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Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague Dawley rats

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ABSTRACT

To investigate toxicity and neoplastic potential from chronic exposure to perfluorooctanesulfonate (PFOS), a two-year toxicity and cancer bioassay was conducted with potassium PFOS (K^+PFOS) in male and female Sprague Dawley rats via dietary exposure at nominal K^+PFOS concentrations of 0, 0.5, 2, 5, and 20 μg/g (ppm) diet for up to 104 weeks. Additional groups were fed 20 ppm for the first 52 weeks, after which they were fed control diet through study termination (20 ppm Recovery groups). Scheduled interim sacrifices occurred on Weeks 4, 14, and 53, with terminal sacrifice between Weeks 103 and 106. K^+PFOS appeared to be well-tolerated, with some reductions in body weight occurring in treated rats relative to controls over certain study periods. Male rats experienced a statistically significant decreased trend in mortality with significantly increased survival to term at the two highest treatment levels. Decreased serum total cholesterol, especially in males, and increased serum urea nitrogen were consistent clinical chemistry observations that were clearly related to treatment. The principal non-neoplastic effect associated with K^+PFOS exposure was in livers of males and females and included hepatocellular hypertrophy, with proliferation of endoplasmic reticulum, vacuolation, and increased eosinophilic granulation of the cytoplasm. Statistically significant increases in hepatocellular adenoma were observed in males ($p = 0.046$) and females ($p = 0.039$) of the 20 ppm treatment group, and all of these tumors were observed in rats surviving to terminal sacrifice. The only hepatocellular carcinoma observed was in a 20 ppm dose group female. There were no treatment-related findings for thyroid tissue in rats fed K^+PFOS through study termination; however, male rats in the 20 ppm Recovery group had statistically significantly increased thyroid follicular cell adenoma, which was considered spurious. There was no evidence of kidney or bladder effects. In rats, the dietary dose estimated as the lower 95% confidence limit of the benchmark dose for a 10% increase in hepatic tumors was 8 ppm for both sexes. Recent mechanistic studies suggest a PPARα/CAR/PXR-mediated mode of action for the liver tumors observed in the present two-year study.

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1. Introduction

In the late 1990s, the fluorinated surfactant perfluorooctanesulfonate (PFOS) was discovered to be broadly disseminated in biomonitoring samples taken from humans (Hansen et al., 2001) and wildlife (Giesy and Kannan, 2001). The exceptional stability of PFOS to environmental and metabolic degradation and its observed low elimination rates in most species once absorbed (Chang et al., 2012; Olsen et al., 2007) impart to PFOS the properties of a bioaccumulative agent (Martin et al., 2003a,b). These properties of PFOS led a major manufacturer, 3M Company, to voluntarily cease production by the end of 2002 of PFOS and compounds that may degrade to PFOS and have stimulated strict regulatory controls on production and use of these materials in many countries (Renner, 2001). Furthermore, these properties have led to intensive investigation of the potential environmental and toxicological impacts of PFOS (Lau et al., 2007).

Because of its persistence both in the environment and in the body once absorbed, the potential health consequences of chronic exposure to PFOS deserve careful examination. The potential health hazards from exposure to PFOS have been investigated in numerous mechanistic, toxicological, and human epidemiological studies (Lau et al., 2007). PFOS has been observed to lack the properties of a genotoxic agent based on the results of a variety of genotoxicity assays. Hepatocellular hypertrophy and hypolipidemia are common findings in toxicological studies of PFOS. As noted in two articles also published in this issue (Elcombe et al., 2012a,b), PFOS

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Table 1
Kaplan–Meier estimated probability of mortality through 105 weeks in male and female rats fed control diet or diets containing up to 20 μg/g (ppm) K^+PFOS.

	Dietary K^+PFOS concentration (μg K^+PFOS/g diet)													
	Males							Females						
	0	0.5	2	5	20	20 R[a]	20 R vs. 20[b]	0	0.5	2	5	20	20 R[a]	20 R vs. 20[b]
Total rats in group	70[c,d]	60[c]	60[c]	60[c]	70[c,d]	40[e]		70[c,d]	60[c]	60[c]	60[c]	70[c,d]	40[e]	
Estimated mortality	.778	.800	.660	**.500**[f]	**.565**	.750		.520	.700	**.820**	.700	.498	.575	
Standard error	.059	.057	.067	**.071**	**.070**	.068		.071	.065	**.054**	.065	.071	.078	
p-Value	***.0005*–**[g]	.9849–	.1820–	**.0115**–	**.0292**–	.7372–	.0980+	.3394–	.1700+	**.0015**+	.2338+	.8644–	.9407+	.6113+

[a] The male and female 20 Rec. groups (20 ppm recovery groups) were given K^+PFOS in diet for the first 52 weeks, after which they were given control diet.
[b] *p*-Value given represents the comparison of the 20 ppm recovery group mortality to the 20 ppm group mortality.
[c] Five rats/sex/group were sacrificed at Weeks 4 and 14 for hepatocellular proliferation rate measurements, biochemical analyses (palmitoyl-CoA oxidation), and histopathology (Week 14 only).
[d] Ten rats/sex/group were designated for interim sacrifice. These rats were sacrificed after at least 52 weeks of treatment.
[e] Rats in the 20 ppm Recovery (Rec.) group were treated for 52 weeks, then treatment was discontinued, and the rats were observed for reversibility, persistence, or delayed occurrence of toxic effects for 52 weeks post-treatment. During recovery, the rats received basal diet only.
[f] Bolded mortality estimates are statistically significant from the control (p (two-sided) < 0.05) based on the Cox-Tarone test, except for the italicized value in the control (0 μg K^+PFOS/g diet) column, which represents the *p*-value of the test for trend across groups (excluding the 20 ppm recovery groups).
[g] The one-sided *p*-value of the test for trend across groups (excluding the 20 ppm recovery group). Minus and plus signs after *p*-values represent the direction of change, i.e. increased (+) or decreased (−) mortality.

has been shown to produce responses consistent with the activation of the xenosensor nuclear receptors NR1C1 (PPARα, peroxisome proliferator-activated receptor α), NR1I3 (CAR, constitutive androstane receptor), and NR1I2 (PXR, pregnane X receptor). The role of activation of xenosensor nuclear receptors such as PPARα, CAR, and PXR in producing hepatomegaly and liver tumors in rodents has been well-established (Klaunig et al., 2003; Lake, 2009).

In this article, the results of a chronic toxicity and carcinogenicity study of potassium PFOS (K^+PFOS) in Sprague Dawley rats that were fed K^+PFOS in their diets for up to two years are summarized. Although this study was completed in 2002, and the full report has been made available to the public via the United States Environmental Protection Agency Administrative Record 226 (Thomford, 2002), this report has been prepared to make the key findings more accessible.

The study reported herein included scheduled interim sacrifices and observations after 4, 14, and 53 weeks of dietary administration of K^+PFOS to rats as well as the incorporation of male and female stop-dose recovery groups that received the highest dietary dose for the first year of the study followed by control diet. The 4- and 14-week interim observations have been published previously (Seacat et al., 2003a,b), and only microscopic histological findings, clinical chemistry data, and PFOS anion concentration data in serum and liver from the 4- and/or 14-week interim observations will be presented herein for the sake of completeness.

Human occupational and general population epidemiological studies have not revealed consistent associations of PFOS exposure with cancer outcomes. The results of the two-year bioassay reported herein provide valuable additional information for evaluation of potential cancer risk from chronic exposure to PFOS.

2. Materials and methods

2.1. Test material, diet preparation and analysis

A representative production lot of potassium perfluorooctanesulfonate (K^+PFOS, FC-95, Lot 217, 86.9% pure) was provided by 3M Company (Saint Paul, MN). Details concerning impurities in this representative lot were published previously (Seacat et al., 2003a). Lesser homologs constituted the majority of the impurities, with perfluorohexanesulfonate (C6 homolog, PFHxS) present in highest proportion in the sample at 4.73%. Perfluorinated carboxylic acids (C4, C5, and C8) constituted 0.71% of the sample. Metals (calcium, magnesium, sodium, nickel, and iron) were present at 1.45%, and inorganic fluoride constituted 0.59%. Acetone vehicle for preparation of diets was purchased from Spectrum Chemical Manufacturing Corp. (Lots LH0253 and NS0231, New Brunswick, NJ and Gardena, CA, respectively).

PMI Nutrition International Certified Rodent Diet 5002 meal (Saint Louis, MO, USA) was used for the base diet. Details of diet preparation and analysis were published previously (Seacat et al., 2003a). Dietary concentrations of K^+PFOS were based on the test material as supplied and were not corrected for purity. Diets containing nominal concentrations of 0.5, 2, 5, and 20 ppm K^+PFOS were prepared for stability and homogeneity analyses prior to initiation of treatment. During the course of the study, diets were prepared at least once every four weeks and were stored at room temperature in covered containers until dispensed into feeding jars. Analyses of perfluorooctanesulfonate anion (PFOS) concentration in diets by LC–MS/MS following ion-pair extraction (Hansen et al., 2001) were performed within 7 days of mixing. Control rats received basal diet sham-treated with acetone.

2.2. Laboratory rats, husbandry, and treatment

Male and female Crl:CD®(SD)IGS BR rats were obtained from Charles River Laboratories (Raleigh, North Carolina). Rats were quarantined for 13 days for health observations before being placed on study. At initiation of treatment, the rats were approximately 41 days of age, and body weights in males and females ranged from 135 to 226 g and 128 to 182 g, respectively. Rats were individually identified with a unique number encoded on an implanted RFID microchip tag. Rats were housed individually during the treatment period in stainless-steel caging, except when health problems dictated placement of individual rats in polycarbonate caging. Food and water were provided *ad libitum*. Rooms housing rats were maintained at 22 ± 4 °C, with a relative humidity of 50 ± 20% and a 12-h light/dark cycle. The laboratory facility was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Procedures involving live rats were reviewed and approved by the Institutional Animal Care and Use Committee of the laboratory facility and conformed to Institute for Laboratory Animal Research Guidelines (ILAR, 1996).

Rats were assigned to treatment groups using a computerized blocking procedure. Body-weight variations did not exceed two standard deviations of the mean body weight for each sex at randomization, and the mean body weights between groups for each sex were not statistically significantly different. Rats were assigned to treatment groups as depicted in Table 1. Rats in the control, 0.5 ppm, 2 ppm, 5 ppm, and 20 ppm groups received the test diet for up to 104 weeks. Due to their mortality rate, female rats in the 2 ppm received the test diet for up to 103 weeks. Rats in the 20 ppm recovery group (20 ppm Rec. group) received the test diet for 52 weeks followed by control diet through study termination.

2.3. Clinical observations, body weights, and food consumption

Rats were observed twice daily (a.m. and p.m.) for mortality and moribundity. Once prior to treatment and weekly thereafter, each rat was removed from its cage and examined, and any abnormal findings or an indication of normal health status was recorded. In addition, the time of onset, location, size, appearance, and progression of each grossly visible or palpable mass was recorded. Individual body weights were recorded at the time of randomization for group assignment, weekly for Weeks 1 through 17, once every 4 weeks thereafter, and at termination. Gravimetric data for individual food consumption were recorded weekly for Weeks 1 through 16 and once every 4 weeks thereafter.

2.4. Clinical pathology, hematology, urinalysis, and urine chemistry

Blood and urine samples were collected from 10 rats/sex/group in all groups except for the 20 ppm Rec. group during Weeks 27 and 53, and blood also was collected for cholesterol and triglycerides from all surviving rats at the terminal sacrifice.

Rats were fasted overnight (approximately 16 h) before blood sampling, during which time urine was collected chilled. After overnight fasting, blood was collected

from rats in random order from a jugular vein into collection tubes either containing potassium EDTA anticoagulant for hematology tests or containing no anticoagulant to obtain serum for clinical chemistry tests. In addition, blood films were made and held for possible future examination from all sacrificed rats, including rats sacrificed at unscheduled intervals.

2.4.1. Clinical chemistry

For clinical chemistry, the following parameters were evaluated using a Kinetic/Hitachi® 704,911 instrument (Roche Diagnostics): glucose; urea nitrogen; creatinine; total protein; albumin; globulin; total cholesterol; total bilirubin; alanine aminotransferase; gamma glutamyltransferase; aspartate aminotransferase; calcium; inorganic phosphorus; sodium; potassium; and chloride.

2.4.2. Hematology

For hematology, the following parameters were evaluated according to manufacturer's specifications on a Hitachi 704 (Bohringer Mannheim Corporation, Indianapolis, IN): red blood cell (erythrocyte) count; white blood cell (leukocyte) count (total); platelet count; hemoglobin; hematocrit; mean corpuscular volume; mean corpuscular hemoglobin; and mean corpuscular hemoglobin concentration. Differential leukocyte counts and blood cell morphology were evaluated by standard methods. Reticulocyte smears were made and held for possible future examination.

2.4.3. Urinalysis and urine chemistry

Urinalysis parameters evaluated included: appearance; volume (graduated cylinder); specific gravity (AO/TS refractometer); and microscopic examination of urinary sediment. Multistix (Bayer Diagnostics, Tarrytown, NY) were used according to manufacturer's directions for measurement of the following urinary parameters: pH; protein; bilirubin; urobilinogen; blood; glucose; ketones; sodium; and potassium. In addition, 16-h excretion of sodium and potassium was measured.

2.5. Necropsy procedures

Necropsies were scheduled at the following intervals: during Week 53 after 52 weeks of dietary exposure (10 rats/sex/group in the control and 20 ppm groups) and at study termination after 104 weeks of treatment (all groups). However, due to reduced numbers in the female 2 ppm dose group near study termination, this group was necropsied after 103 weeks of treatment. Otherwise, rats that were moribund or found dead were necropsied, with moribund rats being anesthetized with carbon dioxide, weighed, exsanguinated prior to necropsy. At scheduled necropsies, rats were fasted overnight, anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied in random order. Also during scheduled necropsies, serum (obtained from approximately 2 mL blood taken from a jugular vein) and liver samples were obtained from 5 rats/sex/group in the control and 20 ppm groups during Week 53 and all surviving rats during the terminal sacrifices and stored frozen (−60 to −80 °C) pending PFOS anion concentration determination as described previously (Seacat et al., 2003a). Included in all necropsies were macroscopic examinations of: external features of the carcass including all body orifices; the abdominal, thoracic, and cranial cavities; organs and tissues.

At the scheduled sacrifice during Week 53, the following organs (when present) were weighed (paired organs were weighed separately): adrenals; brain; kidneys; liver; lung; ovaries (females); spleen; testes (males); thyroids with parathyroid; and uterus with cervix (females). Organ to body weight percentages and organ to brain weight ratios were calculated.

The following tissues were collected and preserved in 10% neutral buffered formalin: adrenals; brain; cecum; cervix; colon; duodenum; epididymides; esophagus; eyes; femur with bone marrow (articular surface of the distal end); Harderian gland; heart; ileum; jejunum; kidneys; noted lesions; liver; lung with mainstem bronchi; lymph node (mesenteric); mammary gland (females only); ovaries; pancreas; pituitary; prostate; rectum; salivary glands (mandibular); sciatic nerve; seminal vesicles; skeletal muscle (thigh); skin; spinal cord (cervical, thoracic, and lumbar); spleen; sternum with bone marrow; stomach; testes; thymus; thyroids with parathyroid; trachea; urinary bladder; uterus; and vagina.

In addition, at the terminal sacrifice, sections of the heart and liver were collected from 10 rats/sex in control, 5 ppm, 20 ppm, and 20 ppm Rec. groups and preserved in 2.0% paraformaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer. These tissues were processed and embedded in epoxy blocks for examination by electron microscopy.

2.6. Histopathology

All tissues from rats in the control and 20 ppm groups as well as from any rat that died or was sacrificed at unscheduled intervals were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. In addition, from each rat in the 0.5, 2 and 5 ppm groups and in the 20 ppm Rec. group sacrificed during scheduled terminal necropsy, all noted lesions, liver, lungs, kidneys, pancreas, thyroid, testes, mammary glands (females), and urinary bladder were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Additional histological procedures included BrdU immunohistochemistry, Oil Red O staining, and electron microscopy, and pathology peer review. BrdU immunohistochemistry as well as H&E microscopic examination was performed on sections of liver and duodenum from the 5 rats/sex/group in the control and 20 ppm groups from the Week 53 scheduled necropsy that were previously implanted with BrdU osmotic pumps. From samples obtained at the terminal necropsy, liver sections from 5 rats/sex/group from the control and 20 ppm groups were stained with Oil Red O stain and examined microscopically and epoxy blocks for 5 rats/sex/group were processed and evaluated by electron microscopy.

A pathology peer review was conducted which included a review of all tissues from 10% of the control, 20 ppm, and 20 ppm Rec. males and females in addition to a review of all male livers, thyroids, and pancreatic tissues. Differences noted during the pathology peer review were examined and discussed by the reviewing pathologist, the study pathologist, and a third pathologist and resolved with unanimous agreement.

2.7. Statistical analysis

Levene's test (Levene, 1960) was used to test for variance homogeneity. One-way ANOVA (Winer, 1971) was used to analyze body weights, body weight changes, food consumption, continuous clinical pathology values, and organ weight data. If the ANOVA was significant, Dunnett's t test (Dunnett, 1964) was used for pairwise comparisons between treated and control groups (two-tailed, significance at $p < 0.05$).

Evaluations of trend and heterogeneity of survival data were performed using the Cox-Tarone binary regression method using the National Cancer Institute (NCI) Life Table Package (Thomas et al., 1977). Rats sacrificed during scheduled interim sacrifices were not included in the analysis. Continuity-corrected one-sided tail probabilities for trend and two-sided tail probabilities for group comparisons were evaluated at the 5.0% significance level.

Non-neoplastic and neoplastic lesions were chosen for statistical analyses if the incidence in at least one treated group was increased or decreased by at least two occurrences over the control group. Non-neoplastic lesions were analyzed by the Cochran-Armitage test for trend and the Fisher Irwin exact test for control versus treatment comparisons (Thakur et al., 1985). One-sided tail probabilities for trend and group comparisons were evaluated at the 5.0% significance level.

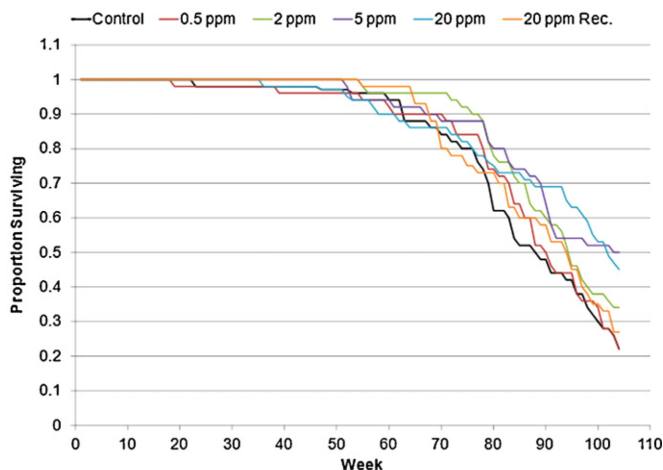
For neoplastic lesions, tumors that were not assigned to be the cause of death by the study pathologist (incidental tumors) were analyzed by logistic regression of tumor prevalence (Dinse and Lagakos, 1983). The fatal and palpable tumors were analyzed by the Cox-Tarone binary regression method using the time of death or the first palpation time (as applicable) as a surrogate for the tumor onset time. In the case of any particular tumor type where the study pathologist assigned the tumor in question being the cause of death of a subset of the animals and the rest of the animals were assumed to be dead of other competing risks, IARC type (Peto et al., 1980) cause of death analysis was performed. Tumor types where cause of death was undetermined were treated as incidental for statistical evaluation. The score statistics and their respective variances from the above tests were then used to compute the combined evidence as described by (Gart et al., 1986). In addition, in the cases where there was lack of convergence for the asymptotic test of the logistic regression method, the exact probability of the significance ts at risk was obtained by using LogXact Turbo (Cytel Software Corporation, Version 1.1, Cambridge, Massachusetts, 1993) and combined with the probability of Cox-Tarone test, if necessary. Where appropriate, criteria for combination of benign and malignant neoplastic incidences were applied to evaluate the combined incidences (McConnell et al., 1986).

Benchmark doses (BMDs) for increased incidence of liver tumors were estimated for male and female rats. Briefly, for each sex, the numbers of rats with hepatocellular adenoma/carcinoma and the effective number of animals at risk were entered into the U.S. Environmental Protection Agency benchmark dose software program (BMDS). The effective numbers of rats at risk were estimated using the Poly-3 approach (Bailer and Portier, 1988). Estimates of the benchmark dose were obtained using the multistage model. Goodness-of-fit *p*-values greater than 0.1 were considered adequate; whereas, goodness-of-fit *p*-values less than or equal to 0.1 were considered to represent statistically significant deviance from the multistage model. The benchmark response rate used was 10%. Both the benchmark dose (BMD_{10}) and the lower 95% confidence limit of the benchmark dose ($BMDL_{10}$) were estimated.

3. Results

3.1. Diet homogeneity and stability

Diet preparations containing K^+PFOS were homogeneous and stable. Inherent variability and lack of sufficient sensitivity at low levels in the analytical method employed at the time resulted in homogeneity, stability, and routine analysis data that were in many cases outside of the standard limits of ±15%. The overall mean concentrations within each dose level ranged from 102.3 to 108.7 percent of target concentration.



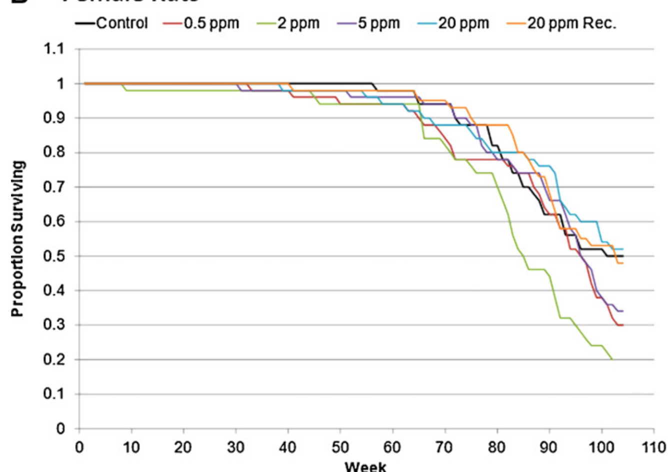


Fig. 1. Kaplan–Meier adjusted survival through 104 weeks in control rats (black line), rats fed K+PFOS in their diets at nominal concentrations of 0.5 (red line), 2 (green line), 5 (purple line), or 20 (blue line) μg/g diet (ppm), and rats fed 20 ppm in their diet for 52 weeks and control diet thereafter (20 ppm Recovery (Rec.) group (orange line)). In males (panel A), there was a statistically significant decreasing trend in mortality (increasing trend in survival) across the 0, 0.5, 2, 5, and 20 ppm male dietary dose groups (excludes 20 ppm Rec. group) based on Kaplan–Meier estimated probabilities of mortality through 105 weeks. Statistically significant decreased mortality based on Kaplan–Meier estimated probabilities of mortality through 105 weeks was observed in the 5 and 20 ppm male dietary dose groups. In female rats (panel B), there were no statistically significant trends in mortality across the 0, 0.5, 2, 5, and 20 ppm female dietary dose groups (excludes 20 ppm Rec. group) based on Kaplan–Meier estimated probabilities of mortality through 105 weeks. However, females in the 2 ppm dietary dose group experienced statistically significant increased mortality based on Kaplan–Meier estimated probabilities of mortality through 105 weeks.

3.2. *Clinical observations and survival*

There were no clinical observations attributed to exposure to K+PFOS. The types of clinical observations noted were commonly observed in laboratory rats as they age on long-term safety studies. There was also no effect of the K+PFOS on the incidence of palpable masses.

Survival curves for male and female rats, adjusted by Kaplan–Meier procedure for rats sacrificed at scheduled interim intervals, are presented in Fig. 1, panels A and B, respectively. Final Kaplan–Meier estimates of mortality through 105 weeks are presented in Table 1. Estimated mortality was *decreased* with statistical significance in males fed 5 and 20 ppm K+PFOS throughout the study when compared to control male survival, resulting in a statistically significant dose-related increased trend in survival. There was not a statistically significant dose-related trend in survival in female rats fed K+PFOS, and female rats in the 2 ppm group experienced a statistically significant decrease in survival compared to control females.

3.3. *Food consumption and K+PFOS dietary intake*

Food consumption data are presented graphically in the supplemental material as Supplemental Figs. 1 and 2 for males and females, respectively. Although not always statistically significant, males in the 20 ppm groups tended to consume less food during Weeks 1 through 24. Food consumption was similar for males given 20 ppm compared to males fed control diet during Weeks 28 through 104. Statistically significantly lower food consumption was noted for females in the 20 ppm groups during Weeks 2 through 36. In all the other treated groups, food consumption for males and females was similar compared to rats fed control diet.

The grand (overall) mean daily dietary intakes of K+PFOS (mg/kg/day) as well as minimum and maximum mean intakes are presented in Table 2. Overall mean daily intake across doses was highly linear with increasing dietary K+PFOS concentration ($R^2 = 0.9999$) and ranged across doses from 0.024 to 1.144 mg/kg/day for males and 0.029 to 1.385 mg/kg/day for females.

3.4. *Body weight*

Body weight data for male and female rats are presented graphically in the supplemental material as Supplemental Figs. 3 and 4, respectively. Males in the 20 ppm and 20 ppm Rec. groups experienced statistically significantly lower mean body weights compared to control males during Weeks 9 through 37. Females in the 20 ppm group had statistically significantly lower body weights compared to control females during Weeks 3 through 101. Mean body weights for females in the 20 ppm Rec. group were statistically significantly lower than those for control females during Weeks 3 through 61, but their mean body weights approached the weights of the control females when placed on control diet after Week 52.

For the nine male rats in each of the control and 20 ppm treatment groups that were sacrificed at the 53-week scheduled interval and for which organ weights were obtained, body weights in the males were similar between the control and 20 ppm group, with values of 713.4 ± 105.5 g and 668.6 ± 142.5 g, respectively. Mean body weights for the 10 control and 10 20 ppm group females at the 53-week sacrifice were statistically significantly different, with values of 414.5 ± 101.9 g and 331.9 ± 39.7 g, respectively.

At scheduled terminal sacrifice, there were no statistically significant differences in mean body weights between K+PFOS-treated rats and controls. For males, mean ± SD body weights at terminal sacrifice were 746 ± 165 ($n = 11$), 734 ± 154 ($n = 11$), 809 ± 130 ($n = 17$), 768 ± 130 ($n = 25$), 755 ± 136 ($n = 23$), and 719 ± 151 ($n = 11$) for the control, 0.5 ppm, 2 ppm, 5 ppm, 20 ppm, and 20 ppm Rec. groups, respectively. Corresponding data for female rats at terminal sacrifice were 516 ± 106 ($n = 24$), 523 ± 117 ($n = 15$), 566 ± 151 ($n = 12$), 518 ± 86 ($n = 17$), 447 ± 101 ($n = 26$), and 542 ± 138 ($n = 19$) respectively by treatment group.

3.5. *Organ weights at Week 53 interim sacrifice*

Organ weight data for scheduled sacrifices prior to Week 53 (Weeks 4 and 14) were previously reported (Seacat et al., 2003a). Tabulated data for the Week 53 sacrifice for organs showing statistically significant differences in one or more of these organ-weight parameters between control and 20 ppm dose group are provided in the supplementary material (Supplementary Table 1).

Table 2
Overall (grand) mean daily intake of K^+PFOS (mg/kg/day) by male and female rats fed diets containing K^+PFOS for up to 104 weeks.

	Mean daily intake of K^+PFOS (mg/kg/day) by sex and dietary dose group (μg K^+PFOS/g diet (ppm))									
	Male					Female				
	0.5	2	5	20	20 Rec.[a]	0.5	2[b]	5	20	20 Rec.
Grand mean[c]	0.024	0.098	0.242	0.984	1.144	0.029	0.120	.299	1.251	1.385
SD[d]	0.010	0.037	0.093	0.351	0.385	0.010	0.038	0.095	0.304	0.276
Minimum	0.015	0.064	0.153	0.643	0.732	0.015	0.073	0.186	0.838	1.047
Maximum	0.057	0.226	0.570	2.205	2.336	0.052	0.213	0.559	2.149	2.160

[a] The 20 Rec. (20 ppm recovery) male and female groups received a nominal concentration 20 μg K^+PFOS/g diet for the first 52 weeks of the study, after which they were given control diet.
[b] The 2 ppm female dietary dose group was fed K^+PFOS for 103 weeks as opposed to 104 weeks.
[c] The grand mean represents the overall mean of weekly determined mean K^+PFOS consumption values for each group.
[d] The standard deviation of the mean of the weekly determined mean K^+PFOS consumption values for each group.

In the males, absolute and relative (to body weight and to brain weight) liver weights were increased in the 20 ppm group. In addition, absolute and relative (to body weight and to brain weight) spleen weights were decreased in 20-ppm-group males. Significantly decreased left thyroid/parathyroid weights were considered to be spurious due to the absence of a contralateral effect and lack of difference in organ-to-body-weight ratios between control and treated male rats.

For female rats, in view of the significant decrease in body weight given 20 ppm, significant increases in organ to body weight percentages for brain, kidney, liver, and spleen may be of no toxicological importance. Decreased absolute weights in the left adrenal gland and bilateral adrenal to brain weight ratios may also represent changes secondary to the body weight loss.

3.6. Clinical pathology

3.6.1. Clinical chemistry

There were relatively few statistically significant or otherwise notable differences between the control and treated groups for clinical chemistry results. The results for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol are presented in Fig. 2 for male and female rats. The results for serum glucose, urea nitrogen, and creatinine are presented in Fig. 3 for males and females. Data for measurements made at Weeks 4 and 14, previously reported by Seacat et al. (2003a), are included in the figures for the sake of completeness. Those differences that were dose-dependent and consistent over time were considered to be associated with K^+PFOS treatment. Between Week 14 and Week 53, reductions in serum total cholesterol in males of the 20 ppm dose group and mild increases in urea nitrogen in males and females of the two higher dose groups likely represent treatment-related changes.

The statistically significant increases in ALT in 20 ppm males that were observed on Weeks 14 and 53 were accompanied by a large increase in relative standard deviations, driven by one and two individual increases in ALT at 14 and 53 weeks, respectively. Mean ± SD values for male ALT ($n = 10$/group) on Week 14 for the 0, 0.5, 2, 5, and 20 ppm groups were 36 ± 7, 41 ± 6.3, 41 ± 4.7, 44 ± 13.6, and 65 ± 53. At 14 weeks, the statistic for the 20 ppm group was affected by a high individual value of 213, which, if censured from the analysis, would result in a mean ± SD of 49 ± 10.2. Similarly, mean ± SD values for male ALT ($n = 10$/group, except for 20 ppm group where $n = 9$) on Week 53 for the 0, 0.5, 2, 5, and 20 ppm groups were 54 ± 66, 62 ± 52, 40 ± 7.5, 44 ± 8.3, and 83 ± 84. The large standard deviations in the control and 0.5 ppm groups were affected by high individual values of 241 and 205, respectively. Censuring the individual control value of 241 would yield a mean ± SD of 34 ± 6. In the 20 ppm group at 53 weeks, the mean ± SD ALT value was affected by two high individual values of 292 and 140. Censuring these values would yield a mean ± SD of 45 ± 5.1. Thus, while ALT was increased with statistical significance in 20 ppm group males on Weeks 14 and 53 when compared to the control group, the finding is influenced heavily by one or two high individual values, and the observation of an individual value of 241 in the control group on Week 53 and lack of effect on AST raises some question as to the overall toxicological significance of the finding with respect to treatment.

Mean serum total cholesterol was reduced in male rats fed 20 ppm K^+PFOS with statistical significance as compared to control males on Weeks 14, 27, and 53. Statistically significant reductions in serum cholesterol occurred in female rats only on Week 27 in the 2, 5, and 20 ppm groups. Although not statistically significant, cholesterol appeared lower in 20 ppm dose group females on Week 53, and in males and females fed 20 ppm when measured at terminal sacrifice. The lowering of serum cholesterol is consistent with data from other studies with PFOS (Bijland et al., 2011) and likely represents a treatment-related effect.

Statistically significantly decreased mean serum glucose as compared to control means were observed on Weeks 4 and 53 in 20 ppm group males, and, in females, on Week 53 in the 2 ppm group, on Weeks 14 and 53 in the 5 ppm group, and on Week 53 in the 20 ppm group.

Serum urea nitrogen (UN) was statistically significantly increased in 20 ppm dose group males and females relative to controls on Weeks 14, 27, and 53. The 5 ppm dose group males and females also had statistically significantly elevated UN on Week 53, as did the 2 ppm dose group males. There were no correlative microscopic renal findings for the minor change in urea nitrogen and serum creatinine was unchanged relative to controls, with the exception of the Week 14 value for 2 ppm group females, which was elevated with statistical significance. Thus, the data for UN likely are associated with mild dehydration as a result of non-renal-related morbidity.

All statistically significant differences between the control and treated groups for other clinical pathology parameters were considered incidental. Most of these differences did not affect animals fed the highest dose level, and none of these differences were consistent over time.

3.6.2. Urinalysis and urine chemistry

There were no differences in urinalysis or urine chemistry that were considered to be related to K^+PFOS exposure between control rats and fed K^+PFOS when evaluated during study Weeks 27 and 53. The only statistically significant differences in urinalysis parameters between the control and K^+PFOS-treated groups occurred among males during Week 53. These were: at the 2 ppm dose, mean urinary pH of 7.1 ± 0.42 compared to the control mean of 6.6 ± 0.32 and mean sodium ion concentration of 23 ± 9 mmol/L in urine lower compared to the control

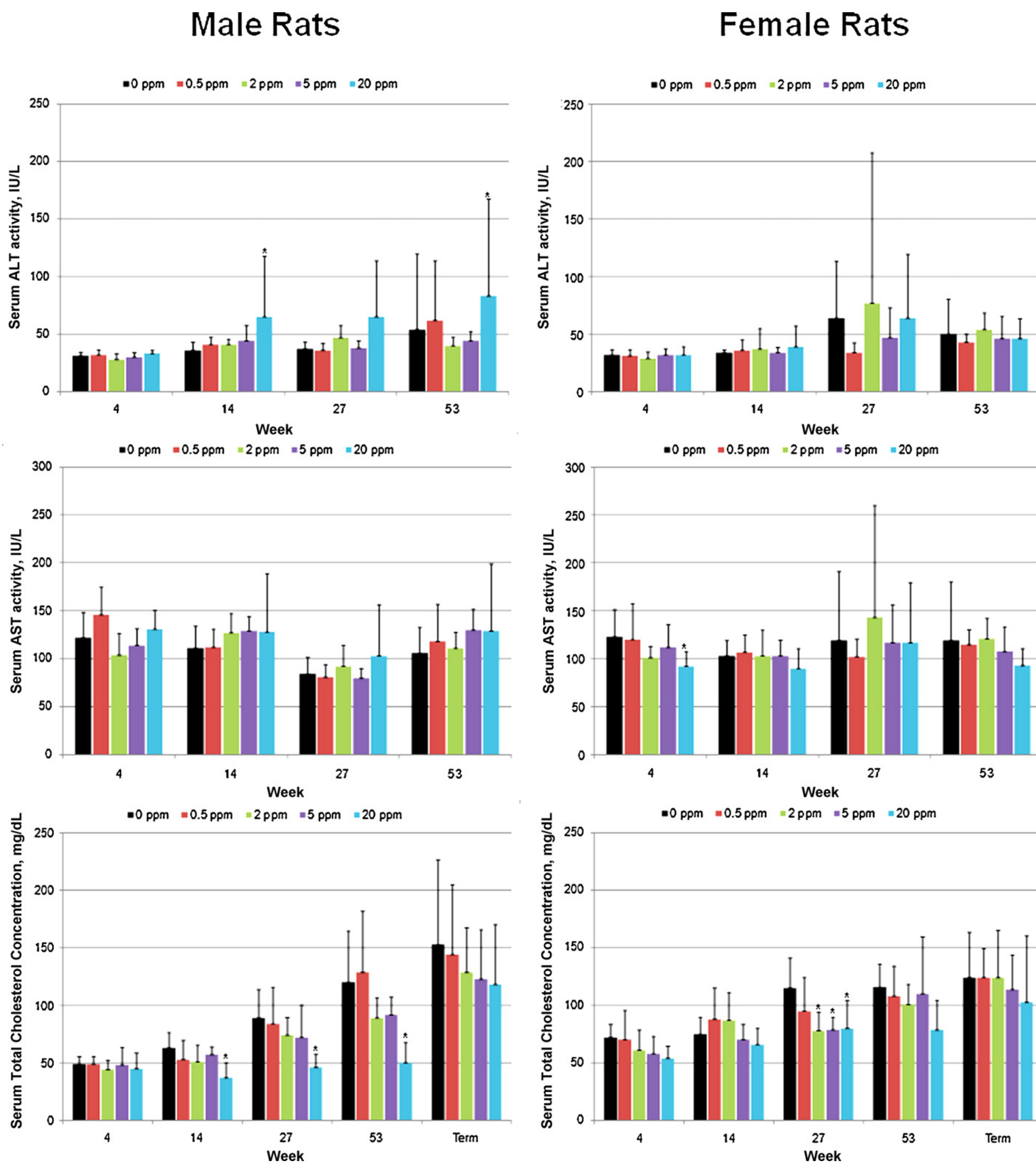


Fig. 2. Mean serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol concentrations in male and female rats. Samples were obtained at scheduled sacrifices on study Weeks 4, 14, 27, 53, and at term (total cholesterol only). Rats were fed K^+PFOS in their diets at nominal concentrations of 0 (control rats, black bars), 0.5 (red bars), 2 (green bars), 5 (purple bars), or 20 (blue bars) μg/g diet (ppm). Error bars represent the positive standard deviation. In male rats, the statistically significant increases in mean ALT in 20 ppm dose group that were observed on Weeks 14 and 53 were accompanied by a large increase in relative standard deviations, driven by one and two individual increases in ALT at 14 and 53 weeks, respectively. Also among the controls and 0.5 ppm dose group on Week 53 were single individual rats with high ALT values in the range of those individual values observed in 20 ppm group males on Weeks 14 and 53 that affected the relative standard deviations of the mean. Mean AST was unaffected by treatment and mean serum total cholesterol was reduced in male rats fed 20 ppm K^+PFOS with statistical significance as compared to control males on Weeks 14, 27, and 53. In female rats, ALT was unaffected by treatment. Compared to time-matched control, mean AST was reduced in 20 ppm dose group females with statistical significance on Week 4. There were statistically significant reductions in mean serum cholesterol occurred in female rats on Week 27 in the 2, 5, and 20 ppm dose groups. Although not statistically significant, cholesterol appeared lower in 20 ppm dose group females on Week 53 and at terminal sacrifice. An asterix represents mean values that are statistically significant as compared to the time-matched control mean, $p \leq 0.05$.

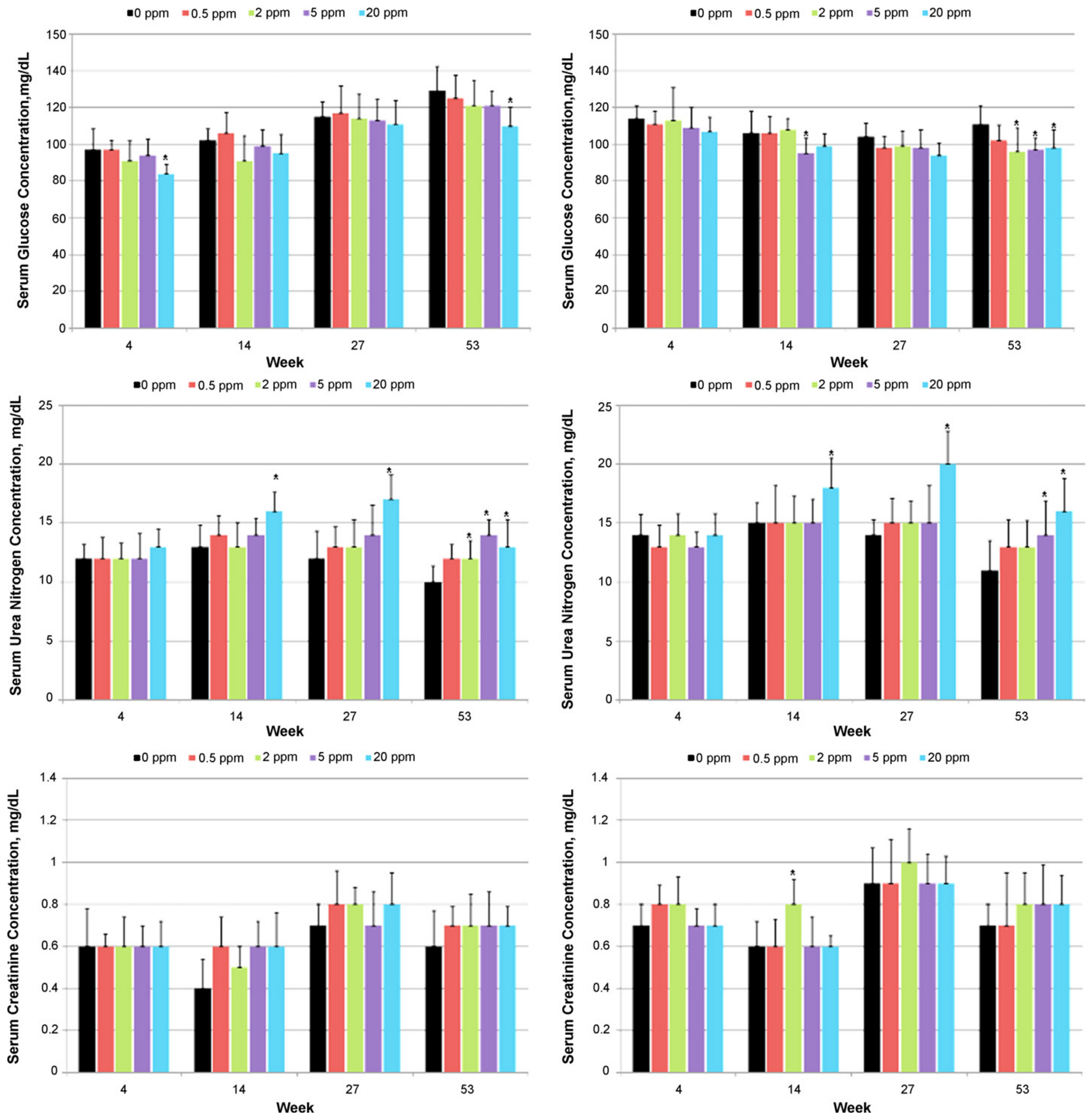


Fig. 3. Mean serum concentrations of glucose, urea nitrogen (UN), and creatinine in male and female rats. Samples were obtained at scheduled sacrifices on study Weeks 4, 14, 27, and 53. Rats were fed K⁺PFOS in their diets at nominal concentrations of 0 (control rats, black bars), 0.5 (red bars), 2 (green bars), 5 (purple bars), or 20 (blue bars) μg/g diet (ppm). Error bars represent the positive standard deviation. In males, mean serum glucose was decreased with statistical significance in 20 ppm dietary dose group rats compared to control rats on study Weeks 4 and 53. Mean serum UN was statistically significantly increased in 20 ppm dose group males relative to controls on Weeks 14, 27, and 53 and in 2 and 5 ppm dose group males on Week 53. Mean serum creatinine was unaffected by treatment in male rats. In females, mean serum glucose was decreased with statistical significance relative to the controls on Week 53 in the 2 ppm group, on Weeks 14 and 53 in the 5 ppm dose group, and on Week 53 in the 20 ppm dose group. Mean serum UN was statistically significantly increased in 20 ppm dose group females relative to controls on Weeks 14, 27, and 53 and in 5 ppm dose group females on Week 53. Mean serum creatinine was slightly elevated with statistical significance in the 2 ppm dose group on Week 14. An asterix represents mean values that are statistically significant as compared to the time-matched control mean, $p \leq 0.05$.

mean of 40 ± 15 mmol/L; at the 0.5 and 5 ppm doses, excretion of potassium ion of 0.87 ± 0.28 mmol and 0.76 ± 0.24 mmol in the 0.5 and 5.0 ppm groups, respectively, versus control mean of 1.19 ± 0.34.

3.6.3. Hematology

There were no hematological changes that were considered related to treatment with K^+PFOS. With one exception, the few statistically significant differences from control in mean hematology parameter values occurred at dietary K^+PFOS concentrations less than 20 ppm. These were not consistent over time and were of small magnitude. The exception was the increase in mean segmented neutrophil number previously reported by (Seacat et al., 2003a) in males of the 20 ppm group at 14 weeks. This also was considered to be an incidental occurrence.

3.7. Anatomic pathology

3.7.1. Macroscopic observations

For unscheduled deaths from study initiation through Week 53, large, mottled, or diffusely dark livers were noted in 2/3 males and 1/1 females given 20 ppm; otherwise, there were no other gross observations that could be attributed to dietary exposure to K^+PFOS. There were no clear or consistent gross observations at the Week 53 interim sacrifice that could be attributed to the administration of the test material. Rats in the 20 ppm Rec. group had no consistent gross findings that could be attributed to treatment with K^+PFOS. Unscheduled deaths from Week 53 through study term during Week 105 were not associated with consistent gross pathological findings. At the terminal sacrifice, the livers of rats given 5 or 20 ppm exhibited a slight increase in macroscopic findings, including enlarged, mottled, diffusely darkened, or focally lightened livers.

3.7.2. Non-neoplastic microscopic lesions

The only non-neoplastic microscopic findings attributable to K^+PFOS treatment were observed in the liver. Results of statistical evaluations for microscopic histological findings in the liver are presented in Tables 3 and 4 for males and females, respectively, and include the data from the scheduled sacrifices during Weeks 14 and 53 as well as unscheduled sacrifices and the terminal sacrifice. The 20 ppm group male rats that were sacrificed as scheduled during Week 53 had increased incidence and severity of centrilobular hepatocytic hypertrophy and vacuolation. Generally, in the females given 20 ppm and sacrificed during Week 53, only centrilobular hypertrophy was seen, and the change was less severe than that noted in the males. In addition, minimal to slight centrilobular hepatocytic pigment was found in the females given 20 ppm. There were no statistically significant increases in hepatocellular S-phase labeling index (cell proliferation index) as measured by bromodeoxyuridine (BrdU) immunohistochemistry. There were no other histomorphologic changes that could be associated with the administration of the test material. Liver findings in several unscheduled deaths prior to Week 53 in rats fed 20 ppm K^+PFOS in diet resembled those seen in animals sacrificed at Week 53.

Liver findings of the rats that died on test or moribund sacrifice rats from Week 54 through 105 were generally similar to those seen in the terminally sacrificed animals. Increased hepatocellular centrilobular hypertrophy, eosinophilic hepatocytic granules, and centrilobular hepatocytic pigment were noted in the rats given 20 ppm. Increased hepatocellular centrilobular hypertrophy also was observed in rats given 5 ppm.

For rats sacrificed at the scheduled terminal sacrifice, hepatotoxicity, characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in rats fed 5 or 20 ppm K^+PFOS. An increase in eosinophilic clear cell altered foci and cystic hepatocellular degeneration was noted in the males given 2, 5, or 20 ppm. There were no clear differences in the Oil Red O stained sections between control and 20 ppm dose group rats.

The data suggest that the hepatotoxicity resolved in the year following cessation of K^+PFOS treatment in the 20 ppm stop-dose rats sacrificed at term.

Electron microscopic evaluation of liver from 5 rats/sex in each of the control and 20 ppm groups identified smooth endoplasmic reticulum hyperplasia and hepatocellular hypertrophy as the prominent features found to be different between the 20 ppm group and the control rats. Other changes included a slight increase in the amount of glycogen in treated animals compared with controls.

The only other statistically significant findings occurred in the pancreas; however, these were not believed to be related to K^+PFOS treatment. In 2 ppm treatment group males, there was an increase in the incidence of interstitial fat infiltration when compared to the control incidence. The latter finding was not dose-related, and the incidence in other K^+PFOS groups was quite similar to the control incidence. In female rats, all K^+PFOS groups had higher incidence of decreased zymogen granules without dose–response. This was most likely due to a chance lower incidence in the control females.

3.7.3. Neoplastic lesions

Neoplastic observations reaching statistical significance (increased or decreased incidence or trend) occurred in the liver and thyroid tissues of male rats (Table 5). Statistically significant increases in hepatocellular adenoma of the liver (7/60, $p = 0.046$) were observed in the 20 ppm group. The male 20 ppm Rec. group had no hepatocellular adenoma, and thus had a statistically significantly decreased incidence of hepatocellular adenoma when compared to the 20 ppm group males.

Statistically significantly increased incidences for thyroid follicular cell adenoma when compared to either the control or the 20 ppm group occurred in the 20 ppm Rec. group males. Although thyroid follicular cell carcinoma was not increased in incidence in the 20 ppm Rec. compared to the control group or the 20 ppm group, the significant increased incidence of thyroid follicular cell adenoma resulted in a significant increase in the combined thyroid follicular cell tumors (adenoma and carcinoma) in the 20 ppm Rec. group rats as compared to that of the 20 ppm group. There was no significant trend in thyroid tumors across treatment groups. Although the increased incidence of thyroid follicular cell tumors in the 20 ppm Rec. group was outside the range of historical control values from the laboratory, there was no other microscopic evidence of thyroid abnormality.

Neoplastic observations reaching statistical significance (increased or decreased incidence or trend) occurred in the liver, thyroid, and mammary tissues of female rats (Table 6). Statistically significant increases in hepatocellular adenoma of the liver (5/60, $p = 0.039$) were observed in female rats in the 20 ppm group. The occurrence of one hepatocellular carcinoma (not statistically significant) in a 20 ppm group female resulted in a statistically significant increase in combined hepatocellular adenoma and carcinoma in females of the 20 ppm group. All hepatocellular tumors in females of the 20 ppm group were incidental and noted at the terminal sacrifice.

For females, a statistically significant increase as compared to the control group for combined thyroid follicular cell adenoma and carcinoma was observed in the 5 ppm group (3/50, $p = 0.0471$) but not in the 20 ppm group (Table 6). Thyroid C cell adenoma and thyroid C cell combined adenoma and carcinoma showed

Table 3
Results of the statistical analyses of incidence of non-neoplastic microscopic findings in livers of male rats (statistically significant observations in bold). Data included are from the scheduled sacrifices during Weeks 14 and 53, unscheduled sacrifices, and the terminal sacrifice.

Finding	Incidence[a] (top row) and significance (*p*-value)[b] by K^+PFOS dietary dose group						
	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm Rec.[c]	20 ppm Rec. vs. 20 ppm
Infiltrate, lymphohistiocytic							
	51/65	43/55	44/55	45/55	45/65	31/40	
	.1836–	.5720-	.5090+	.4111+	.1592–	NA	.2450+
Hypertrophy, hepatocellular centrilobular							
	0/65	2/55	4/55	22/55	42/65	3/40	
	.0000+**	.2080+	**.0415+***	**.0000+****	**.0000+****	.0527+	**.0000–****
Granular cytoplasm, eosinophilic, centrilobular							
	0/65	0/55	0/55	0/55	14/65	0/40	
	.0000+**	NA	NA	NA	**.0000+****	NA	**.0007–****
Pigment hepatocellular, centrilobular							
	0/65	0/55	0/55	0/55	6/65	0/40	
	.0006+**	NA	NA	NA	**.0139+***	NA	.0513–
Necrosis, individual hepatocyte							
	5/65	4/55	6/55	5/55	14/65	4/40	
	.0106+*	NA	NA	NA	**.0224+***	.4699+	.1024–
Vacuolation, hepatocellular midzonal/centrilobular							
	3/65	3/55	6/55	13/55	19/65	3/40	
	.0000+**	NA	.1696+	**.0024+****	**.0001+****	.4152+	**.0060–****
Altered hepatocellular, clear/eosinophilic cell							
	20/65	21/55	23/55	24/55	24/65	16/40	
	.1948+	NA	.1431+	.1026+	.2892+	.2243+	.4553+
Focus, altered hepatocellular, basophilic							
	10/65	7/55	16/55	17/55	13/65	8/40	
	.0663+	.4414–	.0556+	**.0352+***	.3232+	.3616+	NA
Degeneration, cystic							
	5/65	15/55	19/55	17/55	22/65	15/40	
	.0007+**	**.0041+****	**.0003+****	**.0011+****	**.0002+****	**.0002+****	.4306+
Necrosis, coagulative							
	1/65	4/55	1/55	6/55	2/65	4/40	
	.2563+	.1346+	NA	**.0351+***	NA	.0683+	.1471+
Hematopoiesis, extramedullary							
	4/65	3/55	8/55	3/55	7/65	5/40	
	.2161+	NA	.1111+	NA	.2652+	.2187+	NA
Hyperplasia, bile duct							
	20/65	20/55	25/55	24/55	25/65	21/40	
	.1351+	NA	.0713+	.1026+	.2305+	**.0224+***	.1141+
Fibrosis, peribiliary							
	8/65	7/55	11/55	7/55	14/65	4/40	
	.1057+	NA	.1842+	NA	.1209+	.4901–	.1024–
Hypertrophy, hepatocellular periportal							
	4/65	1/55	7/55	2/55	2/65	5/40	
	.3303–	.2387–	.1773+	.4222–	.3400–	.2187+	.0719+
Infiltrate, macrophage, pigmented							
	6/65	5/55	3/55	4/55	5/65	2/40	
	.3554–	NA	.3357–	.4813–	NA	.3491–	.4588–
Vacuolation, hepatocellular							
	6/65	2/55	3/55	1/55	7/65	3/40	
	.4677+	.1976–	.3357–	.0881–	NA	NA	.4254–
Degeneration/necrosis centrilobular							
	1/65	0/55	0/55	1/55	5/65	1/40	
	.0132+*	NA	NA	NA	.1039+	NA	.2566–
Glycogen, hepatocellular, increased							
	1/65	3/55	0/55	0/55	1/65	0/40	
	.2206–	.2491+	NA	NA	NA	NA	NA
Angiectasis							
	1/65	1/55	2/55	0/55	2/65	0/40	
	NA	NA	NA	NA	NA	NA	.3810–
Lipidosis subcapsular							
	0/65	0/55	3/55	1/55	0/65	0/40	
	.4317+	NA	.0934+	NA	NA	NA	NA

[a] Number of observations per number of rats observed.
[b] The *p*-values given in the control column are for trend and those for individual K^+PFOS dietary dose groups are as compared to control. The *p*-value in the last column is for the statistical comparison of the 20 ppm Recovery (Rec.) group response with the 20 ppm group response. The minus sign (–) indicates an effect in the decreased direction. The plus sign (+) indicates an effect in the decreased direction. The symbols * and ** represent comparisons that are statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. NA indicates that the response was not analyzed.
[c] The 20 ppm dietary dose recovery group (20 ppm Rec.) was given 20 ppm K^+PFOS in diet for up to 53 weeks, after which the group was fed control diet.

statistically significant *decreased* ($p = 0.0336$) incidence in the 20 ppm group females when compared to control.

For females, there were statistically significant *decreased* trends in the incidences of mammary fibroadenoma and mammary combined adenoma and fibroadenoma. These were due to significantly lower incidences in the respective 20 ppm group when compared to that of the control. However, the 0.5 ppm group females for these exhibited statistically significant increases in combined adenoma/fibroadenoma as compared to the control. In addition, a statistically significant *decreased* incidence of mammary carcinoma

Table 4

Results of the statistical analyses of incidence of non-neoplastic microscopic findings in livers of female rats (statistically significant observations in bold). Data included are from the scheduled sacrifices during Weeks 14 and 53, unscheduled sacrifices, and the terminal sacrifice.

Finding	Incidence[a] (top row) and significance (*p*-value)[b] by K⁺PFOS dietary dose group						
	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm Rec.[c]	20 ppm Rec. vs. 20 ppm
Infiltrate, lymphohistiocytic							
	42/65	42/55	38/55	41/55	56/65	32/40	
	.0080+**	NA	.3738+	NA	**.0038+****	.0709+	.2852−
Hypertrophy, hepatocellular, centrilobular							
	2/65	1/55	4/55	16/55	52/65	2/40	
	.0000+**	NA	.2641+	**.0001+****	**.0000+****	NA	**.0000**−**
Granular cytoplasm, eosinophilic, centrilobular							
	0/65	0/55	0/55	7/55	36/65	1/40	
	.0000+**	NA	NA	**.0034+****	**.0000+****	.3810+	**.0000**−**
Pigment, hepatocellular, centrilobular							
	0/65	0/55	0/55	1/55	36/65	0/40	
	.0000+**	NA	NA	NA	**.0000+****	NA	**.0000**−**
Necrosis, individual hepatocyte							
	7/65	6/55	6/55	6/55	15/65	3/40	
	.0359+*	NA	NA	NA	**.0500+***	.4254−	**.0329**−*
Vacuolation, hepatocellular, midzonal/centrilobular							
	1/65	1/55	0/55	1/55	4/65	1/40	
	.0753+	NA	NA	NA	.1826+	NA	.3660−
Vacuolation, hepatocellular, periportal							
	15/65	15/55	22/55	16/55	22/65	11/40	
	.1052+	NA	**.0358+***	NA	.1217+	.3877+	.3235−
Focus, altered hepatocellular, clear/eosinophilic cell							
	27/65	21/55	18/55	17/55	21/65	13/40	
	.0985−	.4263−	.2109−	.1553−	.1818−	.2367−	NA
Focus, altered hepatocellular, basophilic							
	16/65	20/55	13/55	21/55	20/65	11/40	
	.2314+	.1153+	.5367−	.0801+	.2785+	.4573+	.4487−
Degeneration, cystic							
	0/65	1/55	1/55	2/55	4/65	1/40	
	.0187+*	NA	NA	.2080+	.0596+	.3810+	.3660−
Necrosis, coagulative							
	3/65	2/55	4/55	2/55	2/65	2/40	
	.3857−	NA	NA	NA	NA	NA	NA
Hematopoiesis, extramedullary							
	10/65	17/55	15/55	8/55	7/65	5/40	
	.0721−	**.0352+***	.0851+	.5528−	.3020−	.4579−	NA
Hyperplasia, bile duct							
	23/65	25/55	19/55	17/55	30/65	17/40	
	.3266+	.1749+	.5389−	.3738−	.1421+	.2999+	.4358−
Fibrosis, Peribiliary							
	9/65	13/55	4/55	9/55	10/65	4/40	
	.4341−	.1264+	.1960−	NA	NA	.3987−	.3168−
Hypertrophy, hepatocellular, periportal							
	12/65	10/55	9/55	4/55	3/65	7/40	
	.0026−**	.5796−	.4778−	.0614−	**.0127**−*	NA	**.0344+***
Infiltrate, macrophage, pigmented							
	2/65	3/55	5/55	6/55	23/65	7/40	
	.0000+**	NA	.1567+	.0889+	**.0000+****	**.0147+***	**.0383**−*
Vacuolation, hepatocellular							
	3/65	2/55	0/55	3/55	3/65	2/40	
	.4582+	NA	.1555−	NA	NA	NA	NA
Cyst, biliary							
	3/65	1/55	1/55	1/55	4/65	2/40	
	.3708+	.3749−	.3749−	.3749−	NA	NA	NA
Inflammation, subacute							
	2/65	0/55	0/55	0/55	1/65	0/40	
	.2760−	.2913−	.2913−	.2913−	NA	.3810−	NA
Angiectasis							
	6/65	1/55	2/55	1/55	1/65	2/40	
	.0231−*	.0881−	.1976−	.0881−	.0574−	.3491−	.3232+
Congestion							
	2/65	2/55	1/55	0/55	0/65	0/40	
	.0451−*	NA	NA	.2913−	.2481−	.3810−	NA

[a] Number of observations per number of rats observed.

[b] The *p*-values given in the control column are for trend and those for individual K⁺PFOS dietary dose groups are as compared to control. The *p*-value in the last column is for the statistical comparison of the 20 ppm Recovery (Rec.) group response with the 20 ppm group response. The minus sign (−) indicates an effect in the decreased direction. The plus sign (+) indicates an effect in the decreased direction. The symbols * and ** represent comparisons that are statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. NA indicates that the response was not analyzed.

[c] The 20 ppm dietary dose recovery group (20 ppm Rec.) was given 20 ppm K⁺PFOS in diet for up to 53 weeks, after which the group was fed control diet.

Table 5
Statistical analyses of neoplastic lesions in male rats (statistically significant observations in bold).

Organ	Incidence[a] (top row) and significance (p-value)[b] by K+PFOS dietary dose group						
Finding	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm Rec.[c]	20 ppm Rec. vs. 20 ppm
Liver							
Hepatocellular, adenoma							
Fatal incidence	0	0	1	0	0	0	
Incidental incidence	0	3	2	1	7	0	
Total incidence rate	0/60	3/50	3/50	1/50	7/60	0/40	
One-sided p-value	**.0276+***	.1345+	.0689+	NA	**.0456** + *E	NA	**.0240**−*
Thyroid							
Follicular cell, adenoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	3	5	4	4	4	9	
Total incidence rate	3/60	5/49	4/50	4/49	4/59	9/39	
One-sided p-value	.4998−	.3967+	NA	NA	NA	**.0280+***	**.0121+***
Follicular cell, carcinoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	3	1	1	2	1	1	
Total incidence rate	3/60	1/49	1/50	2/49	1/59	1/39	
One-sided p-value	.2210−	.3855−	.2448−	NA	.1734−	.4280−	NA
Follicular cell, adenoma/carcinoma (combined)							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	6	6	5	5	5	10	
Total incidence rate	6/60	6/49	5/50	5/49	5/59	10/39	
One-sided p-value	.2520−	NA	NA	NA	NA	.0970+	**.0125+***

[a] Number of observations per number of rats observed.
[b] The p-values given in the control column are for trend and those for individual K+PFOS dietary dose groups are as compared to control. The p-value in the last column is for the statistical comparison of the 20 ppm Recovery (Rec.) group response with the 20 ppm group response. The minus sign (−) indicates an effect in the decreased direction. The plus sign (+) indicates an effect in the decreased direction. The symbol * represents comparisons that are statistically significant at $p \leq 0.05$. NA indicates that the response was not analyzed. E indicates that the exact permutation test was used.
[c] The 20 ppm dietary dose recovery group (20 ppm Rec.) was given 20 ppm K+PFOS in diet for up to 53 weeks, after which the group was fed control diet.

was found for the 20 ppm Rec. group when compared to the 20 ppm group. There were no other significant group effects in mammary carcinoma.

There were no other statistically significant neoplastic findings for any other anatomic site evaluated in males or females fed K+PFOS in diet when compared with respective control group rats.

3.8. Serum and liver concentrations of PFOS

The results of quantitative determination of serum and liver PFOS concentrations are presented in Table 7, which, for the sake of completeness, includes data from the 4 and 14 week observations previously reported by (Seacat et al., 2003a). Both serum and liver concentrations of PFOS increased in approximate proportion to dose at all time points measured in male and female rats. The concentrations also increased in approximate proportion to length of dosing between Weeks 4 and 14; however, Week 53 concentrations in the 20 ppm group were similar to those measured on Week 14, suggesting that steady state may have been approached after 14 weeks on diet in the 20 ppm dose group. Samples obtained at sacrifice on Week 105 showed much greater individual variation in serum PFOS. In males, serum concentrations measured at terminal sacrifice were 33%, 44%, 51%, and 47% of those measured on Week 14 in the 0.5, 2, 5, and 20 ppm groups, and liver concentrations were 33%, 36%, 19%, and 33% of Week 14 values in the same dose groups, respectively. This decline was likely due to chronic progressive nephritis leading to increased urinary excretion of PFOS across all treatment groups. Individual serum PFOS concentrations correlated significantly ($p < 0.05$, Spearman's Rho) with the incidence and severity of chronic progressive nephritis within all male groups, including controls, except for the 20 ppm Rec. group males ($p = 0.0513$, $n = 4$). In females, correlation of serum PFOS with incidence and severity of chronic progressive nephritis was less apparent, being statistically significant only at the 2 ppm dose level; although, the p-values for the 5 ppm and 20 ppm Rec. group were approximately 0.06. This correlation was less apparent in females as reflected in the lesser extent of decline in serum and liver PFOS concentrations between Week 14 and terminal sacrifice. Serum concentrations measured at terminal sacrifice were 63%, 74%, 116%, and 104% of those measured on Week 14 in the 0.5, 2, 5, and 20 ppm groups, and liver concentrations were 67%, 80%, 35%, and 60% of Week 14 values in the same dose groups, respectively. Serum and liver concentrations from the 20 ppm recovery group rats measured at terminal sacrifice on Week 106 were a small percentage of the concentrations in 20 ppm group rats as measured on Week 105.

3.9. Benchmark dose estimates for increased liver tumor incidence

All benchmark dose estimations for increased liver tumor incidence yielded adequate goodness-of-fit for the multistage model. For male rats, dietary doses corresponding to the estimated BMD_{10} and $BMDL_{10}$ were 18.2 ppm and 7.9 ppm ($p = 0.24$), respectively. Corresponding values for female rats were 16.7 ppm and 8.0 ppm ($p = 0.54$), respectively. BMD_{10} and $BMDL_{10}$ values expressed as serum PFOS concentration after 14 weeks of dosing were also estimated. For males, these values were 135 μg/mL and 62 μg/mL ($p = 0.23$), respectively. For females, they were 193 μg/mL and 92 μg/mL ($p = 0.54$), respectively.

4. Discussion

During the course of the two-year chronic toxicity study reported herein, K+PFOS provided at a nominal dietary concetrations up to 20 ppm in diet was relatively well tolerated. Survival was unaffected for females at the two highest dose groups, and was actually increased for males in the two highest dose groups.

Table 6
Statistical analyses of neoplastic lesions in female rats (statistically significant observations in bold).

Organ	Incidence[a] (top row) and significance (*p*-value)[b] by K⁺PFOS dietary dose group						
Finding	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm Rec.[c]	20 ppm Rec. vs. 20 ppm
Liver							
Hepatocellular, adenoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	1	1	1	5	2	
Total incidence rate	0/60	1/50	1/49	1/50	5/60	2/40	
One-sided *p*-value	**.0153+***	NA	NA	NA	**.0386+***	.2040+	.3050−
Hepatocellular, carcinoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	0	0	0	1	0	
Total incidence rate	0/60	0/50	0/49	0/50	1/60	0/40	
Note: Incidences across groups do not meet selection criterion.							
Hepatocellular, adenoma/carcinoma (combined)							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	1	1	1	6	2	
Total incidence rate	0/60	1/50	1/49	1/50	6/60	2/40	
One-sided *p*-value	**.0057+****	NA	NA	NA	**.0204+***	.2040+	.2114−
Thyroid							
Follicular cell, adenoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	0	0	2	1	1	
Total incidence rate	0/60	0/50	0/49	2/50	1/60	1/40	
One-sided *p*-value	.1002+	NA	NA	.1054+	NA	.2387−	NA
Follicular cell, carcinoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	0	0	1	0	0	
Total incidence rate	0/60	0/50	0/49	1/50	0/60	0/40	
Note: Incidences across groups do not meet selection criterion.							
Follicular cell, adenoma/carcinoma (combined)							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	0	0	3	1	1	
Total incidence rate	0/60	0/50	0/49	3/50	1/60	1/40	
One-sided *p*-value	.0790+	NA	NA	**.0471+***	NA	.1539−	NA
C-Cell, adenoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	12	6	6	8	5	6	
Total incidence rate	12/60	6/50	6/49	8/50	5/60	6/40	
One-sided *p*-value	.0703−	.1478−	.1705−	.2103−	**.0336−***	.2392−	.3548+
C-Cell, carcinoma							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	0	1	0	0	0	1	
Total incidence rate	0/60	1/50	0/49	0/50	0/60	1/40	
Note: Incidences across groups do not meet selection criterion.							
C-Cell, adenoma/carcinoma (combined)							
Fatal incidence	0	0	0	0	0	0	
Incidental incidence	12	7	6	8	5	7	
Total incidence rate	12/60	7/50	6/49	8/50	5/60	7/40	
One-sided *p*-value	.0560−	.2284−	.1705−	.2103−	**.0336−***	.3380−	.2399+
Mammary							
Fibroadenoma							
Total incidence rate	20/60	27/50	19/48	24/50	11/60	15/40	
One-sided *p*-value	**.0152−***	**.0337+***	.1125+	.2521+	**.0235−***	.4099−	.0573+
Adenoma							
Total incidence rate	7/60	6/50	5/48	7/50	4/60	4/40	
One-sided *p*-value	.2359−	NA	.4086+	NA	.2315−	NA	.5055+
Fibroadenoma/adenoma							
Total incidence rate	23/60	30/50	22/48	26/50	15/60	16/40	
One-sided *p*-value	**.0239−***	**.0318+***	.0527+	.2753+	**.0488−***	NA	.1451+
Carcinoma							
Total incidence rate	11/60	12/50	15/48	11/50	14/60	4/40	
One-sided *p*-value	.3569	.3996+	.0515+	NA	.3373+	.0606−	**.0161−***

[a] Number of observations per number of rats observed.
[b] The *p*-values given in the control column are for trend and those for individual K⁺PFOS dietary dose groups are as compared to control. The *p*-value in the last column is for the statistical comparison of the 20 ppm Recovery (Rec.) group response with the 20 ppm group response. The minus sign (−) indicates an effect in the decreased direction. The plus sign (+) indicates an effect in the decreased direction. The symbols * and ** represent comparisons that are statistically significant at $p \leq 0.05$ and $p < 0.01$, respectively. NA indicates that the response was not analyzed.
[c] The 20 ppm dietary dose recovery group (20 ppm Rec.) was given 20 ppm K⁺PFOS in diet for up to 53 weeks, after which the group was fed control diet.

Serum PFOS concentrations reached were in the range of or significantly exceeded reported higher-end human occupational serum PFOS concentrations. The serum concentrations achieved at the lowest dietary dose of 0.5 ppm approximated those reported for more highly exposed fluorochemical production workers (Olsen et al., 2003c). At the 2 ppm dose level, serum concentrations rose to and exceeded the highest reported human occupational serum concentration of 12.8 μg/mL (Olsen et al., 1999), and serum PFOS concentrations at the 20 ppm dose level were at least an order of magnitude higher than the highest reported human serum PFOS

Table 7
Mean PFOS concentrations ± standard deviation in serum (μg/mL) and liver (μg/g) for male and female rats. Sample size was $N = 5$ unless specified in parenthesis after each value.

Week	Matrix	Dietary K^+PFOS concentration, μg K^+PFOS/g diet					
		0	0.5	2	5	20	20 Rec.[a]
Males							
4	Serum	<LOQ[b]	0.91 ± 0.62	4.33 ± 1.16	7.57 ± 2.17	41.80 ± 7.92	–[c]
	Liver	0.104 ± 0.067	11.00 ± 2.31	31.30 ± 5.84	47.60 ± 12.50	282.0 ± 45.30	–
14	Serum	<LOQ[d]	4.04 ± 0.80	17.10 ± 1.22	43.90 ± 4.90	148.0 ± 13.80	–
	Liver	0.459 ± 0.057	23.80 ± 3.45	74.00 ± 6.16	358.0 ± 28.80	568.0 ± 107.0	–
53	Serum	0.025 ± 0.018	–	–	–	146.0 ± 33.5 (*4*)	–
	Liver	0.635 ± 1.040 (*10*)	–	–	–	435.0 ± 96.9 (*9*)	–
102	Serum	–	–	–	–	–	–
	Liver	–	–	–	–	–	–
105	Serum	0.012 ± 0.010 (*11*)	1.31 ± 1.30 (*10*)	7.60 ± 8.60 (*17*)	22.50 ± 23.50 (*25*)	69.3 ± 57.9 (*22*)	–
	Liver	0.114 ± 0.148 (*11*)	7.83 ± 7.34 (*10*)	26.40 ± 20.40 (*17*)	70.50 ± 63.10 (*25*)	189.0 ± 141.0 (*22*)	–
106	Serum	–	–	–	–	–	2.42 ± 5.09 (*10*)
	Liver	–	–	–	–	–	3.12 ± 5.97 (*10*)
Females							
4	Serum	0.026 ± 0.007	1.61 ± 0.21	6.62 ± 0.50	12.60 ± 1.73	54.00 ± 7.34	–
	Liver	0.107 ± 0.049	8.71 ± 0.55	25.00 ± 6.11	83.00 ± 14.10	373.0 ± 44.1	–
14	Serum	2.67 ± 4.58	6.96 ± 0.99 (*4*)	27.30 ± 2.34	64.40 ± 5.48	223.0 ± 22.40	–
	Liver	12.00 ± 22.40	19.20 ± 3.77	69.20 ± 3.46	370.0 ± 22.30	635.0 ± 49.00	–
53	Serum	0.395 ± 0.777	–	–	–	–	–
	Liver	0.923 ± 1.77 (*10*)	–	–	–	560.0 ± 180.0 (*10*)	–
102	Serum	–	–	20.20 ± 13.30 (*9*)	–	–	–
	Liver	–	–	55.10 ± 31.50 (*9*)	–	–	–
105	Serum	0.084 ± 0.134 (*24*)	4.35 ± 2.78 (*15*)	–	75.00 ± 45.70 (*15*)	233.0 ± 124.0 (*25*)	–
	Liver	0.185 ± 0.184 (*24*)	12.90 ± 6.81 (15)	–	131.0 ± 61.40 (*15*)	381.0 ± 176.0 (*25*)	–
106	Serum	–	–	–	–	–	9.51 ± 8.70 (*17*)
	Liver	–	–	–	–	–	12.90 ± 10.40 (*17*)

[a] Recovery.
[b] LOQ = limit of quantitation = 0.009 μg/mL.
[c] Not available.
[d] LOQ = limit of quantitation = 0.046 μg/mL.

concentration. Rats have shown higher liver-to-serum PFOS concentration ratios than non-human primates (Chang et al., 2012) and humans (Olsen et al., 2003b). Concentrations of PFOS anion in serum and liver appeared to be in approximate proportion to dose and time through 14 weeks of dietary exposure. Comparison of 20 ppm dose group PFOS concentrations in serum and liver with those measured from the samples taken during Week 53 suggests that steady state was attained or approached after 14 weeks on diet. However, serum PFOS concentrations in samples from terminally sacrificed males were approximately one-half those from Week 14, and liver PFOS concentrations in terminally sacrificed males were approximately one-third or less those from Week 14. Serum PFOS in terminally sacrificed male rats appeared to correlate with the incidence and severity of chronic progressive nephritis. PFOS concentrations in serum and liver of females trended higher than males of the same dose group and time point. The presence of PFOS in the serum and liver of control rats was indicative of the widespread environmental presence of PFOS in the time period that the study was conducted and likely related to contamination of fish meal included in the basal PMI 5002 diet.

Liver was the principal target of dietary exposure. The liver effects, as evidenced by either serum clinical chemistry or microscopic observations, were largely limited to centrilobular findings of hypertrophy, eosinophilic hepatocytic granules, hepatocytic pigment, hepatocytic vacuolation, and an increase in hepatocellular adenoma in the highest dietary dose group (20 ppm). Transmission electron microscopic examination of control and 20 ppm dose group liver sections revealed that the principal difference was the occurrence in 20 ppm dosed rats of hepatocytes with minimal to mild hypertrophy and mild to moderate increase in smooth endoplasmic reticulum. There were also slight increases in glycogen and lipid, the latter occurring as vacuoles in the cytoplasm. Oil Red O staining of hepatocytes light microscopic sections of control and highest PFOS dose treated rats did not reveal a clear difference between the control and treated groups. All liver tumors identified were found in rats surviving to terminal sacrifice. Statistically significant increases in benign hepatocellular adenoma were observed at the highest dose tested (20 ppm in diet) for males and females. Due to the elevations at the 20 ppm dose level, hepatocellular adenoma was also positive for trend. The only hepatocellular carcinoma observed was from a 20 ppm female rat. Although this was not in and of itself considered significant, it contributed to the statistical evaluation of combined hepatocellular adenoma/carcinoma. For females, combined hepatocellular adenoma/carcinoma incidence was also increased in the 20 ppm dose group relative to controls and was also increased for trend across groups.

Because liver was clearly identified as the principal target organ of PFOS in the study reported herein, the study exposure conditions allow relevant extrapolation to human exposure based on serum concentration as a measure of accumulated body burden. The observation in this and other studies that rats have higher liver-to-serum PFOS concentration ratios than humans or non-human primates suggests that the use of serum PFOS concentrations as a measure of exposure in extrapolating from rat data to assess human health risk is likely conservative with respect to effects originating from liver response to PFOS exposure. Several years before initiation of the study reported herein, Sohlenius et al. (1993) reported that PFOS given to mice in their diets caused changes consistent with those caused by peroxisome proliferators. In the study reported herein, clear evidence for K^+PFOS-mediated increased hepatocellular proliferation and peroxisomal proliferation with respect to control rats was not found at the times evaluated (see Seacat et al. (2003a,b) for 4- and 14-week observations). Although the presence of increased eosinophilic granules in the cytoplasm of hepatocytes from K^+PFOS-treated rats may be consistent with an increase in peroxisomes (Umeda et al., 2004), transmission electron microscopic examination of hepatocytes from terminally sacrificed

rats did not demonstrate a clear increase in peroxisomal bodies. However, a number of more recent studies have confirmed PFOS as an agonist for and activator of PPARα, as well as CAR and PXR (Bijland et al., 2011; Bjork et al., 2011; Elcombe et al., 2012a,b; Takacs and Abbott, 2007; Wolf et al., 2008) suggesting that the hepatomegaly and benign liver tumors observed after chronic dietary exposure of Sprague Dawley rats to PFOS may be due to activation of these nuclear receptors.

In humans, the possible association of PFOS exposure with cancer outcomes has been studied in occupationally exposed workers (Alexander and Olsen, 2007; Alexander et al., 2003) and the general population (Eriksen et al., 2009). In a cohort mortality study of 2083 workers with occupational exposure to PFOS and materials that can be metabolized to PFOS, Alexander et al. reported null results for causes of death selected *a priori* as diseases of interest, which included liver cancer (2 deaths versus 1.2 expected). However, an excess of death from bladder cancer was observed in the highly exposed group, based on 3 cases. Due to a relatively low case-fatality rate, mortality data do not fully describe the extent of bladder cancer risk in a population (NCI, 2011). Therefore, Alexander and Olsen (2007) subsequently conducted a bladder cancer incidence study of this same cohort and concluded that the results provided little support for an association between bladder cancer and PFOS exposure; although, the study population size limited a conclusive exposure-response analysis. In a large case-cohort study covering a 13-year follow-up after enrollment between 1993 and 1997, Eriksen et al. (2009) did not find significant linear trends in adjusted incidence rate ratios related to *a priori* cancers of the prostate, bladder, pancreas, and liver and study enrollment plasma PFOS concentrations. Thus, the human epidemiological data that are available to date do not provide evidence suggestive of a risk of cancer from exposure to PFOS.

With respect to bladder cancer, there were no findings in the rat study reported herein suggestive of potential bladder effects. Urinalysis and urine chemistry results were essentially normal, and microscopic evaluation of the bladder did not reveal findings consistent with potential bladder neoplasia (see Supplementary Table 2). In an inhalation study conducted with the volatile starting material perfluorooctanesulfonyl fluoride, which hydrolyzes to PFOS, and designed specifically to detect factors known to be associated with the development of bladder cancer, no changes indicative of potential bladder effects or increased risk of bladder tumors were observed in rats (Kenny, 2005). Thus, the human epidemiological data and rat chronic data that are available to date do not provide evidence suggestive of a risk of bladder cancer from exposure to PFOS.

Shankar et al. (2011) recently analyzed the National Health Nutrition Examination Survey (NHANES) cross-sectional 1999–2008 database and suggested that general population PFOS concentrations were associated with chronic kidney disease, based on estimated glomerular filtration (eGFR) rate of less than 60 mL/min/1.73 m^2. The findings from Shankar et al. are not consistent with cross-sectional medical surveillance results of 469 PFOS production workers (mean serum PFOS concentration approximately 1000 ng/mL) among whom no significant differences for BUN, serum creatinine, and urinalyses were associated with serum PFOS concentrations; although GFRs were not calculated (Olsen et al., 2003a). In reviewing clinical chemistry data, organ weight data, and microscopic pathology, no evidence for renal effects was observed in the study reported herein. Shankar et al. suggested that their data were plausible based on toxicological findings from Cui et al. (2009) in which rats given 20 mg/kg PFOS for up to 28 days experienced a mean body weight decrease of 33% and mortalities were allowed to occur over a 2-week period, reaching 100% mortality prior to scheduled study termination. Turbidness and swelling of the epithelia of the proximal convoluted tubules and mild congestion in the renal cortex and medulla were noted by Cui et al. at the 20 mg/kg dose but no kidney effects were observed in rats given 5 mg/kg for 28 days. Serum PFOS concentrations at the 5 mg/kg dose were three orders of magnitude higher than the range of concentrations in the 2003–2004 NHANES data. Thus, taking the available toxicological database for PFOS into consideration, including the study reported herein, there are no compelling data or arguments that support a causal basis for increased kidney disease risk from occupational or general population levels of exposure to PFOS.

In the study reported herein there were no anatomical indications of a response of the thyroid to dietary treatment with K^+PFOS, including thyroid weight and microscopic histological changes, with the possible exception of the male 20 ppm Rec. group. The observation of a statistically significant increased incidence of thyroid follicular cell adenoma in the 20 ppm Rec. group males without observation of similar increases in males and/or females of the 20 ppm group is paradoxical and may represent a chance occurrence. Further studies reported in this issue have not identified plausible causative explanations for the finding (Elcombe et al., 2012a,b). Thus, the finding in the 20 ppm Rec. group males remains unexplained and most likely spurious. In the females, the only statistically significant increase in thyroid follicular tumors was in the 5 ppm dose group for combined adenoma and carcinoma ($p = 0.05$). This was based on the occurrence of one carcinoma and two adenomas among 50 rats at that dose, neither tumor type being statistically significant when not combined. These tumors are known to occur in historical controls, and the lack of both dose–response and non-neoplastic thyroid effects in female rats suggests that the thyroid follicular cell tumors at the 5 ppm dose were a spurious finding.

In conclusion, the two-year chronic toxicity and cancer bioassay conducted with K^+PFOS in Sprague Dawley rats via dietary exposure that is reported herein identified liver as the principal site of tissue response to K^+PFOS. Non-neoplastic hepatic responses to treatment included hepatocellular hypertrophy characterized by expansion of the smooth endoplasmic reticulum, increased lipid and glycogen deposits, vacuolation, and increased eosinophilic granulation of the cytoplasm of males and females. The only neoplastic response attributed to treatment was an increased incidence hepatocellular adenoma in males and females of the highest dietary treatment group (20 ppm), and these tumors were incidental observations in rats surviving to terminal sacrifice. The only hepatocellular carcinoma observed was in a 20 ppm dose group female. In male and female rats, the dietary dose corresponding to the estimated lower 95% confidence interval of the benchmark dose for a 10% increased incidence of liver tumors was 8 ppm. The liver effects observed are consistent with those that would be expected from activation of the xenosensor nuclear receptors NR1C1 (PPARα), NR1I3 (CAR), and NR1L2 (PXR), as has been demonstrated in articles in this issue (Elcombe et al., 2012a,b). Human epidemiological data do not provide support for cancer risk from exposure to PFOS.

Conflict of interest statement

John L. Butenhoff, Shu-Ching Chang, and Geary W. Olsen are employees of 3M Company, a former manufacturer of K^+PFOS and the company supporting the work reported in the article. Peter J. Thomford does not have competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tox.2012.01.003.

References

Alexander, B.H., Olsen, G.W., Burris, J.M., Mandel, J.H., Mandel, J.S., 2003. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occup. Environ. Med. 60, 722–729.

Alexander, B.H., Olsen, G.W., 2007. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Ann. Epidemiol. 17, 471–478.

Bailer, A.J., Portier, C.J., 1988. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics 44, 417–431.

Bijland, S., Rensen, P.C.N., Pieterman, E.J., Maas, A.C.E., van der Hoorn, J.W., Van Erk, M.J., Havekes, L.M., van Dijk, K.W., Chang, S.-C., Ehresman, D.J., Butenhoff, J.L., Princen, H.M.G., 2011. Perfluoroalkyl sulfonates cause alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-leiden CETP mice. Toxicol. Sci. 123, 290–303.

Bjork, J.A., Butenhoff, J.L., Wallace, K.B., 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. Toxicology 288, 8–17.

Chang, S.-C., Noker, P.E., Gorman, G.S., Gibson, S.J., Hart, J.A., Ehresman, D.J., Butenhoff, J.L., 2012. Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. Reprod. Toxicol., doi:10.1016/j.reprotox.2011.07.002, in press.

Cui, L., Zhou, Q., Liao, C., Fu, J., Jiang, G., 2009. Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch. Environ. Contam. Toxicol. 56, 338–349.

Dinse, G.E., Lagakos, S.W., 1983. Regression analysis of tumor prevalence data. J. Roy. Stat. Soc. C (Appl. Stat.) 32, 236–248.

Dunnett, C.W., 1964. New tables for multiple comparisons with a control. Biometrics 20, 482–491.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.C., Ehresman, D.J., Butenhoff, J.L., 2012a. Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. Toxicology 293, 16–29.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.C., Ehresman, D.J., Noker, P.E., Butenhoff, J.L., 2012b. Evaluation of hepatic and thyroid responses in male Sprague Dawley rats for up to eighty-four days following seven days of dietary exposure to potassium perfluorooctanesulfonate. Toxicology 293, 30–40.

Eriksen, K.T., Sorensen, M., McLaughlin, J.K., Lipworth, L., Tjonneland, A., Overvad, K., Raaschou-Nielsen, O., 2009. Perfluorooctanoate and Perfluorooctanesulfonate Plasma Levels and Risk of Cancer in the General Danish Population. J. Natl. Cancer Inst.

Gart, J.J., Krewski, D., Lee, P.N., Tarone, R.E., Wahrendorf, J., 1986. Statistical Methods in Cancer Research: The Design and Analysis of Long-Term Animal Experiments, vol. 3, no. 79. IARC Scientific Publications/Oxford University Press, New York.

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

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

ILAR, 1996. Guide for the Care and Use of Laboratory Animals. National Research Council, Institute of Laboratory Animal Resources. National Academy Press, Washington, DC.

Kenny, T.J., 2005. Final report: Perfluorooctanesulfonyl fluoride (POSF; T-7661.4) toxicity study by inhalation administration to CD rats for 13 weeks followed by a 4 week recovery period (Huntingdon Life Sciences LTD, Cambridgeshire, England). Available from United States Environmental Protection Agency Administrative Record 226, Document AR-226-3573.

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit. Rev. Toxicol. 33, 655–780.

Lake, B.G., 2009. Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582–596.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.

Levene, H., 1960. Robust tests for equality of variances. In: Olkin, I. (Ed.), Contributions to Probability and Statistics. Stanford University Press, Stanford, pp. 278–292.

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C., 2003a. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 22, 196–204.

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C., 2003b. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 22, 189–195.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., Boorman, G.A., 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76, 283–289.

NCI, 2011. National Cancer Institute, Surveillance Epidemiology and End Results. SEER Stat Fact Sheets: Bladder. Incidence and Mortality. URL: http://seer.cancer.gov/statfacts/html/urinb.html#incidence-mortality (accessed 09.06.11).

Olsen, G.W., Burris, J.M., Mandel, J.H., 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., Burris, J.M., Burlew, M.M., Mandel, J.H., 2003a. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. J. Occup. Environ. Med. 45, 260–270.

Olsen, G.W., Hansen, K.J., Stevenson, L.A., Burris, J.M., Mandel, J.H., 2003b. Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. Environ. Sci. Technol. 37, 888–891.

Olsen, G.W., Logan, P.W., Hansen, K.J., Simpson, C.A., Burris, J.M., Burlew, M.M., Vorarath, P.P., Venkateswarlu, P., Schumpert, J.C., Mandel, J.H., 2003c. An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: biomonitoring. AIHA J. (Fairfax, Va) 64, 651–659.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115, 1298–1305.

Peto, R., Pike, M.C., Day, N.E., Gray, R.G., Lee, P.N., Parish, S., Peto, J., Richards, S., Wahrendorf, J., 1980. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer, Lyon.

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., Butenhoff, J.L., 2003a. Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. Toxicology 183, 117–131.

Seacat, A.M., Thomford, P.J., Hansen, K.J., Clemen, L.A., Eldridge, S.R., Elcombe, C.R., Butenhoff, J.L., 2003b. Erratum to "sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats" [Toxicology 183 (2003) 117–131]. Toxicology 192, 263–264.

Shankar, A., Xiao, J., Ducatman, A., 2011. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am. J. Epidemiol. 174, 893–900.

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

Takacs, M.L., Abbott, B.D., 2007. Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. Toxicol. Sci. 95, 108–117.

Thakur, A.K., Berry, K.J., Mielke Jr., P.W., 1985. A FORTRAN program for testing trend and homogeneity in proportions. Comput. Programs Biomed. 19, 229–233.

Thomas, D.G., Breslow, N., Gart, J.J., 1977. Trend and homogeneity analyses of proportions and life table data. Comput. Biomed. Res. 10, 373–381.

Thomford, P.J., 2002. Final report: 104-week dietary chronic toxicity and carcinogenicity with perfluroorctanesulfonic acid postassium salt (PFOS; T-6295) in rats (Covance Laboratory Inc, Madison, WI). Available from United States Environmental Protection Agency Administrative Record 226, Document AR-226-1051a.

Umeda, Y., Aiso, S., Arito, H., Nagano, K., Matsushima, T., 2004. Induction of peroxisome proliferation in the liver of biphenyl-fed female mice. J. Occup. Health 46, 486–488.

Winer, B.J., 1971. Analysis of covariance. In: Statistical Principals in Experimental Design, second ed. McGraw-Hill, New York, pp. 752–812.

Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C., Abbott, B.D., 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol. Sci. 106, 162–171.