AR226-1811

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY

Title: Comparative Molecular Biology of Perfluorooctanesulfonate (PFOS, T-6295), N-ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE, T-6316), N-Ethyl perfluorooctanesulfonamide (N-EtFOSA, T-6868), Perfluorooctanesulfonamido acetate (FOSAA, T-7071), and/or Perfluorooctanesulfonamide (FOSA, T-7132) of in Rats and Guinea Pigs following Oral dosing.

Final Report
Date: May 25, 2004

Study Numbers. T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
Strategic Toxicology Study Number: DT-15-B

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In-Life Start Date	**Protocol:**	**11/16/1998**
In-Life End Date	**Protocol:**	**11/20/1998**
In-Life Start Date	**Amendment #1:**	**03/01/1999**
In-Life End Date	**Amendment #1:**	**03/05/1999**
In-Life Start Date	**Amendment #2:**	**02/19/2001**
In-Life End Date	**Amendment #2:**	**02/23/2001**

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Summary

Adult male and female Sprague-Dawley rats and Harlan guinea pigs received oral gavage doses of vehicle control (2% Tween 80), 40 mg/kg/day (mkd) perfluorooctanesulfonate (PFOS, T-6295), 40 mkd or 160 mkd N-Ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE, T-6316), or 40 mg/kg/day N-Ethyl perfluorooctanesulfonamide (N-EtFOSA, T-6868) for four days and were humanely sacrificed on the fifth day. Male Sprague-Dawley rats received oral gavage doses of vehicle control (propylene glycol), 160 mkd perfluorooctanesulfonamidoacetate (FOSAA, M556, T-7071), or 40 mkd perfluorooctansulfonamide (FOSA, T-7132) for four days and were humanely sacrificed on the fifth day.

All animals survived. The percent initial body weights were significantly decreased in rats by 40 mkd PFOS and 160 mkd N-EtFOSE significantly reduced body weight compared to controls to 85% and 87% initial body weight, at average liver PFOS concentrations of > 600 μg/g. 40 mkd FOSA and 160 mkd FOSAA significantly reduced body weight to 93% and 95 % of initial body weight, respectively, at average liver PFOS concentrations of 140 μg/g and 200 μg/g, respectively. Neither 40 mkd N-EtFOSE of 40 mkd N-EtFOSA had a significant effect on body weight in rats. In the rat, liver to body weight ratios were significantly increased by 40 mkd PFOS at average liver PFOS concentrations of > 600 μg/g. Increased liver to body weight ratios were found in rats from all other dose groups, but these changes were not significant compared to the control.

The guinea pig percent body weight was significantly decreased by 160 mkd N-EtFOSE and 40 mkd PFOS to 91% and 93% initial body weight, at average liver PFOS concentrations of 419 μg/g and 171 μg/g, respectively. Guinea pig liver to body weight ratios were unchanged. Guinea pig kidney to body ratios were significantly increased by treatment in all the dose groups measured, i.e. 40 mkd N-EtFOSE, N-EtFOSA, or PFOS.

In rats, cholesterol (CHOL), triglycerides (TRIG), alkaline phosphatase (ALKP), and aspartate aminotransferase (AST) were significantly lowered in rats treated with 40 mkd PFOS and 160 mkd N-EtFOSE. Significant decreases in serum potassium (K^+) occurred in rats treated with 40 mkd PFOS, 40 mkd N-EtFOSE and 40 mkd N-EtFOSA. In rats, 40 mkd PFOS caused a significant increase in hepatic palmitoyl CoA oxidase (PCoAO) activity, but 160 N-EtFOSE did not. Exposure of rats to 160 mkd N-Et-FOSE caused a doubling of the specific activity of lauryl CoA oxidase (LCoAO) and a 2-fold increase in the concentration of mRNA encoding for PCoAO in rat liver, but catalase activity and catalase mRNA were unchanged. Exposure of rats to 40 mkd PFOS caused a 2-fold increase in LCoAO activity and a 3 to 6-fold increase in PCoAO mRNA expression in rats. Treatment with 40 mkd FOSA or 160 mkd FOSAA caused a significant increase in hepatic cytochrome P450 content and Acyl CoA oxidase activity in male rats.

PFOS was apparently a more potent peroxisome proliferator than N-EtFOSE. PFOS significantly induced PCoAO activity whereas N-EtFOSE increased, but did not significantly induce PCoAO activity, even though both treatments achieved similarly high liver PFOS concentrations of greater than 600 μg/Kg. PCoAO activity was not

determined for the 40 mkd FOSA and 160 mkd FOSAA treated male rats, but a 2-fold induction of acyl CoA oxidase activity occurred at relatively lower average liver PFOS concentrations of 193 μg/g and 140 μg/g , respectively, with an equivalent or greater fraction of the total liver fluorochemical was contributed by the parent compound or the metabolite FOSA from the FOSAA treated rats. These data suggest that FOSA may be an equally potent peroxisome proliferator to PFOS, but not the N-acetyl metabolites of N-Et FOSE.

In the guinea pig, potassium (K^+) values were significantly reduced by 40 mkd PFOS and N-EtFOSA. Neither 40 mkd PFOS or N-EtFOSA, nor 40 mkd or 160 mkd N-EtFOSE caused peroxisome proliferation in either gender of guinea pigs.

These results showed that PFOS, N-EtFOSE, FOSA and FOSAA all caused indications of peroxisome proliferation in rats, but not in guinea pigs. These results are in concordance with effects of classical peroxisome proliferators, in that the response is specific to certain species.

Introduction:

The objective of this study was to investigate and compare the molecular mechanisms of peroxisome proliferation in rats and guinea pigs. Three compounds derived from perfluorooctane sulfonate and known to cause peroxisome proliferation in the rat were initially tested, perfluorooctanesulfonate (PFOS), N-Ethyl perfluorooctanesulfonamide (N-EtFOSA) and N-Ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE). By amendment, two other compounds, perfluorooctanesulfonamido acetate (FOSAA) and perfluorooctanesulfonamide (FOSA) were added to the study. The ultimate metabolite of N-EtFOSA, N-EtFOSE, FOSAA and FOSA was presumed to be PFOS, however there is some debate about that. The hypothesis that these fluorochemicals would induce peroxisome proliferation in the rat, but not the guinea pig, was based on several lines of evidence indicating that guinea pigs and primates are resistant to peroxisome proliferation. However, the molecular and biochemical mechanisms that differentiate the response of these species to peroxisome proliferators for the perfluorosulfonamides was unclear. The specific aims of this study were to:

1. To elucidate the molecular response in both rats and guinea pigs by measuring the induction of mRNA of genes that are associated with peroxisome proliferation.
2. To measuring the hepatic activity of peroxisomal enzyme systems and fatty acid binding proteins.
3. To perform standard toxicity tests of serum clinical chemistry and to examine the liver for histological changes which may indicate an explanation for the species differences seen in response to these compounds.
4. To correlate any observed alterations of the above functions to liver and serum levels of perfluorosulfonamides and their metabolites.

This study was part of a series of investigations designed to understand the molecular and biochemical mechanisms for the effects of these compounds observed in-vivo. This study was carried-out in collaboration with other investigators. Dr. Ken Wallace, University of Minnesota Duluth, who has been engaged in studies designed to understand the effects of these perfluorosulfonamides on mitochondrial bioenergetics, performed molecular and biochemical analyses of the induction of genes associated with peroxisomal proliferation and/or cell replication. Dr. Kris Hansen and Lisa A. Stevenson of the 3M Environmental Technology and Safety Services lab performed quantitative serum and liver perfluorosulfonamide metabolite analyses. Dr. Lin Xu performed quantitative liver perfluorosulfonamide metabolite analyses on some samples in Dr. Marion Anders lab at the University of Rochester.

Study Timelines:

This study (DT15-B) was conducted in three parts, the protocol and protocol amendment numbers 1 and 2. The protocol had an in-life start date of 11/16/98 and an in-life end date of 11/20/98. Amendment number 1 had an in-life start date of 3/1/99 and an in-life end date of 3/5/99. Amendment number 2 had an in-life start date of 2/19/01 and an in-life end date of 2/23/01. Amendment #3 was procedural only.

Regulatory Compliance:
This was an exploratory study and thus classified as non-GLP as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:
The sponsor provided samples of all fluorochemicals to the investigators. Analytical documentation of the starting material was the responsibility of the sponsor. A chemical composition specification sheet was kept on file. Compounds were stable at room temperature. Test material was stored tightly sealed at room temperature.

The T-numbers, chemical names, abbreviations used for these samples, and the chemical structures of each of the compounds that were tested in this study are given below. The currently accepted abbreviations for each compound are in bold

1. Vehicle control: 2% Tween 80, or propylene glycol.

2. T-6295: Perfluorooctane sulfonic acid, potassium salt (perfluorooctanesulfonate), **PFOS**, FC-95, Formula: $C_8F_{17}SO_3^-$ K^+, MW = 538.1 g/mole).

3. T-6316: N-ethylperfluorooctane sulfonamido ethanol, (narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol), **N-EtFOSE**, EtFOSE, FC-10, Formula: $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$, MW = 571.06).

4. T-6868: N-ethyl perfluorooctane sulfonamide, (perfluorooctane sulfonyl ethylamide), PFOSA (as stated in the protocol, but not used for this compound in this report), **N-EtFOSA**, PFOSEA (abbreviation used by the 3M Environmental lab), FX-12, Formula $C_8F_{17}SO_2NHC_2H_5$, MW = 527.2).

5. T-7071: Perfluorooctanesulfonamido acetate, **FOSAA**, M556, (Formula $C_8F_{17}SO_2NHCH_2COO^-$, MW – 556 g/mole)

6. T- 7132: Perfluorooctanesulfonamide , **FOSA**, PFOSA (abbreviation used by the 3M Environmental Lab), Formula $C_8F_{17}SO_2NH_2$, FOSA, MW = 499.06 g/mole).

7. Wyeth-14643 (**WY**, MW = 323.79 g/mol) was obtained from Chemsyn Science Laboratories, Lexena, KS. Wyeth-14643 (WY), was added to DT15A as positive control dose group for hepatic peroxisome proliferation.

Methods

The methods and dose groups for each part of this study are summarized below. Detailed methods are given in the protocol and amendments. Deviations to the protocol are listed in Appendix 1.

DT15-B Protocol Procedures

The protocol had an in-life start date of 11/16/98 and an in-life end date of 11/20/98. Under the protocol, eight male and eight female Sprague Dawley Rats, 10-12 weeks old weighing approximately 250-300 grams at the time of initiation were obtained from Harlan Laboratories, Inc. Eight male and eight female Hartley Guinea Pigs 10-12 weeks old and weighing approximately 600 to 750 grams at the time of initiation were obtained from Harlan Laboratories, Inc. Each dose group contained 2 animals/sex/species. The dose groups were vehicle control (2% Tween 80), 40 mg/kg/day N-Et FOSE, 40 mg/kg/day N-EtFOSA, and 40 mg/kg/day PFOS. A 20 mg/mL suspension of each test compound was prepared in 2% Tween 80 in a glass tissue grinder. The animals received four consecutive daily doses. The day of the first dose was designated day zero, thus the doses were administered on days 0,1,2 and 3 of the study. Rats received the test compound suspended in 2% Tween 80 or the vehicle control by oral gavage at a volume of 2 ml/kg body weight. The guinea pigs received their daily oral dose volume of 2 ml/kg by droplet in the back of the mouth. The animals were humanely sacrificed on day 4

Amendment Number 1 Procedures

Amendment number 1 had an in-life start date of 3/1/99 and an in-life end date of 3/5/99. The purpose of amendment 1 was to add groups of both male and female rats and guinea pigs treated with vehicle control, N-EtFOSE (T-6316) or PFOS (T-6295). The histological and clinical chemistry results of the treatment groups under the protocol were not remarkably different than in the controls, and the tissues for northern blot analysis were delayed during shipping and were degraded. Therefore, amendment 1 was designed to replace the specimens that were lost and elevate the dose of N-EtFOSE administered to achieve a more effective level.

Twelve rats and twelve guinea pigs total (6 male, 6 female/species) were used under the protocol amendment #1. Each dose group contained 2 animals/sex/species.
The dose groups were vehicle control (2% Tween 80), 160 mg/kg/day N-Et FOSE and 40 mg/kg/day PFOS.

For N-EtFOSE, a suspension of 80 mg/ml N-EtFOSE in 2% Tween 80 was prepared and a volume of 2 ml/kg was administered by oral gavage to the rats and by droplet in the back of the mouth to the guinea pigs on days 0 through day 3 of the study.
This dose was comparable to the cumulative dose of N-EtFOSE that induced peroxisomal PCoAO activity in the rat in a 4 week feeding study with 300 ppm N-EtFOSE (Ref. 3M Medical Dept. T-6316.1), and was less than half of the LD50.

For PFOS, a suspension of 20 mg/ml of PFOS in 2% Tween 80 was prepared, and a volume of 2 ml/kg was administered by oral gavage to the rats and by droplet in the back of the mouth to the guinea pigs on day zero through day 3 of the study. The cumulative dose of PFOS delivered under protocol amendment #1 was ~160 mg/kg, as was used in the protocol. The cumulative dose was below the LD50 for PFOS and well above the threshold for inducing peroxisome proliferation in the rat.

Amendment Number 2 Procedures

The purpose of amendment 2 was to add groups male rats treated with vehicle control, perfluorooctanesulfonamidoacetate (FOSAA) or perfluorooctanesulfonamide (FOSA) at doses equivalent to the doses of N-EtFOSE and PFOS, respectively, in protocol amendment #1 in order to compare the effects and metabolite profiles of these compounds in rats at equivalent doses. Amendment number 2 had an in-life start date of 2/19/01 and an in-life end date of 2/23/01. Nine male rats were used under the protocol amendment #2. Each dose group contained 3 male Sprague Dawley Rats, 10-12 weeks old weighing approximately 250-300 grams at the time of initiation were obtained from Harlan Laboratories, Inc. The dose groups were vehicle control (propylene glycol), 160 mg/kg/day FOSAA, and 40 mg/kg/day FOSA. The vehicle control was delivered by oral gavage at a volume of 5 ml/kg body weight on days zero through day 3 of the study. A larger volume of 5 mL/Kg was used than in protocol amendment #1 because only rats were dosed by oral gavage, whereas the guinea pigs cannot be dosed by oral gavage.

For FOSAA, a suspension of 32 mg/ml FOSAA in propylene glycol was prepared and a volume of 5 ml/kg was administered by oral gavage to the rats on days 0-3. This dose achieved a cumulative dose of 640 mg/kg FOSAA.

For FOSA, a dose of 40 mg/kg body weight was administered via gavage to rats on day zero through day 3 of the study. A suspension of 8 mg/ml of FOSA in propylene glycol was prepared, and a volume of 5 ml/kg was administered by oral gavage to the rats. This dose achieved a cumulative dose of 160 mg/kg after four days of dosing. The dose of FOSA was the same as the dose of PFOS administered under amendment #1 of this protocol. The LD50 for FOSA is not known, but the cumulative dose of FOSA administered under this protocol was below the LD50 for PFOS of 251 mg/kg for PFOS in corn oil, as a point of reference.

Specimen Handling:

Liver and sera were collected and frozen rapidly after euthanasia according to the details described in the protocol and amendments. A one to two gram aliquot of the liver samples shipped in dry ice to the analytical laboratories listed in the protocol and amendments. The identification of each liver sample sent to each lab is listed in Appendix 2.

Analytical methods

Certain liver samples were sent for analysis by previously published methods for P450 content, Lauroyl CoA oxidase activity (Poosch and Yamazaki 1986) and protein content (Bradford 1976) in the laboratory of Ken Wallace Dept of Biochemistry and Molecular Biology at the University of MN by the following methods:

Enzyme Sample Preparation – The enzyme fraction consisted of the 6,000 g supernatant of a 10% (wt/vol) homogenate of 0.5-1.0 g frozen liver tissue in 300 mM mannitol-10 mM HEPES-1 mM EGTA (pH 7.2). Protein concentration was estimated according to the method of Bradford using commercial bovine serum albumin as standard.

L-CoA Oxidase Assay – The equivalent of ca., 5 µg/ml tissue homogenate was suspended in 60 mM KH_2PO_4-0.02 % Triton X100 (pH 7.4) containing 1 mM *p*-hydroxyphenylacetate (PHPA), 4 units/ml peroxidase, 20 µM FAD, and 60 µM lauryl-CoA (LCoA). The reactions were allowed to incubate at 37°C for 30 min in a shaking water bath and terminated by adding 3 volumes of 2 mM KCN in 100 mM sodium carbonate (pH 10.5). The concentration of H_2O_2 generated during the reaction was estimated from the fluorescence of PHAP as measured with an excitation wavelength of 317 nm and emission at 405 nm. The fluorescence was calibrated with commercial H_2O_2 and the results are expressed as nmol peroxide generated/min/mg mitochondrial protein. Protein was quantitated by the Bradford method.

Catalase Assay - The activity of catalase was estimated by a modification of the original method published by Claiborne and Fridovich (J. Biol. Chem. **254**, 4245-52, 1979), which is based on the direct measurement of H_2O_2 disappearance as quantified spectrophotometrically at 240 nm. In this procedure, the tissue sample was diluted in 50 mM potassium phosphate (pH 7.0). The medium was warmed to 27°C and the reaction initiated by adding 10.3 mM H_2O_2. The progress of the reaction was monitored at 240 nm for 5 min. Catalase activity was estimated from the initial linear rate (E^{240}=43.6 $mM^{-1}cm^{-1}$) and expressed as units/mg protein (Table 1). One unit of activity is defined as that amount of enzyme which catalyzes the decomposition of 1 µmole of H_2O_2 per min.

Northern Blot Analyses - Quantitation of mRNA for both acylCoA oxidase (ACoAO) and catalase were performed by Northern blot analysis of quick frozen liver samples from treated rats and guinea pigs. Approximately 1 g of frozen liver was powderized in liquid nitrogen using a mortar/pestle. Total RNA was recovered using the PERFECT RNA™ isolation kit and the concentration quantified spectrophotometrically at 260nm. The RNA was electrophoresed on a 1% agarose gel, blot transferred to a cellulose membrane and hybridized to the corresponding randomly [^{32}P] labeled oligonucleotides that were PCR amplified from primers to ca., 350 base sequence of the respective rat liver gene. mRNA

band density was quantified autoradiographically using phospho-imaging software.

Certain Liver samples were sent for analysis for the parent compound (s) and the metabolites by the 3M Environmental Technology and Safety Services using published methods (Hansen et al. 2001). The details of the methods used and the results of the analyses that were completed were issued in an analytical report from the 3M Environmental Lab on May 8, 2003 (3M Study No. FACT-TOX –107, 3M Laboratory LIMS No.E01-0129). The results of analyses that were completed are included in this report and integrated with the body weight and liver weight data. The abbreviations that the 3M Environmental lab used to identify compounds were different than the ones listed above, and are found associated with the primary data tables from the lab. The lab analyzed for a metabolite, perfluorooctanesulfonamido(ethyl)acetate (PFOSAA), that was never dosed. The abbreviations used for each analyte that the lab used as standards and analyzed for in liver samples are given below, followed by the chemical formula and the abbreviation used in this report.

PFOS = Perfluorooctanesulfonamide (Formula $C_8F_{17}SO_3$-, PFOS)

PFOSA = Perfluorooctanesulfonamide (Formula $C_8F_{17}SO_2NH_2$, FOSA)

PFOSAA = Perfluorooctanesulfonamido(ethyl)acetate. (This compound was also referred to as Perfluorooctanesulfonamidoacetate in the raw data tables of FACT TOX 107). (Formula $C_8F_{17}SO_2N(C_2H_5)CH_2COO^-$, N-EtFOSAA).

EtFOSE = narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol (Formula $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$, N-EtFOSE)

M556 = Perfluorooctanesulfonamidoacetate (Formula $C_8F_{17}SO_2NHCH_2COO^-$ FOSAA)

PFOSEA = Perfuorooctane sulfonyl ethylamide (Formula $C_8F_{17}SO_2NHC_2H_5$, N-EtFOSA)

Certain other liver samples were analyzed for parent compounds and metabolites of the fluorocarbons by LC-MS/MS by Dr. Lin Xu in the laboratory of M. W. Anders Department of Pharmacology and Physiology, University of Rochester using previously published methods (Hansen et al. 2001) . The results are reported in this report and integrated with the body weight and liver weight data.

Certain other liver samples were sent to Covance in Madison WI and were analyzed for palmitoyl Co-A Oxidase activity as an indicator of peroxisome proliferation using a validated method based on published methods (Lazarow 1981). The principle of the assay is that in the presence of palmitoyl-Co-A, the third step of the b-oxidation spiral involves the reduction of NAD to NADH, which can be measured spectrophotometrically at 340 nm. The results were reported the 3M on October 22, 2002 in a letter report from Covance and the data is included in this report. No formal report was written by Covance.

Results

Biological Parameters

All animals survived to the end of the study. No gross observations were noted during the in-life phase or at necropsy.

The cumulative doses ranged from approximately 33 to 53 mg fluorochemical in rats, and from approximately 67 to 123 mg fluorochemical in guinea pigs receiving 40 mg/kg/day. The 160 mg/Kg/day dose group animals received proportionally higher cumulative doses (Table 1). The body weights of the guinea pigs that were dosed at different times were greatly different, which accounted for the wide range in cumulative dose in the guinea pigs. Individual and summary cumulative dose data are shown in Appendix 3.

Average body weights decreased significantly over the dosing period for female rats given 40 mkd PFOS and 160 mkd N-EtFOSE (Table 2). Average body weights decreased significantly over the dosing period for male rats given 40 mkd PFOS or FOSA and 160 mkd N-EtFOSE or FOSAA (M556). Individual and summary body weight data are shown in Appendix 4.

The male rats given N-EtFOSE at 40 mkd had significantly increased liver weights (Table 3). Male rats given PFOS at 40 mkd had a significant increase in liver to body weight ratios. Male rats treated with PFOS, N-EtFOSE and N-EtFOSA at 40 mkd all had significantly lowered kidney weights and kidney to body weight ratios. Female kidney weight and kidney to body weight ratios were not significantly different from control values. Kidney weight data was not obtained for the other dose groups. Individual and summary body weight data are shown in Appendix 5.

Male and female percent of initial body weights on day four were combined for each dose group and analyzed together. The combined rat percent body weight was significantly decreased by 40 mkd PFOS, FOSA, and FOSAA, and by 160 mkd N-EtFOSE (Table 4). The combined guinea pig percent body weight was significantly decreased by PFOS, N-EtFOSA and N-EtFOSA at 40 mkd, and by N-EtFOSE at 160 mkd.

Organ weight ratios (liver to body weight and kidney to bodyweight where available) were combined for males and females independently from each dose group in all parts of the study and analyzed together. Combining the relative organ weights was done to increase the N for each dose group for analysis and is justified because the organ weights have been normalized by body weight. PFOS at 40 mkd significantly increased liver weight to body weight ratios in the rat combined data (Table 5). The combined male and female guinea pig liver to body weight ratios were not significantly different from the control group values for any of the treatments given. Significantly increased kidney to body weight ratios were found in guinea pigs treated with N-EtFOSE, N-EtFOSA, or PFOS at 40 mkd.

Liver Fluorochemical Concentrations

The liver fluorochemical concentrations in rats and guinea pig livers are summarized from the analyses performed at the University of Rochester (Table 6), and at the 3M Environmental lab (Table 7). The individual and summary liver fluorochemical concentrations data are presented in Appendix 6. The 3M Environmental lab analyzed liver samples from the PFOS 40 mkd and the N-EtFOSE 160 mkd rat dose groups and did not measure fluorochemical levels in the guinea pig livers. Aliquots of the livers that were analyzed by the 3M Environmental lab were also analyzed by Drag Andres lab at the University of Rochester. The individual rat liver data showed that one lot of the samples analyzed at Rochester had a high background of FOSA in the control group. The Rochester analyses tended to have higher PFOS and FOSA determinations than the 3M Environmental lab, however the values for PFOS are listed as ± 35% accuracy, for FOSAA ± 50% accuracy, and for FOSA, FOSAA, EtFOSE and N-EtFOSA the values are listed as qualitative only in the final report (FACT TOX 170). Therefore, the differences in the measurement of PFOS and FOSA fall within the experimental error inherent with the methods used.

The percent of the dose that was in the liver was calculated for each dose group from the amount of the total liver PFOS containing species, derived from the sum of the concentrations of all fluorochemical species detected in the liver and designated TLPFOSX, and for the amount of PFOS itself in the liver. The amount (mg) of all fluorochemical species detected in the liver was derived from the TLPFOSX times the liver weight. The percent of fluorochemical dosed present in the liver was derived for both the TL PFOSX and for PFOS itself for the values derived from both the University of Rochester lab and the 3M Environmental lab (Appendix 6, B and D). In rats dosed with PFOS, the percent of PFOS dosed present in the liver as PFOS ranged from about 17 to 23 percent in both male and female rats, and was consistent between labs. In guinea pigs dosed with PFOS, the percentage of the total amount of PFOS dosed that was present in the livers was far less than in the rat, between 3% and 5%. Guinea pig livers were only analyzed at the University of Rochester so a comparison between labs for the fluorochemical content in the livers of guinea pigs cannot be determined. However, it can be seen from the data presented in Tables B and D in Appendix 6 that the TL PFOSX values derived at the University of Rochester and at the 3M Environmental for rats treated with 160 mg/kg/day N-EtFOSE are about the same at each lab.

The University of Rochester analyzed both rat and guinea pig livers from animals treated with either 40 mkd PFOS, N-EtFOSE or N-EtFOSA, or 160 mkd N-EtFOSE. The TLPFOSX in guinea pigs treated with 160 mkd N-EtFOSE was lower than in the rat, particularly in the males guinea pigs. The percent of the dose that was present in the liver as PFOSX was approximately 2-fold the percent of the dose present in the liver as PFOS itself, in both guinea pigs and in most rats treated with N-EtFOSE and FOSA. However, the female rats treated with 160 mkd N-EtFOSE had greater than 70% of the TLPFOSX as PFOS in their livers. Conversely, the percentage of the dose that was present as PFOS in the liver of rats treated with 160 mkd FOSAA had high levels of FOSAA itself and FOSA, with low levels of PFOS.

Clinical Chemistry

Serum clinical chemistries for rats and guinea pigs are presented in Appendix 7. Clinical chemistry values were combined from males and females in each species and statistics were performed.

In rats, cholesterol (CHOL), triglycerides (TRIG), alkaline phosphatase (ALKP), and aspartate aminotransferase (AST) were significantly lowered in rats treated with 40 mkd PFOS and 160 mkd N-EtFOSE. Significant decreases in serum potassium (K^+) occurred in rats treated with 40 mkd PFOS, 40 mkd N-EtFOSE and 40 mkd N-EtFOSA, with the most significant decreases occurring in the 40 mkd PFOS dose group. Albumin (Alb) and total protein (TP) were significantly increased by 40 mkd PFOS, and creatinine (CREAT) was significantly increased in the 40 mkd PFOS and 40 mkd N-EtFOSA dose groups. These changes in clinical chemistry are consistent with previous studies with PFOS and N-EtFOSE. None of the other clinical chemistry parameters were significantly different from control values in rats.

In the guinea pigs combined male and female clinical chemistry analysis, there were no significant changes in cholesterol, triglycerides, alkaline phosphatase, or aspartate aminotransferase. Albumin, total protein and creatinine were not significantly changed. Potassium (K^+) values for male and female the guinea pigs combined were significantly reduced by 40 mkd PFOS and 40 mkd N-EtFOSA, similar to the rat. Alanine aminotransferase (ALT) was significantly increased by treatment of guinea pigs with 40 mkd PFOS. . None of the other clinical chemistry parameters were significantly different from control values in rats.

Palmitoyl CoA oxidase (PCoAO) activity

Hepatic palmitoyl CoA oxidase (PCoAO) activity data for males and females from each dose group was combined and analyzed together. PFOS at 40 mg/kg/day for four days caused a significant increase in hepatic PCoAO activity in rats (Table 8). N-EtFOSE at 160 mkd for 4 days did not significantly increase hepatic PCoAO in rats. No significant change occurred in the guinea pig. Individual and summary PCoAO values for males and females separately are shown in Appendix 8.

Peroxisomal enzyme activity and gene expression

The effects of four days of oral dosing of 160 mg/kg/day N-EtFOSE or 40 mg/kg/day PFOS for four on catalase and acylCoA oxidase gene expression and enzyme activity in liver tissue from exposed rats and guinea pigs are presented in Appendix 9. These data were presented as a poster at the 2001 Society of Toxicology meeting (Wallace *et al.* 2001). Acute exposure of rats to 160 mkd N-Et-FOSE caused a doubling of the specific activity of LCoAO and a 2-fold increase in the concentration of mRNA encoding for

PcoAO in liver from both male and female rats (Appendix 9). Catalase activity and catalase mRNA were unchanged by exposure to 160 mkd N-Et-FOSE in livers from both sexes. Exposure of rats to 40 mkd PFOS caused a 2-fold increase in LCoAO activity for both sexes, and possibly a slight increase in catalase activity in liver from male, but not female, rats. Acute exposure of rats to 40 mkd PFOS caused a 3- to 6-fold increase in PCoAO mRNA expression that was more pronounced in female compared to male rats.

Exposure of guinea pigs to N-Et-FOSE and PFOS did not stimulate LCoAO activity or catalase activity in either sex. The guinea pig mRNA encoding for PCoAO was undetectable, even following exposure to N-Et-FOSE or PFOS (Appendix 9).

Treatment of rats with 40 mkd FOSA caused significant increases in hepatic cytochrome P450 content and Acyl CoA oxidase activity (Appendix 10). Treatment of rats with 160 mkd FOSAA caused significant increases in hepatic cytochrome P450 content and Acyl CoA oxidase activity (Appendix 11). The induced the expression of these of these proteins in liver of rats indicated that FOSA and FOSAA were peroxisome proliferators in rats. For the 40 mkd FOSA and 160 mkd FOSAA treated male rats, a 2-fold induction of acyl CoA oxidase activity occurred at average liver PFOS concentrations of 193 μg/g and 140 μg/g, respectively. These liver PFOS concentrations were lower than the liver concentrations in the PFOS treatment group.

In FOSA treated rats, the parent compound, FOSA, contributed an equivalent or greater fraction of the total liver fluorochemical as did the FOSA metabolite PFOS and each of these fluorochemical species represented approximately 0.3% of the dose in the liver. In contrast, the FOSA-glucuronide present in FOSA treated rats contributed only a small fraction of the TLPFOSX (Appendix 10).

In FOSAA treated rats, the metabolite FOSA, and the parent compound, FOSAA, contributed an 2 to 3 times, respectively, the amount of the total liver fluorochemical than did the metabolite PFOS, and represented approximately 0.14% and 0.24% of the dose in the liver, respectively (Appendix 11).

Taken together, these data suggest that FOSA may be an equally potent peroxisome proliferator to PFOS in rats, and that the N-acetyl metabolites of N-Et FOSE (N-EtFOSAA, and FOSAA) are weaker peroxisome proliferators than either PFOS or PFOSA. The compounds FOSA and FOSAA, were not tested in guinea pigs.

Discussion

The analytical data showed that FOSA was consistently identified as a metabolite of PFOS, whether PFOS was administered directly or formed as a metabolite, but the source of the amino group is not readily apparent. FOSA was present in some of the control samples submitted for analyses at a given time, but not in other groups of control samples submitted at different times. The formation of PFOSA from PFOS treated animals was determined in all of the livers of all PFOS treated animals that were analyzed at the University of Rochester, and at the 3M environmental lab for the same samples, which

had no background PFOS of PFOSA in the concurrent control from either lab. Clarification of the formation of the FOSA following treatment with PFOS should be obtained in further studies. Whatever its route of formation, FOSA was metabolized to FOSA *N*-glucuronide.

N-EtFOSE alcohol gives rise to a range of major and minor metabolites. FOSE alcohol could arise from the *N*-deethylation of *N*-EtFOSE alcohol, and *N*-EtFOSAA could arise by the oxidation of the alcohol to the carboxylic acid. Glucuronidation of the parent *N*-EtFOSE alcohol would give the observed *N*-EtFOSE alcohol glucuronides. FOSAA could be formed by the *N*-deethylation of *N*-EtFOSAA or by the oxidation of FOSE alcohol, or both. FOSA could be formed by the *N*-deethylation of *N*-EtFOSA or by the removal of the carboxymethyl group of FOSAA as glyoxylate. FOSA *N*-glucuronide may be formed by the glucuronidation of FOSA. Loss of the carboxymethyl group from FOSAA would give FOSA or loss of the glycine moiety would give PFOS directly.

The correlation of a few of the most significant toxic endpoints to liver PFOS concentrations were analyzed by dose group in rats and guinea pigs (Appendix 12). The decrease in the percent initial body weight was most strongly effected in rats by PFOS at 40 mkd and N-EtFOSE at 160 mkd to 85% and 87% initial body weight, at average liver PFOS concentrations of > 600 μg/g. The percent initial body weight was also decreased in rats by FOSA at 40 mkd and FOSAA (M556) at 160 mkd to 93% and 95 % of initial body weight , respectively, at average liver PFOS concentrations of approximately 140 to 200 μg/g. A greater fraction of the total fluorochemical in the liver of the FOSAA treated animals was present as the parent compound or as the metabolite FOSA. Similarly, the FOSA treated animals had a high liver concentration of the unmetabolized parent compound. Thus these perfluorosulfonamide fluorochemical species likely contributed to the body weight effect to a greater degree than the metabolite PFOS in the FOSAA and FOSA treatment groups.

The rank order of the effect of the different treatment groups on increases liver weight to body weight in rats was PFOS at 40 mkd > FOSA at 40 mkd > N-EtFOSE at 160 mkd > FOSAA at 160 mkd occurring at average liver PFOS concentrations of > 600 μg/g for PFOS and N-EtFOSE, apprximately193 μg/g for PFOSA, and 140 μg/g for FOSAA treated animals, respectively. All dose groups had increased liver to body weight ratios, but only the liver to body weight ratios of the PFOS treated rats were significantly increased compared to control values by Dunnett's t-test Correlation of the clinical chemistry endpoints of potassium, cholesterol and triglycerides to liver PFOS concentrations in rats are not shown due to the limited number of data points for each determination.

The decrease in the percent initial body weight was most strongly effected in guinea pigs by N-EtFOSE at 160 mkd and PFOS at 40 mkd and to 91% and 93% initial body weight, at average liver PFOS concentrations of 419 and 171 μg/g, respectively. A greater fraction of the total fluorochemical in the liver of the N-EtFOSE treated guinea pigs was present as the metabolites FOSAA and N-EtFOSAA than occurred in rats. Thus, these

perfluorooctanesulfonamide fluorochemical species likely contributed to the body weight effect in guinea pigs. These data suggest that rats metabolize N-EtFOSE to PFOS more quickly than guinea pigs, perhaps due to the inducibility of cytochrome P450s in rats, as were shown in this report for rats, but was not determined for guinea pigs.

The percent initial body weight was also decreased in guinea pigs to 94% initial body weight by N-Et FOSA at 40 mkd and growth was held to 99% initial body weight by 40 mkd N-EtFOSE, at average liver PFOS concentrations of approximately 100 μg/g in the N-EtFOSA treatment group and 66 μg/g in the N-EtFOSE treatment group, respectively. Only PFOS and FOSA were analyzed for in the liver of the N-EtFOSA treated guinea pigs. However, the 40 mkd N-EtFOSE treatment group guinea pig livers had a large fraction of the total fluorochemical in the liver present as the metabolites N-EtFOSAA and FOSAA which when combined, were equal to or greater than the concentration of PFOS in the liver and thus may have made a significant contribution to the observed effects on body weight.

The liver weight to body weight ratios in guinea pigs, in contrast to rats, were all decreased, although non of these changes were significantly different than control. The rank order of the decreased liver to body weight effect in guinea pigs was N-EtFOSA at 40 mkd > N-EtFOSE at 40 mkd > PFOS at 40 mkd > N-EtFOSE at 160 mkd. This order is roughly the inverse order of the decreased body weight effect in guinea pigs for each of these dose groups. Given the lack of the liver responses in guinea pigs of either hepatomegaly or induction of peroxisome proliferating enzymes coupled with the fact that an Intraperitoneal injection of 100 mg/Kg PFOS caused death in guinea pigs bu not in rats (See DT15 A), suggests that the peroxisome proliferation in the rat is a protective mechanism.

Correlation of the clinical chemistry endpoints in guinea pigs showed that the rank order of the effect of the different treatment groups on decreased serum potassium levels was PFOS at 40 mkd > N-EtFOSA at 40 mkd, occurring at average liver PFOS concentrations of 148 μg/g and 100μg/g, respectively. In contrast, treatment of guinea pigs with 40 mkd N-EtFOSE had no effect on the serum potassium levels at average liver PFOS concentrations of approximately 45 μg/g in female and 88 μg/g in male guinea pigs. Cholesterol and triglycerides concentrations in guinea pigs were not significantly different than control values, and showed no particular trend between dose groups or strong correlation to liver PFOS concentrations.

Conclusions

All treatments caused increased liver to body weight ratios in rats but not guinea pigs, with the increase caused by PFOS being the greatest among all the fluorochemicals tested. The evidence revealed the classical signs of peroxisome proliferation in rats, but not guinea pigs, caused by these acute exposures. These data provide strong evidence that: 1) N-Et-FOSE and PFOS stimulate both the transcriptional and translational expression of acylCoA oxidase in rats *in vivo*, and 2) there is a marked difference in the

response of rats and guinea pigs to *in vivo* exposure to these two fluorochemicals. These results are very consistent with the suggestion that these fluorochemical compounds are "peroxisome proliferators" in rats and, much like what has been demonstrated for the classical "peroxisome proliferator" chemicals, guinea pigs are resistant to this effect of fluorochemical exposures.

Signature Page

Prepared by:

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Study Director

Summary Tables

Table 1. Average Cumulative Dose

Variable = Cumulative_Dose_(mg)

SPECIES GP	DOSE_GRO		SEX F	SEX M
	CONT	N	4	4
		Mean	0.0	0.0
		SD	0.0	0.0
	N-EtFOSA40mkd	N	2	2
		Mean	98.6	122.5
		SD	3.9	0.0
	N-EtFOSE160mkd	N	2	2
		Mean	163.4	163.8
		SD	4.3	4.5
	N-EtFOSE40mkd	N	2	2
		Mean	102.2	117.6
		SD	0.6	4.0
	PFOS40mkd	N	4	4
		Mean	66.7	75.0
		SD	32.4	41.8
R	M556-160mkd	N	Missing	3
		Mean	Missing	166.1
		SD	Missing	1.5
	CONT	N	4	7
		Mean	0.0	0.0
		SD	0.0	0.0
	FOSA40mkg	N	Missing	3
		Mean	Missing	40.0
		SD	Missing	1.3
	N-EtFOSA40mkd	N	2	2
		Mean	36.6	51.2
		SD	1.5	0.5
	N-EtFOSE160mkd	N	2	2
		Mean	124.3	179.5
		SD	2.9	6.3
	N-EtFOSE40mkd	N	2	2
		Mean	39.1	52.6
		SD	1.4	0.1
	PFOS40mkd	N	4	4
		Mean	33.2	46.8
		SD	3.7	3.2

Table 2. Summary of Body weights and BW changes

			SEX					
			F			M		
SPECIES	DOSE_GRO		BWD0 (g)	BWD4(g)	%BW_D0	IBWD0 (g)	BWD4(g)	%BW_D0
GP	CONT	N	4	4	4	4	4	4
		Mean	451	474	107	469	503	110
		SD	234	223	6	246	240	6
	N-EtFOSA40mkd	N	2	2	2	2	2	2
		Mean	617	587	95	770	726	94
		SD	18	25	1	1	4	1
	N-EtFOSE160mkd	N	2	2	2	2	2	2
		Mean	253	233	92	255	233	92
		SD	6	4	4	9	10	1
	N-EtFOSE40mkd	N	2	2	2	2	2	2
		Mean	635	629	99	736	734	100
		SD	0	2	0	30	31	0
	PFOS40mkd	N	4	4	4	4	4	4
		Mean	425	398	94	480	429	91
		SD	213	195	2	278	235	8
R	M556-160mkd	N	Missing	Missing	Missing	3	3	3
		Mean	Missing	Missing	Missing	258	246†	96†
		SD	Missing	Missing	Missing	8	18	9
	CONT	N	4	4	4	7	7	7
		Mean	204	211	103	277	297	107
		SD	7	7	2	19	18	2
	FOSA40mkg	N	Missing	Missing	Missing	3	3	3
		Mean	Missing	Missing	Missing	253	235†	93†
		SD	Missing	Missing	Missing	6	16	4
	N-EtFOSA40mkd	N	2	2	2	2	2	2
		Mean	214	208	97	303	310	102
		SD	8	4	2	4	1	1
	N-EtFOSE160mkd	N	2	2	2	2	2	2
		Mean	201	175†	87†	286	252†	88†
		SD	5	1	3	8	29	8
	N-EtFOSE40mkd	N	2	2	2	2	2	2
		Mean	228	229	101	312	316	101
		SD	6	3	4	1	1	0
	PFOS40mkd	N	4	4	4	4	4	4
		Mean	205	177†	86†	290	251†	87†
		SD	14	19	4	12	15	3

†Significantly different from control values by Dunnett's t-test

Table 4. Percent Initial Body Weight, Combined Data

SPECIES	DOSE GRO		%BW D0
GP	CONT	N	8
		Mean	108
		SD	6
	N-EtFOSA40mkd	N	4
		Mean	95†
		SD	1
	N-EtFOSE160mkd	N	4
		Mean	92†
		SD	2
	N-EtFOSE40mkd	N	4
		Mean	99†
		SD	1
	PFOS40mkd	N	8
		Mean	93†
		SD	5
R	CONT	N	11
		Mean	106
		SD	3
	N-EtFOSE40mkd	N	4
		Mean	101
		SD	2
	N-EtFOSA40mkd	N	4
		Mean	100
		SD	3
	M556-160mkd	N	3
		Mean	96†
		SD	9
	FOSA40mkg	N	3
		Mean	93†
		SD	4
	N-EtFOSE160mkd	N	4
		Mean	87†
		SD	5
	PFOS40mkd	N	8
		Mean	86†
		SD	3

†Significantly different from control values by Dunnett's t-test

Table 5. Liver- and Kidney- to Body Weight Ratios, Combined Data.

SPECIES	DOSE_GRO		LW/BW ratio	KW/BW ratio
GP	CONT	N	8	4
		Mean	0.041	0.006
		SD	0.004	0.001
	N-EtFOSA40mkd	N	4	4
		Mean	0.034	0.008†
		SD	0.003	0.000
	N-EtFOSE160mkd	N	4	0
		Mean	0.038	Missing
		SD	0.002	Missing
	N-EtFOSE40mkd	N	4	4
		Mean	0.034	0.008†
		SD	0.002	0.000
	PFOS40mkd	N	8	4
		Mean	0.036	0.008†
		SD	0.004	0.000
R	M556-160mkd	N	3	0
		Mean	0.044	Missing
		SD	0.003	Missing
	CONT	N	11	4
		Mean	0.040	0.009
		SD	0.003	0.000
	FOSA40mkg	N	3	0
		Mean	0.046	Missing
		SD	0.003	Missing
	N-EtFOSA40mkd	N	4	4
		Mean	0.042	0.008
		SD	0.004	0.001
	N-EtFOSE160mkd	N	4	0
		Mean	0.045	Missing
		SD	0.003	Missing
	N-EtFOSE40mkd	N	4	4
		Mean	0.045	0.008
		SD	0.003	0.001
	PFOS40mkd	N	8	4
		Mean	0.050†	0.009
		SD	0.004	0.000

†Significantly different from control values by Dunnett's t-test

Table 6. Liver Perfluorosulfonamides and Metabolite Values from Rats and Guinea Pigs. Analyses performed at the University of Rochester.

(All units are μg/g)

SPECIES	SEX	DOSE_GRP		PFOS__ROC	FOSA__ROC	FOSAA__R	ETFOSAA_ROC	FOSE_ALC_ROC	EtFOSE_ROC	NETFOSE_gluc_	FOSA_gluc
GP	F	CONT	N	0	0	0	0	0	0	0	0
			Mean	NA	NA	NA	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA	NA	NA	NA
		N-EtFOSA 40mkd	N	2	2	0	0	0	0	0	2
			Mean	104.6	0.6	NA	NA	NA	NA	NA	0.2
			SD	30.1	0.8	NA	NA	NA	NA	NA	0.0
		N-EtFOSE 160mkd	N	2	2	2	2	2	2	2	2
			Mean	350.2	16.2	217.9	388.2	28.7	1.9	6.1	0.3
			SD	339.9	7.0	103.0	98.1	0.8	0.7	3.5	0.1
		N-EtFOSE 40mkd	N	2	2	2	2	2	2	2	2
			Mean	44.7	7.1	30.4	105.0	3.2	0.9	1.2	0.3
			SD	45.3	3.9	6.2	27.5	1.4	0.1	0.2	0.0
		PFOS 40mkd	N	3	3	0	0	0	0	0	0
			Mean	140.8	24.3	NA	NA	NA	NA	NA	NA
			SD	110.5	37.5	NA	NA	NA	NA	NA	NA
	M	CONT	N	1	0	0	0	0	0	0	0
			Mean	0.1	NA	NA	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA	NA	NA	NA
		N-EtFOSA 40mkd	N	2	2	0	0	0	0	0	2
			Mean	96.3	0.5	NA	NA	NA	NA	NA	0.1
			SD	23.0	0.2	NA	NA	NA	NA	NA	0.0
		N-EtFOSE 160mkd	N	2	2	2	2	2	2	2	2
			Mean	488.6	8.2	129.4	261.6	16.0	2.3	2.0	0.5
			SD	59.8	4.6	56.4	113.8	8.3	0.2	1.1	0.0
		N-EtFOSE 40mkd	N	2	2	2	2	2	2	2	2
			Mean	88.4	4.9	28.2	54.0	3.5	1.3	0.4	0.4
			SD	84.0	0.3	8.6	1.6	1.6	0.5	0.1	0.0
		PFOS 40mkd	N	4	4	0	0	0	0	0	0
			Mean	194.5	41.7	NA	NA	NA	NA	NA	NA
			SD	131.8	33.1	NA	NA	NA	NA	NA	NA
R	F	CONT	N	1	2	0	0	0	0	0	0
			Mean	0.0	4.4	NA	NA	NA	NA	NA	NA
			SD	NA	0.4	NA	NA	NA	NA	NA	NA
		N-EtFOSE 160mkd	N	2	2	2	2	2	2	2	2
			Mean	891.9	18.1	67.5	201.9	47.2	6.4	0.2	0.2
			SD	37.6	1.1	5.5	33.4	24.6	1.5	0.1	0.0
		PFOS	N	2	2	0	0	0	0	0	0

	40mkd								
		Mean	866.4	18.3	NA	NA	NA	NA	NA	NA
		SD	143.0	4.2	NA	NA	NA	NA	NA	NA
M	M556	N	3	3	3	0	0	0	0	0
	-									
	160mkd									
		Mean	140.3	316.8	555.2	NA	NA	NA	NA	NA
		SD	85.4	114.1	113.8	NA	NA	NA	NA	NA
	CONT	N	2	5	0	0	0	0	0	0
		Mean	0.0	109.2	NA	NA	NA	NA	NA	NA
		SD	0.0	113.6	NA	NA	NA	NA	NA	NA
	FOSA	N	3	3	0	0	0	0	0	3
	40mkg									
		Mean	193.4	177.9	NA	NA	NA	NA	NA	0.4
		SD	26.8	16.2	NA	NA	NA	NA	NA	0.1
	N-	N	2	2	2	2	2	2	2	2
	EtFOSE									
	160mkd									
		Mean	1124.3	18.5	94.3	294.6	12.1	3.8	0.3	0.1
		SD	166.1	9.5	23.7	115.8	2.5	1.2	0.0	0.0
	PFOS	N	2	2	0	0	0	0	0	0
	40mkd									
		Mean	969.9	17.8	NA	NA	NA	NA	NA	NA
		SD	88.2	19.9	NA	NA	NA	NA	NA	NA

Table 7. Summary of Liver Perfluorosulfonamides and Metabolite Values from Rats and Guinea Pigs. Analyses performed at the 3M Environmental Lab.

SPECIES	SEX	DOSE_GRP		PFOS__3M	FOSA_3M	N-EtFOSAA-3M	N-EtFOSE_3M	FOSAA__3M	N-EtFOSA_3M
R	F	CONT	N	1	3	2	0	1	0
			Mean	0.151	0.0	0.2	NA	0.1	NA
			SD	NA	0.0	0.0	NA	NA	NA
		N-EtFOSE 160mkd	N	2	2	2	2	2	1
			Mean	604.000	109.0	244.5	302.5	118.5	0.9
			SD	5.657	11.3	24.7	67.2	13.4	NA
		PFOS 40mkd	N	2	2	2	0	2	0
			Mean	803.500	0.1	0.1	NA	0.1	NA
			SD	67.175	0.0	0.0	NA	0.0	NA
	M	CONT	N	2	2	2	0	1	0
			Mean	0.298	0.0	0.2	NA	0.1	NA
			SD	0.136	0.0	0.1	NA	NA	NA
		N-EtFOSE 160mkd	N	2	2	2	2	2	2
			Mean	908.000	89.0	317.5	147.0	169.5	0.4
			SD	90.510	9.9	17.7	42.4	10.6	0.1
		PFOS 40mkd	N	2	2	2	0	2	0
			Mean	800.000	0.1	0.3	NA	0.1	NA
			SD	16.971	0.0	0.0	NA	0.0	NA

Table 8 Hepatic Palmitoyl CoA Oxidase Activity, Combined Data

SPECIES	DOSE GRO		PCOAO U
GP	CONT	N	8
		Mean	2
		SD	0.8
	N-EtFOSA40mkd	N	4
		Mean	2
		SD	1.0
	N-EtFOSE160mkd	N	3
		Mean	3
		SD	0.6
	N-EtFOSE40mkd	N	4
		Mean	2
		SD	0.6
	PFOS40mkd	N	7
		Mean	3
		SD	1.6
R	CONT	N	7
		Mean	6
		SD	3.2
	N-EtFOSE160mkd	N	4
		Mean	13
		SD	7.6
	PFOS40mkd	N	5
		Mean	16†
		SD	5.1

†Significantly different from control values by Dunnett's t-test

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Appendix 1. Deviations to the Protocol

The 3M Medical Department identification number for N-Ethyl perfluorooctanesulfonamide, T-6868, was not listed in the protocol.

The protocol used abbreviations for N-Ethyl perfluorooctanesulfonamide (T-6868) of PFOSA and FX-12. Protocol amendment number 2 lists the abbreviations for perfluorooctanesulfonamide (T-7132) as FOSA, PFOSA and FOSAmide. The abbreviations used in this report for N-Ethyl perfluorooctanesulfonamide was N-EtFOSA and for perfluorooctanesulfonamide was FOSA.

The protocol stated that the animals would be dosed on days 'zero through 4 of the study". That was a typographical error, as evidenced by the fact that even the example calculation in the protocol was for a 4-day dosing period, not a 5-day dosing period. The actual dosing period was for days zero through three of the study, and the animals were sacrificed n day four of the study.

Animals were not weighed in most cases on day one of the study, and in some cases on day three of the study. In those instances, the previous days' body weights were used for determination of dosing volume.

Protocol amendment #2 stated the Dr. M. Wempe at the University of Rochester would perform the metabolite analysis of the liver samples from animals treated with T-7071.1, T-7132.1. Due to personnel changes, Dr. Xin Lu at the University of Rochester performed these metabolite analyses instead.

Kidney weight was not obtained during necropsy under amendment number 2 on 3/5/99.
Clinical chemistry was not performed on all serum samples.
Histological evaluation of liver, kidney and testis were not performed. Serum perfluorosulfonamides and metabolite values were not obtained.

Induction of mRNA for the following genes associated with peroxisomal proliferation and/or cell replication were not analyzed for in liver: Peroxisome Proliferation Activating Receptor (PPAR), Liver fatty acid binding protein (L-FABP) and Proliferating Cell Nuclear Antigen (PCNA).

Table 3. Summary of Organ weights and Organ to Body Weight Ratios.

			SEX							
			F				M			
SPECIES	DOSE GRP		LW (g)	LW/BW ratio	KW (g)	KW/BW ratio	LW (g)	LW/BW	KW (g)	KW/BW
GP	CONT	N	4	4	2	2	4	4	2	2
		Mean	19.6	0.043	4.3	0.006	19.7	0.039	4.6	0.006
		SD	7.7	0.005	0.1	0.001	10.1	0.003	0.9	0.001
	N-EtFOSA 40mkd	N	2	2	2	2	2	2	2	2
		Mean	18.8	0.032	4.5	0.008	26.7	0.037	5.5	0.008
		SD	1.4	0.001	0.5	0.001	2.6	0.003	0.1	0.000
	N-EtFOSE 160mkd	N	2	2	0	0	2	2	0	0
		Mean	9.3	0.040	Missing	Missing	8.6	0.037	Missing	Missing
		SD	0.5	0.003	Missing	Missing	0.1	0.001	Missing	Missing
	N-EtFOSE 40mkd	N	2	2	2	2	2	2	2	2
		Mean	20.8	0.033	5.2	0.008	26.4	0.036	5.5	0.007
		SD	1.5	0.002	0.0	0.000	1.8	0.001	0.1	0.000
	PFOS 40mkd	N	4	4	2	2	4	4	2	2
		Mean	13.8	0.035	4.1	0.007	16.4	0.038	5.0	0.008
		SD	6.9	0.003	0.1	0.000	9.5	0.004	0.1	0.001
R	M556-160mkd	N	Missing	Missing	Missing	Missing	3	3	0	0
		Mean	Missing	Missing	Missing	Missing	10.8	0.044	Missing	Missing
		SD	Missing	Missing	Missing	Missing	0.0	0.003	Missing	Missing
	CONT	N	4	4	2	2	7	7	2	2
		Mean	8.1	0.038	1.9	0.009	12.1	0.041	2.9	0.009
		SD	0.9	0.003	0.1	0.000	1.4	0.003	0.0	0.000
	FOSA 40mkg	N	Missing	Missing	Missing	Missing	3	3	0	0
		Mean	Missing	Missing	Missing	Missing	10.7	0.046	Missing	Missing
		SD	Missing	Missing	Missing	Missing	0.1	0.003	Missing	Missing
	N-EtFOSA 40mkd	N	2	2	2	2	2	2	2	2
		Mean	8.0	0.038	1.8	0.008	14.1	0.046	2.5	0.008†
		SD	0.4	0.002	0.2	0.001	0.3	0.001	0.1	0.000
	N-EtFOSE 160mkd	N	2	2	0	0	2	2	0	0
		Mean	7.4	0.042	Missing	Missing	12.1	0.048	Missing	Missing
		SD	0.1	0.001	Missing	Missing	1.3	0.000	Missing	Missing
	N-EtFOSE 40mkd	N	2	2	2	2	2	2	2	2
		Mean	9.9	0.043	2.0	0.009	15.0†	0.047	2.7	0.008†
		SD	0.1	0.000	0.2	0.001	1.2	0.004	0.1	0.000
	PFOS 40mkd	N	4	4	2	2	4	4	2	2
		Mean	8.2	0.047†	1.7	0.009	13.2	0.052†	2.2	0.008†
		SD	0.7	0.001	0.1	0.000	1.2	0.004	0.0	0.000

†Significantly different from control values by Dunnett's t-test

Appendix 2 – Analytical Liver Sample Lab Identification.

Study / Record	Tube number	Animal #	sex	Dose group	Necropsy Date	Specimen Type	Amt sent to Covance (g)	Amt sent to Rochester 1-19-01	Amt sent to 3M Env lab (1-19-01)	Amt sent to Univ MN Duluth (3/8/99)
dosed 11/16/98 - 11/19/98. 40 mkd PFOS, N-Et FOSE, FX-12.	1	8R04032	M	Cont	11/20/98	liver	0.451	~ 1 g	~ 1 g	NA
	2	8R04033	M	Cont	11/20/98	liver	0.328	~ 1 g	~ 1 g	NA
	3	8R04041	F	Cont	11/20/98	liver	0.489	~ 1 g	~ 1 g	NA
	4	8G01477	M	Cont	11/20/98	liver	0.709	~ 1 g	~ 1 g	NA
	5	8G01478	M	Cont	11/20/98	liver	0.49	~ 1 g	~ 1 g	NA
	6	8G01485	F	Cont	11/20/98	liver	0.544	~ 1 g	~ 1 g	NA
	7	8G01486	F	Cont	11/20/98	liver	0.468	~ 1 g	~ 1 g	NA
	8	8G01483	M	PFOS	11/20/98	liver	0.901	~ 1 g	~ 1 g	NA
	9	8G01484	M	PFOS	11/20/98	liver	0.638	~ 1 g	~ 1 g	NA
	10	8G01491	F	PFOS	11/20/98	liver	0.387	~ 1 g	~ 1 g	NA
	11	8G01479	M	N-et FOSE	11/20/98	liver	0.374	~ 1 g	~ 1 g	NA
	12	8G01480	M	N-et FOSE	11/20/98	liver	0.817	~ 1 g	~ 1 g	NA
	13	8G01487	F	N-et FOSE	11/20/98	liver	0.484	~ 1 g	~ 1 g	NA
	14	8G01489	F	N-et FOSE	11/20/98	liver	0.786	~ 1 g	~ 1 g	NA
	15	8G01481	M	N-EtFOSA	11/20/98	liver	0.57	~ 1 g	~ 1 g	NA
	16	8G01482	M	N-EtFOSA	11/20/98	liver	0.538	~ 1 g	~ 1 g	NA
	17	8G01488	F	N-EtFOSA	11/20/98	liver	0.483	~ 1 g	~ 1 g	NA
	18	8G01490	F	N-EtFOSA	11/20/98	liver	0.85	~ 1 g	~ 1 g	NA
Dosed 3/1/99-3/4/99 40 mkd PFOS; 160 mkd N-Et FOSE; vehicle control tween 80	19	9R00463	M	Cont	3/5/99	liver	1.025	~ 1 g	~ 1 g	~ 1 g
	20	9R00464	M	Cont	3/5/99	liver	0.76	~ 1 g	~ 1 g	~ 1 g
	21	9R00469	F	Cont	3/5/99	liver	0.747	~ 1 g	~ 1 g	~ 1 g
	22	9R00470	F	Cont	3/5/99	liver	0.816	~ 1 g	~ 1 g	~ 1 g
	23	9R00465	M	N-et FOSE	3/5/99	liver	0.888	~ 1 g	~ 1 g	~ 1 g
	24	9R00466	M	N-et FOSE	3/5/99	liver	0.812	~ 1 g	~ 1 g	~ 1 g
	25	9R00471	F	N-et FOSE	3/5/99	liver	0.593	~ 1 g	~ 1 g	~ 1 g

2%;	26	9R00472	F	N-et FOSE	3/5/99	liver	0.834	~ 1 g	~ 1 g	~ 1 g
	27	9R00467	M	PFOS	3/5/99	liver	0.93	~ 1 g	~ 1 g	~ 1 g
	28	9R00468	M	PFOS	3/5/99	liver	0.81	~ 1 g	~ 1 g	~ 1 g
	29	9R00473	F	PFOS	3/5/99	liver	0.703	~ 1 g	~ 1 g	~ 1 g
	30	9R00474	F	PFOS	3/5/99	liver	0.776	~ 1 g	~ 1 g	~ 1 g
	31	9G00045	M	Cont	3/5/99	liver	0.842	~ 1 g	~ 1 g	~ 1 g
	32	9G00046	M	Cont.	3/5/99	liver	0.78	~ 1 g	~ 1 g	~ 1 g
	33	9G00051	F	Cont	3/5/99	liver	0.422	~ 1 g	~ 1 g	~ 1 g
	34	9G00052	F	Cont	3/5/99	liver	0.727	~ 1 g	~ 1 g	~ 1 g
	35	9G00047	M	N-et FOSE	3/5/99	liver	0.527	~ 1 g	~ 1 g	~ 1 g
	36	9G00048	M	N-et FOSE	3/5/99	liver	0.834	~ 1 g	~ 1 g	~ 1 g
	37	9G00053	F	N-et FOSE	3/5/99	liver	0.812	~ 1 g	~ 1 g	~ 1 g
	38	9G00054	F	N-et FOSE	3/5/99	liver	0.7	~ 1 g	~ 1 g	~ 1 g
	39	9G00049	M	PFOS	3/5/99	liver	0.808	~ 1 g	~ 1 g	~ 1 g
	40	9G00050	M	PFOS	3/5/99	liver	0.815	~ 1 g	~ 1 g	~ 1 g
	41	9G00055	F	PFOS	3/5/99	liver	0.716	~ 1 g	~ 1 g	~ 1 g
	42	9G00056	F	PFOS	3/5/99	liver	0.517	~ 1 g	~ 1 g	~ 1 g
Dosed 2/19/01 160 mkd FOSAA, 40 mkd FOSA , Veh control	1	1R00742	M	Cont	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	2	1R00743	M	Cont	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	3	1R00744	M	Cont	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	7	1R00748	M	FOSAA	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	8	1R00749	M	FOSAA	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	9	1R00750	M	FOSAA	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	4	1R00745	M	FOSA	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	5	1R00746	M	FOSA	2/23/01	liver	NA	~ 1 g	NA	~ 1 g
	6	1R00747	M	FOSA	2/23/01	liver	NA	~ 1 g	NA	~ 1 g

Appendix 3. Cumulative Dose Individual and Summary data.

SPECIES	SEX	DOSE GRO	ID	Cumulative Dose (mg)
GP	F	CONT	8G01485	0
			8G01486	0
			9G00051	0
			9G00052	0
			N	4
			Mean	0.0
			SD	0.0
		N-EtFOSA40mkd	8G01488	96
			8G01490	101
			N	2
			Mean	98.6
			SD	3.9
		N-EtFOSE160mkd	9G00053	166
			9G00054	160
			N	2
			Mean	163.4
			SD	4.3
		N-EtFOSE40mkd	8G01487	103
			8G01489	102
			N	2
			Mean	102.2
			SD	0.6
		PFOS40mkd	8G01491	95
			8G01492	94
			9G00055	38
			9G00056	39
			N	4
			Mean	66.7
			SD	32.4
	M	CONT	8G01477	0
			8G01478	0
			9G00045	0
			9G00046	0
			N	4
			Mean	0.0
			SD	0.0
		N-EtFOSA40mkd	8G01481	123
			8G01482	123
			N	2
			Mean	122.5
			SD	0.0
		N-EtFOSE160mkd	9G00047	167
			9G00048	161
			N	2
			Mean	163.8
			SD	4.5
		N-EtFOSE40mkd	8G01479	115
			8G01480	120

SPECIES	SEX	DOSE_GRO	ID	Cumulative_Dose_(mg)
			N	2
			Mean	117.6
			SD	4.0
		PFOS40mkd	8G01483	115
			8G01484	107
			9G00049	39
			9G00050	39
			N	4
			Mean	75.0
			SD	41.8
R	F	CONT	8R04040	0
			8R04041	0
			9R00469	0
			9R00470	0
			N	4
			Mean	0.0
			SD	0.0
		N-EtFOSA40mkd	8R04044	38
			8R04045	36
			N	2
			Mean	36.6
			SD	1.5
		N-EtFOSE160mkd	9R00471	126
			9R00472	122
			N	2
			Mean	124.3
			SD	2.9
		N-EtFOSE40mkd	8R04042	40
			8R04043	38
			N	2
			Mean	39.1
			SD	1.4
		PFOS40mkd	8R04046	38
			8R04047	35
			9R00473	31
			9R00474	29
			N	4
			Mean	33.2
			SD	3.7
	M	M556-160mkd	1R00748	165
			1R00749	166
			1R00750	168
			N	3
			Mean	166.1
			SD	1.5
SPECIES	SEX	DOSE_GRO	ID	Cumulative_Dose_(mg)
		CONT	1R00742	0
			1R00743	0
			1R00744	0
			8R04032	0
			8R04033	0
			9R00463	0

	9R00464	0
	N	7
	Mean	0.0
	SD	0.0
FOSA40mkg	1R00745	40
	1R00746	39
	1R00747	41
	N	3
	Mean	40.0
	SD	1.3
N-EtFOSA40mkd	8R04036	51
	8R04037	51
	N	2
	Mean	51.2
	SD	0.5
N-EtFOSE160mkd	9R00465	184
	9R00466	175
	N	2
	Mean	179.5
	SD	6.3
N-EtFOSE40mkd	8R04034	53
	8R04035	53
	N	2
	Mean	52.6
	SD	0.1
PFOS40mkd	8R04038	49
	8R04039	50
	9R00467	45
	9R00468	43
	N	4
	Mean	46.8
	SD	3.2

Appendix 4. Body Weight Individual and Summary Data.

SPECIES	SEX	DOSE GRO	ID	IBWD0 G	IBWD1 G	BWD2 G	BWD3 G	BWD4 G	BW GAIN	%BW D0
GP	F	CONT	8G01485	621	Missing	632	642	636	15	102.42
			8G01486	684	Missing	692	689	695	11	101.61
			9G00051	243	Missing	260	Missing	274	31	112.76
			9G00052	257	Missing	272	Missing	289	32	112.45
			N	4	0	4	2	4	4	4
			Mean	451.3	Missing	464.0	665.5	473.5	22.3	107.3
			SD	233.9	Missing	230.0	33.2	223.1	10.8	6.1
		N-EtFOSA40mkd	8G01488	604	Missing	604	583	569	-35	94.21
			8G01490	630	Missing	651	623	604	-26	95.87
			N	2	0	2	2	2	2	2
			Mean	617.0	Missing	627.5	603.0	586.5	-30.5	95.0
			SD	18.4	Missing	33.2	28.3	24.7	6.4	1.2
		N-EtFOSE160mkd	9G00053	257	Missing	263	Missing	230	-27	89.49
			9G00054	249	Missing	252	Missing	236	-13	94.78
			N	2	0	2	0	2	2	2
			Mean	253.0	Missing	257.5	Missing	233.0	-20.0	92.1
			SD	5.7	Missing	7.8	Missing	4.2	9.9	3.7
		N-EtFOSE40mkd	8G01487	635	Missing	648	648	630	-5	99.21
			8G01489	635	Missing	641	633	627	-8	98.74
			N	2	0	2	2	2	2	2
			Mean	635.0	Missing	644.5	640.5	628.5	-6.5	99.0
			SD	0.0	Missing	4.9	10.6	2.1	2.1	0.3
		PFOS40mkd	8G01491	608	Missing	591	579	574	-34	94.41
			8G01492	610	Missing	575	556	558	-52	91.48
			9G00055	234	Missing	242	Missing	221	-13	94.44
			9G00056	246	Missing	244	Missing	237	-9	96.34
			N	4	0	4	2	4	4	4
			Mean	424.5	Missing	413.0	567.5	397.5	-27.0	94.2

			SD	213.1	Missing	196.4	16.3	194.8	19.9	2.0
	M	CONT	8G01477	723	Missing	751	755	754	31	104.29
			8G01478	637	Missing	650	653	662	25	103.92
			9G00045	257	Missing	281	Missing	296	39	115.18
			9G00046	260	Missing	278	Missing	298	38	114.62
			N	4	0	4	2	4	4	4
			Mean	469.3	Missing	490.0	704.0	502.5	33.3	109.5
			SD	245.9	Missing	246.5	72.1	240.2	6.551	6.2
		N-EtFOSA40mkd	8G01481	769	Missing	773	753	729	-40	94.80
			8G01482	771	Missing	773	748	723	-48	93.77
			N	2	0	2	2	2	2	2
			Mean	770.0	Missing	773.0	750.5	726.0	-44.0	94.3
			SD	1.4	Missing	0.0	3.5	4.2	5.7	0.7
		N-EtFOSE160mkd	9G00047	261	Missing	261	Missing	240	-21	91.95
			9G00048	248	Missing	254	Missing	226	-22	91.13
			N	2	0	2	0	2	2	2
			Mean	254.5	Missing	257.5	Missing	233.0	-21.5	91.5
			SD	9.2	Missing	4.9	Missing	9.9	0.7	0.6
		N-EtFOSE40mkd	8G01479	714	Missing	720	725	712	-2	99.72
			8G01480	757	Missing	743	754	756	-1	99.87
SPECIES	SEX	DOSE_GRO	ID	IBWD0_G_	IBWD1_G_	BWD2_G_	BWD3_G_	BWD4_G_	BW_GAIN_	_%BW_D0
			N	2	0	2	2	2	2	2
			Mean	735.5	Missing	731.5	739.5	734.0	-1.5	99.8
			SD	30.4	Missing	16.3	20.5	31.1	0.7	0.1
		PFOS40mkd	8G01483	737	Missing	727	675	668	-69	90.64
			8G01484	702	Missing	653	621	594	-108	84.62
			9G00049	244	Missing	246	Missing	215	-29	88.11
			9G00050	235	Missing	248	Missing	240	5	102.13
			N	4	0	4	2	4	4	4
			Mean	479.5	Missing	468.5	648.0	429.3	-50.3	91.4
			SD	277.5	Missing	257.5	38.2	235.1	49.0	7.6

R	F	CONT	8R04040	205	Missing	274	217	218	13	106.34
			8R04041	213	Missing	279	209	215	2	100.94
			9R00469	201	Missing	202	Missing	210	9	104.48
			9R00470	197	Missing	192	Missing	201	4	102.03
			N	4	0	4	2	4	4	4
			Mean	204.0	Missing	236.8	213.0	211.0	7.0	103.4
			SD	6.8	Missing	46.1	5.7	7.4	5.0	2.4
		N-EtFOSA40mkd	8R04044	220	Missing	283	217	210	-10	95.45
			8R04045	208	Missing	267	205	205	-3	98.56
			N	2	0	2	2	2	2	2
			Mean	214.0	Missing	275.0	211.0	207.5	-6.5	97.0
			SD	8.5	Missing	11.3	8.5	3.5	4.950	2.2
		N-EtFOSE160mkd	9R00471	204	Missing	191	Missing	174	-30	85.29
			9R00472	197	Missing	185	Missing	175	-22	88.83
			N	2	0	2	0	2	2	2
			Mean	200.5	Missing	188.0	Missing	174.5	-26.0	87.1
			SD	4.9	Missing	4.2	Missing	0.7	5.7	2.5
		N-EtFOSE40mkd	8R04042	232	Missing	303	235	227	-5	97.84
			8R04043	223	Missing	285	223	231	8	103.59
			N	2	0	2	2	2	2	2
			Mean	227.5	Missing	294.0	229.0	229.0	1.5	100.7
			SD	6.4	Missing	12.7	8.5	2.8	9.2	4.1
		PFOS40mkd	8R04046	221	Missing	284	212	200	-21	90.50
			8R04047	208	Missing	265	189	178	-30	85.58
			9R00473	202	Missing	191	Missing	175	-27	86.63
			9R00474	188	Missing	176	Missing	153	-35	81.38
			N	4	0	4	2	4	4	4
			Mean	204.8	Missing	229.0	200.5	176.5	-28.3	86.0
			SD	13.7	Missing	53.5	16.3	19.2	5.9	3.7
	M	M556-160mkd	1R00748	254	263	264	258	250	-4	98.43
			1R00749	254	262	265	263	262	8	103.15
			1R00750	267	270	267	247	227	-40	85.02

SPECIES	SEX	DOSE_GRO							
			N	3	3	3	3	3	3	3
			Mean	258.3	265.0	265.3	256.0	246.3	-12.0	95.5
			SD	7.5	4.4	1.5	8.2	17.8	25.0	9.4
			ID	IBWD0_G_	IBWD1_G_	BWD2_G_	BWD3_G_	BWD4_G_	BW_GAIN_	_%BW_D0
		CONT	1R00742	250	259	260	264	272	22	108.80
			1R00743	269	278	285	292	295	26	109.67
			1R00744	256	260	267	268	276	20	107.81
			8R04032	297	Missing	374	320	321	24	108.08
			8R04033	295	Missing	368	305	307	12	104.07
			9R00463	279	Missing	286	Missing	298	19	106.81
			9R00464	294	Missing	303	Missing	313	19	106.46
			N	7	3	7	5	7	7	7
			Mean	277.1	265.7	306.1	289.8	297.4	20.3	107.4
			SD	19.4	10.7	46.5	23.9	18.3	5.0	1.8
		FOSA40mkg	1R00745	250	254	250	239	227	-23	90.80
			1R00746	249	245	243	233	225	-24	90.36
			1R00747	259	265	263	255	254	-5	98.07
			N	3	3	3	3	3	3	3
			Mean	252.7	254.7	252.0	242.3	235.3	-17.3	93.1
			SD	5.5	10.0	10.1	11.4	16.2	10.7	4.3
		N-EtFOSA40mkd	8R04036	305	Missing	371	306	310	5	101.64
			8R04037	300	Missing	367	304	309	9	103.00
			N	2	0	2	2	2	2	2
			Mean	302.5	Missing	369.0	305.0	309.5	7.0	102.3
			SD	3.5	Missing	2.8	1.4	0.7	2.8	1.0
		N-EtFOSE160mkd	9R00465	292	Missing	283	Missing	272	-20	93.15
			9R00466	280	Missing	267	Missing	231	-49	82.50
			N	2	0	2	0	2	2	2
			Mean	286.0	Missing	275.0	Missing	251.5	-34.5	87.8
			SD	8.5	Missing	11.3	Missing	29.0	20.5	7.5
		N-EtFOSE40mkd	8R04034	312	Missing	375	314	316	4	101.28
			8R04035	311	Missing	378	318	315	4	101.29
			N	2	0	2	2	2	2	2

	Mean	311.5	Missing	376.5	316.0	315.5	4.0	101.3
	SD	0.7	Missing	2.1	2.8	0.7	0.000	0.0
PFOS40mkd	8R04038	295	Missing	353	275	257	-38	87.12
	8R04039	303	Missing	363	279	261	-42	86.14
	9R00467	286	Missing	282	Missing	257	-29	89.86
	9R00468	275	Missing	262	Missing	229	-46	83.27
	N	4	0	4	2	4	4	4
	Mean	289.8	Missing	315.0	277.0	251.0	-38.8	86.6
	SD	12.0	Missing	50.5	2.8	14.8	7.3	2.7

Appendix 5. Organ weights, Organ to BW ratios. Individual and Summary data.

SPECIES	SEX	DOSE GRO	ID	LW G	LW BW RA	KW	KW BW RA
GP	F	CONT	8G01485	24.50	0.04	4.30	0.01
			8G01486	27.70	0.04	4.20	0.01
			9G00051	11.90	0.04	Missing	Missing
			9G00052	14.20	0.05	Missing	Missing
			N	4	4	2	2
			Mean	19.575	0.043	4.250	0.006
			SD	7.704	0.005	0.071	0.001
		N-EtFOSA40mkd	8G01488	17.80	0.03	4.10	0.01
			8G01490	19.80	0.03	4.80	0.01
			N	2	2	2	2
			Mean	18.800	0.032	4.450	0.008
			SD	1.414	0.001	0.495	0.001
		N-EtFOSE160mkd	9G00053	9.60	0.04	Missing	Missing
			9G00054	8.90	0.04	Missing	Missing
			N	2	2	0	0
			Mean	9.250	0.040	Missing	Missing
			SD	0.495	0.003	Missing	Missing
		N-EtFOSE40mkd	8G01487	19.70	0.03	5.20	0.01
			8G01489	21.80	0.03	5.20	0.01
			N	2	2	2	2
			Mean	20.750	0.033	5.200	0.008
			SD	1.485	0.002	0.000	0.000
		PFOS40mkd	8G01491	18.50	0.03	4.20	0.01
			8G01492	20.90	0.04	4.00	0.01
			9G00055	8.40	0.04	Missing	Missing

			9G00056	7.40	0.03	Missing	Missing
			N	4	4	2	2
			Mean	13.800	0.035	4.100	0.007
			SD	6.895	0.003	0.141	0.000
	M	CONT	8G01477	32.30	0.04	5.20	0.01
			8G01478	23.30	0.04	3.90	0.01
			9G00045	11.30	0.04	Missing	Missing
			9G00046	11.90	0.04	Missing	Missing
			N	4	4	2	2
			Mean	19.700	0.039	4.550	0.006
			SD	10.052	0.003	0.919	0.001
		N-EtFOSA40mkd	8G01481	28.50	0.04	5.40	0.01
			8G01482	24.80	0.03	5.60	0.01
			N	2	2	2	2
			Mean	26.650	0.037	5.500	0.008
			SD	2.616	0.003	0.141	0.000
		N-EtFOSE160mkd	9G00047	8.70	0.04	Missing	Missing
			9G00048	8.50	0.04	Missing	Missing
			N	2	2	0	0
			Mean	8.600	0.037	Missing	Missing
			SD	0.141	0.001	Missing	Missing
		N-EtFOSE40mkd	8G01479	25.10	0.04	5.40	0.01
			8G01480	27.60	0.04	5.60	0.01
SPECIES	SEX	DOSE_GRO	ID	LW_G_	LW_BW_RA	KW	KW_BW_RA
			N	2	2	2	2
			Mean	26.350	0.036	5.500	0.007
			SD	1.768	0.001	0.141	0.000
		PFOS40mkd	8G01483	27.60	0.04	5.00	0.01

			8G01484	20.60	0.03	4.90	0.01
			9G00049	7.20	0.03	Missing	Missing
			9G00050	10.00	0.04	Missing	Missing
			N	4	4	2	2
			Mean	16.350	0.038	4.950	0.008
			SD	9.463	0.004	0.071	0.001
R	F	CONT	8R04040	9.00	0.04	1.90	0.01
			8R04041	8.80	0.04	1.80	0.01
			9R00469	7.60	0.04	Missing	Missing
			9R00470	7.10	0.04	Missing	Missing
			N	4	4	2	2
			Mean	8.125	0.038	1.850	0.009
			SD	0.922	0.003	0.071	0.000
		N-EtFOSA40mkd	8R04044	7.70	0.04	1.90	0.01
			8R04045	8.20	0.04	1.60	0.01
			N	2	2	2	2
			Mean	7.950	0.038	1.750	0.008
			SD	0.354	0.002	0.212	0.001
		N-EtFOSE160mkd	9R00471	7.50	0.04	Missing	Missing
			9R00472	7.30	0.04	Missing	Missing
			N	2	2	0	0
			Mean	7.400	0.042	Missing	Missing
			SD	0.141	0.001	Missing	Missing
		N-EtFOSE40mkd	8R04042	9.80	0.04	2.10	0.01
			8R04043	9.90	0.04	1.80	0.01
			N	2	2	2	2
			Mean	9.850	0.043	1.950	0.009
			SD	0.071	0.000	0.212	0.001
		PFOS40mk	8R04046	8.90	0.04	1.70	0.01

		d					
			8R04047	8.40	0.05	1.60	0.01
			9R00473	8.30	0.05	Missing	Missing
			9R00474	7.30	0.05	Missing	Missing
			N	4	4	2	2
			Mean	8.225	0.047	1.650	0.009
			SD	0.670	0.001	0.071	0.000
	M	M556-160mkd	1R00748	10.74	0.04	Missing	Missing
			1R00749	10.77	0.04	Missing	Missing
			1R00750	10.79	0.05	Missing	Missing
			N	3	3	0	0
			Mean	10.767	0.044	Missing	Missing
			SD	0.025	0.003	Missing	Missing
SPECIES	SEX	DOSE_GRO CONT	ID	LW_G_	LW_BW_RA	KW	KW_BW_RA
			1R00742	10.73	0.04	Missing	Missing
			1R00743	10.82	0.04	Missing	Missing
			1R00744	10.70	0.04	Missing	Missing
			8R04032	14.20	0.04	2.90	0.01
			8R04033	13.30	0.04	2.90	0.01
			9R00463	12.00	0.04	Missing	Missing
			9R00464	13.10	0.04	Missing	Missing
			N	7	7	2	2
			Mean	12.121	0.041	2.900	0.009
			SD	1.434	0.003	0.000	0.000
		FOSA40mkg	1R00745	10.77	0.05	Missing	Missing
			1R00746	10.81	0.05	Missing	Missing
			1R00747	10.64	0.04	Missing	Missing
			N	3	3	0	0
			Mean	10.740	0.046	Missing	Missing
			SD	0.089	0.003	Missing	Missing
		N-EtFOSA40mkd	8R04036	14.30	0.05	2.40	0.01
			8R04037	13.90	0.04	2.50	0.01
			N	2	2	2	2

	Mean	14.100	0.046	2.450	0.008
	SD	0.283	0.001	0.071	0.000
N-EtFOSE160mkd	9R00465	13.00	0.05	Missing	Missing
	9R00466	11.10	0.05	Missing	Missing
	N	2	2	0	0
	Mean	12.050	0.048	Missing	Missing
	SD	1.344	0.000	Missing	Missing
N-EtFOSE40mkd	8R04034	15.80	0.05	2.70	0.01
	8R04035	14.10	0.04	2.60	0.01
	N	2	2	2	2
	Mean	14.950	0.047	2.650	0.008
	SD	1.202	0.004	0.071	0.000
PFOS40mkd	8R04038	13.50	0.05	2.20	0.01
	8R04039	14.70	0.06	2.20	0.01
	9R00467	11.90	0.05	Missing	Missing
	9R00468	12.50	0.05	Missing	Missing
	N	4	4	2	2
	Mean	13.150	0.052	2.200	0.008
	SD	1.226	0.004	0.000	0.000

Appendix 6. Liver Fluorochemical Data. Individual and Summary data.

A. Rat and Guinea Pig Liver FC concentrations (All units are μg/g). Analyses at the University of Rochester.

SPECIES	SEX	DOSE GRP	ID	PFOS ROC	FOSA ROC	FOSAAR	ETFOSAA ROC	FOSE ALC ROC	EtFOSE ROC	NETFOSE gluc	FOSA gluc
GP	F	CONT	8G01485	NA	NA	NA	NA	NA	NA	NA	NA
			8G01486	NA	NA	NA	NA	NA	NA	NA	NA
			9G00051	NA	NA	NA	NA	NA	NA	NA	NA
			9G00052	NA	NA	NA	NA	NA	NA	NA	NA
			N	0	0	0	0	0	0	0	0
			Mean	NA	NA	NA	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA	NA	NA	NA
		N-EtFOSA 40mkd	8G01488	125.80	1.10	NA	NA	NA	NA	NA	0.26
			8G01490	83.30	0.00	NA	NA	NA	NA	NA	0.23
			N	2	2	0	0	0	0	0	2
			Mean	104.55	0.55	NA	NA	NA	NA	NA	0.25
			SD	30.05	0.78	NA	NA	NA	NA	NA	0.02
		N-EtFOSE 160mkd	9G00053	590.50	11.20	290.70	457.60	28.10	1.43	8.57	0.25
			9G00054	109.80	21.10	145.00	318.80	29.20	2.37	3.58	0.35
			N	2	2	2	2	2	2	2	2
			Mean	350.15	16.15	217.85	388.20	28.65	1.90	6.08	0.30
			SD	339.91	7.00	103.03	98.15	0.78	0.66	3.53	0.07
		N-EtFOSE 40mkd	8G01487	76.70	4.30	26.00	85.50	4.20	0.82	1.02	0.25
			8G01489	12.70	9.80	34.80	124.40	2.20	0.96	1.35	0.29
			N	2	2	2	2	2	2	2	2
			Mean	44.70	7.05	30.40	104.95	3.20	0.89	1.19	0.27
			SD	45.25	3.89	6.22	27.51	1.41	0.10	0.23	0.03
		PFOS 40mkd	8G01491	127.50	67.60	NA	NA	NA	NA	NA	NA
			8G01492	NA	NA	NA	NA	NA	NA	NA	NA

		9G00055	37.50	3.00	NA	NA	NA	NA	NA	NA
		9G00056	257.30	2.20	NA	NA	NA	NA	NA	NA
		N	3	3	0	0	0	0	0	0
		Mean	140.77	24.27	NA	NA	NA	NA	NA	NA
		SD	110.50	37.53	NA	NA	NA	NA	NA	NA
M	CONT	8G01477	NA	NA	NA	NA	NA	NA	NA	NA
		8G01478	NA	NA	NA	NA	NA	NA	NA	NA
		9G00045	0.10	NA	NA	NA	NA	NA	NA	NA
		9G00046	NA	NA	NA	NA	NA	NA	NA	NA
		N	1	0	0	0	0	0	0	0
		Mean	0.10	NA	NA	NA	NA	NA	NA	NA
		SD	NA	NA	NA	NA	NA	NA	NA	NA
	N-EtFOSA 40mkd	8G01481	112.50	0.30	NA	NA	NA	NA	NA	0.12
		8G01482	80.00	0.60	NA	NA	NA	NA	NA	0.16
		N	2	2	0	0	0	0	0	2
		Mean	96.25	0.45	NA	NA	NA	NA	NA	0.14
		SD	22.98	0.21	NA	NA	NA	NA	NA	0.03
	N-EtFOSE 160mkd	9G00047	530.80	11.40	169.30	342.10	21.80	2.46	2.75	0.49
		9G00048	446.30	4.90	89.50	181.10	10.10	2.13	1.26	0.48
		N	2	2	2	2	2	2	2	2
		Mean	488.55	8.15	129.40	261.60	15.95	2.30	2.01	0.49
		SD	59.75	4.60	56.43	113.84	8.27	0.23	1.05	0.01
	N-EtFOSE 40mkd	8G01479	29.00	5.10	34.20	52.90	2.40	0.96	0.31	0.36
		8G01480	147.80	4.70	22.10	55.10	4.60	1.67	0.47	0.40
		N	2	2	2	2	2	2	2	2
		Mean	88.40	4.90	28.15	54.00	3.50	1.32	0.39	0.38
		SD	84.00	0.28	8.56	1.56	1.56	0.50	0.11	0.03
	PFOS 40mkd	8G01483	304.00	69.80	NA	NA	NA	NA	NA	NA
		8G01484	90.80	71.00	NA	NA	NA	NA	NA	NA
		9G00049	312.80	14.50	NA	NA	NA	NA	NA	NA

			9G00050	70.50	11.60	NA	NA	NA	NA	NA	NA
			N	4	4	0	0	0	0	0	0
			Mean	194.53	41.73	NA	NA	NA	NA	NA	NA
			SD	131.80	33.14	NA	NA	NA	NA	NA	NA
R	F	CONT	8R04040	NA	NA	NA	NA	NA	NA	NA	NA
			8R04041	NA	NA	NA	NA	NA	NA	NA	NA
			9R00469	0.00	4.60	NA	NA	NA	NA	NA	NA
			9R00470	NA	4.10	NA	NA	NA	NA	NA	NA
			N	1	2	0	0	0	0	0	0
			Mean	0.00	4.35	NA	NA	NA	NA	NA	NA
			SD	NA	0.35	NA	NA	NA	NA	NA	NA
		N-EtFOSA 40mkd	8R04044	NA	NA	NA	NA	NA	NA	NA	NA
			8R04045	NA	NA	NA	NA	NA	NA	NA	NA
			N	0	0	0	0	0	0	0	0
			Mean	NA	NA	NA	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA	NA	NA	NA
		N-EtFOSE 160mkd	9R00471	865.30	17.30	63.60	178.20	29.80	7.45	0.11	0.23
			9R00472	918.50	18.80	71.40	225.50	64.60	5.34	0.28	0.24
			N	2	2	2	2	2	2	2	2
			Mean	891.90	18.05	67.50	201.85	47.20	6.40	0.20	0.24
			SD	37.62	1.06	5.52	33.45	24.61	1.49	0.12	0.01
		N-EtFOSE 40mkd	8R04042	NA	NA	NA	NA	NA	NA	NA	NA
			8R04043	NA	NA	NA	NA	NA	NA	NA	NA
			N	0	0	0	0	0	0	0	0
			Mean	NA	NA	NA	NA	NA	NA	NA	NA
			SD	NA	NA	NA	NA	NA	NA	NA	NA
		PFOS 40mkd	8R04046	NA	NA	NA	NA	NA	NA	NA	NA
			8R04047	NA	NA	NA	NA	NA	NA	NA	NA
			9R00473	967.50	21.20	NA	NA	NA	NA	NA	NA
			9R00474	765.30	15.30	NA	NA	NA	NA	NA	NA
			N	2	2	0	0	0	0	0	0

		Mean	866.40	18.25	NA	NA	NA	NA	NA	NA
		SD	142.98	4.17	NA	NA	NA	NA	NA	NA
M	M556 -160mkd	1R00748	238.40	447.30	614.40	NA	NA	NA	NA	NA
		1R00749	81.90	235.70	424.00	NA	NA	NA	NA	NA
		1R00750	100.70	267.40	627.10	NA	NA	NA	NA	NA
		N	3	3	3	0	0	0	0	0
		Mean	140.33	316.80	555.17	NA	NA	NA	NA	NA
		SD	85.45	114.12	113.77	NA	NA	NA	NA	NA
	CONT	1R00742	NA	154.10	NA	NA	NA	NA	NA	NA
		1R00743	NA	113.20	NA	NA	NA	NA	NA	NA
		1R00744	NA	273.20	NA	NA	NA	NA	NA	NA
		8R04032	NA	NA	NA	NA	NA	NA	NA	NA
		8R04033	NA	NA	NA	NA	NA	NA	NA	NA
		9R00463	0.00	2.60	NA	NA	NA	NA	NA	NA
		9R00464	0.00	2.80	NA	NA	NA	NA	NA	NA
		N	2	5	0	0	0	0	0	0
		Mean	0.00	109.18	NA	NA	NA	NA	NA	NA
		SD	0.00	113.59	NA	NA	NA	NA	NA	NA
	FOSA 40mkg	1R00745	163.20	195.70	NA	NA	NA	NA	NA	0.44
		1R00746	214.30	174.10	NA	NA	NA	NA	NA	0.39
		1R00747	202.80	163.90	NA	NA	NA	NA	NA	0.34
		N	3	3	0	0	0	0	0	3
		Mean	193.43	177.90	NA	NA	NA	NA	NA	0.39
		SD	26.81	16.24	NA	NA	NA	NA	NA	0.05
	N-EtFOSA 40mkd	8R04036	NA	NA	NA	NA	NA	NA	NA	NA
		8R04037	NA	NA	NA	NA	NA	NA	NA	NA
		N	0	0	0	0	0	0	0	0
		Mean	NA	NA	NA	NA	NA	NA	NA	NA
		SD	NA	NA	NA	NA	NA	NA	NA	NA
	N-EtFOSE 160mkd	9R00465	1241.70	25.20	111.00	376.40	13.90	2.92	0.24	0.09
		9R00466	1006.80	11.70	77.50	212.70	10.30	4.66	0.29	0.08
		N	2	2	2	2	2	2	2	2

	Mean	1124.25	18.45	94.25	294.55	12.10	3.79	0.27	0.09
	SD	166.10	9.55	23.69	115.75	2.55	1.23	0.04	0.01
N-EtFOSE 40mkd	8R04034	NA	NA	NA	NA	NA	NA	NA	NA
	8R04035	NA	NA	NA	NA	NA	NA	NA	NA
	N	0	0	0	0	0	0	0	0
	Mean	NA	NA	NA	NA	NA	NA	NA	NA
	SD	NA	NA	NA	NA	NA	NA	NA	NA
PFOS 40mkd	8R04038	NA	NA	NA	NA	NA	NA	NA	NA
	8R04039	NA	NA	NA	NA	NA	NA	NA	NA
	9R00467	907.50	3.70	NA	NA	NA	NA	NA	NA
	9R00468	1032.30	31.80	NA	NA	NA	NA	NA	NA
	N	2	2	0	0	0	0	0	0
	Mean	969.90	17.75	NA	NA	NA	NA	NA	NA
	SD	88.25	19.87	NA	NA	NA	NA	NA	NA

B. Rat and Guinea Pig Liver FC Percent of Dose Evaluations. Analyses at the University of Rochester.

SPECIES	SEX	DOSE_GRO	ID	TL_PFOSX_ (ROC)_ (ug/g)_	TL_PFOSX_ (ROC)_ (mg)	%_DOSE_ TL_PFOSX_ (ROC_)_ (%)	%_DOSE __PFOS _(ROC) _(%)
GP	F	CONT	8G01485	Missing	Missing	Missing	Missing
			8G01486	Missing	Missing	Missing	Missing
			9G00051	Missing	Missing	Missing	Missing
			9G00052	Missing	Missing	Missing	Missing
			N	0	0	0	0
			Mean	Missing	Missing	Missing	Missing
			SD	Missing	Missing	Missing	Missing
		N-EtFOSA 40mkd	8G01488	Missing	Missing	Missing	2.3
			8G01490	83.5	1.7	1.6	1.6
			N	1	1	1	2
			Mean	83.5	1.7	1.6	2.0
			SD	Missing	Missing	Missing	0.5
		N-EtFOSE 160mkd	9G00053	1388.4	13.3	8.0	3.4
			9G00054	630.2	5.6	3.5	0.6
			N	2	2	2	2
			Mean	1009.3	9.5	5.8	2.0
			SD	536.1	5.5	3.2	2.0
		N-EtFOSE 40mkd	8G01487	198.8	3.9	3.8	1.5
			8G01489	186.5	4.1	4.0	0.3
			N	2	2	2	2
			Mean	192.6	4.0	3.9	0.9
			SD	8.7	0.1	0.1	0.8
		PFOS 40mkd	8G01491	Missing	Missing	Missing	2.5
			8G01492	Missing	Missing	Missing	Missing
			9G00055	40.5	0.3	0.9	0.8

		9G00056	259.5	1.9	4.9	4.9
		N	2	2	2	3
		Mean	150.0	1.1	2.9	2.7
		SD	154.9	1.1	2.8	2.0
M	CONT	8G01477	Missing	Missing	Missing	Missing
		8G01478	Missing	Missing	Missing	Missing
		9G00045	0.1	0.0	Missing	Missing
		9G00046	Missing	Missing	Missing	Missing
		N	1	1	0	0
		Mean	0.1	0.0	Missing	Missing
		SD	Missing	Missing	Missing	Missing
	N-EtFOSA 40mkd	8G01481	112.9	3.2	2.6	2.6
		8G01482	80.8	2.0	1.6	1.6
		N	2	2	2	2
		Mean	96.8	2.6	2.1	2.1
		SD	22.7	0.9	0.7	0.7
	N-EtFOSE 160mkd	9G00047	1081.1	9.4	5.6	2.8
		9G00048	735.8	6.3	3.9	2.4
		N	2	2	2	2
		Mean	908.4	7.8	4.8	2.6
		SD	244.2	2.2	1.2	0.3
	N-EtFOSE 40mkd	8G01479	125.2	3.1	2.7	0.6
		8G01480	236.8	6.5	5.4	3.4
		N	2	2	2	2
		Mean	181.0	4.8	4.1	2.0
		SD	78.9	2.4	1.9	1.9
	PFOS 40mkd	8G01483	Missing	Missing	Missing	7.3
		8G01484	Missing	Missing	Missing	1.7
		9G00049	327.3	2.4	6.0	5.7
		9G00050	82.1	0.8	2.1	1.8
		N	2	2	2	4

			Mean	204.7	1.6	4.1	4.2
			SD	173.4	1.1	2.7	2.8
R	F	CONT	8R04040	Missing	Missing	Missing	Missing
			8R04041	Missing	Missing	Missing	Missing
			9R00469	4.6	0.0	Missing	Missing
			9R00470	4.1	0.0	Missing	Missing
			N	2	2	0	0
			Mean	4.4	0.0	Missing	Missing
			SD	0.4	0.0	Missing	Missing
		N-EtFOSE 160mkd	9R00471	1162.0	8.7	6.9	5.1
			9R00472	1304.7	9.5	7.8	5.5
			N	2	2	2	2
			Mean	1233.3	9.1	7.3	5.3
			SD	100.9	0.6	0.6	0.2
		PFOS 40mkd	8R04046	Missing	Missing	Missing	Missing
			8R04047	Missing	Missing	Missing	Missing
			9R00473	988.7	8.2	26.1	25.5
			9R00474	780.6	5.7	19.6	19.2
			N	2	2	2	2
			Mean	884.7	7.0	22.8	22.4
			SD	147.1	1.8	4.6	4.5
	M	M556- 160mkd	1R00748	1300.1	14.0	8.5	1.6
			1R00749	741.6	8.0	4.8	0.5
			1R00750	995.2	10.7	6.4	0.6
			N	3	3	3	3
			Mean	1012.3	10.9	6.6	0.9
			SD	279.6	3.0	1.8	0.6
		CONT	1R00742	Missing	Missing	Missing	Missing
			1R00743	113.2	1.2	Missing	Missing
			1R00744	Missing	Missing	Missing	Missing
			8R04032	Missing	Missing	Missing	Missing

	8R04033	Missing	Missing	Missing	Missing
	9R00463	2.6	0.0	Missing	Missing
	9R00464	2.8	0.0	Missing	Missing
	N	3	3	0	0
	Mean	39.5	0.4	Missing	Missing
	SD	63.8	0.7	Missing	Missing
FOSA 40mkg	1R00745	359.3	3.9	9.8	4.4
	1R00746	388.8	4.2	10.8	5.9
	1R00747	367.0	3.9	9.4	5.2
	N	3	3	3	3
	Mean	371.7	4.0	10.0	5.2
	SD	15.3	0.2	0.7	0.8
N-EtFOSE 160mkd	9R00465	1771.5	23.0	12.5	8.8
	9R00466	1324.0	14.7	8.4	6.4
	N	2	2	2	2
	Mean	1547.7	18.9	10.5	7.6
	SD	316.4	5.9	2.9	1.7
PFOS40mkd	8R04038	Missing	Missing	Missing	Missing
	8R04039	Missing	Missing	Missing	Missing
	9R00467	911.2	10.8	23.9	23.8
	9R00468	1064.1	13.3	31.0	30.0
	N	2	2	2	2
	Mean	987.7	12.1	27.4	26.9
	SD	108.1	1.7	5.0	4.4

C. Rat Liver PFOSX Analyses at the 3M Environmental Lab.

(All units are µg/g. The 3M Environmental Lab only analyzed rat liver samples)

SPECIES	SEX	DOSE GRO	ID	PFOS 3M	FOSA 3M	N-EtFOSAA-3M	N-EtFOSE 3M	FOSAA 3M	N-EtFOSA 3M
R	F	CONT	8R04040	NA	NA	NA	NA	NA	NA
			8R04041	NA	0.01	NA	NA	NA	NA
			9R00469	NA	0.03	0.23	NA	NA	NA
			9R00470	0.15	0.05	0.21	NA	0.07	NA
			N	1	3	2	0	1	0
			Mean	0.15	0.03	0.22	NA	0.07	NA
			SD	NA	0.02	0.01	NA	NA	NA
		N-EtFOSE160mkd	9R00471	608.00	101.00	262.00	255.00	128.00	0.89
			9R00472	600.00	117.00	227.00	350.00	109.00	NA
			N	2	2	2	2	2	1
			Mean	604.00	109.00	244.50	302.50	118.50	0.89
			SD	5.66	11.31	24.75	67.18	13.44	NA
		PFOS40mkd	8R04046	NA	NA	NA	NA	NA	NA
			8R04047	NA	NA	NA	NA	NA	NA
			9R00473	851.00	0.08	0.12	NA	0.04	NA
			9R00474	756.00	0.08	0.16	NA	0.06	NA
			N	2	2	2	0	2	0
			Mean	803.50	0.08	0.14	NA	0.05	NA
			SD	67.18	0.00	0.03	NA	0.01	NA
	M	CONT	1R00742	NA	NA	NA	NA	NA	NA
			1R00743	NA	NA	NA	NA	NA	NA
			1R00744	NA	NA	NA	NA	NA	NA
			8R04032	NA	NA	NA	NA	NA	NA
			8R04033	NA	NA	NA	NA	NA	NA
			9R00463	0.39	0.05	0.24	NA	0.08	NA
			9R00464	0.20	0.02	0.12	NA	NA	NA
			N	2	2	2	0	1	0
			Mean	0.30	0.04	0.18	NA	0.08	NA
			SD	0.14	0.02	0.08	NA	NA	NA

N-EtFOSE160mkd	9R00465	844.00	96.00	330.00	117.00	177.00	0.37
	9R00466	972.00	82.00	305.00	177.00	162.00	0.50
	N	2	2	2	2	2	2
	Mean	908.00	89.00	317.50	147.00	169.50	0.44
	SD	90.51	9.90	17.68	42.43	10.61	0.09
PFOS40mkd	8R04038	NA	NA	NA	NA	NA	NA
	8R04039	NA	NA	NA	NA	NA	NA
	9R00467	788.00	0.16	0.30	NA	0.12	NA
	9R00468	812.00	0.12	0.25	NA	0.11	NA
	N	2	2	2	0	2	0
	Mean	800.00	0.14	0.28	NA	0.11	NA
	SD	16.97	0.03	0.03	NA	0.01	NA

D. Rat and Guinea Pig Liver FC Percent of Dose Evaluations. Analyses at the 3M environmental Lab.

SPECIES	SEX	DOSE_GRO	ID	TL_PFOSX_(3M)_(ug/g)	TL_PFOSX_(3M)_(mg)	%_DOSE_TL_PFOSX_(3M)_(%)	%_DOSE_PFOS_(3M)_(%)
R	F	CONT	8R04040	Missing	Missing	Missing	Missing
			8R04041	Missing	Missing	Missing	Missing
			9R00469	0.26	0.0	Missing	Missing
			9R00470	0.48	0.0	Missing	Missing
			N	2	2	0	0
			Mean	0.4	0.0	Missing	Missing
			SD	0.2	0.0	Missing	Missing
		N-EtFOSE 160mkd	9R00471	1408.00	10.6	8.4	3.6
			9R00472	1407.00	10.3	8.4	3.6
			N	2	2	2	2
			Mean	1407.5	10.4	8.4	3.6
			SD	0.7	0.2	0.0	0.0

	PFOS 40mkd	8R04046	Missing	Missing	Missing	Missing
		8R04047	Missing	Missing	Missing	Missing
		9R00473	975.00	8.1	25.7	22.5
		9R00474	880.00	6.4	22.1	19.0
		N	2	2	2	2
		Mean	927.5	7.3	23.9	20.7
		SD	67.2	1.2	2.6	2.5
M	M556-160mkd	1R00748	1300.10	14.0	8.5	Missing
		1R00749	741.60	8.0	4.8	Missing
		1R00750	995.20	10.7	6.4	Missing
		N	3	3	3	0
		Mean	1012.3	10.9	6.6	Missing
		SD	279.6	3.0	1.8	Missing
	CONT	1R00742	154.10	1.7	Missing	Missing
		1R00743	113.20	1.2	Missing	Missing
		1R00744	273.20	2.9	Missing	Missing
		8R04032	0.00	0.0	Missing	Missing
		8R04033	0.00	0.0	Missing	Missing
		9R00463	0.79	0.0	Missing	Missing
		9R00464	0.35	0.0	Missing	Missing
		N	7	7	0	0
		Mean	77.4	0.8	Missing	Missing
		SD	107.5	1.2	Missing	Missing
	FOSA 40mkg	1R00745	358.90	3.9	9.8	Missing
		1R00746	388.40	4.2	10.8	Missing
		1R00747	366.70	3.9	9.4	Missing
		N	3	3	3	0
		Mean	371.3	4.0	10.0	Missing
		SD	15.3	0.2	0.7	Missing
	N-EtFOSE	9R00465	1580.00	20.5	11.2	6.0

160mkd					
	9R00466	1675.00	18.6	10.6	6.2
	N	2	2	2	2
	Mean	1627.5	19.6	10.9	6.1
	SD	67.2	1.4	0.4	0.1
PFOS 40mkd	8R04038	Missing	Missing	Missing	Missing
	8R04039	Missing	Missing	Missing	Missing
	9R00467	811.00	9.7	21.2	20.6
	9R00468	804.00	10.1	23.4	23.6
	N	2	2	2	2
	Mean	807.5	9.9	22.3	22.1
	SD	4.9	0.3	1.5	2.1