± 0.2	12.6 ± 0.4	12.5 ± 0.3
0.75	12.5 ± 0.8	11.8 ± 0.6*	12.3 ± 0.8	12.2 ± 0.8*	11.8 ± 1.0*	11.4 ± 0.8*·**
Females						
WBC ($\times 10^3/\mu l$)						
0	10.2 ± 2.5	12.7 ± 4.8	11.1 ± 2.9	9.4 ± 1.9	9.7 ± 2.4	10.4 ± 3.6
0.03	8.7 ± 2.4	9.6 ± 2.4	8.7 ± 1.4	8.7 ± 3.4	9.3 ± 4.5	8.5 ± 2.1
0.15	10.1 ± 2.9	9.1 ± 2.7	8.8 ± 1.8	8.7 ± 1.3	8.1 ± 2.2	9.2 ± 1.6
0.75	9.0 ± 3.1	8.9 ± 2.9	9.3 ± 4.6	8.1 ± 1.9	9.5 ± 3.0	9.7 ± 2.7
N-SEG ($\times 10^3/\mu l$)						
0	4.6 ± 1.1	7.8 ± 3.8	5.8 ± 3.1	4.0 ± 1.3	4.6 ± 3.0	5.0 ± 3.4
0.03	5.0 ± 2.5	5.7 ± 1.9	4.4 ± 1.6	4.4 ± 2.7	5.0 ± 3.6	4.3 ± 1.5
0.15	5.1 ± 2.8	5.5 ± 2.5	4.3 ± 1.8	4.4 ± 0.8	3.7 ± 2.0	4.5 ± 1.7
0.75	4.8 ± 1.7	5.3 ± 3.2	5.6 ± 3.9	4.6 ± 1.8	4.4 ± 2.3	4.2 ± 1.1
LYMPH ($\times 10^3/\mu l$)						
0	4.5 ± 1.4	3.6 ± 1.6	4.3 ± 1.7	4.3 ± 1.6	4.1 ± 1.1	4.3 ± 2.0
0.03	2.9 ± 0.8	2.8 ± 0.8	3.4 ± 1.2	3.2 ± 1.2	2.9 ± 0.6	3.2 ± 0.6
0.15	3.8 ± 0.4	2.9 ± 0.7	3.6 ± 0.9	3.4 ± 0.7	3.4 ± 1.0	3.8 ± 0.6
0.75	3.2 ± 1.7	2.9 ± 1.1	3.0 ± 1.1	2.9 ± 1.0	4.0 ± 2.2	4.6 ± 2.7

TABLE 2—*Continued*

Dose group (mg/kg/day)	Day –27	Day 37	Day 62	Day 91	Day 153	Day 182
N-SEG %						
0	45 ± 8	59 ± 18	50 ± 18	44 ± 13	44 ± 19	46 ± 18
0.03	55 ± 14	60 ± 11	50 ± 15	48 ± 13	50 ± 13	50 ± 9
0.15	48 ± 13	56 ± 17	48 ± 13	51 ± 6	44 ± 5	48 ± 13
0.75	54 ± 11	56 ± 15	56 ± 13	56 ± 13	46 ± 17	46 ± 15
LYMPH %						
0	44 ± 8	30 ± 16	40 ± 16	46 ± 10	44 ± 16	44 ± 15
0.03	35 ± 12	29 ± 6	40 ± 15	39 ± 11	36 ± 16	38 ± 10
0.15	40 ± 8	35 ± 16	42 ± 11	39 ± 7	44 ± 14	43 ± 12
0.75	35 ± 10	35 ± 14	36 ± 14	36 ± 10	43 ± 17	45 ± 15
HCT (%)						
0	39.3 ± 3.9	40.5 ± 2.4	40.2 ± 3.3	40.3 ± 2.8	40.6 ± 3.0	40.8 ± 2.7
0.03	39.5 ± 3.3	40.6 ± 2.9	40.2 ± 2.4	39.2 ± 2.2	40.4 ± 2.44	38.3 ± 5.7
0.15	40.1 ± 2.0	39.7 ± 1.1	42.3 ± 6.7	39.4 ± 1.4	39.9 ± 1.3	39.9 ± 1.8
0.75	41.5 ± 1.7	39.1 ± 2.0*	40.2 ± 2.0	40.3 ± 1.5	39.5 ± 2.0	39.2 ± 2.17
RBC ($\times 10^6/\mu$l)						
0	6.09 ± 0.68	6.41 ± 0.35	6.23 ± 0.77	6.31 ± 0.57	6.60 ± 0.56	6.40 ± 0.46
0.03	6.31 ± 0.52	6.60 ± 0.66	6.48 ± 0.59	6.20 ± 0.47	6.66 ± 0.64	6.15 ± 0.80
0.15	6.54 ± 0.32	6.55 ± 0.34	7.00 ± 1.1	6.50 ± 0.30	6.66 ± 0.26	6.57 ± 0.26
0.75	6.50 ± 0.20	6.21 ± 0.28	6.28 ± 0.40	6.32 ± 0.21	6.33 ± 0.29	6.09 ± 0.36
HGB (g/dl)						
0	12.0 ± 1.0	12.2 ± 0.7	12.3 ± 0.7	12.3 ± 0.8	12.4 ± 0.9	12.1 ± 0.6
0.03	11.5 ± 1.1	11.8 ± 1.0	12.0 ± 0.8	11.7 ± 1.0	12.0 ± 1.0	11.2 ± 1.7
0.15	12.0 ± 0.5	11.8 ± 0.4	12.8 ± 2.2	12.0 ± 0.4	12.1 ± 0.5	11.9 ± 0.6
0.75	12.1 ± 0.5	11.6 ± 0.4	11.8 ± 0.3	11.9 ± 0.4	11.7 ± 0.4	11.3 ± 0.4

Note. Values are given as the mean ± SD, except where noted. $n = 6$/sex for the 0, 0.15, and female 0.75 mg/kg/day dose groups; $n = 4$/sex for the 0.03 mg/kg/day dose group and the male 0.75 mg/kg/day dose group on day 182. The *n* includes values from the recovery group monkeys. WBC, white blood cell count; N-SEG, segmented neutrophil count; LYMPH, lymphocyte count; HCT, hematocrit; RBC, red blood cell count;HGB, hemoglobin.

*Significantly different from pretreatment values (day –27) by a two-tailed, paired Students *t*-test.

**Significantly different from control using Dunnett's *t*-test ($p < 0.05$, 2-tailed, homoscedastic).

were present in the males and females of the 0.75 mg/kg/day dose group. Free T_3 was also lower in these animals based on a single analysis of samples from terminal sacrifice on day 184; however, the Mayo Medical Laboratory was unable to perform this test to confirm the AniLytics results. Even though the lowered free T_3 levels were not confirmed, they are believed to be valid based on the lower total T_3 values. All other variations in thyroid hormone values over the course of the study were not consistent with respect to dose response or over time, including T_4.

The mean estradiol for females was not significantly different from the control value (Table 5). However, 2 of the 6 females had lowered estradiol levels at end of treatment (9.45 and 0.00 pg/dl, respectively). Male estradiol levels in the 0.75 mg/kg/day dose group were significantly lower than controls at the end of treatment, and were significantly lower than pretreatment values as early as day 62. Two out of 4 males had estradiol values of 0 on day 182. The lowered estradiol levels in the 0.75 mg/kg/day dose group were confirmed by analysis of serum samples collected at terminal sacrifice on day 184 (data not shown). Mean testosterone values were not significantly different between treatment groups in males or females (Table 5).

Hepatic Peroxisome Proliferation and Cell Proliferation

Hepatic peroxisome proliferation, as measured by palmitoyl CoA oxidase activity, was significantly increased in the female 0.75 mg/kg/day dose group; however, it did not exceed the criteria for biological significance of a 2-fold increase over the control values (Table 6). Treatment had no significant effect on cell proliferation in the liver, pancreas, or testes after 182 days of treatment as determined by PCNA immunohistochemistry cell labeling index.

Histopathology

No treatment-related effects were observed in any of the tissues examined (liver, thymus, and spinal cord) in the 0.03 and 0.15 mg/kg/day dose groups. By light microscopy, centrilobular vacuolation, hypertrophy, and mild bile stasis were noted in some of the 0.75 mg/kg/day dose group livers. By electron microscopy, lipid-droplet accumulation was seen in two high-dose males and in 2 of 4 female livers (e.g., Figs. 2A and 2B), but not at the lower treatment levels, or after 7 months of recovery (Fig. 2C). Increased glycogen content was also noted at 0.75 mg/kg/day in 1 of 2 males and 2 of 4 females by electron microscopy.

TABLE 3
Effects of PFOS on Clinical Chemistry Values in Cynomolgus Monkeys after 183 Days of Treatment

Dose group (mg/kg/day)	Day –27	Day 37	Day 62	Day 91	Day 153	Day 182
Males						
Cholesterol (mg/dl)						
0	138 ± 34	140 ± 22	153 ± 23	154 ± 25	154 ± 30	152 ± 28
0.03	110 ± 20	118 ± 28	114 ± 25**	126 ± 15	120 ± 16	110 ± 17**
0.15	151 ± 26	146 ± 22	144 ± 20	150 ± 19	149 ± 23	147 ± 24
0.75	138 ± 29	130 ± 18	125 ± 21	112 ± 27*,**	65 ± 20*,**	48 ± 19*,**
HDL (mg/dl)						
0	ND	ND	ND	ND	69 ± 11	63 ± 11
0.03	ND	ND	ND	ND	46 ± 5**	42 ± 4**
0.15	ND	ND	ND	ND	55 ± 13	48 ± 14
0.75	ND	ND	ND	ND	19 ± 7**	13 ± 5**
Triglyceride (mg/dl)						
0	70 ± 13	69 ± 19	62 ± 19	68 ± 20	60 ± 18*	45 ± 9*
0.03	61 ± 35	47 ± 5	60 ± 15	54 ± 10	60 ± 25	33 ± 7
0.15	56 ± 5	58 ± 5	57 ± 13	50 ± 10	65 ± 10	36 ± 10*
0.75	67 ± 11	91 ± 27	76 ± 17	73 ± 21	60 ± 26	30 ± 12*
ALK PHOS (IU/l)						
0	711 ± 253	888 ± 340*	839 ± 322	866 ± 296	792 ± 305	735 ± 319
0.03	700 ± 266	876 ± 384	750 ± 370	789 ± 402	684 ± 348	604 ± 353
0.15	770 ± 210.3	896 ± 267*	806 ± 238	851 ± 265	853 ± 208*	688 ± 178*
0.75	840 ± 360.9	996 ± 417	823 ± 367	807 ± 429	646 ± 384	389 ± 156
ALT/SGPT (IU/l)						
0	32 ± 10	39 ± 20	34 ± 15	35 ± 16	43 ± 27	39 ± 18
0.03	36 ± 11	40 ± 13	35 ± 18	60 ± 56	36 ± 20	29 ± 9
0.15	69 ± 36	50 ± 24*	37 ± 12*	45 ± 22	55 ± 17	47 ± 17
0.75	80 ± 54	46 ± 23	43 ± 16	44 ± 21	52 ± 27	47 ± 20
Total bilirubin (mg/dl)						
0	0.6 ± 0.5	0.8 ± 0.4*	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.4	0.6 ± 0.2
0.03	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1**
0.15	0.6 ± 0.4	0.6 ± 0.3	0.5 ± 0.2*	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1
0.75	0.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1**	0.2 ± 0.1*,**	0.2 ± 0.1**
SBA (mg/dl)						
0	9 ± 2	9 ± 2	9 ± 2	8 ± 1	10 ± 2*	6 ± 0.8*
0.03	9 ± 1	8 ± 1	10 ± 2	8 ± 1	7 ± 1	5 ± 1*
0.15	10 ± 2.2	8 ± 1	10 ± 2	9 ± 3	10 ± 2	6 ± 1*
0.75	9 ± 1	7 ± 2*	10 ± 3	10 ± 4	14 ± 6*	18 ± 9**
CK (IU/l)						
0	1133 ± 535	354 ± 461	132 ± 28*	168 ± 32*	165 ± 28*	210 ± 159*
0.03	936 ± 709	191 ± 58	149 ± 48	175 ± 43	133 ± 43	138 ± 59
0.15	1087 ± 322	184 ± 60*	153 ± 20*	164 ± 36*	193 ± 38*	174 ± 102*
0.75	1094 ± 497	283 ± 150*	203 ± 57*,**	214 ± 7*	352 ± 298*,**	182 ± 65**
SDH (IU/l)						
0	7 ± 2	7 ± 3	7 ± 2	7 ± 3	3 ± 3*	6 ± 4
0.03	3 ± 1	3 ± 2	4 ± 2	4 ± 1	2 ± 2	4 ± 2
0.15	8 ± 5	5 ± 3	5 ± 4	6 ± 4	4 ± 3	6 ± 4
0.75	6 ± 2	2 ± 0*,**	3 ± 1*	2 ± 1*,**	2 ± 1*	3 ± 1
I PHOS (mg/dl)						
0	6.2 ± 0.8	6.7 ± 0.8*	7.1 ± 0.7*	6.8 ± 0.4	6.6 ± 0.3	6.4 ± 0.8
0.03	6.6 ± 0.4	6.9 ± 1.6	6.8 ± 0.9	6.7 ± 0.7	6.3 ± 1.1	5.8 ± 1.0
0.15	7.1 ± 1.0	6.8 ± 1.0	6.6 ± 1.1	6.8 ± 1.1	6.8 ± 1.0	6.2 ± 0.8
0.75	6.9 ± 1.1	6.6 ± 1.3	6.8 ± 1.3	6.4 ± 1.0	6.8 ± 1.7*	6.1 ± 1.2*
Females						
Cholesterol (mg/dl)						
0	149 ± 37	147 ± 27	155 ± 46	166 ± 42*	163 ± 49	160 ± 47
0.03	130 ± 12	124 ± 11*	127 ± 11	134 ± 16	110 ± 22**	122 ± 22
0.15	144 ± 14	133 ± 22	137 ± 20	140 ± 13	130 ± 23	129 ± 22
0.75	154 ± 10	130 ± 26	127 ± 19*	111 ± 27*,**	91 ± 23*,**	82 ± 15*,**

TABLE 3—*Continued*

Dose group (mg/kg/day)	Day –27	Day 37	Day 62	Day 91	Day 153	Day 182
HDL (mg/dl)						
0	ND	ND	ND	ND	59 ± 17	56 ± 16
0.03	ND	ND	ND	ND	47 ± 10	42 ± 9
0.15	ND	ND	ND	ND	41 ± 9**	36 ± 12**
0.75	ND	ND	ND	ND	23 ± 4**	21 ± 7**
Triglyceride (mg/dl)						
0	73 ± 32	71 ± 26	86 ± 49	67 ± 11	80 ± 47	56 ± 13
0.03	62 ± 8	64 ± 12	76 ± 22	61 ± 14	82 ± 29	49 ± 15
0.15	58 ± 12	57 ± 11	64 ± 18	56 ± 18	69 ± 14*	49 ± 16
0.75	61 ± 17	66 ± 23	61 ± 11	60 ± 14	64 ± 14	53 ± 16
ALK PHOS (IU/l)						
0	291 ± 130	362 ± 126*	360 ± 153*	329 ± 129*	315 ± 143	283 ± 137
0.03	201 ± 13	240 ± 49	241 ± 45	233 ± 45	262 ± 42*	239 ± 40
0.15	257 ± 119	319 ± 129	305 ± 134	324 ± 140	308 ± 121	254 ± 85
0.75	310 ± 141	415 ± 189*	398 ± 201*	388 ± 150*	336 ± 147*	316 ± 172
ALT/SGPT (IU/l)						
0	140 ± 77	59 ± 30*	53 ± 17*	54 ± 18*	77 ± 43	87 ± 86
0.03	103 ± 29	70 ± 13	77 ± 16*	79 ± 27	80 ± 53	72 ± 26
0.15	110 ± 41	68 ± 31*	60 ± 29*	67 ± 43*	66 ± 34*	53 ± 36*
0.75	123 ± 54	46 ± 8*	42 ± 6*	45 ± 13*	48 ± 10*	44 ± 10*
Total bilirubin (mg/dl)						
0	0.5 ± 0.3	0.6 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.3
0.03	0.5 ± 0.2	0.5 ± 0.3	0.6 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.2
0.15	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.1*	0.5 ± 0.1	0.4 ± 0.1*
0.75	0.6 ± 0.4	0.4 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
SBA (mg/dl)						
0	7 ± 2	8 ± 1	9 ± 2	8 ± 2	9 ± 1	6 ± 1
0.03	6 ± 2	9 ± 3	10 ± 4*	8 ± 1	8 ± 2*	5 ± 2
0.15	12 ± 12	8 ± 3	10 ± 3	10 ± 6	10 ± 3	8 ± 2
0.75	9 ± 1	7 ± 1*	9 ± 2	11 ± 7	15 ± 14	7 ± 3
CK (IU/l)						
0	979 ± 384	240 ± 147*	262 ± 124*	786 ± 947*	235 ± 105*	212 ± 123*
0.03	484 ± 157	378 ± 262	276 ± 68*	220 ± 39*	255 ± 132	823 ± 1282
0.15	844 ± 306	198 ± 101*	194 ± 76*	209 ± 82*	242 ± 117*	182 ± 85*
0.75	1624 ± 1252	331 ± 242	243 ± 130*	416 ± 377*	695 ± 1217	284 ± 119*
SDH (IU/l)						
0	4 ± 2	5 ± 2	4 ± 2	6 ± 2	2 ± 1*	5 ± 3
0.03	3 ± 1	3 ± 1	4 ± 2	4 ± 4	2 ± 1	6 ± 6
0.15	4 ± 2	3 ± 2	4 ± 1	4 ± 3	3 ± 3	7 ± 5
0.75	4 ± 2	2 ± 1**	2 ± 0**	2 ± 1**	2 ± 2	3 ± 2
I PHOS (mg/dl)						
0	6.2 ± 0.6	5.7 ± 0.7	6.0 ± 0.4	5.7 ± 0.7*	5.6 ± 0.3	5.3 ± 0.8*
0.03	4.7 ± 1.0	4.2 ± 0.4**	4.6 ± 0.3**	4.2 ± 0.4**	4.1 ± 0.8	4.4 ± 0.4
0.15	5.9 ± 0.8	5.1 ± 0.7*	5.1 ± 0.5*,**	5.2 ± 0.6*	5.0 ± 1.0*	4.8 ± 1.1*
0.75	5.5 ± 0.5	5.0 ± 0.5	5.0 ± 0.3**	5.1 ± 0.5	4.9 ± 0.7**	5.2 ± 0.8

Note. $n = 6$ for the 0, 0.15, and 0.75 mg/kg/day dose groups; $n = 4$ for the 0.03 mg/kg/day dose group. ND: not determined. HDL, high-density lipoprotein cholesterol; ALK PHOS, alkaline phosphatase; ALT/SGPT; SBA, serum bile acids; CK, creatine kinase; SDH, sorbitol dehydrogenase; I PHOS, inorganic phosphate.

*Significantly different from pretreatment values (day –27) by a two-tailed, paired Student's *t*-test.

**Significantly different from control using Dunnett's *t*-test ($p < 0.05$, 2-tailed, homoscedastic).

Recovery Phase

Serum PFOS concentrations declined during recovery (Fig. 3). Serum PFOS concentrations were higher during the first week of the recovery period than at the end of treatment. The serum PFOS elimination curves appeared to be multiphasic for the 0.75 mg/kg/day dose group recovery monkeys and linear for the 0.15 mg/kg/day dose group recovery monkeys. Toward the end of the one-year recovery period, the slopes of the two recovery group elimination curves were similar, suggesting that the elimination half-lives were approximately 200 days for both dose groups. There was no apparent difference in the rate of serum PFOS

TABLE 4
Effects of PFOS on Thyroid Hormone Values in Cynomolgus Monkeys

Dose group (mg/kg/day)	Day –27	Day 37	Day 62	Day 91	Day 182	Day 184	Day 184[a]
Males							
TSH (μU/ml)							
0	0.00 ± 0.00	0.34 ± 0.39	0.57 ± 0.79	0.00 ± 0.00	0.43 ± 0.52	0.37 ± 0.07	0.55 ± 0.44
0.03	0.00 ± 0.00	0.35 ± 0.27	0.42 ± 0.21	0.00 ± 0.00	0.34 ± 0.30	0.56 ± 0.13	0.56 ± 0.10
0.15	0.05 ± 0.13	0.33 ± 0.39	0.98 ± 1.12	0.03 ± 0.07	0.74 ± 0.75	0.70 ± 0.15**	1.38 ± 0.78
0.75	0.00 ± 0.00	0.21 ± 0.26	0.22 ± 0.39	0.00 ± 0.00	0.93 ± 0.57	0.93 ± 0.57**	1.43 ± 0.25*
Total thyroxine (T_4) (μg/dl)							
0	3.49 ± 0.64	4.06 ± 0.65	2.27 ± 0.67	3.89 ± 0.55	4.38 ± 0.61*	3.25 ± 0.45	3.24 ± 0.35
0.03	4.46 ± 1.48	5.11 ± 0.95	3.91 ± 0.62**	5.30 ± 0.73**	4.72 ± 0.68	3.85 ± 0.58	3.68 ± 0.5
0.15	4.63 ± 1.16	4.00 ± 0.84	3.08 ± 0.71*	4.47 ± 0.87	3.99 ± 0.62	2.71 ± 0.32	3.00 ± 0.18
0.75	4.88 ± 1.24	3.61 ± 0.55*	2.59 ± 0.59*	4.61 ± 0.57	5.34 ± 1.57	3.30 ± 1.54	3.77 ± 1.65
Total triiodothyronine (T_3) (ng/dl)							
0	110 ± 12	273 ± 30*	145 ± 17	153 ± 10	160 ± 7	115 ± 10	146 ± 19.8
0.03	113 ± 13	273 ± 32*	139 ± 14	153 ± 9*	119 ± 31**	110 ± 7	145 ± 18.0
0.15	118 ± 24	255 ± 25*	142 ± 21	147 ± 18*	125 ± 15**	94 ± 6**	129 ± 4.8
0.75	133 ± 11	239 ± 25*	121 ± 16**	118 ± 22**	66 ± 27*,**	43 ± 25*,**	76 ± 22*,**
Free T_4 (ng/dL)							
0	ND[b]	ND	ND	ND	ND	1.01 ± 0.15	1.4 ± 0.07
0.03	ND	ND	ND	ND	ND	1.21 ± 0.20	1.53 ± 0.19
0.15	ND	ND	ND	ND	ND	0.94 ± 0.11	1.47 ± 0.15
0.75	ND	ND	ND	ND	ND	1.12 ± 0.25	1.50 ± 0.10
Free T_3 (pg/ml)							
0	ND	ND	ND	ND	ND	4.21 ± 0.85	ND
0.03	ND	ND	ND	ND	ND	5.13 ± 0.39	ND
0.15	ND	ND	ND	ND	ND	4.33 ± 0.41	ND
0.75	ND	ND	ND	ND	ND	2.45 ± 0.80**	ND
Female							
TSH (μU/ml)							
0	0.21 ± 0.51	0.73 ± 0.94	1.19 ± 1.72	0.00 ± 0.00	0.73 ± 1.12	0.53 ± 0.31	1.02 ± 0.69
0.03	0.00 ± 0.00	0.24 ± 0.32	0.46 ± 0.86	0.00 ± 0.00	0.68 ± 0.82	0.43 ± 0.09	2.01 ± 2.09
0.15	0.34 ± 0.80	0.61 ± 0.70	0.82 ± 1.18	0.02 ± 0.04	1.27 ± 1.52	0.47 ± 0.20	1.33 ± 1.13
0.75	0.01 ± 0.02	0.59 ± 0.62	0.74 ± 0.85	0.00 ± 0.00	0.84 ± 0.79	1.03 ± 0.50**	1.86 ± 1.29
Total thyroxine (T_4) (μg/dl)							
0	6.46 ± 1.71	5.82 ± 1.22	3.53 ± 0.86*	4.77 ± 0.75	5.66 ± 0.89	4.14 ± 1.31	3.78 ± 1.07
0.03	7.06 ± 1.79	5.06 ± 1.18	4.07 ± 1.08*	6.00 ± 0.72	4.33 ± 1.46	3.48 ± 0.72	2.80 ± 1.9
0.15	7.13 ± 1.17	3.68 ± 0.65*,**	2.77 ± 0.88*	3.77 ± 0.70*	3.91 ± 0.62*,**	2.88 ± 0.82	3.23 ± 0.50
0.75	7.10 ± 1.30	4.23 ± 0.90*,**	3.08 ± 1.05*	4.92 ± 0.81*	5.61 ± 1.00	3.43 ± 1.05	3.80 ± 0.67
Total triiodothyronine (T_3) (ng/dl)							
0	119 ± 39	230 ± 30*	115 ± 28	143 ± 36	135 ± 31	106 ± 19	148 ± 21.6
0.03	171 ± 80	244 ± 24	136 ± 29	147 ± 19	120 ± 24	92 ± 15	139 ± 11.5
0.15	138 ± 42	214 ± 21*	114 ± 19	110 ± 10**	97 ± 8*,**	80 ± 10*,**	116 ± 16.8
0.75	155 ± 74	196 ± 6	96 ± 9	88 ± 6*,**	85 ± 12*,**	58 ± 5*,**	99 ± 16.8*
Free T_4 (ng/dL)							
0	ND[b]	ND	ND	ND	ND	1.06 ± 0.30	1.53 ± 0.28
0.03	ND	ND	ND	ND	ND	1.01 ± 0.27	1.70 ± 0.26
0.15	ND	ND	ND	ND	ND	0.90 ± 0.22	1.35 ± 0.21
0.75	ND	ND	ND	ND	ND	1.08 ± 0.30	1.57 ± 0.31
Free T_3 (pg/mL)							
0	ND	ND	ND	ND	ND	4.05 ± 0.98	ND
0.03	ND	ND	ND	ND	ND	3.59 ± 0.50	ND
0.15	ND	ND	ND	ND	ND	3.27 ± 0.42	ND
0.75	ND	ND	ND	ND	ND	2.82 ± 0.29**	ND

Note. Values are given as the mean ± SD, except where noted; $n = 6$ for the 0, 0.15, and 0.75 mg/kg/day dose groups, and $n = 4$/sex for the 0.03 mg/kg/day dose group and the male 0.75 mg/kg/day dose group on day 182. The *n* includes values from the recovery group monkeys.

[a]Confirmatory analyses of thyroid hormone values performed at Mayo Clinical Laboratories, $n = 3$ or 4 for all dose groups.

[b]ND: not determined.

*Significantly different from pretreatment values (day –27) by a two-tailed, paired Student's *t*-test ($p < 0.05$).

**Significantly different from control using Dunnett's *t*-test ($p \leq 0.05$, 2-tailed, homoscedastic).

TABLE 5
Effects of PFOS on Estradiol and Testosterone Values in Cynomolgus Monkeys

Dose group (mg/kg/day)	Day –27	Day 37	Day 62	Day 91	Day 182
Male estradiol (pg/ml)					
0	26.7 ± 5.9	24.0 ± 8.0	26.9 ± 9.3	18.7 ± 10.8	23.0 ± 11.5
0.03	32.1 ± 6.1	27.1 ± 10.2	29.6 ± 11.5	32.1 ± 6.1	24.1 ± 14.2
0.15	25.8 ± 2.7	21.2 ± 6.7	27.2 ± 8.2	19.2 ± 3.0*	23.2 ± 7.4
0.75	29.7 ± 5.1	31.8 ± 23.9	14.0 ± 9.8*,**	10.9 ± 8.9*	0.8 ± 1.0*,**
Female estradiol (pg/ml)					
0	91.5 ± 64.8	58.8 ± 20.8	84.2 ± 37.4	99.3 ± 66.2	148.5 ± 110.1
0.03	61.2 ± 11.2	56.8 ± 8.2	82.1 ± 17.1*	111.8 ± 112.0	125.2 ± 101.2
0.15	61.2 ± 26.1	105.2 ± 140.9	92.0 ± 63.1	80.1 ± 64.6	70.6 ± 62.7
0.75	89.9 ± 63.9	61.0 ± 26.0	195.8 ± 222.7	126.4 ± 140.7	39.9 ± 33.6
Male testosterone (ng/ml)					
0	1.88 ± 1.15	2.68 ± 4.01	1.09 ± 0.72	1.32 ± 0.79	1.38 ± 1.05
0.03	1.54 ± 1.12	3.48 ± 4.53	1.54 ± 1.32	3.48 ± 4.89	2.55 ± 3.67
0.15	1.84 ± 1.87	1.00 ± 0.56	1.15 ± 0.85	1.18 ± 0.51	1.13 ± 0.97
0.75	1.72 ± 1.34	9.42 ± 14.86	1.22 ± 0.73	1.46 ± 0.92	1.16 ± 0.96
Female testosterone (ng/ml)					
0	0.28 ± 0.18	0.25 ± 0.13	0.19 ± 0.10	0.43 ± 0.34	0.18 ± 0.21
0.03	0.28 ± 0.14	0.30 ± 0.07	0.34 ± 0.09	0.33 ± 0.03	0.21 ± 0.08
0.15	0.25 ± 0.25	0.2 ± 0.16	0.16 ± 0.19	0.14 ± 0.16	0.05 ± 0.06
0.75	0.25 ± 0.18	0.15 ± 0.08	0.30 ± 0.38	0.27 ± 0.17	0.12 ± 0.06

Note. Values are given as the mean ± SD, except where noted; $n = 6$ for the 0, 0.15, and 0.75 mg/kg/day dose groups, and $n = 4$ for the 0.03 dose group, except where noted.

*Significantly different from pretreatment values (day –27) by a two-tailed, paired Student's *t*-test ($p < 0.05$).

**Significantly different from control using Dunnett's *t*-test ($p \leq 0.05$, 2-tailed, homoscedastic).

decrease between males and females in either the 0.75 mg/kg/day or the 0.15 mg/kg/day dose groups over the one-year recovery period.

TABLE 6
Effects of PFOS on Hepatic Palmitoyl CoA Oxidase Activity (PCOAO) and Cell Proliferation (PCNA) in Cynomolgus Monkeys after 182 Days of Treatment

Dose (mg/kg/day)	0	0.03	0.15	0.75
PCOAO (IU/G)				
Male	5.0 ± 1.5	5.0 ± 1.0	6.0 ± 0.6	7.0 ± 1.4[a]
Female	4.0 ± 0.8	4.0 ± 1.3	6.0 ± 1.0	6.0 ± 0.8*
PCNA labeling index				
Male				
Liver[b]	0.18 ± 0.09	0.02 ± 0.02	0.06 ± 0.03	0.02 ± 0.00[a]
Pancreas[c]	3.5 ± 0.5	3.3 ± 0.3	3.5 ± 0.3	3.0 ± 0.0[a]
Testis[d]	14.6 ± 3.1	17.6 ± 2.2	12.9 ± 1.8	13.7 ± 6.0[a]
Female				
Liver[b]	0.06 ± 0.05	0.11 ± 0.04	0.06 ± 0.03	0.08 ± 0.08
Pancreas[c]	3.5 ± 0.5	3.3 ± 0.3	3.3 ± 0.3	3.0 ± 0.0

Note. Values are given as the mean ± SD, except where noted; $n = 4$, except where noted.

[a] $n = 2$, values are given as the average ± range.

[b] Percentage of hepatocytes in S-Phase.

[c] Scored subjectively with 4 = islets and acinar stained heavily (> 50% labeled); 3 = acninar ≫ islets.

[d] Percentage of proliferating leydig cells.

*Significantly different from control by Dunnett's *t*-test ($p \leq 0.05$).

The liver PFOS concentration decreased substantially during the recovery period when compared to liver concentrations in monkeys sacrificed at the end of treatment. After 7 months of recovery, liver PFOS concentrations from liver biopsies of the 0.75 mg/kg/day dose group were 142 ppm and 138 ppm for the males, and 175 ppm and 421 ppm for the females. For these same 0.75 mg/kg/day dose group recovery monkeys, liver PFOS concentrations one year after cessation of treatment were 23 ppm and 71 ppm for the males and 43 ppm and 58 ppm for the females, respectively. Liver obtained from the 0.15 mg/kg/day biopsy samples taken one year after the cessation of treatment contained 8 ppm and 10 ppm PFOS for the males, and 25 ppm and 18 ppm PFOS for the females. These values averaged 19 ± 8% of the mean liver PFOS concentrations measured in those dose groups at the end of treatment (Table 1).

Serum cholesterol concentrations in the 0.75 mg/kg/day recovery group had returned to pretreatment levels within 36 days of cessation of treatment. During that time, the two males went from 59 and 69 mg/dl to 140 and 134 mg/dl, respectively, and the two females went from 88 and 66 mg/dl to 135 and 131 mg/dl, respectively. HDL cholesterol values returned to control levels within 61 days of cessation of treatment in the 0.75 mg/kg/day and the 0.15 mg/kg /day dose group monkeys.

All hormone values returned to normal during recovery between 33 and 61 days after cessation of treatment, and remained so to the end of the one-year recovery period. In males, total T_3 for the control, 0.15, and 0.75 mg/kg/day dose

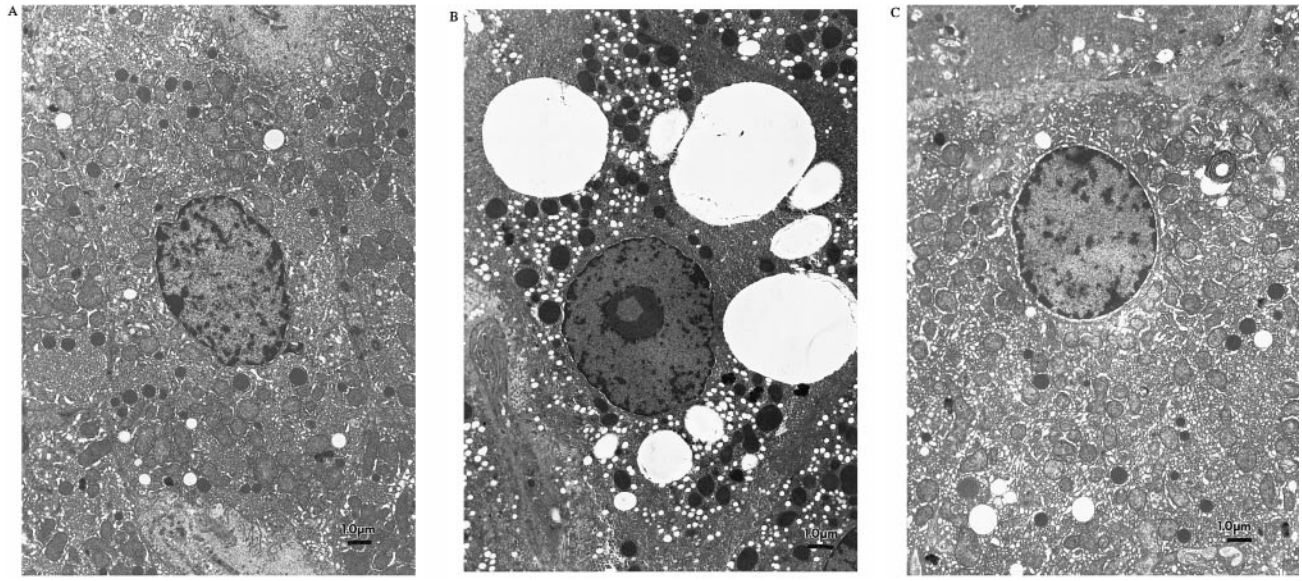

FIG. 2. Electron microscopy. Control and high dose liver after 183 days of treatment and 210 days of recovery. Magnification: the measurement bar on each photomicrograph equals 1 micron (0.001 mm); original magnification ×10,920. (A) Control male liver, day 184. Centrilobular region of a normal hepatocyte showing normal cytoplasmic organelles. Numerous dense lysosomes and/or peroxisomes intermixed with mitochondria and abundant endoplasmic reticulum are present; 29 peroxisomes counted. (B) PFOS 0.75 mg/kg/day male, day 184. Centrilobular region of hepatocyte showing moderately severe hepatocellular lipid droplet accumulation, containing several very large clear lipid droplets and many smaller lipid droplets. Several residual bodies are present. Many glycogen rosettes are present in cytoplasm. Peroxisomes are scarce (3 peroxisomes counted). (C) PFOS 0.75 mg/kg/day male, day 393 (211 days of recovery). Centrilobular hepatocyte showing normal ultrastructural findings in hepatocytes; 22 peroxisomes counted.

groups were 171 ± 17, 160 ± 7, and 147 ± 25 ng/dl (n = 2/dose group), respectively, 61 days after cessation of treatment. In females, total T_3 for the control, 0.15, and 0.75 mg/kg/day dose groups were 133 ± 6, 135 ± 11, and 121 ± 2 ng/dl (n = 2/dose group) respectively, 61 days after cessation of treatment.

In males, the mean estradiol values for male monkeys for the 0, 0.15, and 0.75 mg/kg/day dose groups were 28.7 ± 17.1, 24.6 ± 1.1, and 22.2 ± 6.2 pg/ml (day 245, n = 2/dose group), respectively.

Complete recovery of histopathological indications were observed in the 0.75 mg/kg/day dose group liver tissue by light or electron microscopy of the liver biopsy samples collected after 7 months (211 days) of recovery (Fig. 2C). Liver samples collected at necropsy after one year (371 days) of recovery were also considered ultrastructurally normal by electron microscopy. Hepatocellular hypertrophy and vacuolation, noted after 182 days of treatment in some high-dose animals, were not identified in recovery samples, indicating complete reversal of the liver changes.

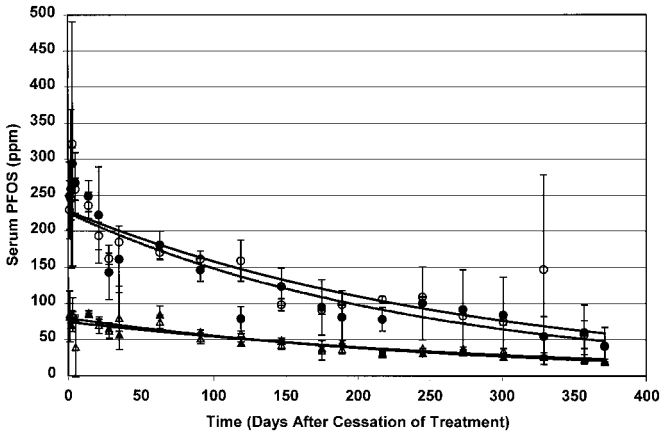

FIG. 3. Average serum PFOS concentration in recovery group cynomolgus monkeys after the cessation of treatment. Male (filled symbols, dashed lines) and female (open symbols, solid lines) cynomolgus monkeys treated with 0.15 (triangles) or 0.75 (circles) mg PFOS/kg/day.

DISCUSSION

The current study in cynomolgus monkeys was designed to improve the understanding of potential early effects of PFOS in primates on repeated dosing and adds significantly to the limited information available from a previous study in rhesus monkeys (Goldenthal *et al.*, 1978b). The effects were measured in a different species, at lower dose levels over a longer time period, and observations included many endpoints that were not measured in the rhesus study. For example, clinical chemistry, hormone level, and serum PFOS measurements were made at repeated intervals, and electron microscopy, liver PFOS determination, and cell proliferation data were obtained from tissue samples at sacrifice. Finally, data from recovery

group monkeys were obtained for one year after the cessation of treatment.

The cause of death or morbidity for the two males terminated on days 155 and 179, respectively, cannot be unequivocally determined from the available information. The monkey that died on day 155 showed indications of a pulmonary inflammation that appeared to be an acute exacerbation of a chronic lesion. Hepatocellular hypertrophy with significant vacuolation, similar to that found in the other 0.75 mg/kg/day dose group monkeys, was also present. Body weight changes did not seem related, since one monkey gained 14% and one animal lost 13% of their pretreatment body weight.

The clinical picture for the monkey that was sacrificed in moribund condition on day 179 included hyperkalemia and indications of myolysis (elevated creatine kinase and creatinine) as well as some liver involvement (elevated alkaline phosphatase, sorbitol dehydrogenase, and serum bile acids). PFOS has been shown to decrease HMG CoA reductase (Haughom and Spydevold, 1992), a proposed mechanism for the hypolipidemic effect that is shared by the statin class of cholesterol-lowering drugs. The increase in creatine kinase and hyperkalemia seen in this monkey are consistent with what has been reported for certain HMG CoA reductase inhibitors (statins), especially when given in combination with fibrates (De Pinieux *et al.,* 1996; Omar *et al.,* 2001). Statins have been shown to decrease ubiquinone and increase the lactate/pyruvate ratio (De Pinieux *et al.,* 1996; Flint *et al.,* 1997). One could speculate that this monkey may have suffered from rhabdomyolysis and lactic acidosis as a result of reductions in ubiquinone.

Two outcomes of the previous rhesus monkey study with PFOS (Goldenthal *et al.,* 1978b) that warrant discussion: mortality and cholesterol lowering. Male and female Rhesus monkeys (2 animals/sex per group) received 0, 0.5, 1.5, or 4.5 mg/kg/day potassium PFOS by gavage for up to 90 days. With regard to mortality, the Rhesus monkeys treated with 4.5 mg/kg/day died or were sacrificed *in extremis* between weeks 5 and 7 after symptoms including anorexia, emesis, decreased activity, rigidity, convulsions, trembling, prostration, and loss of body weight. The corresponding cumulative doses associated with mortality (158–220 mg/kg) are somewhat higher than those of the two high-dose male cynomolgus monkeys from the current study that either died or were sacrificed (93 and 107 mg/kg). With regard to cholesterol lowering, cholesterol values were noticeably lower in the 1.5 mg/kg/day dose group rhesus monkeys, and were significantly decreased in the 4.5 mg/kg/day dose group after one month of dosing. Corresponding cumulative doses of PFOS at one month for the 1.5 mg/kg/day and 4.5 mg/kg/day rhesus monkeys would be about 45 mg/kg and 135 mg/kg, respectively. In the current cynomolgus monkey study, significant decreases in cholesterol from predose values occurred in the 0.75 mg/kg/day dose group at serum PFOS concentrations above 100 ppm and corresponded to cumulative doses of 50 mg/kg in males and 34 mg/kg in females. This decrease in serum total cholesterol observed in cynomolgus monkeys from the study reported herein was the earliest reliable measure of clinical response to PFOS.

The lower HDL values (as compared to controls) observed in the 0.75 mg/kg/day males and females, in the 0.15 mg/kg/day females and in the 0.03 mg/kg/day males, may have been related to lower total cholesterol or to an effect on HDL itself. In the case of the 0.75 mg/kg/day dose group, it is more likely a treatment-related effect resulting from lowered total cholesterol. In the case of the 0.03 mg/kg/day males it appeared to be a result of inherently lower cholesterol in these monkeys as compared to control, and was not believed to be an effect of treatment. Furthermore, all individual HDL levels in the 0.03 mg/kg/day males were within the reference range of 30 to 150 mg/dl for cynomolgus monkeys (Wagner *et al.,* 1999), and there was no HDL effect in the 0.15 mg/kg/day dose group males.

In the case of the 0.15 mg/kg/day females at the end of dosing, 2 out of 6 HDL values were slightly below the reference range of 30–150 mg/dl, and the group-mean HDL was significantly lower than time-related female control values. HDL values are usually proportional to total cholesterol values. Due to the small number of monkeys and the lack of prestudy and interim HDL values for individuals prior to day 153, the biological significance of lower HDL without an apparent lowering of total cholesterol are difficult to interpret. If individual changes in HDL did occur over the dosing period without proportionate changes in total cholesterol, lowering of HDL could be argued to be a more sensitive indicator of treatment than lowering of total cholesterol. Since all HDL and cholesterol levels returned to normal during recovery, and due to the lack of prestudy and interim HDL values, the significance of the lower HDL values in the 0.15 mg/kg dose group females is uncertain.

HDL can be affected by a variety of factors. HDL levels are under the influence of estradiol in female cynomolgus monkeys (Adams *et al.,* 1985); therefore, there may be a relationship between the lower estradiol and HDL levels observed in the 0.75 mg/kg/day dose group. The potential role of PPAR delta-mediated reverse cholesterol transport pathways (Oliver *et al.,* 2001) is currently being investigated as a possible explanation for this observed effect.

Although the serum bilirubin levels in the 0.75 mg/kg/day dose group male were significantly lower than controls, these values were within the published range for cynomolgus monkeys of 0.3 $\pm$ 0.29 mg/dl (Wolford *et al.,* 1986). Furthermore, the average control bilirubin values ranged much higher (0.6 to 0.8 mg/dl) than the reference mean, and were highly variable. Although we did not analyze for urinary excretion of urobilinogen, analysis of fecal urobilinogen during week 23 of treatment from the 0.75 mg/kg/day males did not reveal any significant differences from control levels (data not shown). Thus, the modest decrease in bilirubin in the 0.75 mg/kg/day

dose group males remains unclear. The increase in serum bile acids that occurred in the 0.75 mg/kg/day dose group males on day 182 could be considered to have been indicative of mild bile stasis associated with hepatic hypertrophy; however, this was not consistent with the lowered serum bilirubin observed in this dose group.

The lack of a mitotic response in liver, as measured by PCNA suggests that the observed accumulation of PFOS did not cause hepatocellular proliferation. Pancreas and testes were investigated for their proliferative response because peroxisome proliferating compounds have been shown to cause adenoma formation in these tissues in rodents (Biegel *et al.*, 2001; Cattley *et al.*, 1998; Cook *et al.*, 1992; Just *et al.*, 1989; Pastoor *et al.*, 1987). The lack of an increase in the mitotic index in these organs suggests that PFOS does not cause a similar response in primates.

The results of this study indicated lipid accumulation occurred in the liver, without peroxisome proliferation. While the mechanisms of toxicity are not completely understood, studies in rodents have indicated that PFOS interferes with fatty acid and cholesterol metabolism through inhibition of HMG CoA reductase and acyl CoA cholesterol acyl transferase (Haughom and Spydevold, 1992). Peroxisome proliferation has been demonstrated in rats and mice treated with PFOS (Ikeda *et al.*, 1987; Kennedy *et al.*, 1998; Sohlenius *et al.*, 1993), but had not been previously measured in primates. *In vitro*, PFOS has been shown to compete with fatty acids for transport proteins (Luebker *et al.*, in press) and to interfere with mitochondrial bioenergetics (Starkov and Wallace, 2002). The findings from this study were consistent with evidence that primates do not respond with hepatic peroxisomal proliferation to known peroxisome proliferators (Pugh *et al.*, 2000). Furthermore, the peroxisome proliferators examined by Pugh *et al.* did not cause effects on body weight, liver weight, and cholesterol that were observed with PFOS. Thus, we believe the hepatic effects of PFOS in this study are not related to peroxisome proliferation.

The decrease in total T_3 in both males and females of the 0.75 mg/kg/day dose group at the end of the treatment period was consistent with the slight (approximately 2-fold) compensatory increase in TSH observed in these monkeys (Ingbar, 1985), although the absence of a corresponding decrease in T_4 was puzzling. Normal-range values for T_3 were not available for cynomolgus monkeys; however, with the exception of one male with a T_3 of 34 ng/100 ml, all values for T_3 were within the normal ranges of 54–115 ng/100 ml and 65–295 ng/100 ml for male and female rhesus monkeys, respectively (Belchetz *et al.*, 1978; DePaolo and Masaro, 1989). The clinical relevance of the lowered T_3 values was not apparent in this study since there was no indication of a clinical hypothyroid response. All TSH values were within the reference range (Belchetz *et al.*, 1978), hyperlipidemia was not present, and there were no changes in thyroid gland histology. The thyroid hormone values in the 0.75 mg/kg/day dose group did resemble some aspects of nonthyroidal illness syndrome, which is associated with many types of illnesses, in which lowered T_3 values are the most common abnormality (Chopra, 1997; DeGroot, 1999).

The lower estradiol values found in the 0.75 mg/kg/day dose group male and female monkeys are not well understood. Since testosterone was not decreased in males, it is possible that the lowered estradiol is a result of a feed-back mechanism aimed at preserving testosterone levels in males, e.g., decreased aromatase activity, or by a direct inhibition of aromatase. The relatively low estradiol values in females may be proportional to lower HDL (Adams *et al.*, 1985).

The recovery group animals revealed that the effects of PFOS on clinical chemistry, hormones, and liver histology are reversible. This reversal was accompanied by significant decreases in serum and liver PFOS. After one year of recovery, average serum and liver concentrations of PFOS in the combined 0.15 and 0.75 mg/kg/day dose groups were 25 $\pm$ 6% and 19 $\pm$ 8%, respectively, of values measured of the end of the 6-month treatment period.

Factors that may have resulted in the plateauing of serum PFOS concentrations in the high-dose monkeys may have included a limitation on the absorption rate of PFOS, saturation of serum carrier-protein sites, increased uptake of PFOS from serum by the liver, and increased biliary or urinary excretion of unbound PFOS. The fact that the reduction in serum cholesterol corresponds with the plateauing of serum PFOS concentration suggests that there may be a causal correlation between these two observations. If the plateauing of PFOS serum concentrations is related to an increase in free versus bound PFOS in serum with a corresponding increased uptake by liver, the increase in the liver concentration of PFOS may be related to lowering of serum cholesterol. Inhibition of enterohepatic circulation of cholesterol could also contribute to the observed lowering of serum cholesterol. The recovery of serum cholesterol 36 days after cessation of dosing, while PFOS levels were still elevated in serum and liver, would seem to suggest that serum PFOS is not a major contributor to the observed lowering of cholesterol. The cessation of daily dosing may work to remove an inhibition of cholesterol absorption from the gut. Also, the cessation of daily dosing may allow for the sequestration of PFOS, removing its reported effect of decreasing HMG CoA reductase activity (Haughom and Spydevold, 1992).

Acknowledging the uncertainty concerning the significance of lowered HDL observed in females in the 0.15 mg/kg/day dose group females (see discussion above), no toxicologically significant effects were observed after 182 days of dosing at 0.03 and 0.15 mg PFOS/kg/day. Therefore, the authors consider 0.15 mg/kg/day to represent a no-observed-adverse-effect level (NOAEL) in cynomolgus monkeys under the conditions of this study.

Due to the poor elimination of PFOS in monkeys and humans, serum PFOS concentrations can be used as an indication of body burden and integrated exposure over time. A comparison of serum concentrations associated with no ad-

verse health effects in the cynomolgus monkeys from this study with serum concentrations measured in various human population subgroups can be used as an estimate of potential risk. The serum PFOS concentrations achieved after 6 months of dosing at 0.15 mg/kg/day were 83 ± 25 ppm and 67 ± 11 ppm for males and females, respectively. This compares to serum levels in exposed workers that have averaged approximately 2 ppm (Olsen *et al.*, 1999). Extensive monitoring of this exposed worker population has not revealed any associations with hepatic effects or changes in cholesterol. Hansen *et al.* (2001) reported a mean PFOS serum concentration of 0.028 ± 0.014 ppm in human blood samples from 65 nonoocupationally-exposed donors and several commercial suppliers. Therefore, compared to serum concentrations associated with no adverse effects in cynomolgus monkeys, a margin of safety approximating 3 orders of magnitude appears to exist for nonoccupational human populations.

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