

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.

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Sponsor: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul MN 55133-3220

Study Location(s): 1. 3M Strategic Alternative Toxicology Laboratory
3M Center, Building 270-SB-181
Saint Paul, MN 55133-3220

2. University of Minnesota, Duluth
Dept. of Biochemistry and Molecular Biology
School of Medicine
10 University Drive
Duluth, MN 55812-2496

Study Director: Andrew M. Seacat Ph.D., DABT
Toxicology Specialist
3M Medical Dept. Corporate Toxicology and Regulatory Services

Study Toxicologist: Deanna Luebker M.S
Senior Toxicologist
3M Medical Dept.

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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 perfluorooctanesulfonamide (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-EtFOSE 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 KH₂PO₄-0.02 % 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 H₂O₂ 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 H₂O₂ 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 H₂O₂ 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 H₂O₂. The progress of the reaction was monitored at 240 nm for 5 min. Catalase activity was estimated from the initial linear rate ($E^{240}=43.6 \text{ mM}^{-1}\text{cm}^{-1}$) 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 H₂O₂ 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 [³²P] 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 = Perfluorooctane 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 β -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 $\pm 35\%$ accuracy, for FOSAA $\pm 50\%$ 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 expression 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 or 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, approximately 193 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 none 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 but 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 100 µg/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

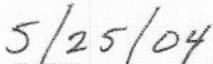
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response of rats and guinea pigs to *in vivo* exposure to these two fluorochemicals. These results are very consistent with the suggestion that these fluorochemical compounds are “peroxisome proliferators” in rats and, much like what has been demonstrated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

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Signature Page

Prepared by:

	
Andrew M. Seacat, Ph.D., DABT. Study Director	Date

Summary Tables

Table 1. Average Cumulative Dose

Variable = Cumulative Dose (mg)				
SPECIES	DOSE_GRO		SEX	
			F	M
GP	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

SPECIES	DOSE_GRO		SEX		BWD0 (g)	BWD4 (g)	%BW_D0	IBWD0 (g)	BWD4 (g)	%BW_D0
			F	M						
GP	CONT	N	4	4	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	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	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	2	2
		Mean	635	629	99	736	734	100		
		SD	0	2	0	30	31	0		
	PFOS40mkd	N	4	4	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	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	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	2	2
		Mean	228	229	101	312	316	101		
		SD	6	3	4	1	1	0		
	PFOS40mkd	N	4	4	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 3. Summary of Organ weights and Organ to Body Weight Ratios.

SPECIES	DOSE GRP		SEX		LW/BW ratio	KW (g)	KW/BW ratio	LW (g)	LW/BW ratio	KW (g)	KW/BW ratio
			F	M							
			LW (g)	LW/BW ratio							
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

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
R	CONT	N	8
		Mean	93 [†]
		SD	5
	PFOS40mkd	N	8
		Mean	93 [†]
		SD	5
	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
R	PFOS40mkd	N	8	4
		Mean	0.036	0.008 [†]
		SD	0.004	0.000
	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)

SPEC IES	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

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M	40mkd	Mean	866.4	18.3	NA	NA	NA	NA	NA	NA
		SD	143.0	4.2	NA	NA	NA	NA	NA	NA
		N	3	3	3	0	0	0	0	0
	M556	Mean	140.3	316.8	555.2	NA	NA	NA	NA	NA
		SD	85.4	114.1	113.8	NA	NA	NA	NA	NA
		N	2	5	0	0	0	0	0	0
	CONT	Mean	0.0	109.2	NA	NA	NA	NA	NA	NA
		SD	0.0	113.6	NA	NA	NA	NA	NA	NA
		N	3	3	0	0	0	0	0	3
	FOSA 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	2	2	2	2	2	2	2	2
	N- 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
		N	2	2	0	0	0	0	0	0
	PFOS 40mkd	Mean	969.9	17.8	NA	NA	NA	NA	NA	NA
		SD	88.2	19.9	NA	NA	NA	NA	NA	NA
		N								
		Mean								
		SD								
		N								
		Mean								
		SD								
		N								

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

SPEC IES	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	2	2	2	2	2	1
			N- EtFOSE 160mkd						
			Mean	604.000	109.0	244.5	302.5	118.5	0.9
		PFOS 40mkd	SD	5.657	11.3	24.7	67.2	13.4	NA
			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	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	2	2	2	2	2	2
			N- EtFOSE 160mkd						
			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
			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	
R	PFOS40mkd	N	7	
		Mean	3	
		SD	1.6	
	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

g

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 on 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).

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	9G0004 5	M	Cont	3/5/99	liver	0.842	~ 1 g	~ 1 g	~ 1 g
	32	9G0004 6	M	Cont.	3/5/99	liver	0.78	~ 1 g	~ 1 g	~ 1 g
	33	9G0005 1	F	Cont	3/5/99	liver	0.422	~ 1 g	~ 1 g	~ 1 g
	34	9G0005 2	F	Cont	3/5/99	liver	0.727	~ 1 g	~ 1 g	~ 1 g
	35	9G0004 7	M	N-et FOSE	3/5/99	liver	0.527	~ 1 g	~ 1 g	~ 1 g
	36	9G0004 8	M	N-et FOSE	3/5/99	liver	0.834	~ 1 g	~ 1 g	~ 1 g
	37	9G0005 3	F	N-et FOSE	3/5/99	liver	0.812	~ 1 g	~ 1 g	~ 1 g
	38	9G0005 4	F	N-et FOSE	3/5/99	liver	0.7	~ 1 g	~ 1 g	~ 1 g
	39	9G0004 9	M	PFOS	3/5/99	liver	0.808	~ 1 g	~ 1 g	~ 1 g
	40	9G0005 0	M	PFOS	3/5/99	liver	0.815	~ 1 g	~ 1 g	~ 1 g
	41	9G0005 5	F	PFOS	3/5/99	liver	0.716	~ 1 g	~ 1 g	~ 1 g
	42	9G0005 6	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

SRPT T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
DT15-B

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

SRPT T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
DT15-B

	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

SRPT T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
DT15-B

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
				8G01488	604	Missing	604	583	569	-35	94.21
				N- EtFOSA40mkd							
				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
				9G00053	257	Missing	263	Missing	230	-27	89.49
				N- EtFOSE160mkd							
				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
				8G01487	635	Missing	648	648	630	-5	99.21
				N- EtFOSE40mkd							
				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
				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

M	SD	213.1	Missing	196.4	16.3	194.8	19.9	2.0
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
	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-	8R04044	220	Missing	283	217	210	-10	95.45
		EtFOSA40mkd								
			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-	9R00471	204	Missing	191	Missing	174	-30	85.29
		EtFOSE160mkd								
			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-	8R04042	232	Missing	303	235	227	-5	97.84
		EtFOSE40mkd								
			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
		PFOSE40mkd	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 CONT	N	Mean	SD	IBWD0_G_	IBWD1_G_	BWD2_G_	BWD3_G_	BWD4_G_	BW_GAIN_	%BW_D0
			3	258.3	265.0	265.3	265.0	256.0	246.3	3	-12.0	95.5
			7.5	7.5	4.4	1.5	8.2	8.2	17.8	3	25.0	9.4
			250	250	259	260	264	264	272	22	22	108.80
			269	269	278	285	292	292	295	26	26	109.67
			256	256	260	267	268	268	276	20	20	107.81
			297	297	Missing	374	320	320	321	24	24	108.08
			295	295	Missing	368	305	305	307	12	12	104.07
			279	279	Missing	286	Missing	Missing	298	19	19	106.81
			294	294	Missing	303	Missing	Missing	313	19	19	106.46
			7	7	3	7	5	5	7	7	7	7
			277.1	277.1	265.7	306.1	289.8	289.8	297.4	20.3	20.3	107.4
			19.4	19.4	10.7	46.5	23.9	23.9	18.3	5.0	5.0	1.8
			250	250	254	250	239	239	227	-23	-23	90.80
			249	249	245	243	233	233	225	-24	-24	90.36
			259	259	265	263	255	255	254	-5	-5	98.07
			3	3	3	3	3	3	3	3	3	3
			252.7	252.7	254.7	252.0	242.3	242.3	235.3	-17.3	-17.3	93.1
			5.5	5.5	10.0	10.1	11.4	11.4	16.2	10.7	10.7	4.3
			305	305	Missing	371	306	306	310	5	5	101.64
			300	300	Missing	367	304	304	309	9	9	103.00
			2	2	0	2	2	2	2	2	2	2
			302.5	302.5	Missing	369.0	305.0	305.0	309.5	7.0	7.0	102.3
			3.5	3.5	Missing	2.8	1.4	1.4	0.7	2.8	2.8	1.0
			292	292	Missing	283	Missing	Missing	272	-20	-20	93.15
			280	280	Missing	267	Missing	Missing	231	-49	-49	82.50
			2	2	0	2	0	0	2	2	2	2
			286.0	286.0	Missing	275.0	Missing	Missing	251.5	-34.5	-34.5	87.8
			8.5	8.5	Missing	11.3	Missing	Missing	29.0	20.5	20.5	7.5
			312	312	Missing	375	314	314	316	4	4	101.28
			311	311	Missing	378	318	318	315	4	4	101.29
			2	2	0	2	2	2	2	2	2	2

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	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	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		
				8G01488	17.80		0.03		4.10	0.01		
				N- EtFOSA40 mkd								
				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		
				9G00053	9.60		0.04		Missing	Missing		
				N- EtFOSE16 Omkd								
				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		
				8G01487	19.70		0.03		5.20	0.01		
				N- EtFOSE40 mkd								
				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		
				PFOS40mk d								
				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		

M	CONT	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
		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
	N- EtFOSA40 mkd	SD	10.052	0.003	0.919	0.001
		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
		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
	N- EtFOSE16 0mkd	SD	0.141	0.001	Missing	Missing
		8G01479	25.10	0.04	5.40	0.01
		8G01480	27.60	0.04	5.60	0.01
		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
		PFOS40mk	8G01483	27.60	5.00	0.01
		d				
		SEX				
SPECIES	DOSE_GRO	8G01480	27.60	0.04	5.60	0.01
		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
		PFOS40mk	8G01483	27.60	5.00	0.01
		d				
		SEX				

R	F	CONT	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
			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- EtFOSA40 mkd	N	4	4	2	2
			Mean	8.125	0.038	1.850	0.009
			SD	0.922	0.003	0.071	0.000
			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
			9R00471	7.50	0.04	Missing	Missing
			N- EtFOSE16 Omkd				
		N- EtFOSE40 mkd	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
			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	8.90	0.04	1.70	0.01

d	M	M556- 160mkd	SEX	DOSE_GRO CONT_	ID	8.40	0.05	1.60	0.01
					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
					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
					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
					FOSA40mk	10.77	0.05	Missing	Missing
					g				
					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
					8R04036	14.30	0.05	2.40	0.01
					N-				
					EtFOSA40				
					mkd				
					8R04037	13.90	0.04	2.50	0.01
					N	2	2	2	2

N- EtFOSE16 Omkd	Mean	14.100	0.046	2.450	0.008
	SD	0.283	0.001	0.071	0.000
	9R00465	13.00	0.05	Missing	Missing
N- EtFOSE40 mkd	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
PFOS40mk d	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
	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.

SPEC IES	SEX	DOSE GRP	ID	PFOS ROC	FOSA ROC	FOSAA R	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

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

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R	F	CONT	9G000050	70.50	11.60	NA	NA	NA	NA	NA	NA	NA
				4	4	0	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
				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
		N-EtFOSE 40mkd	8R04044	NA	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
				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	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
				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
		PFOS 40mkd	8R04042	NA	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
				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

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

SRPT T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
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N-EtFOSE 40mkd	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
	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
	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
PFOS 40mkd	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
	8R04034	NA	NA	NA	NA	NA	NA	NA	NA
	8R04035	NA	NA	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_PFOSEX_ (ROC) _ (ug/g) _	TL_PFOSEX_ (ROC) _ (mg)	% DOSE_ TL_PFOSEX_ (ROC) _ (%)	% DOSE_ _PFOSEX_ (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
			8G01488	Missing	Missing	Missing	2.3
		N-EtFOSA 40mkd	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
		N-EtFOSE 40mkd	SD	536.1	5.5	3.2	2.0
			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

M	CONT	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
		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

R	F	CONT	Mean	204.7	1.6	4.1	4.2
			SD	173.4	1.1	2.7	2.8
			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
			1R00742	Missing	Missing	Missing	Missing
			1R00743	113.2	1.2	Missing	Missing
			1R00744	Missing	Missing	Missing	Missing
			8R04032	Missing	Missing	Missing	Missing
		CONT					

FOSA 40mkg	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
	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
N-EtFOSE 160mkg	Mean	371.7	4.0	10.0	5.2
	SD	15.3	0.2	0.7	0.8
	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
	8R04038	Missing	Missing	Missing	Missing
	8R04039	Missing	Missing	Missing	Missing
	9R00467	911.2	10.8	23.9	23.8
PFOS40mkg	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	NA	NA	NA	NA	NA
				8R04041	NA	0.01	0.01	NA	NA	NA	NA	NA	NA	NA	NA
				9R00469	NA	0.03	0.03	0.23	NA	NA	NA	NA	NA	NA	NA
				9R00470	0.15	0.05	0.05	0.21	NA	NA	0.07	NA	NA	NA	NA
				N	1	3	3	2	0	0	1	0	0	0	0
				Mean	0.15	0.03	0.03	0.22	NA	NA	0.07	NA	NA	NA	NA
				SD	NA	0.02	0.02	0.01	NA	NA	NA	NA	NA	NA	NA
				9R00471	608.00	101.00	101.00	262.00	255.00	128.00	0.89	NA	NA	NA	NA
				9R00472	600.00	117.00	117.00	227.00	350.00	109.00	NA	NA	NA	NA	NA
				N	2	2	2	2	2	2	1	NA	NA	NA	NA
			N-EtFOSE160mkd	Mean	604.00	109.00	109.00	244.50	302.50	118.50	0.89	NA	NA	NA	NA
				SD	5.66	11.31	11.31	24.75	67.18	13.44	NA	NA	NA	NA	NA
				8R04046	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				8R04047	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				9R00473	851.00	0.08	0.08	0.12	NA	0.04	NA	NA	NA	NA	NA
				9R00474	756.00	0.08	0.08	0.16	NA	0.06	NA	NA	NA	NA	NA
				N	2	2	2	2	0	2	0	0	0	0	0
				Mean	803.50	0.08	0.08	0.14	NA	0.05	NA	NA	NA	NA	NA
				SD	67.18	0.00	0.00	0.03	NA	0.01	NA	NA	NA	NA	NA
				1R00742	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
M		CONT		1R00743	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				1R00744	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				8R04032	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				8R04033	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				9R00463	0.39	0.05	0.05	0.24	NA	0.08	NA	NA	NA	NA	NA
				9R00464	0.20	0.02	0.02	0.12	NA	NA	NA	NA	NA	NA	NA
				N	2	2	2	2	0	1	0	0	0	0	0
				Mean	0.30	0.04	0.04	0.18	NA	0.08	NA	NA	NA	NA	NA
				SD	0.14	0.02	0.02	0.08	NA	NA	NA	NA	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	8R04046	Missing	Missing	Missing	Missing
					Missing
40mkd	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	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
	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
FOSA 40mkg	Mean	77.4	0.8	Missing	Missing
	SD	107.5	1.2	Missing	Missing
	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
	9R00465	1580.00	20.5	11.2	6.0
N-EtFOSE					

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

SRPT T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
DT15-B

E. Technical Report: Liver Fluorocarbon Metabolites – University of Rochester.

Title:

Summary of Quantitative Analysis of Fluorocarbon Metabolites in Rat Liver Samples

Lin Xu and M. W. Anders

This is a brief summary of the analysis of liver samples from rats given a range of fluorocarbons. The parent and metabolites of the fluorocarbons were determined in liver samples by LC-MS/MS. The results are presented below.

Three groups of liver samples were analyzed: The first group (Group 1) consisted of livers from control rats, from rats given 40 mg/kg/day FOSA orally, and from rats given 160 mg/kg/day FOSAA (M556) orally. The concentrations of parent compounds and their metabolites in livers were measured (Table 1). The data show that PFOS was the major metabolite found in the livers of rats given FOSA; FOSA *N*-glucuronide was identified as a minor metabolite. Two metabolites were identified in the livers of rats given FOSAA (M556): PFOS and FOSA. It is noteworthy that the control animals (1R00742, 1R00743, and 1R00743) contained significant concentrations of FOSA. A parallel analysis of livers from Fischer 344 rats maintained in the University of Rochester Vivarium did not show detectable concentrations of FOSA.

The second group (Group 2) of rats was treated in November, 1998. Group 2 contained livers from control rats, from rats given 40 mg/kg/day PFOS orally, from rats given 40 mg/kg/day *N*-EtFOSE alcohol orally, and from rats given 40 mg/kg/day *N*-EtFOSA orally. The concentrations of parent compounds and identified metabolites are shown in Table 2. The data show that livers from rats given PFOS contained parent compound PFOS; FOSA was also found in these samples. The livers from rats given *N*-EtFOSE alcohol contained several metabolites. The major metabolites were *N*-EtFOSAA, PFOS, and FOSAA (M556). The minor metabolites were FOSA, FOSE alcohol, *N*-EtFOSE alcohol glucuronide, and FOSA *N*-glucuronide. PFOS was the major metabolite in livers from rats given *N*-EtFOSA; FOSA and FOSA *N*-glucuronide were identified as minor metabolites. In contrast to Group 1, the livers from control rats did not contain detectable concentrations of parent compounds or metabolites.

The third group (Group 3) was treated in March, 1999. Group 3 contained livers from control rats, from rats given 40 mg/kg/day PFOS orally, and from rats given 160 mg/kg/day *N*-EtFOSE alcohol orally. The concentrations of the chemicals and metabolites are shown in Table 3. As with Group 2, PFOS and low concentrations of FOSA were identified in livers from rats given PFOS. In the livers of rats given *N*-EtFOSE alcohol, *N*-EtFOSAA, PFOS, and FOSAA (M556) were identified as major metabolites, and the minor metabolites identified were FOSA, FOSE alcohol, *N*-EtFOSE alcohol glucuronide, and FOSA *N*-glucuronide. Similar to Group 1, control animals showed detectable hepatic concentrations of FOSA.

Discussion: These data allow conclusions about the overall metabolic fate of the fluorocarbons studied (see Scheme), but do not allow inferences about the exact routes of metabolite formation. 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. 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.

SRPT T-6295.8, T-6316.4, T-6868.2, T-7071.1, T-7132.1
DT15-B

The fate of FOSAA appears relatively straightforward. Loss of the carboxymethyl group would give FOSA or loss of the glycine moiety would give PFOS directly.

Table 1. Hepatic Concentrations of Fluorocarbons and Fluorocarbon Metabolites in Livers of Rats Given FOSA (40 mg/kg/day) or FOSAA (M556) (160 mg/kg/day) (Group 1)

Sample #	Animal #	Sex	Treatment	Weight of liver sample (g)	PFOS (ppm)	FOSA (ppm)	FOSAA (ppm)	FOSA N-glucuronide (ppm)
1	1R00742	M	Control	0.4600	0.0	154.1	0.0	0.00
2	1R00743	M	Control	0.9236	0.0	113.2	0.0	0.00
3	1R00744	M	Control	1.1287	0.0	273.2	0.0	0.00
4	1R00745	M	FOSA	0.2402	163.2	195.7	n.m.	0.44
5	1R00746	M	FOSA	0.7905	214.3	174.1	n.m.	0.39
6	1R00747	M	FOSA	0.8305	202.8	163.9	n.m.	0.34
7	1R00748	M	FOSAA	0.5520	238.4	447.3	614.4	0.00
8	1R00749	M	FOSAA	0.9373	81.9	235.7	424.0	0.00
9	1R00750	M	FOSAA	0.5320	100.7	267.4	627.1	0.00

Note: n.m. = not measured.

Table 2: Hepatic Concentrations of Fluorocarbons and Fluorocarbon Metabolites in Livers from Rats Given PFOS (40 mg/kg/day), N-EtFOSE alcohol (40 mg/kg/day), or N-EtFOSA (40 mg/kg/day) (Group 2)

Sample #	Animal #	Sex	Treatment	Weight of liver samples (g)	PFOS (ppm)	FOSA (ppm)	FOSA (ppm)	N-EtFOSA (ppm)	FOSE alcohol (ppm)	N-EtFOSE alcohol (ppm)	N-EtFOSE alcohol glucuronide (ppm)	FOSA N-glucuronide (ppm)
1	8R04032	M	Control	0.7516	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
2	8R04033	M	Control	1.1	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
3	8R04041	F	Control	0.413	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
4	8G01477	M	Control	1.081	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
5	8G01478	M	Control	0.6009	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
6	8G01485	F	Control	1.206	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
7	8G01486	F	Control	0.8228	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
8	8G01483	M	PFOS	0.7756	304.0	69.8	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
9	8G01484	M	PFOS	0.789	90.8	71.0	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
10	8G01491	F	PFOS	1.1795	127.5	67.6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
11	8G01479	M	N-EtFOSE alcohol	0.7712	29.0	5.1	34.2	52.9	2.4	0.96	0.31	0.36
12	8G01480	M	N-EtFOSE alcohol	0.7744	147.8	4.7	22.1	55.1	4.6	1.67	0.47	0.40
13	8G01487	F	N-EtFOSE alcohol	0.7137	76.7	4.3	26.0	85.5	4.2	0.82	1.02	0.25
14	8G01489	F	N-EtFOSE alcohol	0.4796	12.7	9.8	34.8	124.4	2.2	0.96	1.35	0.29
15	8G01481	M	N-EtFOSA	0.707	112.5	0.3	0.0	0.0	0.0	n.m.	0.00	0.12
16	8G01482	M	N-EtFOSA	0.993	80.0	0.6	0.0	0.0	0.0	n.m.	0.00	0.16
17	8G01488	F	N-EtFOSA	0.6395	125.8	1.1	0.0	0.0	0.0	n.m.	0.00	0.26
18	8G01490	F	N-EtFOSA	0.754	83.3	0.0	0.0	0.0	0.0	n.m.	0.00	0.23

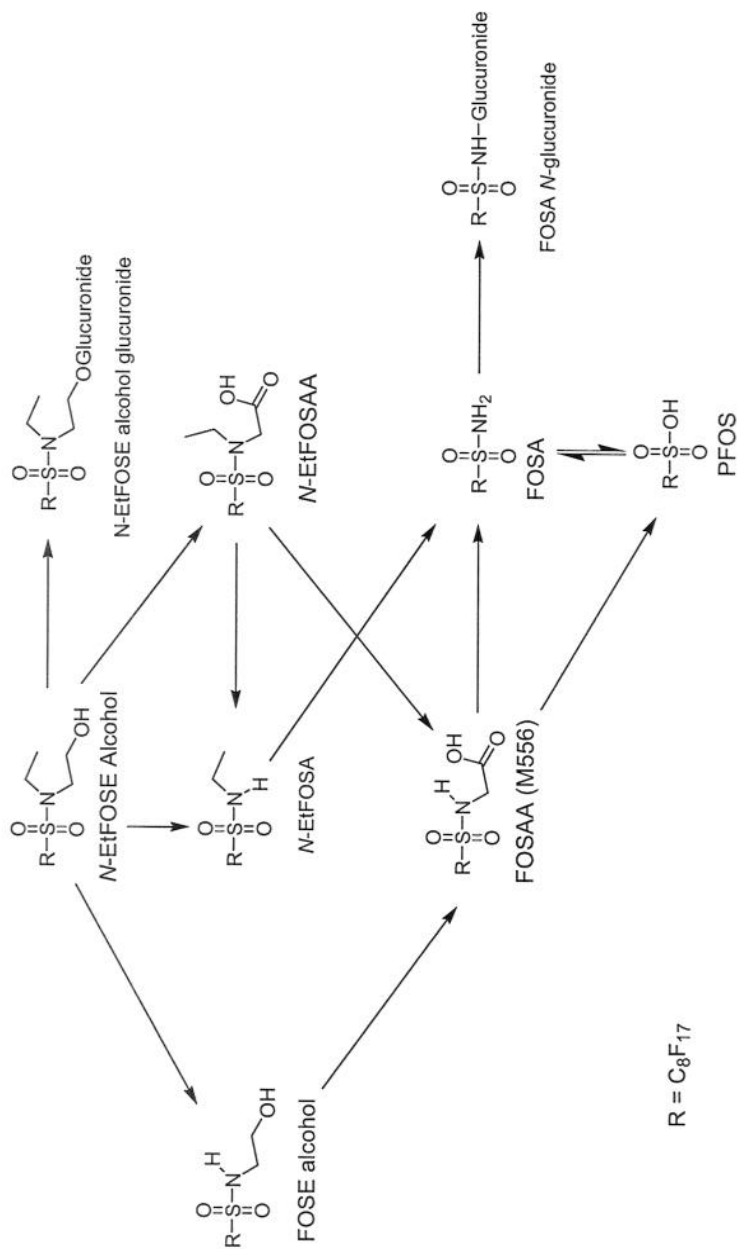
Note: n.m. = not measured.

Table 3. Hepatic Concentrations of Fluorocarbons and Fluorocarbon Metabolites in Livers of Rats Given *N*-EtFOSE alcohol (160 mg/kg/day) or PFOS (40 mg/kg/day) (Group 3)

Sample #	Animal #	Sex	Treatment	Weight of liver samples (g)	PFOS (ppm)	FOSA (ppm)	FOSAA (ppm)	<i>N</i> -EtFOSAA (ppm)	FOSE alcohol (ppm)	<i>N</i> -EtFOSE alcohol (ppm)	<i>N</i> -EtFOSE alcohol glucuronide (ppm)	PFOSA glucuronide (ppm)
19	9R00463	M	Control	0.4459	0.0	2.6	n.m.	n.m.	n.m.	n.m.	0.00	0.00
20	9R00464	M	Control	0.903	0.0	2.8	n.m.	n.m.	n.m.	n.m.	0.00	0.00
21	9R00469	F	Control	0.8444	0.0	4.6	n.m.	n.m.	n.m.	n.m.	0.00	0.00
22	9R00470	F	Control	0.5802	0.0	4.1	n.m.	n.m.	n.m.	n.m.	0.00	0.00
23	9R00465	M	<i>N</i> -EtFOSE alcohol	0.1707	1241.7	25.2	111.0	376.4	13.9	2.92	0.24	0.09
24	9R00466	M	<i>N</i> -EtFOSE alcohol	0.885	1006.8	11.7	77.5	212.7	10.3	4.66	0.29	0.08
25	9R00471	F	<i>N</i> -EtFOSE alcohol	0.304	865.3	17.3	63.6	178.2	29.8	7.45	0.11	0.23
26	9R00472	F	<i>N</i> -EtFOSE alcohol	0.2791	918.5	18.8	71.4	225.5	64.6	5.34	0.28	0.24
27	9R00467	M	PFOS	0.5589	907.5	3.7	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
28	9R00468	M	PFOS	0.411	1032.3	31.8	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
29	9R00473	F	PFOS	0.6576	967.5	21.2	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
30	9R00474	F	PFOS	0.4673	765.3	15.3	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
31	9G00045	M	Control	0.1608	0.0	0.1	0.0	0.0	0.0	n.m.	n.m.	n.m.
32	9G00046	M	Control	0.3882	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
33	9G00051	F	Control	0.712	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
34	9G00052	F	Control	0.59	0.0	0.0	0.0	0.0	0.0	n.m.	0.00	0.00
35	9G00047	M	<i>N</i> -EtFOSE alcohol	0.7258	530.8	11.4	169.3	342.1	21.8	2.46	2.75	0.49
36	9G00048	M	<i>N</i> -EtFOSE alcohol	0.713	446.3	4.9	89.5	181.1	10.1	2.13	1.26	0.48
37	9G00053	F	<i>N</i> -EtFOSE alcohol	0.9192	590.5	11.2	290.7	457.6	28.1	1.43	8.57	0.25
38	9G00054	F	<i>N</i> -EtFOSE alcohol	0.44	109.8	21.1	145.0	318.8	29.2	2.37	3.58	0.35
39	9G00049	M	PFOS	0.787	312.8	14.5	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
40	9G00050	M	PFOS	0.43	70.5	11.6	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
41	9G00055	F	PFOS	0.9398	37.5	3.0	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
42	9G00056	F	PFOS	0.5605	257.3	2.2	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.

Note: n.m. = not measured.

Metabolism of Fluorocarbons in Rats



Appendix 7: Clinical Chemistry.

Individual and Summary Clinical Chemistry Data.

SPEC	SEX	DOSE GRP	ID	CHOL mg/dL ¹	CA mg/dL	PHOS mg/dL	TBIL mg/dL	ALB g/dL	TP g/dL	BUN mg/dL	GLU mg/dL	ALKP U/L	AST U/L	ALT U/L	LDH U/L	Na+ mmol/L	K+ mmol/L	Cl- mmol/L	CREA mg/dl	TRIG mg/dL
GP	F	CONT	8G0148 5	63	13.1	9.0	0.2	2.7	5.6	14	416	156	46	50	257	141	NR	103	0.5	124
			8G0148 6	62	11.9	9.7	0.1	2.4	5.0	13	202	117	45	46	118	144	12.0	102	0.4	132
			9G0005 1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
			9G0005 2	23	NR	NR	0.2	1.9	4.3	14	310	202	37	42	133	141	12.9	104	0.3	78
			N Mean	3 49.2	2 12.50	2 9.35	3 0.17	3 2.33	3 4.97	3 13.7	3 309.3	3 158.3	3 42.7	3 46.0	3 169.	3 142.0	2 12.45	3 103.0	3 0.40	3 111.3
			SD	23.1	0.85	0.49	0.06	0.40	0.65	0.6	107.0	42.5	4.9	4.0	76.3	1.7	0.64	1.0	0.10	29.1
		N- EtFOSE 40mkd	8G0148 8	67	11.6	8.7	0.2	2.6	5.7	17	198	126	38	49	160	138	5.8	100	0.5	107
			8G0149 0	116	12.6	8.6	0.2	2.9	6.3	13	263	122	38	56	158	138	6.6	100	0.5	143
			N Mean	2 91.5	2 12.10	2 8.65	2 0.20	2 2.75	2 6.00	2 15.0	2 230.5	2 124.0	2 38.0	2 52.5	2 159.	2 138.0	2 6.20	2 100.0	2 0.50	2 125.0
			SD	34.6	0.71	0.07	0.00	0.21	0.42	2.8	46.0	2.8	0.0	4.9	1.4	0.0	0.57	0.0	0.00	25.5
		N- EtFOSE 40mkd	8G0148 7	70	11.7	9.5	0.1	2.5	5.5	17	182	121	32	34	117	141	11.0	99	0.6	96
			8G0148 9	23	12.7	9.2	0.2	2.4	5.0	10	286	125	32	43	166	141	13.2	104	0.4	117
			N Mean	2 46.3	2 12.20	2 9.35	2 0.15	2 2.45	2 5.25	2 13.5	2 234.0	2 123.0	2 32.0	2 38.5	2 141.	2 141.0	2 12.10	2 101.5	2 0.50	2 106.5
			SD	33.6	0.71	0.21	0.07	0.07	0.35	4.9	73.5	2.8	0.0	6.4	34.6	0.0	1.56	3.5	0.14	14.8
		PFOS 40mkd	8G0149 1	75	12.4	10.2	0.1	2.6	5.6	15	326	163	77	60	286	133	6.1	100	0.5	127

M	8G0149	71	11.9	7.4	0.4	2.6	5.6	10	315	110	44	55	344	134	4.1	100	0.5	101
	2																	
	9G0005	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	5																	
	9G0005	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	6																	
	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Mean	73.0	12.15	8.80	0.23	2.60	5.60	12.5	320.5	136.5	60.5	57.5	315.0	133.5	5.10	100.0	0.50	114.0
	SD	2.8	0.35	1.98	0.25	0.00	0.00	3.5	7.8	37.5	23.3	3.5	41.0	0.7	1.41	0.0	0.00	18.4
CONT	8G0147	23	11.2	8.3	0.3	2.5	5.3	15	154	110	64	45	303	144	12.1	103	0.5	168
	7																	
	8G0147	48	11.7	9.2	0.1	2.4	5.1	16	211	194	49	52	113	140	NR	103	0.4	183
	8																	
	9G0004	23	NR	NR	0.1	1.7	4.0	16	275	329	36	52	141	141	14.2	107	0.3	84
	5																	
	9G0004	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	6																	
	N	3	2	2	3	3	3	3	3	3	3	3	3	3	2	3	3	3
	Mean	31.0	11.45	8.75	0.15	2.20	4.80	15.7	213.3	211.0	49.7	49.7	185.7	141.7	13.15	104.3	0.40	145.0
N- EtFOSE 40mkd	SD	14.7	0.35	0.64	0.13	0.44	0.70	0.6	60.5	110.5	14.0	4.0	102.6	2.1	1.48	2.3	0.10	53.4
	8G0148	55	12.8	9.7	0.1	2.6	5.5	12	485	147	54	51	208	135	7.5	97	0.6	201
	1																	
	8G0148	49	11.8	8.9	0.1	2.6	5.5	16	194	128	47	57	218	136	6.7	101	0.7	103
	2																	
	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Mean	52.0	12.30	9.30	0.10	2.60	5.50	14.0	339.5	137.5	50.5	54.0	213.0	135.5	7.10	99.0	0.65	152.0
	SD	4.2	0.71	0.57	0.00	0.00	0.00	2.8	205.8	13.4	4.9	4.2	7.1	0.7	0.57	2.8	0.07	69.3
N- EtFOSE 40mkd	8G0147	23	12.8	9.0	0.3	2.6	5.4	13	449	163	117	62	514	138	12.7	98	0.5	154
	9																	
	8G0148	23	12.4	9.6	0.1	2.3	4.8	16	372	108	41	47	151	140	11.9	103	0.5	126
	0																	
	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Mean	22.5	12.60	9.30	0.18	2.45	5.10	14.5	410.5	135.5	79.0	54.5	332.5	139.0	12.30	100.5	0.50	140.0
	SD	0.0	0.28	0.42	0.18	0.21	0.42	2.1	54.4	38.9	53.7	10.6	256.7	1.4	0.57	3.5	0.00	19.8

R	F	CONT	PFOS 40mkd	8G0148	52	12.6	9.1	0.1	3.0	6.2	16	342	102	49	57	131	133	4.1	95	0.8	118
				3																	
				8G0148	54	11.6	7.6	0.2	2.9	6.2	17	131	115	47	62	155	138	2.7	101	0.7	94
				4																	
				9G0004	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
				9																	
				9G0005	23	NR	NR	0.3	1.8	4.5	16	248	270	70	66	348	134	6.4	103	0.3	85
				0																	
				N	3	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3
				Mean	42.8	12.10	8.35	0.20	2.57	5.63	16.3	240.3	162.3	55.3	61.7	211.	135.0	4.40	99.7	0.60	99.0
R	F	CONT	N- EtFOSE 40mkd	SD	17.6	0.71	1.06	0.10	0.67	0.98	0.6	105.7	93.5	12.7	4.5	119.	2.6	1.87	4.2	0.26	17.1
				0												0					
				8R04040	96	12.9	10.5	0.4	4.3	7.1	15	426	267	86	76	374	149	6.8	98	0.6	75
				8R04041	77	12.1	10.7	0.3	3.8	6.7	13	351	214	84	69	343	149	5.7	98	0.5	57
				9R00469	86	NR	NR	0.4	3.1	5.9	20	188	253	80	72	416	148	7.2	105	0.4	81
				9R00470	81	NR	NR	0.4	3.6	6.5	19	215	274	94	78	307	148	7.4	104	0.5	62
				N	4	2	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4
				Mean	85.0	12.50	10.60	0.38	3.70	6.55	16.8	295.0	252.0	86.0	73.8	360.	148.5	6.78	101.3	0.50	68.8
				SD	8.2	0.57	0.14	0.05	0.50	0.50	3.3	112.8	26.8	5.9	4.0	46.3	0.6	0.76	3.8	0.08	11.1
				8R04044	82	11.8	8.2	0.3	3.8	7.0	14	328	283	69	66	139	151	4.5	106	0.7	53
R	F	CONT	N- EtFOSE 160mkd	8R04045	88	12.2	8.3	0.3	4.1	7.4	19	315	234	80	70	180	150	4.6	102	0.6	70
				N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
				Mean	85.0	12.00	8.25	0.30	3.95	7.20	16.5	321.5	258.5	74.5	68.0	159.	150.5	4.55	104.0	0.65	61.5
				SD	4.2	0.28	0.07	0.00	0.21	0.28	3.5	9.2	34.6	7.8	2.8	29.0	0.7	0.07	2.8	0.07	12.0
				9R00471	NR	NR	NR	0.4	NR	NR	24	NR	184	60	77	NR	NR	NR	NR	NR	35
				9R00472	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
				N	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	0	1
				Mean	NR	NR	NR	0.40	NR	NR	24.0	NR	184.0	60.0	77.0	NR	NR	NR	NR	NR	35.0
				SD	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
				8R04042	70	11.3	9.5	0.2	3.5	6.7	14	248	236	71	56	219	147	5.1	105	0.6	40
R	F	CONT	N- EtFOSE 40mkd	8R04043	89	12.0	10.1	0.3	3.7	6.8	18	318	229	77	87	222	147	5.7	100	0.5	105

[illegible]

EtFOSE 40mkd		8R04035	70	11.0	9.8	0.2	3.4	6.6	19	243	258	68	66	272	150	4.8	101	0.6	70
		N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		Mean	66.5	11.60	10.40	0.30	3.55	6.80	17.5	286.5	262.0	68.5	66.5	347.	151.5	5.00	100.0	0.55	92.0
		SD	4.9	0.85	0.85	0.14	0.21	0.28	2.1	61.5	5.7	0.7	0.7	106.	2.1	0.28	1.4	0.07	31.1
														1					
PFOS 40mkd		8R04038	63	12.3	10.6	0.6	4.6	7.8	21	365	203	71	90	527	148	3.1	100	0.8	55
		8R04039	49	12.2	9.6	0.4	4.5	7.8	20	346	160	77	93	482	150	3.0	103	0.7	54
		9R00467	59	NR	NR	0.4	4.0	7.1	17	379	227	59	83	274	147	4.6	101	0.6	42
		9R00468	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
		N	3	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3
		Mean	57.0	12.25	10.10	0.47	4.37	7.57	19.3	363.3	196.7	69.0	88.7	427.	148.3	3.57	101.3	0.70	50.3
		SD	7.2	0.07	0.71	0.12	0.32	0.40	2.1	16.6	33.9	9.2	5.1	135.	1.5	0.90	1.5	0.10	7.2
														0					

1. The LOD for CHOL was 45 mg/dL. When CHOL < LOD, half of LOD (22.5 mg/dL) was entered to perform statistics

A. Statistics on Clinical Chemistries for rats, male and female values combined.

CHOL mg/dL By Dose Group (mg/Kg/day)

Oneway Anova Summary of Fit	
RSquare	0.701781
RSquare Adj	0.631612
Root Mean Square Error	10.64141
Mean of Response	72.52273
Observations (or Sum Wgts)	22

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	4530.1636	1132.54	10.0013
Error	17	1925.0750	113.24	Prob>F
C Total	21	6455.2386	307.39	0.0002

Means for Oneway Anova				
Level	Number	Mean	Std Error	
CONT	8	84.8750	3.762	
N-EtFOSA40mkd	4	81.0000	5.321	
N-EtFOSE160mkd	1	50.0000	10.641	
N-EtFOSE40mkd	4	73.0000	5.321	
PFOS40mkd	5	50.1000	4.759	

Std Error uses a pooled estimate of error variance

Means and Std Deviations				
Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	84.8750	7.6052	2.6888
N-EtFOSA40mkd	4	81.0000	5.4772	2.7386
N-EtFOSE160mkd	1	50.0000	?	?
N-EtFOSE40mkd	4	73.0000	11.1654	5.5827
PFOS40mkd	5	50.1000	16.2496	7.2670

Means Comparisons					
Dif=Mean[i]-Mean[j]	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSE160mkd
CONT	0.0000	3.8750	11.8750	34.7750	34.8750
N-EtFOSA40mkd	-3.8750	0.0000	8.0000	30.9000	31.0000

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DT15

N-EtFOSE40mkd	-11.8750	-8.0000	0.0000	22.9000	23.0000
PFOS40mkd	-34.7750	-30.9000	-22.9000	0.0000	0.1000
N-EtFOSE160mkd	-34.8750	-31.0000	-23.0000	-0.1000	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

		t			
		2.10980			
Abs(Dif)-LSD	CONT	N-EtFOSE40mkd	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSE160mkd
CONT	-11.2256	-9.8736	-1.8736	21.9758	11.0618
N-EtFOSE40mkd	-9.8736	-15.8755	-7.8755	15.8392	5.8987
N-EtFOSE40mkd	-1.8736	-7.8755	-15.8755	7.8392	-2.1013
PFOS40mkd	21.9758	15.8392	7.8392	-14.1994	-24.4942
N-EtFOSE160mkd	11.0618	5.8987	-2.1013	-24.4942	-31.7509

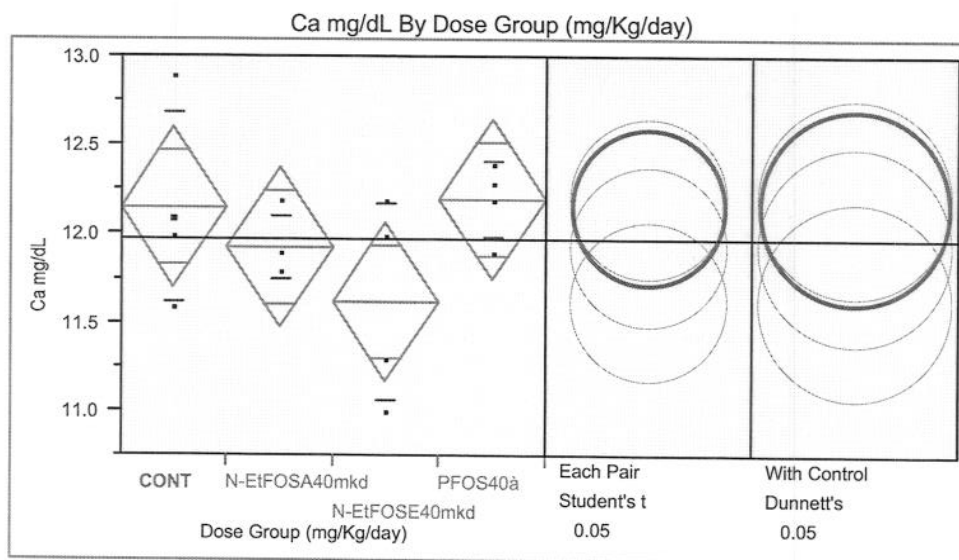
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.74189
Abs(Dif)-LSD	CONT
CONT	-14.5888
N-EtFOSE40mkd	-13.9926
N-EtFOSE40mkd	-5.9926
PFOS40mkd	18.1412
N-EtFOSE160mkd	3.9275

Positive values show pairs of means that are significantly different.

Cholesterol in rats male and female combined for DT15. Sig reduction by 40 mkd PFOS and 160 mkd N-EtFOSE.



Oneway Anova
Summary of Fit

RSquare	0.28157
RSquare Adj	0.101962
Root Mean Square Error	0.418828
Mean of Response	11.975
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.8250000	0.275000	1.5677
Error	12	2.1050000	0.175417	Prob>F
C Total	15	2.9300000	0.195333	0.2484

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	4	12.1500	0.20941
N-EtFOSA40mkd	4	11.9250	0.20941
N-EtFOSE40mkd	4	11.6250	0.20941
PFOS40mkd	4	12.2000	0.20941

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	4	12.1500	0.544671	0.27234
N-EtFOSA40mkd	4	11.9250	0.189297	0.09465
N-EtFOSE40mkd	4	11.6250	0.567891	0.28395
PFOS40mkd	4	12.2000	0.216025	0.10801

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd
PFOS40mkd	0.000000	0.050000	0.275000	0.575000
CONT	-0.05	0.000000	0.225000	0.525000
N-EtFOSA40mkd	-0.275	-0.225	0.000000	0.300000
N-EtFOSE40mkd	-0.575	-0.525	-0.3	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.17882

Abs(Dif)-LSD	PFOS40mkd	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd
PFOS40mkd	-0.64527	-0.59527	-0.37027	-0.07027
CONT	-0.59527	-0.64527	-0.42027	-0.12027
N-EtFOSA40mkd	-0.37027	-0.42027	-0.64527	-0.34527
N-EtFOSE40mkd	-0.07027	-0.12027	-0.34527	-0.64527

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

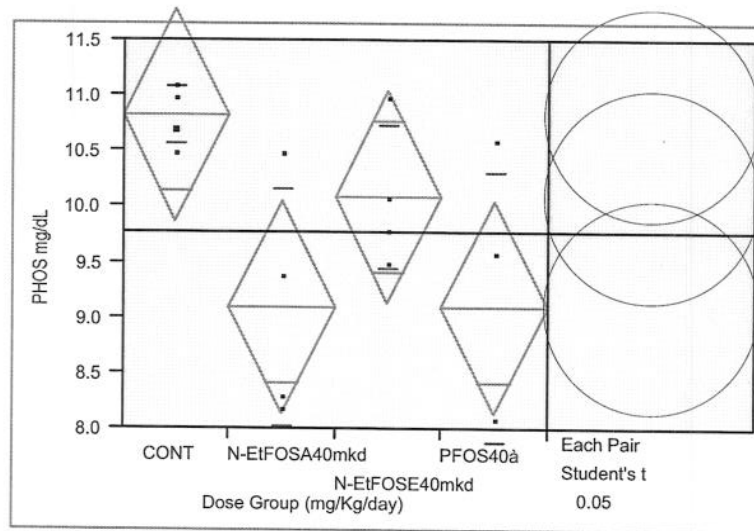
|d|

2.68294

Abs(Dif)-LSD	CONT
PFOS40mkd	-0.74457
CONT	-0.79457
N-EtFOSA40mkd	-0.56957
N-EtFOSE40mkd	-0.26957

Positive values show pairs of means that are significantly different.

PHOS mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.471871
RSquare Adj	0.339839
Root Mean Square Error	0.889171
Mean of Response	9.78125
Observations (or Sum Wgts)	16

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	8.476875	2.82562	3.5739
Error	12	9.487500	0.79063	Prob>F
C Total	15	17.964375	1.19763	0.0470

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	4	10.8250	0.44459
N-EtFOSA40mkd	4	9.1000	0.44459
N-EtFOSE40mkd	4	10.1000	0.44459
PFOS40mkd	4	9.1000	0.44459

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	4	10.8250	0.27538	0.13769
N-EtFOSA40mkd	4	9.1000	1.08012	0.54006
N-EtFOSE40mkd	4	10.1000	0.64807	0.32404
PFOS40mkd	4	9.1000	1.22474	0.61237

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	0.00000	0.72500	1.72500	1.72500
N-EtFOSE40mkd	-0.72500	0.00000	1.00000	1.00000
N-EtFOSA40mkd	-1.72500	-1.00000	0.00000	0.00000
PFOS40mkd	-1.72500	-1.00000	0.00000	0.00000

Alpha=

0.05

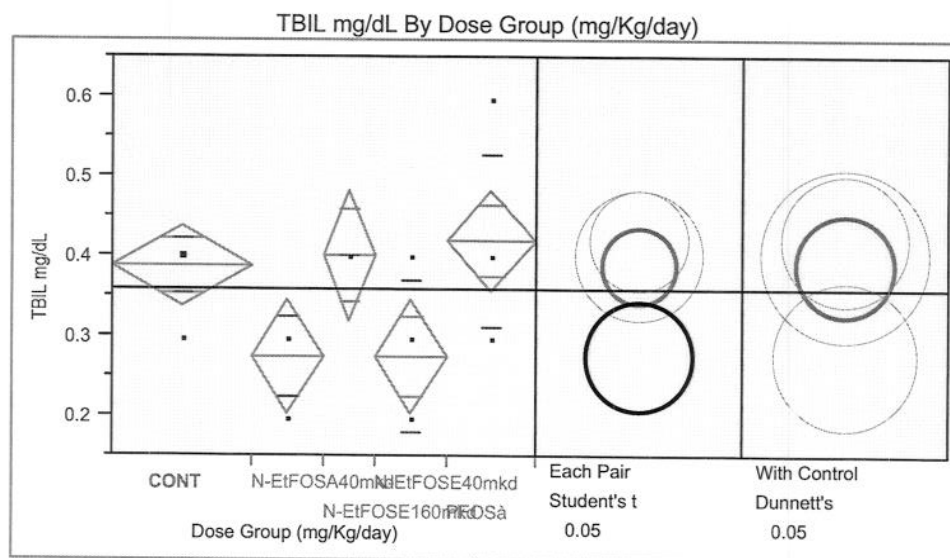
Comparisons for each pair using Student's t

t				
2.17882				
Abs(Dif)-LSD	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	-1.36991	-0.64491	0.35509	0.35509

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N-EtFOSE40mkd	-0.64491	-1.36991	-0.36991	-0.36991
N-EtFOSA40mkd	0.35509	-0.36991	-1.36991	-1.36991
PFOS40mkd	0.35509	-0.36991	-1.36991	-1.36991

Positive values show pairs of means that are significantly different.
Male and female rats combined Ca and Phos clinical chemistries. No sig change from control.



Oneway Anova
Summary of Fit

RSquare	0.485514
RSquare Adj	0.377201
Root Mean Square Error	0.069491
Mean of Response	0.358333
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	0.08658333	0.021646	4.4825
Error	19	0.09175000	0.004829	Prob>F
C Total	23	0.17833333	0.007754	0.0102

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	0.387500	0.02457
N-EtFOSA40mkd	4	0.275000	0.03475
N-EtFOSE160mkd	3	0.400000	0.04012
N-EtFOSE40mkd	4	0.275000	0.03475
PFOS40mkd	5	0.420000	0.03108

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	0.387500	0.035355	0.01250
N-EtFOSA40mkd	4	0.275000	0.050000	0.02500
N-EtFOSE160mkd	3	0.400000	0.000000	0.00000
N-EtFOSE40mkd	4	0.275000	0.095743	0.04787
PFOS40mkd	5	0.420000	0.109545	0.04899

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSE160mkd	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd
PFOS40mkd	0.000000	0.020000	0.032500	0.145000	0.145000
N-EtFOSE160mkd	-0.02	0.000000	0.012500	0.125000	0.125000
CONT	-0.0325	-0.0125	0.000000	0.112500	0.112500
N-EtFOSA40mkd	-0.145	-0.125	-0.1125	0.000000	0.000000
N-EtFOSE40mkd	-0.145	-0.125	-0.1125	0.000000	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSE160mkd	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd
PFOS40mkd	-0.09199	-0.08622	-0.05042	0.047433	0.047433
N-EtFOSE160mkd	-0.08622	-0.11875	-0.08597	0.013915	0.013915
CONT	-0.05042	-0.08597	-0.07272	0.023434	0.023434
N-EtFOSA40mkd	0.047433	0.013915	0.023434	-0.10284	-0.10284
N-EtFOSE40mkd	0.047433	0.013915	0.023434	-0.10284	-0.10284

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|

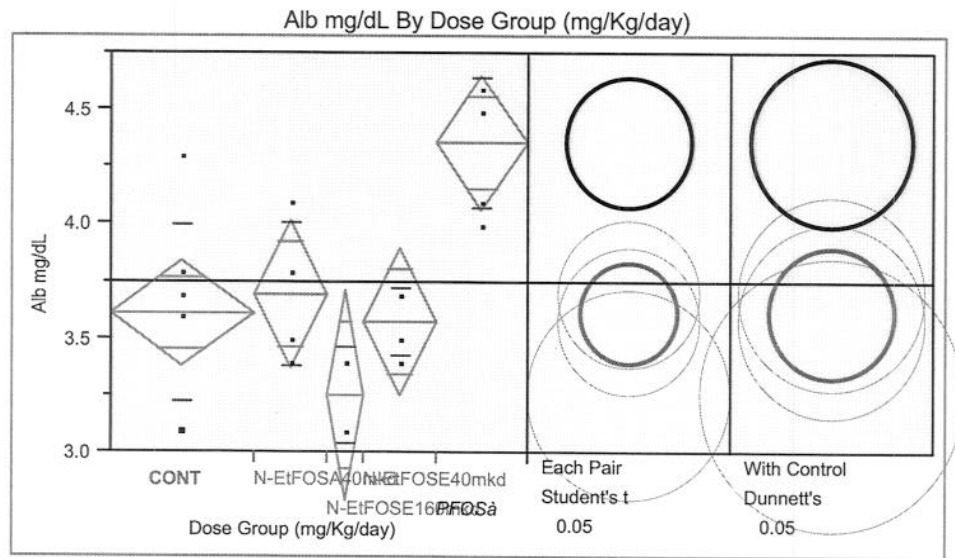
2.70438

Abs(Dif)-LSD	CONT
PFOS40mkd	-0.07464
N-EtFOSE160mkd	-0.11473
CONT	-0.09396
N-EtFOSA40mkd	-0.00258
N-EtFOSE40mkd	-0.00258

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DT15

Positive values show pairs of means that are significantly different.

Above is for rat TBIL, male and femal combined data. no sig changes.



Oneway Anova
Summary of Fit

RSquare	0.595877
RSquare Adj	0.506072
Root Mean Square Error	0.315634
Mean of Response	3.752174
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	2.6441413	0.661035	6.6352
Error	18	1.7932500	0.099625	Prob>F
C Total	22	4.4373913	0.201700	0.0018

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	3.61250	0.11159
N-EtFOSA40mkd	4	3.70000	0.15782
N-EtFOSE160mkd	2	3.25000	0.22319
N-EtFOSE40mkd	4	3.57500	0.15782
PFOS40mkd	5	4.36000	0.14116

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	3.61250	0.387068	0.13685
N-EtFOSA40mkd	4	3.70000	0.316228	0.15811
N-EtFOSE160mkd	2	3.25000	0.212132	0.15000
N-EtFOSE40mkd	4	3.57500	0.150000	0.07500
PFOS40mkd	5	4.36000	0.288097	0.12884

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	0.00000	0.66000	0.74750	0.78500	1.11000
N-EtFOSA40mkd	-0.66000	0.00000	0.08750	0.12500	0.45000
CONT	-0.74750	-0.08750	0.00000	0.03750	0.36250
N-EtFOSE40mkd	-0.78500	-0.12500	-0.03750	0.00000	0.32500
N-EtFOSE160mkd	-1.11000	-0.45000	-0.36250	-0.32500	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	-0.41939	0.215166	0.369464	0.340166	0.555195
N-EtFOSA40mkd	0.215166	-0.4689	-0.31858	-0.3439	-0.12428
CONT	0.369464	-0.31858	-0.33156	-0.36858	-0.16174
N-EtFOSE40mkd	0.340166	-0.3439	-0.36858	-0.4689	-0.24928
N-EtFOSE160mkd	0.555195	-0.12428	-0.16174	-0.24928	-0.66312

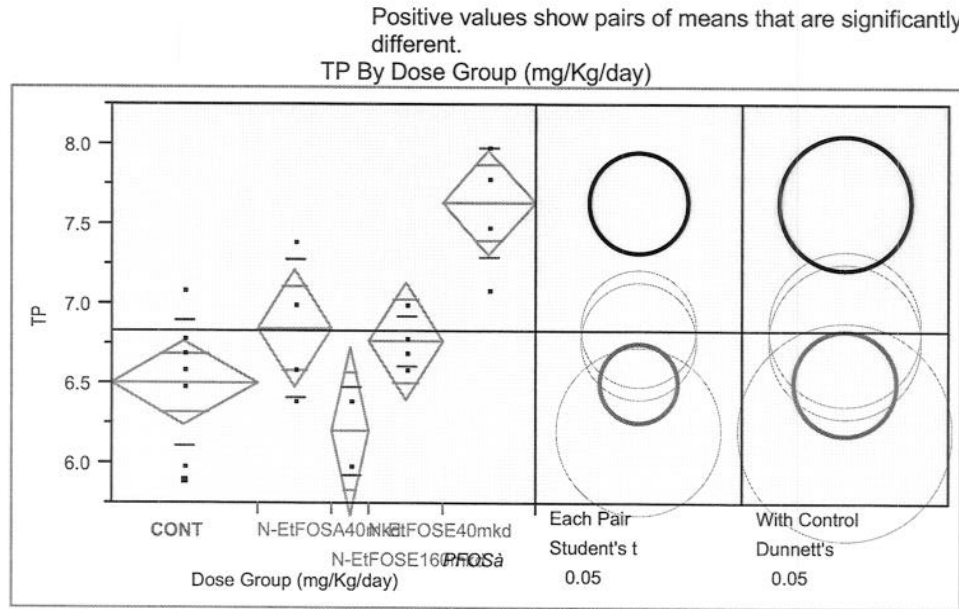
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|

2.72184

Abs(Dif)-LSD	CONT
PFOS40mkd	0.257735
N-EtFOSA40mkd	-0.43859
CONT	-0.42955
N-EtFOSE40mkd	-0.48859
N-EtFOSE160mkd	-0.31668



Oneway Anova Summary of Fit

RSquare	0.674822
RSquare Adj	0.60256
Root Mean Square Error	0.361958
Mean of Response	6.834783
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	4.8939239	1.22348	9.3386
Error	18	2.3582500	0.13101	Prob>F
C Total	22	7.2521739	0.32964	0.0003

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	6.51250	0.12797
N-EtFOSA40mkd	4	6.85000	0.18098
N-EtFOSE160mkd	2	6.20000	0.25594
N-EtFOSE40mkd	4	6.77500	0.18098
PFOSA40mkd	5	7.64000	0.16187

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	6.51250	0.397986	0.14071
N-EtFOSA40mkd	4	6.85000	0.443471	0.22174
N-EtFOSE160mkd	2	6.20000	0.282843	0.20000
N-EtFOSE40mkd	4	6.77500	0.170783	0.08539
PFOSA40mkd	5	7.64000	0.350714	0.15684

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOSA40mkd	N-EtFOSA40mkd	N-EtFOSE40mkd	CONT	N-EtFOSE160mkd
PFOSA40mkd	0.00000	0.79000	0.86500	1.12750	1.44000
N-EtFOSA40mkd	-0.79000	0.00000	0.07500	0.33750	0.65000
N-EtFOSE40mkd	-0.86500	-0.07500	0.00000	0.26250	0.57500
CONT	-1.12750	-0.33750	-0.26250	0.00000	0.31250
N-EtFOSE160mkd	-1.44000	-0.65000	-0.57500	-0.31250	0.00000

T-7071.1 FR
DT15

Alpha=

0.05

Comparisons for each pair using Student's t

	t				
	2.10091				
Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	N-EtFOSE40mkd	CONT	N-EtFOSE160mkd
PFOS40mkd	-0.48095	0.279880	0.354880	0.693982	0.803769
N-EtFOSA40mkd	0.279880	-0.53771	-0.46271	-0.12817	-0.00856
N-EtFOSE40mkd	0.354880	-0.46271	-0.53771	-0.20317	-0.08356
CONT	0.693982	-0.12817	-0.20317	-0.38022	-0.28868
N-EtFOSE160mkd	0.803769	-0.00856	-0.08356	-0.28868	-0.76044

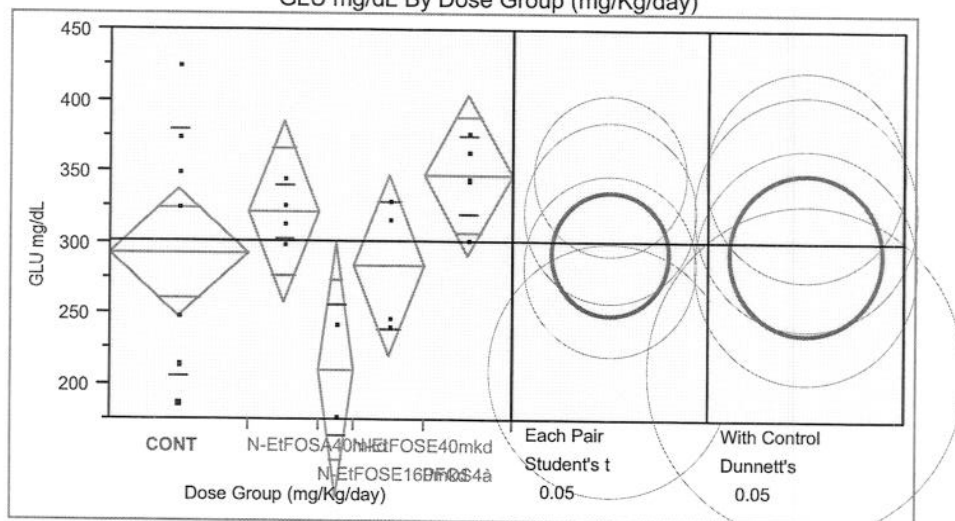
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.72184
Abs(Dif)-LSD	
PFOS40mkd	0.565854
N-EtFOSA40mkd	-0.2658
N-EtFOSE40mkd	-0.3408
CONT	-0.4926
N-EtFOSE160mkd	-0.46636

Positive values show pairs of means that are significantly different.

GLU mg/dL By Dose Group (mg/Kg/day)



Oneway Anova

Summary of Fit

RSquare	0.312227
RSquare Adj	0.159389
Root Mean Square Error	60.99861
Mean of Response	301.6087
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	30404.528	7601.13	2.0429
Error	18	66974.950	3720.83	Prob>F
C Total	22	97379.478	4426.34	0.1312

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	293.500	21.566

N-EtFOSA40mkd	4	322.500	30.499
N-EtFOSE160mkd	2	211.000	43.133
N-EtFOSE40mkd	4	284.750	30.499
PFOS40mkd	5	347.600	27.279

Std Error uses a pooled estimate of error variance

Means and Std Deviations				
Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	293.500	87.8879	31.073
N-EtFOSA40mkd	4	322.500	19.9416	9.971
N-EtFOSE160mkd	2	211.000	46.6690	33.000
N-EtFOSE40mkd	4	284.750	45.6317	22.816
PFOS40mkd	5	347.600	28.6671	12.820

Means Comparisons					
Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	0.000	25.100	54.100	62.850	136.600
N-EtFOSA40mkd	-25.100	0.000	29.000	37.750	111.500
CONT	-54.100	-29.000	0.000	8.750	82.500
N-EtFOSE40mkd	-62.850	-37.750	-8.750	0.000	73.750
N-EtFOSE160mkd	-136.600	-111.500	-82.500	-73.750	0.000

Alpha=

0.05

Comparisons for each pair using Student's t

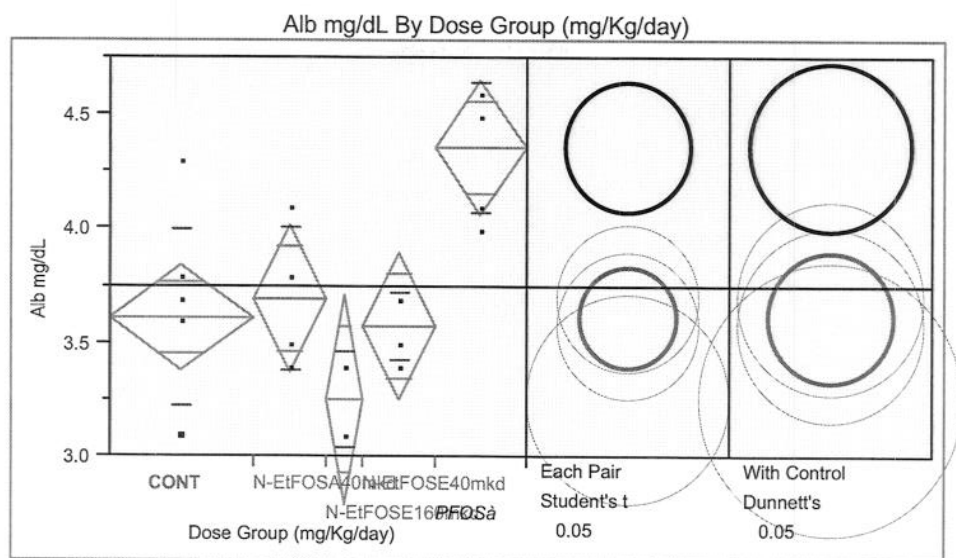
t					
2.10091					
Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	-81.051	-60.867	-18.958	-23.117	29.380
N-EtFOSA40mkd	-60.867	-90.617	-49.477	-52.867	0.517
CONT	-18.958	-49.477	-64.076	-69.727	-18.813
N-EtFOSE40mkd	-23.117	-52.867	-69.727	-90.617	-37.233
N-EtFOSE160mkd	29.380	0.517	-18.813	-37.233	-128.152

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.72184	
Abs(Dif)-LSD	CONT
PFOS40mkd	-40.5507
N-EtFOSA40mkd	-72.6711
CONT	-83.0141
N-EtFOSE40mkd	-92.9211
N-EtFOSE160mkd	-48.7569

Positive values show pairs of means that are significantly different.



Oneway Anova
Summary of Fit

RSquare	0.595877
RSquare Adj	0.506072
Root Mean Square Error	0.315634
Mean of Response	3.752174
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	2.6441413	0.661035	6.6352
Error	18	1.7932500	0.099625	Prob>F
C Total	22	4.4373913	0.201700	0.0018

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	3.61250	0.11159
N-EtFOSA40mkd	4	3.70000	0.15782
N-EtFOSE160mkd	2	3.25000	0.22319
N-EtFOSE40mkd	4	3.57500	0.15782
PFOS40mkd	5	4.36000	0.14116

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	3.61250	0.387068	0.13685
N-EtFOSA40mkd	4	3.70000	0.316228	0.15811
N-EtFOSE160mkd	2	3.25000	0.212132	0.15000
N-EtFOSE40mkd	4	3.57500	0.150000	0.07500
PFOS40mkd	5	4.36000	0.288097	0.12884

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	0.00000	0.66000	0.74750	0.78500	1.11000
N-EtFOSA40mkd	-0.66000	0.00000	0.08750	0.12500	0.45000
CONT	-0.74750	-0.08750	0.00000	0.03750	0.36250
N-EtFOSE40mkd	-0.78500	-0.12500	-0.03750	0.00000	0.32500
N-EtFOSE160mkd	-1.11000	-0.45000	-0.36250	-0.32500	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	-0.41939	0.215166	0.369464	0.340166	0.555195
N-EtFOSA40mkd	0.215166	-0.4689	-0.31858	-0.3439	-0.12428
CONT	0.369464	-0.31858	-0.33156	-0.36858	-0.16174
N-EtFOSE40mkd	0.340166	-0.3439	-0.36858	-0.4689	-0.24928
N-EtFOSE160mkd	0.555195	-0.12428	-0.16174	-0.24928	-0.66312

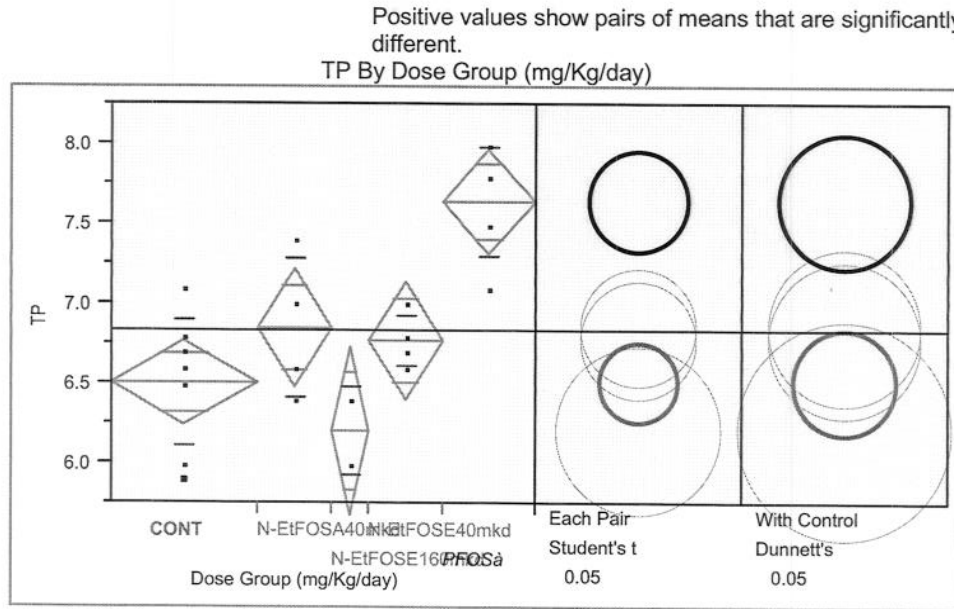
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|

2.72184

Abs(Dif)-LSD	CONT
PFOS40mkd	0.257735
N-EtFOSA40mkd	-0.43859
CONT	-0.42955
N-EtFOSE40mkd	-0.48859
N-EtFOSE160mkd	-0.31668



Oneway Anova Summary of Fit

RSquare	0.674822
RSquare Adj	0.60256
Root Mean Square Error	0.361958
Mean of Response	6.834783
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	4.8939239	1.22348	9.3386
Error	18	2.3582500	0.13101	Prob>F
C Total	22	7.2521739	0.32964	0.0003

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	6.51250	0.12797
N-EtFOSA40mkd	4	6.85000	0.18098
N-EtFOSE160mkd	2	6.20000	0.25594
N-EtFOSE40mkd	4	6.77500	0.18098
PFOSA40mkd	5	7.64000	0.16187

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	6.51250	0.397986	0.14071
N-EtFOSA40mkd	4	6.85000	0.443471	0.22174
N-EtFOSE160mkd	2	6.20000	0.282843	0.20000
N-EtFOSE40mkd	4	6.77500	0.170783	0.08539
PFOSA40mkd	5	7.64000	0.350714	0.15684

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOSA40mkd	N-EtFOSA40mkd	N-EtFOSE40mkd	CONT	N-EtFOSE160mkd
PFOSA40mkd	0.00000	0.79000	0.86500	1.12750	1.44000
N-EtFOSA40mkd	-0.79000	0.00000	0.07500	0.33750	0.65000
N-EtFOSE40mkd	-0.86500	-0.07500	0.00000	0.26250	0.57500
CONT	-1.12750	-0.33750	-0.26250	0.00000	0.31250
N-EtFOSE160mkd	-1.44000	-0.65000	-0.57500	-0.31250	0.00000

T-7071.1 FR
DT15

Alpha=

0.05
Comparisons for each pair using Student's t
t

	2.10091				
Abs(Dif)-LSD					
PFOS40mkd	-0.48095	N-EtFOSA40mkd	0.279880	CONT	0.693982
N-EtFOSA40mkd	0.279880		-0.53771		0.803769
N-EtFOSE40mkd	0.354880		-0.46271		-0.00856
CONT	0.693982		-0.53771		-0.08356
N-EtFOSE160mkd	0.803769		-0.20317		-0.28868
			-0.38022		-0.28868
			-0.08356		-0.76044

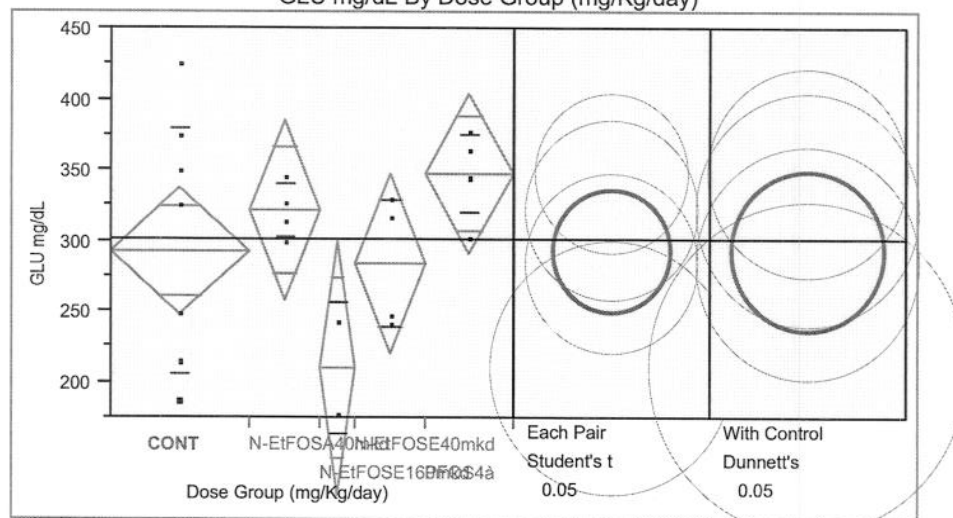
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.72184
Abs(Dif)-LSD	
PFOS40mkd	0.565854
N-EtFOSA40mkd	-0.2658
N-EtFOSE40mkd	-0.3408
CONT	-0.4926
N-EtFOSE160mkd	-0.46636

Positive values show pairs of means that are significantly different.

GLU mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.312227
RSquare Adj	0.159389
Root Mean Square Error	60.99861
Mean of Response	301.6087
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	30404.528	7601.13	2.0429
Error	18	66974.950	3720.83	Prob>F
C Total	22	97379.478	4426.34	0.1312

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	293.500	21.566

N-EtFOSA40mkd	4	322.500	30.499
N-EtFOSE160mkd	2	211.000	43.133
N-EtFOSE40mkd	4	284.750	30.499
PFOS40mkd	5	347.600	27.279

Std Error uses a pooled estimate of error variance

Means and Std Deviations				
Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	293.500	87.8879	31.073
N-EtFOSA40mkd	4	322.500	19.9416	9.971
N-EtFOSE160mkd	2	211.000	46.6690	33.000
N-EtFOSE40mkd	4	284.750	45.6317	22.816
PFOS40mkd	5	347.600	28.6671	12.820

Means Comparisons					
Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	0.000	25.100	54.100	62.850	136.600
N-EtFOSA40mkd	-25.100	0.000	29.000	37.750	111.500
CONT	-54.100	-29.000	0.000	8.750	82.500
N-EtFOSE40mkd	-62.850	-37.750	-8.750	0.000	73.750
N-EtFOSE160mkd	-136.600	-111.500	-82.500	-73.750	0.000

Alpha=

0.05

Comparisons for each pair using Student's t

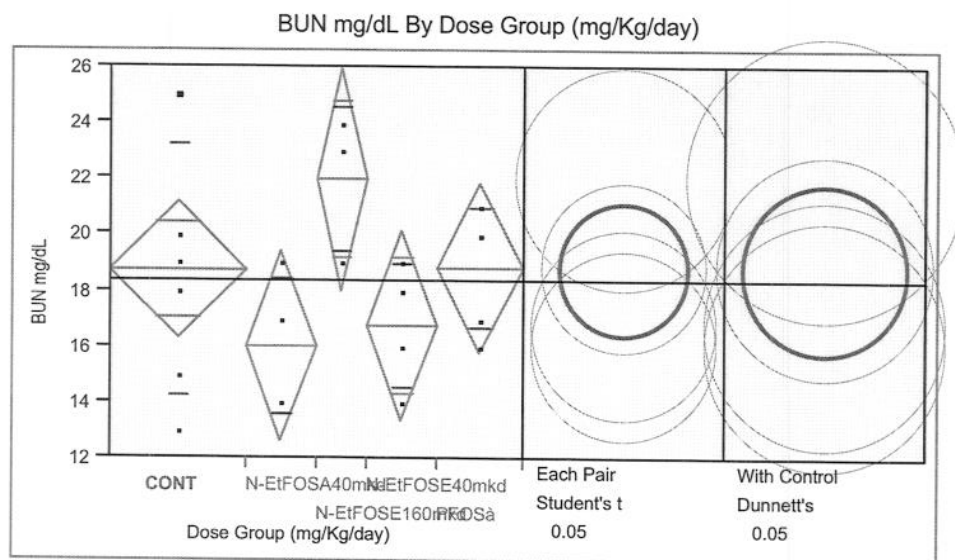
t					
2.10091					
Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	-81.051	-60.867	-18.958	-23.117	29.380
N-EtFOSA40mkd	-60.867	-90.617	-49.477	-52.867	0.517
CONT	-18.958	-49.477	-64.076	-69.727	-18.813
N-EtFOSE40mkd	-23.117	-52.867	-69.727	-90.617	-37.233
N-EtFOSE160mkd	29.380	0.517	-18.813	-37.233	-128.152

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.72184	
Abs(Dif)-LSD	CONT
PFOS40mkd	-40.5507
N-EtFOSA40mkd	-72.6711
CONT	-83.0141
N-EtFOSE40mkd	-92.9211
N-EtFOSE160mkd	-48.7569

Positive values show pairs of means that are significantly different.



Oneway Anova
Summary of Fit

RSquare	0.264802
RSquare Adj	0.110024
Root Mean Square Error	3.301116
Mean of Response	18.375
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	74.57500	18.6438	1.7108
Error	19	207.05000	10.8974	Prob>F
C Total	23	281.62500	12.2446	0.1892

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	18.7500	1.1671
N-EtFOSA40mkd	4	16.0000	1.6506
N-EtFOSE160mkd	3	22.0000	1.9059
N-EtFOSE40mkd	4	16.7500	1.6506
PFOS40mkd	5	18.8000	1.4763

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	18.7500	4.49603	1.5896
N-EtFOSA40mkd	4	16.0000	2.44949	1.2247
N-EtFOSE160mkd	3	22.0000	2.64575	1.5275
N-EtFOSE40mkd	4	16.7500	2.21736	1.1087
PFOS40mkd	5	18.8000	2.16795	0.9695

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSE160mkd	PFOS40mkd	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd
N-EtFOSE160mkd	0.00000	3.20000	3.25000	5.25000	6.00000
PFOS40mkd	-3.20000	0.00000	0.05000	2.05000	2.80000
CONT	-3.25000	-0.05000	0.00000	2.00000	2.75000
N-EtFOSE40mkd	-5.25000	-2.05000	-2.00000	0.00000	0.75000
N-EtFOSA40mkd	-6.00000	-2.80000	-2.75000	-0.75000	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.09301

Abs(Dif)-LSD	N-EtFOSE160mkd	PFOS40mkd	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd
N-EtFOSE160mkd	-5.64139	-1.84581	-1.42760	-0.02704	0.72296
PFOS40mkd	-1.84581	-4.36980	-3.88889	-2.58488	-1.83488
CONT	-1.42760	-3.88889	-3.45463	-2.23104	-1.48104
N-EtFOSE40mkd	-0.02704	-2.58488	-2.23104	-4.88559	-4.13559
N-EtFOSA40mkd	0.72296	-1.83488	-1.48104	-4.13559	-4.88559

Positive values show pairs of means that are significantly different.

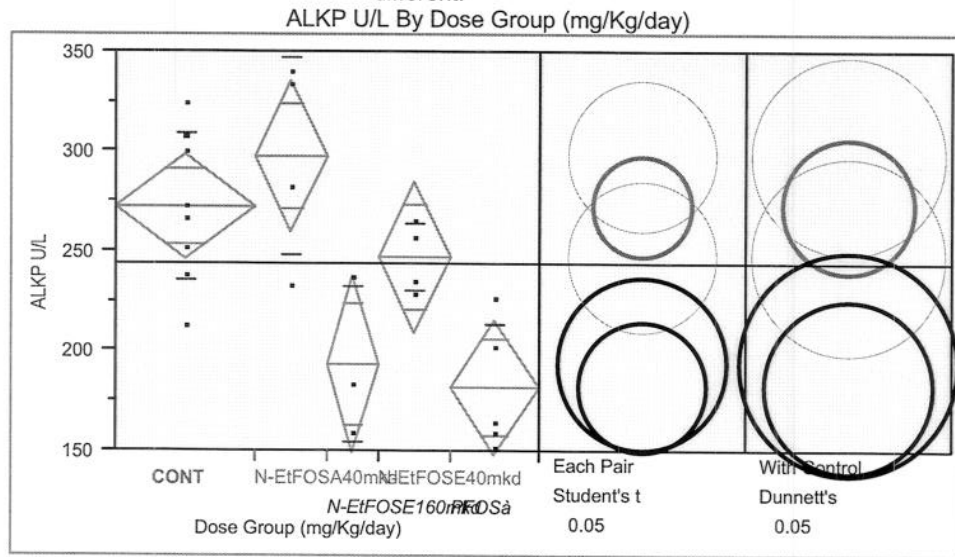
Comparisons with a control using Dunnett's Method

|d|

2.70438

Abs(Dif)-LSD	CONT
N-EtFOSE160mkd	-2.79392
PFOS40mkd	-5.03944
CONT	-4.46373
N-EtFOSE40mkd	-3.46693
N-EtFOSA40mkd	-2.71693

Positive values show pairs of means that are significantly different.



Oneway Anova Summary of Fit

RSquare	0.63982
RSquare Adj	0.563993
Root Mean Square Error	36.65097
Mean of Response	243.875
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	45338.050	11334.5	8.4379
Error	19	25522.575	1343.3	Prob>F
C Total	23	70860.625	3080.9	0.0004

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	272.625	12.958
N-EtFOSA40mkd	4	298.250	18.325
N-EtFOSE160mkd	3	194.000	21.160
N-EtFOSE40mkd	4	247.250	18.325
PFOSA40mkd	5	181.600	16.391

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	272.625	37.3896	13.219
N-EtFOSA40mkd	4	298.250	50.1290	25.065
N-EtFOSE160mkd	3	194.000	39.9500	23.065
N-EtFOSE40mkd	4	247.250	17.5760	8.788
PFOSA40mkd	5	181.600	31.9343	14.281

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd	PFOSA40mkd
N-EtFOSA40mkd	0.000	25.625	51.000	104.250	116.650
CONT	-25.625	0.000	25.375	78.625	91.025
N-EtFOSE40mkd	-51.000	-25.375	0.000	53.250	65.650
N-EtFOSE160mkd	-104.250	-78.625	-53.250	0.000	12.400
PFOSA40mkd	-116.650	-91.025	-65.650	-12.400	0.000

T-7071.1 FR
DT15

Alpha=

0.05
Comparisons for each pair using Student's t

	2.09301				
Abs(Dif)-LSD	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd	PFOS40mkd
N-EtFOSA40mkd	-54.2427	-21.3506	-3.2427	45.6612	65.1908
CONT	-21.3506	-38.3554	-21.6006	26.6915	47.2931
N-EtFOSE40mkd	-3.2427	-21.6006	-54.2427	-5.3388	14.1908
N-EtFOSE160mkd	45.6612	26.6915	-5.3388	-62.6341	-43.6217
PFOS40mkd	65.1908	47.2931	14.1908	-43.6217	-48.5162

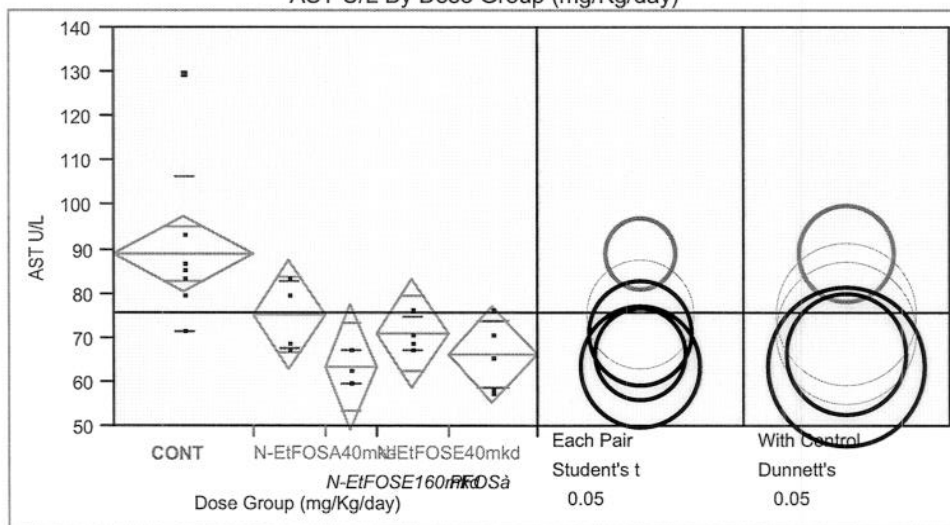
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.70438
Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-35.0721
CONT	-49.5590
N-EtFOSE40mkd	-35.3221
N-EtFOSE160mkd	11.5218
PFOS40mkd	34.5190

Positive values show pairs of means that are significantly different.

AST U/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.468919
RSquare Adj	0.357112
Root Mean Square Error	11.9777
Mean of Response	75.875
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	2406.7833	601.696	4.1940
Error	19	2725.8417	143.465	Prob>F
C Total	23	5132.6250	223.158	0.0134

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	89.1250	4.2348

N-EtFOSA40mkd	4	75.2500	5.9889
N-EtFOSE160mkd	3	63.6667	6.9153
N-EtFOSE40mkd	4	71.2500	5.9889
PFOS40mkd	5	66.2000	5.3566

Std Error uses a pooled estimate of error variance

Means and Std Deviations				
Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	89.1250	17.7074	6.2605
N-EtFOSA40mkd	4	75.2500	7.9739	3.9870
N-EtFOSE160mkd	3	63.6667	4.0415	2.3333
N-EtFOSE40mkd	4	71.2500	4.0311	2.0156
PFOS40mkd	5	66.2000	8.0436	3.5972

Means Comparisons					
Dif=Mean[i]-Mean[j]	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSE160mkd
CONT	0.0000	13.8750	17.8750	22.9250	25.4583
N-EtFOSA40mkd	-13.8750	0.0000	4.0000	9.0500	11.5833
N-EtFOSE40mkd	-17.8750	-4.0000	0.0000	5.0500	7.5833
PFOS40mkd	-22.9250	-9.0500	-5.0500	0.0000	2.5333
N-EtFOSE160mkd	-25.4583	-11.5833	-7.5833	-2.5333	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

t					
2.09301					
Abs(Dif)-LSD	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSE160mkd
CONT	-12.5347	-1.4768	2.5232	8.6332	8.4862
N-EtFOSA40mkd	-1.4768	-17.7268	-13.7268	-7.7671	-7.5638
N-EtFOSE40mkd	2.5232	-13.7268	-17.7268	-11.7671	-11.5638
PFOS40mkd	8.6332	-7.7671	-11.7671	-15.8553	-15.7748
N-EtFOSE160mkd	8.4862	-7.5638	-11.5638	-15.7748	-20.4691

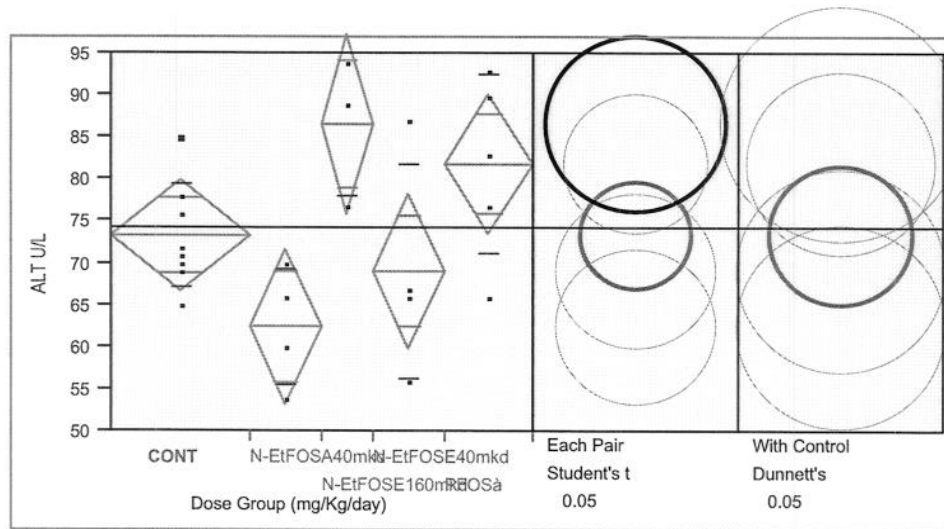
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.70438	
Abs(Dif)-LSD	CONT
CONT	-16.1961
N-EtFOSA40mkd	-5.9611
N-EtFOSE40mkd	-1.9611
PFOS40mkd	4.4586
N-EtFOSE160mkd	3.5287

Positive values show pairs of means that are significantly different.

ALT U/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.478735
RSquare Adj	0.368994
Root Mean Square Error	9.014511
Mean of Response	74.20833
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	1417.9917	354.498	4.3624
Error	19	1543.9667	81.261	Prob>F
C Total	23	2961.9583	128.781	0.0114

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	73.2500	3.1871
N-EtFOSA40mkd	4	62.5000	4.5073
N-EtFOSE160mkd	3	86.6667	5.2045
N-EtFOSE40mkd	4	69.0000	4.5073
PFOS40mkd	5	81.8000	4.0314

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	73.2500	6.2278	2.2019
N-EtFOSA40mkd	4	62.5000	7.0000	3.5000
N-EtFOSE160mkd	3	86.6667	8.7369	5.0442
N-EtFOSE40mkd	4	69.0000	12.9872	6.4936
PFOS40mkd	5	81.8000	10.8028	4.8311

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSE160mkd	PFOS40mkd	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd
N-EtFOSE160mkd	0.0000	4.8667	13.4167	17.6667	24.1667
PFOS40mkd	-4.8667	0.0000	8.5500	12.8000	19.3000
CONT	-13.4167	-8.5500	0.0000	4.2500	10.7500
N-EtFOSE40mkd	-17.6667	-12.8000	-4.2500	0.0000	6.5000
N-EtFOSA40mkd	-24.1667	-19.3000	-10.7500	-6.5000	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

T-7071.1 FR
DT15

		2.09301			
Abs(Dif)-LSD	N-EtFOSE160mkd	PFOS40mkd	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd
N-EtFOSE160mkd	-15.4052	-8.9122	0.6433	3.2564	9.7564
PFOS40mkd	-8.9122	-11.9328	-2.2061	0.1433	6.6433
CONT	0.6433	-2.2061	-9.4337	-7.3039	-0.8039
N-EtFOSE40mkd	3.2564	0.1433	-7.3039	-13.3413	-6.8413
N-EtFOSA40mkd	9.7564	6.6433	-0.8039	-6.8413	-13.3413

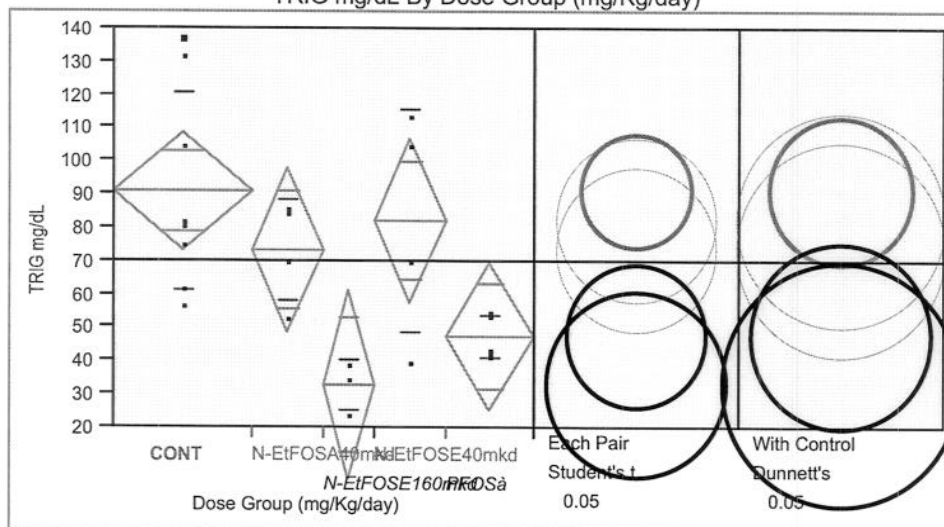
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.70438
Abs(Dif)-LSD	CONT
N-EtFOSE160mkd	-3.0877
PFOS40mkd	-5.3480
CONT	-12.1893
N-EtFOSE40mkd	-10.6788
N-EtFOSA40mkd	-4.1788

Positive values show pairs of means that are significantly different.

TRIG mg/dL By Dose Group (mg/Kg/day)



Oneway Anova Summary of Fit

RSquare	0.502305
RSquare Adj	0.397527
Root Mean Square Error	23.94016
Mean of Response	70.41667
Observations (or Sum Wgts)	24

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	10990.342	2747.59	4.7940
Error	19	10889.492	573.13	Prob>F
C Total	23	21879.833	951.30	0.0076

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	91.3750	8.464
N-EtFOSA40mkd	4	73.5000	11.970
N-EtFOSE160mkd	3	32.6667	13.822
N-EtFOSE40mkd	4	82.2500	11.970

PFOS40mkd 5 47.6000 10.706

Std Error uses a pooled estimate of error variance
Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	91.3750	30.2982	10.712
N-EtFOSA40mkd	4	73.5000	15.5027	7.751
N-EtFOSE160mkd	3	32.6667	7.7675	4.485
N-EtFOSE40mkd	4	82.2500	33.9644	16.982
PFOS40mkd	5	47.6000	6.3482	2.839

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE160mkd
CONT	0.0000	9.1250	17.8750	43.7750	58.7083
N-EtFOSE40mkd	-9.1250	0.0000	8.7500	34.6500	49.5833
N-EtFOSA40mkd	-17.8750	-8.7500	0.0000	25.9000	40.8333
PFOS40mkd	-43.7750	-34.6500	-25.9000	0.0000	14.9333
N-EtFOSE160mkd	-58.7083	-49.5833	-40.8333	-14.9333	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t
t

Abs(Dif)-LSD	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE160mkd
CONT	-25.0535	-21.5591	-12.8091	15.2096	24.7858
N-EtFOSE40mkd	-21.5591	-35.4310	-26.6810	1.0372	11.3135
N-EtFOSA40mkd	-12.8091	-26.6810	-35.4310	-7.7128	2.5635
PFOS40mkd	15.2096	1.0372	-7.7128	-31.6904	-21.6596
N-EtFOSE160mkd	24.7858	11.3135	2.5635	-21.6596	-40.9122

Positive values show pairs of means that are significantly different.

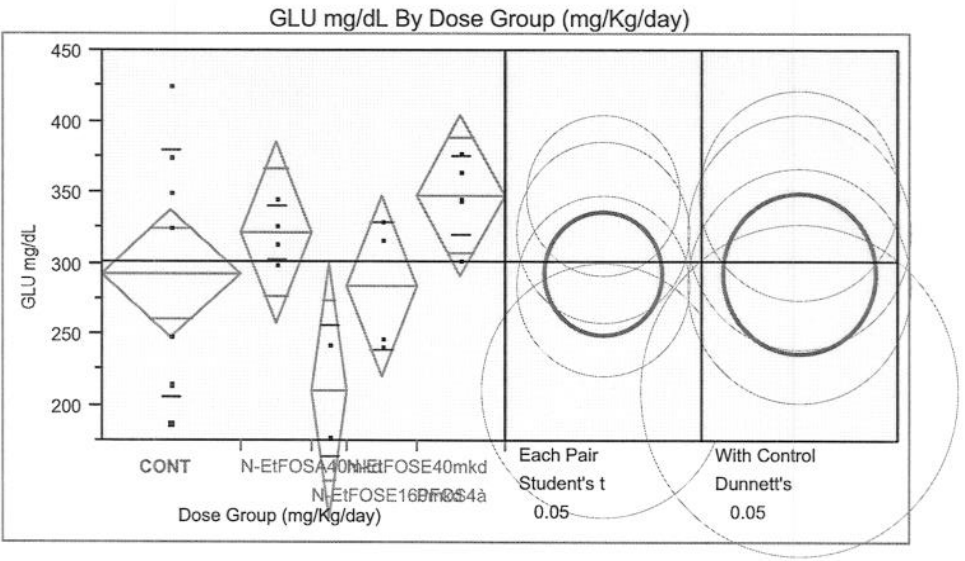
Comparisons with a control using Dunnett's Method

d	
2.70438	
Abs(Dif)-LSD	CONT
CONT	-32.3716
N-EtFOSE40mkd	-30.5220
N-EtFOSA40mkd	-21.7720
PFOS40mkd	6.8657
N-EtFOSE160mkd	14.8770

Positive values show pairs of means that are significantly different.

Combined rat data for males and females.

Sig decreases in ALKP, AST and TRIG by 40 mkd PFOS and 160 mkd N-EtFOSE in rats.



Oneway Anova
Summary of Fit

RSquare	0.312227
RSquare Adj	0.159389
Root Mean Square Error	60.99861
Mean of Response	301.6087
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	30404.528	7601.13	2.0429
Error	18	66974.950	3720.83	Prob>F
C Total	22	97379.478	4426.34	0.1312

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	293.500	21.566
N-EtFOSA40mkd	4	322.500	30.499
N-EtFOSE160mkd	2	211.000	43.133
N-EtFOSE40mkd	4	284.750	30.499
PFOS40mkd	5	347.600	27.279

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	293.500	87.8879	31.073
N-EtFOSA40mkd	4	322.500	19.9416	9.971
N-EtFOSE160mkd	2	211.000	46.6690	33.000
N-EtFOSE40mkd	4	284.750	45.6317	22.816
PFOS40mkd	5	347.600	28.6671	12.820

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	0.000	25.100	54.100	62.850	136.600
N-EtFOSA40mkd	-25.100	0.000	29.000	37.750	111.500
CONT	-54.100	-29.000	0.000	8.750	82.500
N-EtFOSE40mkd	-62.850	-37.750	-8.750	0.000	73.750
N-EtFOSE160mkd	-136.600	-111.500	-82.500	-73.750	0.000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	N-EtFOSE160mkd
PFOS40mkd	-81.051	-60.867	-18.958	-23.117	29.380
N-EtFOSA40mkd	-60.867	-90.617	-49.477	-52.867	0.517
CONT	-18.958	-49.477	-64.076	-69.727	-18.813
N-EtFOSE40mkd	-23.117	-52.867	-69.727	-90.617	-37.233
N-EtFOSE160mkd	29.380	0.517	-18.813	-37.233	-128.152

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

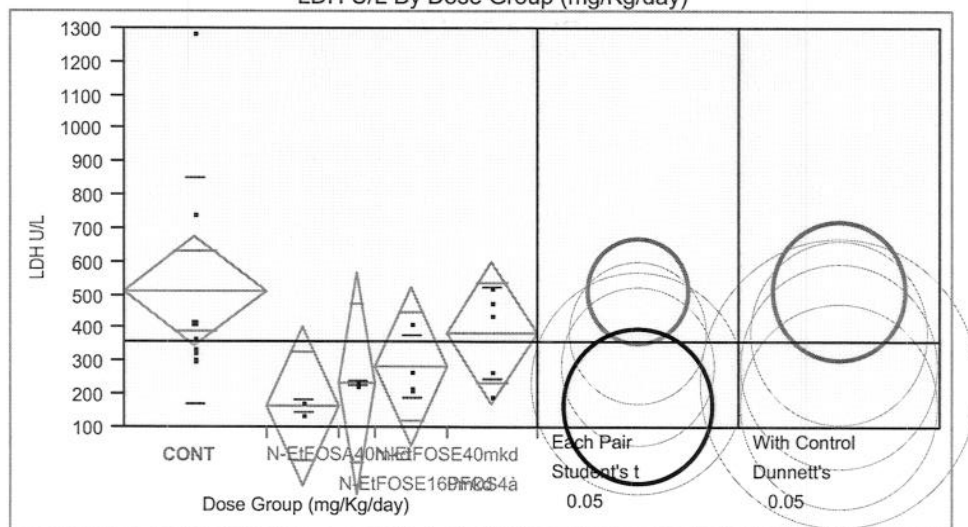
|d|

2.72184

Abs(Dif)-LSD	CONT
PFOS40mkd	-40.5507
N-EtFOSA40mkd	-72.6711
CONT	-83.0141
N-EtFOSE40mkd	-92.9211
N-EtFOSE160mkd	-48.7569

Positive values show pairs of means that are significantly different.

LDH U/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.295198
RSquare Adj	0.138575
Root Mean Square Error	228.9437
Mean of Response	361.6957
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	395163.0	98790.8	1.8848
Error	18	943473.8	52415.2	Prob>F
C Total	22	1338636.9	60847.1	0.1569

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	514.625	80.94
N-EtFOSA40mkd	4	169.000	114.47
N-EtFOSE160mkd	2	234.000	161.89
N-EtFOSE40mkd	4	283.750	114.47
PFOS40mkd	5	384.600	102.39

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	514.625	345.236	122.06
N-EtFOSA40mkd	4	169.000	20.017	10.01
N-EtFOSE160mkd	2	234.000	9.899	7.00
N-EtFOSE40mkd	4	283.750	95.318	47.66
PFOS40mkd	5	384.600	141.952	63.48

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	PFOS40mkd	N-EtFOSE40mkd	N-EtFOSE160mkd	N-EtFOSA40mkd
CONT	0.000	130.025	230.875	280.625	345.625
PFOS40mkd	-130.025	0.000	100.850	150.600	215.600
N-EtFOSE40mkd	-230.875	-100.850	0.000	49.750	114.750
N-EtFOSE160mkd	-280.625	-150.600	-49.750	0.000	65.000
N-EtFOSA40mkd	-345.625	-215.600	-114.750	-65.000	0.000

T-7071.1 FR
DT15

Alpha=

0.05
Comparisons for each pair using Student's t
t
2.10091

Abs(Dif)-LSD	CONT	PFOS40mkd	N-EtFOSE40mkd	N-EtFOSE160mkd	N-EtFOSA40mkd
CONT	-240.495	-144.181	-63.670	-99.631	51.080
PFOS40mkd	-144.181	-304.205	-221.808	-251.825	-107.058
N-EtFOSE40mkd	-63.670	-221.808	-340.111	-366.799	-225.361
N-EtFOSE160mkd	-99.631	-251.825	-366.799	-480.990	-351.549
N-EtFOSA40mkd	51.080	-107.058	-225.361	-351.549	-340.111

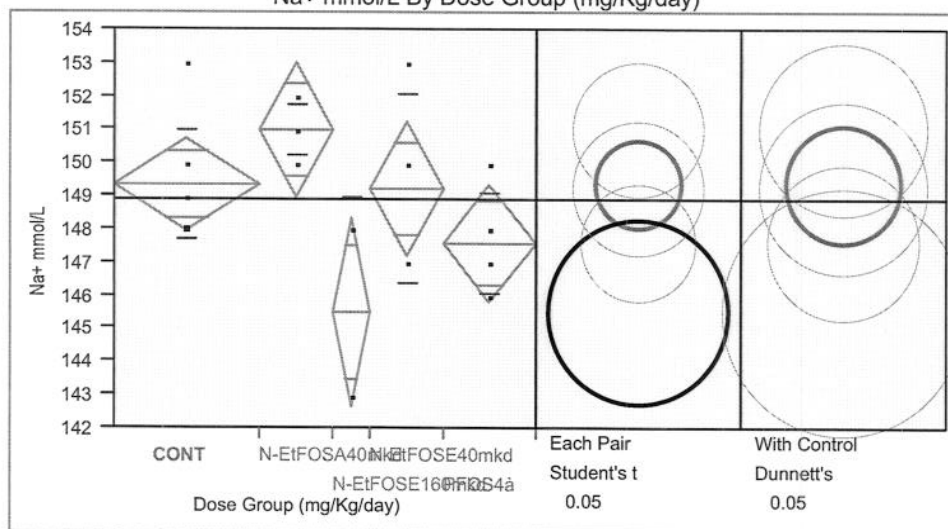
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

Abs(Dif)-LSD	CONT
CONT	-311.574
PFOS40mkd	-225.224
N-EtFOSE40mkd	-150.723
N-EtFOSE160mkd	-212.016
N-EtFOSA40mkd	-35.973

Positive values show pairs of means that are significantly different.

Na⁺ mmol/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.429799
RSquare Adj	0.303087
Root Mean Square Error	1.94829
Mean of Response	148.913
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	51.50109	12.8753	3.3919
Error	18	68.32500	3.7958	Prob>F
C Total	22	119.82609	5.4466	0.0310

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	149.375	0.6888

N-EtFOSA40mkd	4	151.000	0.9741
N-EtFOSE160mkd	2	145.500	1.3776
N-EtFOSE40mkd	4	149.250	0.9741
PFOS40mkd	5	147.600	0.8713

Std Error uses a pooled estimate of error variance

Means and Std Deviations				
Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	149.375	1.68502	0.5957
N-EtFOSA40mkd	4	151.000	0.81650	0.4082
N-EtFOSE160mkd	2	145.500	3.53553	2.5000
N-EtFOSE40mkd	4	149.250	2.87228	1.4361
PFOS40mkd	5	147.600	1.51658	0.6782

Means Comparisons					
Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSE160mkd
N-EtFOSA40mkd	0.00000	1.62500	1.75000	3.40000	5.50000
CONT	-1.62500	0.00000	0.12500	1.77500	3.87500
N-EtFOSE40mkd	-1.75000	-0.12500	0.00000	1.65000	3.75000
PFOS40mkd	-3.40000	-1.77500	-1.65000	0.00000	2.10000
N-EtFOSE160mkd	-5.50000	-3.87500	-3.75000	-2.10000	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

t					
2.10091					
Abs(Dif)-LSD	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSE160mkd
N-EtFOSA40mkd	-2.89431	-0.88155	-1.14431	0.65421	1.95520
CONT	-0.88155	-2.04659	-2.38155	-0.55847	0.63906
N-EtFOSE40mkd	-1.14431	-2.38155	-2.89431	-1.09579	0.20520
PFOS40mkd	0.65421	-0.55847	-1.09579	-2.58875	-1.32460
N-EtFOSE160mkd	1.95520	0.63906	0.20520	-1.32460	-4.09318

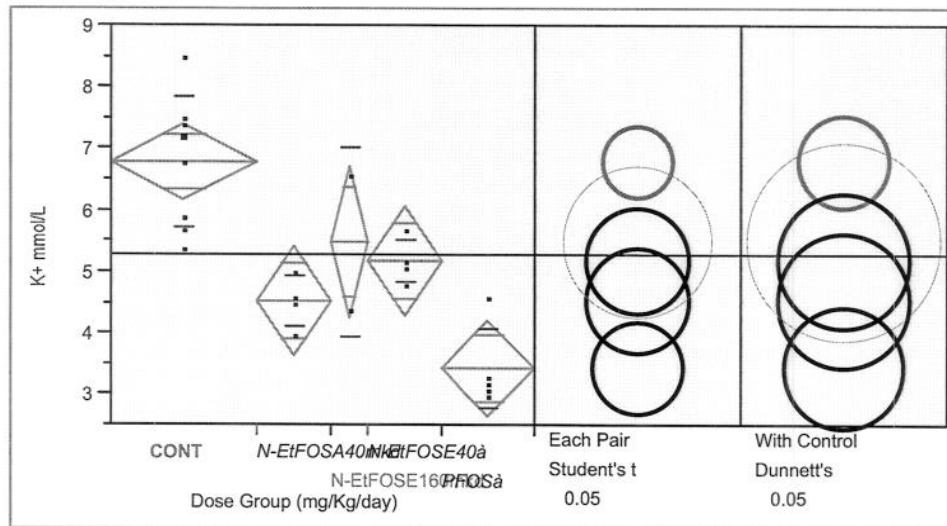
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.72184	
Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-1.62237
CONT	-2.65146
N-EtFOSE40mkd	-3.12237
PFOS40mkd	-1.24813
N-EtFOSE160mkd	-0.31733

Positive values show pairs of means that are significantly different.

K+ mmol/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.744756
RSquare Adj	0.688035
Root Mean Square Error	0.848512
Mean of Response	5.282609
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	37.813543	9.45339	13.1302
Error	18	12.959500	0.71997	Prob>F
C Total	22	50.773043	2.30787	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	6.80000	0.29999
N-EtFOSA40mkd	4	4.52500	0.42426
N-EtFOSE160mkd	2	5.50000	0.59999
N-EtFOSE40mkd	4	5.20000	0.42426
PFOS40mkd	5	3.44000	0.37947

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	6.80000	1.06100	0.3751
N-EtFOSA40mkd	4	4.52500	0.41130	0.2056
N-EtFOSE160mkd	2	5.50000	1.55563	1.1000
N-EtFOSE40mkd	4	5.20000	0.37417	0.1871
PFOS40mkd	5	3.44000	0.65803	0.2943

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	N-EtFOSE160mkd	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	0.00000	1.30000	1.60000	2.27500	3.36000
N-EtFOSE160mkd	-1.30000	0.00000	0.30000	0.97500	2.06000
N-EtFOSE40mkd	-1.60000	-0.30000	0.00000	0.67500	1.76000
N-EtFOSA40mkd	-2.27500	-0.97500	-0.67500	0.00000	1.08500
PFOS40mkd	-3.36000	-2.06000	-1.76000	-1.08500	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t
t

		2.10091			
Abs(Dif)-LSD	CONT	N-EtFOSE160mkd	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	-0.89132	-0.10930	0.50836	1.18336	2.34374
N-EtFOSE160mkd	-0.10930	-1.78265	-1.24382	-0.56882	0.56853
N-EtFOSE40mkd	0.50836	-1.24382	-1.26052	-0.58552	0.56417
N-EtFOSA40mkd	1.18336	-0.56882	-0.58552	-1.26052	-0.11083
PFOS40mkd	2.34374	0.56853	0.56417	-0.11083	-1.12744

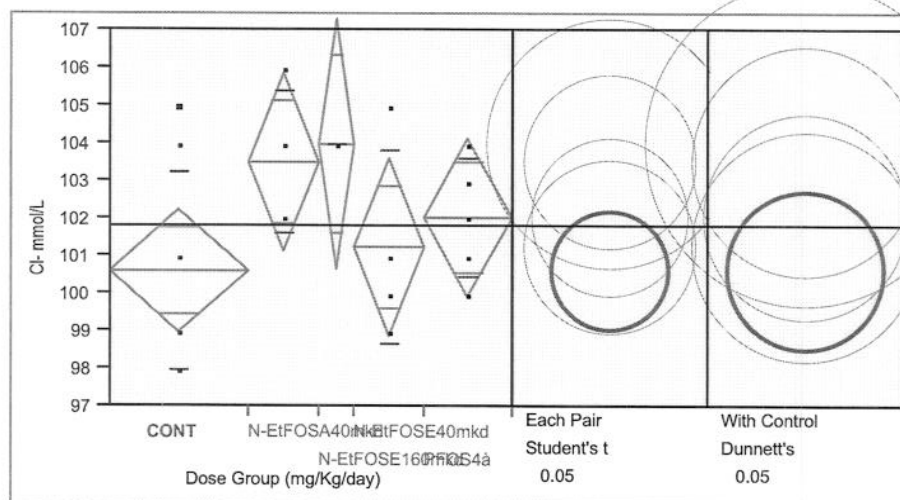
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.72184
Abs(Dif)-LSD	CONT
CONT	-1.15476
N-EtFOSE160mkd	-0.52583
N-EtFOSE40mkd	0.18572
N-EtFOSA40mkd	0.86072
PFOS40mkd	2.04338

Positive values show pairs of means that are significantly different.

Cl- mmol/L By Dose Group (mg/Kg/day)



Oneway Anova Summary of Fit

RSquare	0.26878
RSquare Adj	0.106287
Root Mean Square Error	2.256164
Mean of Response	101.8261
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	33.67935	8.41984	1.6541
Error	18	91.62500	5.09028	Prob>F
C Total	22	125.30435	5.69565	0.2043

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	100.625	0.7977
N-EtFOSA40mkd	4	103.500	1.1281
N-EtFOSE160mkd	2	104.000	1.5953
N-EtFOSE40mkd	4	101.250	1.1281

PFOS40mkd 5 102.000 1.0090

Std Error uses a pooled estimate of error variance

Means and Std Deviations				
Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	100.625	2.66927	0.9437
N-EtFOSA40mkd	4	103.500	1.91485	0.9574
N-EtFOSE160mkd	2	104.000	0.00000	0.0000
N-EtFOSE40mkd	4	101.250	2.62996	1.3150
PFOS40mkd	5	102.000	1.58114	0.7071

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSE160mkd	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSE160mkd	0.00000	0.50000	2.00000	2.75000	3.37500
N-EtFOSA40mkd	-0.50000	0.00000	1.50000	2.25000	2.87500
PFOS40mkd	-2.00000	-1.50000	0.00000	0.75000	1.37500
N-EtFOSE40mkd	-2.75000	-2.25000	-0.75000	0.00000	0.62500
CONT	-3.37500	-2.87500	-1.37500	-0.62500	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.10091

Abs(Dif)-LSD	N-EtFOSE160mkd	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSE160mkd	-4.73999	-3.60496	-1.96576	-1.35496	-0.37229
N-EtFOSA40mkd	-3.60496	-3.35168	-1.67968	-1.10168	-0.02764
PFOS40mkd	-1.96576	-1.67968	-2.99784	-2.42968	-1.32721
N-EtFOSE40mkd	-1.35496	-1.10168	-2.42968	-3.35168	-2.27764
CONT	-0.37229	-0.02764	-1.32721	-2.27764	-2.37000

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

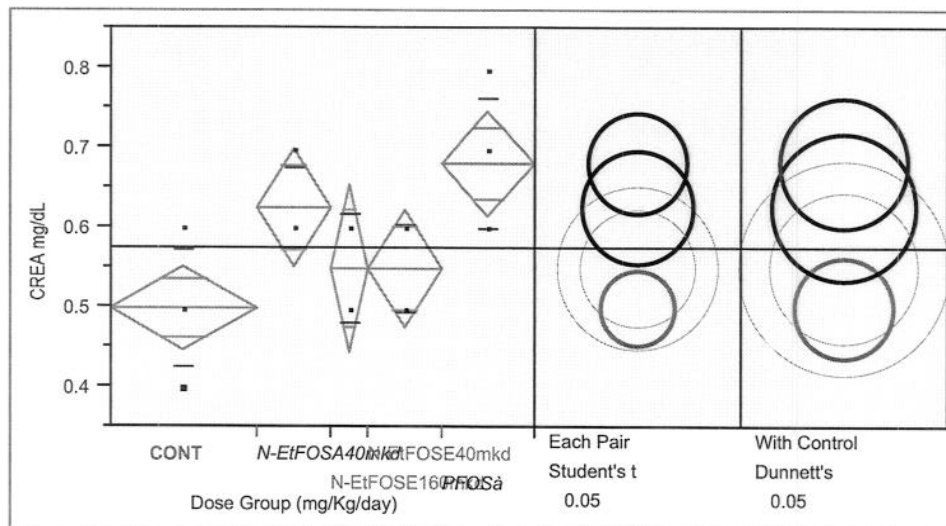
|d|

2.72184

Abs(Dif)-LSD	CONT
N-EtFOSE160mkd	-1.47982
N-EtFOSA40mkd	-0.88552
PFOS40mkd	-2.12586
N-EtFOSE40mkd	-3.13552
CONT	-3.07046

Positive values show pairs of means that are significantly different.

CREA mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.557128
RSquare Adj	0.458712
Root Mean Square Error	0.070907
Mean of Response	0.573913
Observations (or Sum Wgts)	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	0.11384783	0.028462	5.6609
Error	18	0.09050000	0.005028	Prob>F
C Total	22	0.20434783	0.009289	0.0039

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	8	0.500000	0.02507
N-EtFOSA40mkd	4	0.625000	0.03545
N-EtFOSE160mkd	2	0.550000	0.05014
N-EtFOSE40mkd	4	0.550000	0.03545
PFOS40mkd	5	0.680000	0.03171

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	8	0.500000	0.075593	0.02673
N-EtFOSA40mkd	4	0.625000	0.050000	0.02500
N-EtFOSE160mkd	2	0.550000	0.070711	0.05000
N-EtFOSE40mkd	4	0.550000	0.057735	0.02887
PFOS40mkd	5	0.680000	0.083666	0.03742

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	N-EtFOSE160mkd	N-EtFOSE40mkd	CONT
PFOS40mkd	0.000000	0.055000	0.130000	0.130000	0.180000
N-EtFOSA40mkd	-0.055	0.000000	0.075000	0.075000	0.125000
N-EtFOSE160mkd	-0.13	-0.075	0.000000	0.000000	0.050000
N-EtFOSE40mkd	-0.13	-0.075	0.000000	0.000000	0.050000
CONT	-0.18	-0.125	-0.05	-0.05	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

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		2.10091			
Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	N-EtFOSE160mkd	N-EtFOSE40mkd	CONT
PFOS40mkd	-0.09422	-0.04493	0.005364	0.030069	0.095075
N-EtFOSA40mkd	-0.04493	-0.10534	-0.05401	-0.03034	0.033776
N-EtFOSE160mkd	0.005364	-0.05401	-0.14897	-0.12901	-0.06777
N-EtFOSE40mkd	0.030069	-0.03034	-0.12901	-0.10534	-0.04122
CONT	0.095075	0.033776	-0.06777	-0.04122	-0.07448

Positive values show pairs of means that are significantly different.

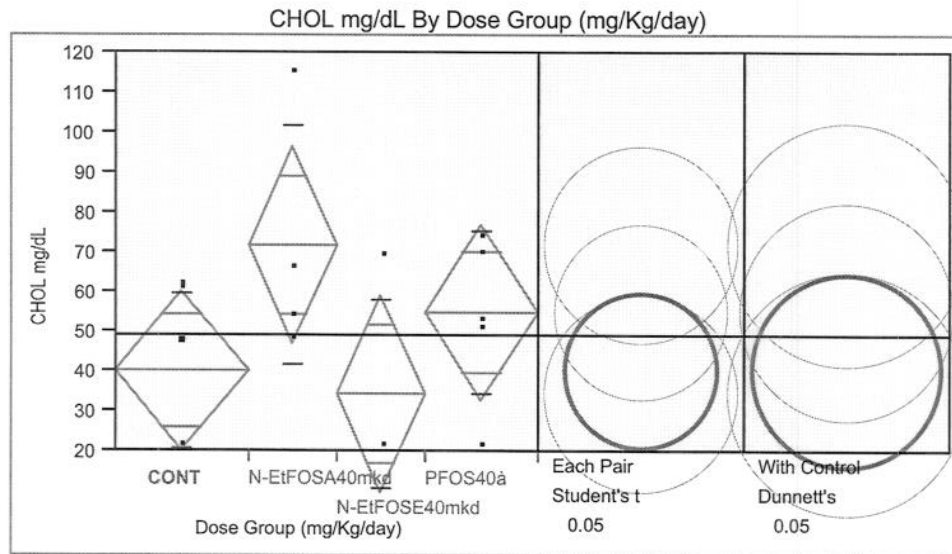
Comparisons with a control using Dunnett's Method

	d
	2.72184
Abs(Dif)-LSD	CONT
PFOS40mkd	0.069975
N-EtFOSA40mkd	0.006814
N-EtFOSE160mkd	-0.10258
N-EtFOSE40mkd	-0.06819
CONT	-0.0965

Positive values show pairs of means that are significantly different.

Clinical chemistries for all rats (males and females combined in DT15B. Shows significant decrease in potassium by 40 mkd PFOS, N-EtFOSE and N-EtFOSA, with the most significant changes occurring in the 40 mkd PFOS dose group. Creatinine (CREAT) was significantly increased in the 40 mkd PFOS and 40 mkd N-EtFOSA dose groups.

B. Statistics on Clinical Chemistries for Guinea Pigs, male and female values combined.



Oneway Anova
Summary of Fit

RSquare	0.303785
RSquare Adj	0.164542
Root Mean Square Error	23.365
Mean of Response	49.44737
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	3573.102	1191.03	2.1817
Error	15	8188.846	545.92	Prob>F
C Total	18	11761.947	653.44	0.1327

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	40.0833	9.539
N-EtFOSA40mkd	4	71.7500	11.682
N-EtFOSE40mkd	4	34.3750	11.682
PFOS40mkd	5	54.9000	10.449

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	40.0833	19.9785	8.156
N-EtFOSA40mkd	4	71.7500	30.4344	15.217
N-EtFOSE40mkd	4	34.3750	23.7500	11.875
PFOS40mkd	5	54.9000	20.7497	9.280

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	PFOS40mkd	CONT	N-EtFOSE40mkd
N-EtFOSA40mkd	0.0000	16.8500	31.6667	37.3750
PFOS40mkd	-16.8500	0.0000	14.8167	20.5250
CONT	-31.6667	-14.8167	0.0000	5.7083
N-EtFOSE40mkd	-37.3750	-20.5250	-5.7083	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.13144

Abs(Dif)-LSD	N-EtFOSA40mkd	PFOS40mkd	CONT	N-EtFOSE40mkd
N-EtFOSA40mkd	-35.2147	-16.5576	-0.4798	2.1603
PFOS40mkd	-16.5576	-31.4970	-15.3394	-12.8826
CONT	-0.4798	-15.3394	-28.7527	-26.4382
N-EtFOSE40mkd	2.1603	-12.8826	-26.4382	-35.2147

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|

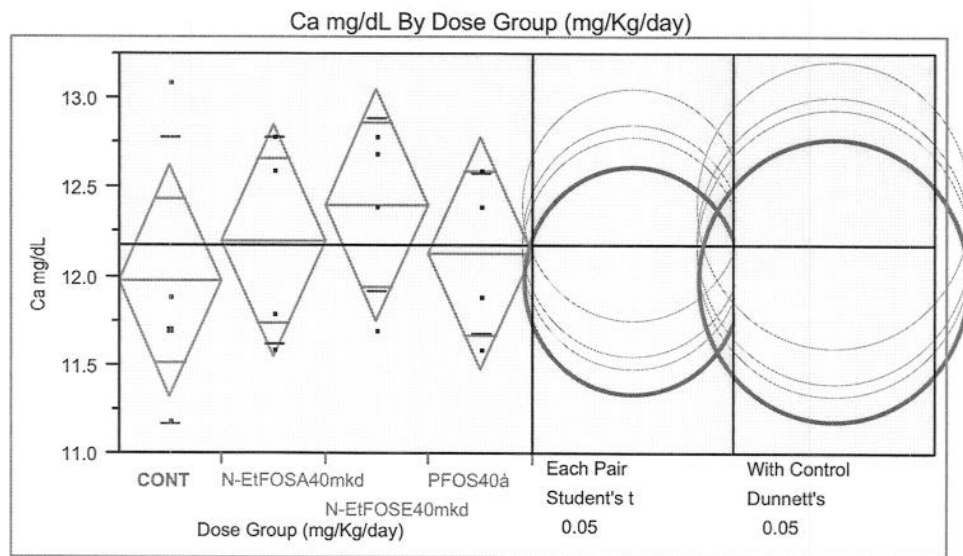
2.62898

Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-7.9838
PFOS40mkd	-22.3787
CONT	-35.4644
N-EtFOSE40mkd	-33.9421

Positive values show pairs of means that are significantly different.

Cholesterol, Male and female guinea pig combined.

T-7071.1 FR
DT15



Oneway Anova
Summary of Fit

RSquare	0.079281
RSquare Adj	-0.1509
Root Mean Square Error	0.602426
Mean of Response	12.175
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.3750000	0.125000	0.3444
Error	12	4.3550000	0.362917	Prob>F
C Total	15	4.7300000	0.315333	0.7938

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	4	11.9750	0.30121
N-EtFOSA40mkd	4	12.2000	0.30121
N-EtFOSE40mkd	4	12.4000	0.30121
PFOS40mkd	4	12.1250	0.30121

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	4	11.9750	0.805709	0.40285
N-EtFOSA40mkd	4	12.2000	0.588784	0.29439
N-EtFOSE40mkd	4	12.4000	0.496655	0.24833
PFOS40mkd	4	12.1250	0.457347	0.22867

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd	CONT
N-EtFOSE40mkd	0.000000	0.200000	0.275000	0.425000
N-EtFOSA40mkd	-0.2	0.000000	0.075000	0.225000
PFOS40mkd	-0.275	-0.075	0.000000	0.150000
CONT	-0.425	-0.225	-0.15	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd	CONT
Abs(Dif)-LSD				
N-EtFOSE40mkd	-0.92813	-0.72813	-0.65313	-0.50313
N-EtFOSA40mkd	-0.72813	-0.92813	-0.85313	-0.70313
PFOS40mkd	-0.65313	-0.85313	-0.92813	-0.77813
CONT	-0.50313	-0.70313	-0.77813	-0.92813

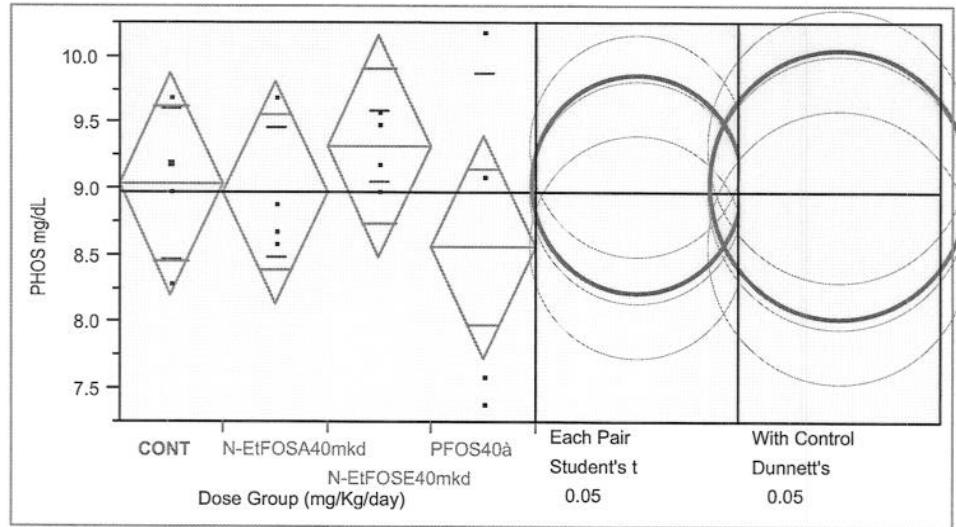
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.68294
Abs(Dif)-LSD	
N-EtFOSE40mkd	-0.71788
N-EtFOSA40mkd	-0.91788
PFOS40mkd	-0.99288
CONT	-1.14288

Positive values show pairs of means that are significantly different.

PHOS mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.137384
RSquare Adj	-0.07827
Root Mean Square Error	0.776343
Mean of Response	8.98125
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	1.1518750	0.383958	0.6371
Error	12	7.2325000	0.602708	Prob>F
C Total	15	8.3843750	0.558958	0.6054

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	4	9.05000	0.38817
N-EtFOSA40mkd	4	8.97500	0.38817
N-EtFOSE40mkd	4	9.32500	0.38817
PFOS40mkd	4	8.57500	0.38817

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	4	9.05000	0.58023	0.29011
N-EtFOSA40mkd	4	8.97500	0.49917	0.24958
N-EtFOSE40mkd	4	9.32500	0.27538	0.13769
PFOS40mkd	4	8.57500	1.32256	0.66128

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSE40mkd	CONT	N-EtFOSA40mkd	PFOS40mkd
N-EtFOSE40mkd	0.000000	0.275000	0.350000	0.750000
CONT	-0.275	0.000000	0.075000	0.475000
N-EtFOSA40mkd	-0.35	-0.075	0.000000	0.400000
PFOS40mkd	-0.75	-0.475	-0.4	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

Abs(Dif)-LSD	N-EtFOSE40mkd	CONT	N-EtFOSA40mkd	PFOS40mkd
N-EtFOSE40mkd	-1.19608	-0.92108	-0.84608	-0.44608

T-7071.1 FR
DT15

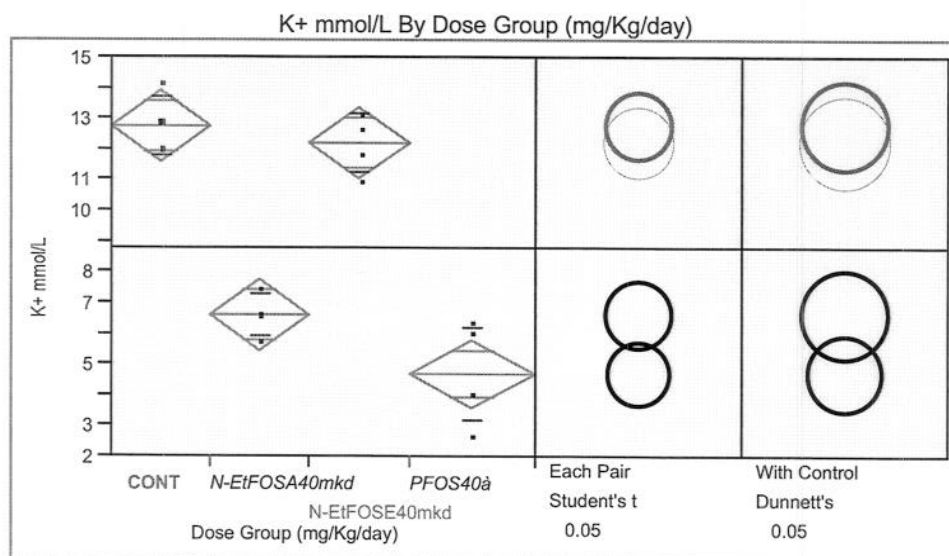
CONT	-0.92108	-1.19608	-1.12108	-0.72108
N-EtFOSA40mkd	-0.84608	-1.12108	-1.19608	-0.79608
PFOS40mkd	-0.44608	-0.72108	-0.79608	-1.19608

Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

d	
2.68294	
Abs(Dif)-LSD	CONT
N-EtFOSE40mkd	-1.19782
CONT	-1.47282
N-EtFOSA40mkd	-1.39782
PFOS40mkd	-0.99782

Positive values show pairs of means that are significantly different.

the two analyses directly Above are for guinea pig Ca and PHOS, male and female combined. No sig changes occurred



Oneway Anova
Summary of Fit

RSquare	0.926687
RSquare Adj	0.909768
Root Mean Square Error	1.140108
Mean of Response	8.823529
Observations (or Sum Wgts)	17

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	213.59259	71.1975	54.7738
Error	13	16.89800	1.2998	Prob>F
C Total	16	230.49059	14.4057	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	4	12.8000	0.57005
N-EtFOSA40mkd	4	6.6500	0.57005
N-EtFOSE40mkd	4	12.2000	0.57005
PFOS40mkd	5	4.6800	0.50987

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	4	12.8000	1.01653	0.50827
N-EtFOSA40mkd	4	6.6500	0.69522	0.34761
N-EtFOSE40mkd	4	12.2000	0.96264	0.48132
PFOS40mkd	5	4.6800	1.54661	0.69166

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	0.00000	0.60000	6.15000	8.12000
N-EtFOSE40mkd	-0.60000	0.00000	5.55000	7.52000
N-EtFOSA40mkd	-6.15000	-5.55000	0.00000	1.97000
PFOS40mkd	-8.12000	-7.52000	-1.97000	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
Abs(Dif)-LSD				
CONT	-1.74164	-1.14164	4.40836	6.46773
N-EtFOSE40mkd	-1.14164	-1.74164	3.80836	5.86773
N-EtFOSA40mkd	4.40836	3.80836	-1.74164	0.31773
PFOS40mkd	6.46773	5.86773	0.31773	-1.55777

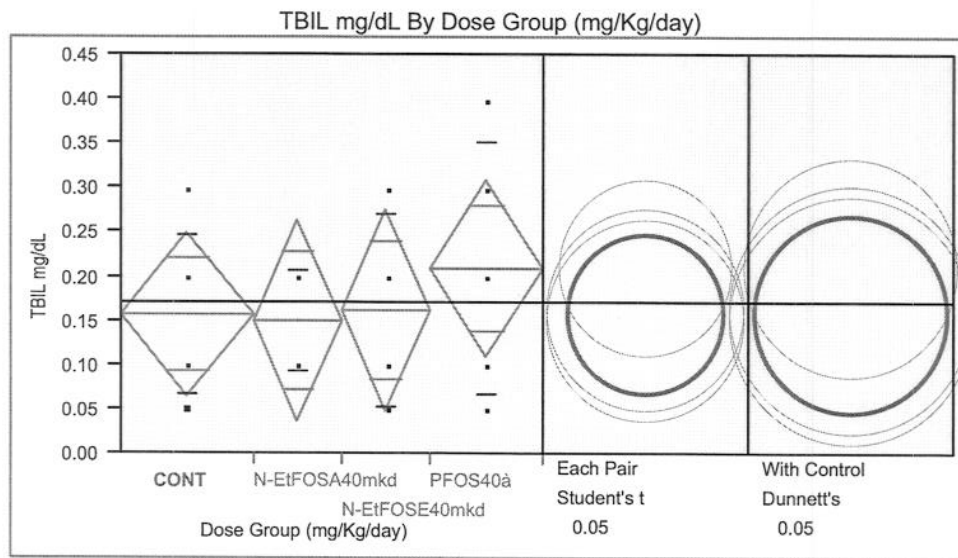
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.64945
Abs(Dif)-LSD	
CONT	-2.13593
N-EtFOSE40mkd	-1.53593
N-EtFOSA40mkd	4.01407
PFOS40mkd	6.09368

Positive values show pairs of means that are significantly different.

Above is for guinea pig combined male and femal potassium levels. Sig reduced by PFOS and N-etFOSA at 40 mg/Kg/day.



Oneway Anova
Summary of Fit

RSquare	0.05849
RSquare Adj	-0.12981
Root Mean Square Error	0.106758
Mean of Response	0.171053
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.01062061	0.003540	0.3106
Error	15	0.17095833	0.011397	Prob>F
C Total	18	0.18157895	0.010088	0.8174

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	0.158333	0.04358
N-EtFOSA40mkd	4	0.150000	0.05338
N-EtFOSE40mkd	4	0.162500	0.05338
PFOS40mkd	5	0.210000	0.04774

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	0.158333	0.091742	0.03745
N-EtFOSA40mkd	4	0.150000	0.057735	0.02887
N-EtFOSE40mkd	4	0.162500	0.110868	0.05543
PFOS40mkd	5	0.210000	0.143178	0.06403

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSE40mkd	CONT	N-EtFOSA40mkd
PFOS40mkd	0.000000	0.047500	0.051667	0.060000
N-EtFOSE40mkd	-0.0475	0.000000	0.004167	0.012500
CONT	-0.05167	-0.00417	0.000000	0.008333
N-EtFOSA40mkd	-0.06	-0.0125	-0.00833	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.13144

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSE40mkd	CONT	N-EtFOSA40mkd
PFOS40mkd	-0.14391	-0.10514	-0.08612	-0.09264
N-EtFOSE40mkd	-0.10514	-0.1609	-0.14271	-0.1484
CONT	-0.08612	-0.14271	-0.13137	-0.13855
N-EtFOSA40mkd	-0.09264	-0.1484	-0.13855	-0.1609

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

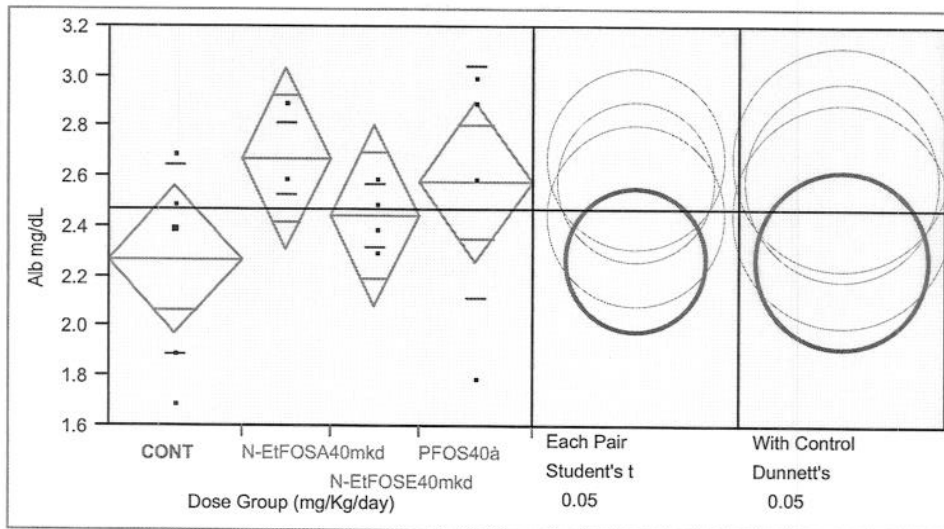
|d|

2.62898

Abs(Dif)-LSD	CONT
PFOS40mkd	-0.11828
N-EtFOSE40mkd	-0.177
CONT	-0.16204
N-EtFOSA40mkd	-0.17283

Positive values show pairs of means that are significantly different.

Alb mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.215626
RSquare Adj	0.058751
Root Mean Square Error	0.340474
Mean of Response	2.473684
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.4780088	0.159336	1.3745
Error	15	1.7388333	0.115922	Prob>F
C Total	18	2.2168421	0.123158	0.2887

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	2.26667	0.13900
N-EtFOSA40mkd	4	2.67500	0.17024
N-EtFOSE40mkd	4	2.45000	0.17024
PFOS40mkd	5	2.58000	0.15226

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	2.26667	0.382971	0.15635
N-EtFOSA40mkd	4	2.67500	0.150000	0.07500
N-EtFOSE40mkd	4	2.45000	0.129099	0.06455
PFOS40mkd	5	2.58000	0.471169	0.21071

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSA40mkd	0.000000	0.095000	0.225000	0.408333
PFOS40mkd	-0.095	0.000000	0.130000	0.313333
N-EtFOSE40mkd	-0.225	-0.13	0.000000	0.183333
CONT	-0.40833	-0.31333	-0.18333	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

Abs(Dif)-LSD	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSA40mkd	-0.51315	-0.39181	-0.28815	-0.0601

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DT15

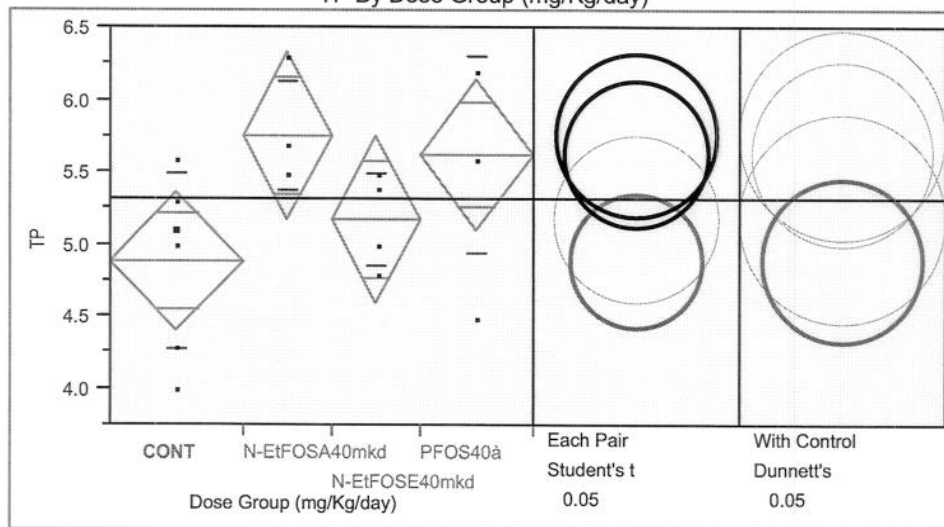
PFOS40mkd	-0.39181	-0.45897	-0.35681	-0.1261
N-EtFOSE40mkd	-0.28815	-0.35681	-0.51315	-0.2851
CONT	-0.0601	-0.1261	-0.2851	-0.41898

Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-0.16945
PFOS40mkd	-0.22868
N-EtFOSE40mkd	-0.39445
CONT	-0.51679

Positive values show pairs of means that are significantly different.

TP By Dose Group (mg/Kg/day)



Oneway Anova Summary of Fit

RSquare	0.3468
RSquare Adj	0.21616
Root Mean Square Error	0.550989
Mean of Response	5.321053
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	2.4177456	0.805915	2.6546
Error	15	4.5538333	0.303589	Prob>F
C Total	18	6.9715789	0.387310	0.0863

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	4.88333	0.22494
N-EtFOSA40mkd	4	5.75000	0.27549
N-EtFOSE40mkd	4	5.17500	0.27549
PFOS40mkd	5	5.62000	0.24641

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	4.88333	0.611283	0.24956

N-EtFOSA40mkd	4	5.75000	0.378594	0.18930
N-EtFOSE40mkd	4	5.17500	0.330404	0.16520
PFOS40mkd	5	5.62000	0.694262	0.31048

Means Comparisons				
Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSA40mkd	0.000000	0.130000	0.575000	0.866667
PFOS40mkd	-0.13	0.000000	0.445000	0.736667
N-EtFOSE40mkd	-0.575	-0.445	0.000000	0.291667
CONT	-0.86667	-0.73667	-0.29167	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

t				
2.13144				
Abs(Dif)-LSD	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSA40mkd	-0.83043	-0.65781	-0.25543	0.108594
PFOS40mkd	-0.65781	-0.74276	-0.34281	0.025532
N-EtFOSE40mkd	-0.25543	-0.34281	-0.83043	-0.46641
CONT	0.108594	0.025532	-0.46641	-0.67804

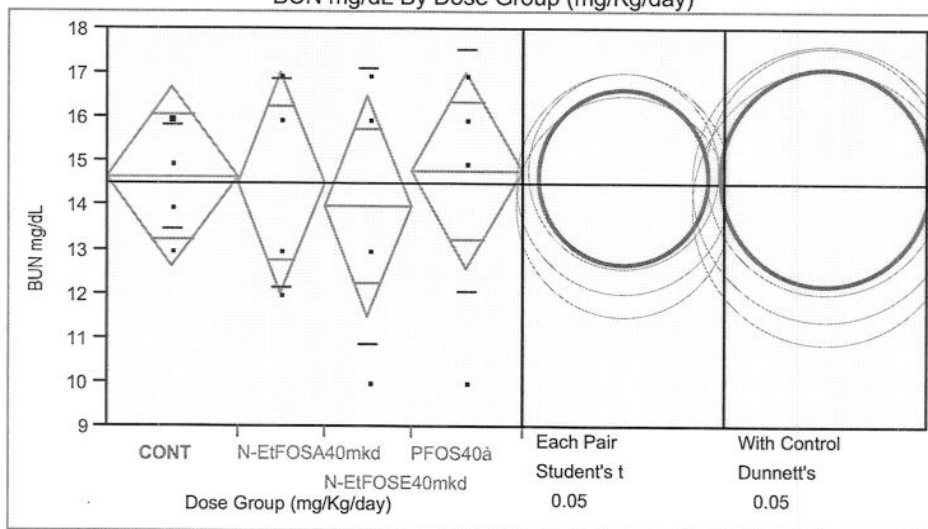
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-0.06836
PFOS40mkd	-0.14047
N-EtFOSE40mkd	-0.64336
CONT	-0.83632

Positive values show pairs of means that are significantly different.

BUN mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.018487
RSquare Adj	-0.17782
Root Mean Square Error	2.382342
Mean of Response	14.52632
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	1.603509	0.53450	0.0942
Error	15	85.133333	5.67556	Prob>F
C Total	18	86.736842	4.81871	0.9621

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	14.6667	0.9726
N-EtFOSA40mkd	4	14.5000	1.1912
N-EtFOSE40mkd	4	14.0000	1.1912
PFOS40mkd	5	14.8000	1.0654

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	14.6667	1.21106	0.4944
N-EtFOSA40mkd	4	14.5000	2.38048	1.1902
N-EtFOSE40mkd	4	14.0000	3.16228	1.5811
PFOS40mkd	5	14.8000	2.77489	1.2410

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd
PFOS40mkd	0.000000	0.133333	0.300000	0.800000
CONT	-0.133333	0.000000	0.166667	0.666667
N-EtFOSA40mkd	-0.3	-0.16667	0.000000	0.500000
N-EtFOSE40mkd	-0.8	-0.66667	-0.5	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.13144

Abs(Dif)-LSD	PFOS40mkd	CONT	N-EtFOSA40mkd	N-EtFOSE40mkd
PFOS40mkd	-3.21150	-2.94144	-3.10631	-2.60631
CONT	-2.94144	-2.93168	-3.11105	-2.61105
N-EtFOSA40mkd	-3.10631	-3.11105	-3.59056	-3.09056
N-EtFOSE40mkd	-2.60631	-2.61105	-3.09056	-3.59056

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

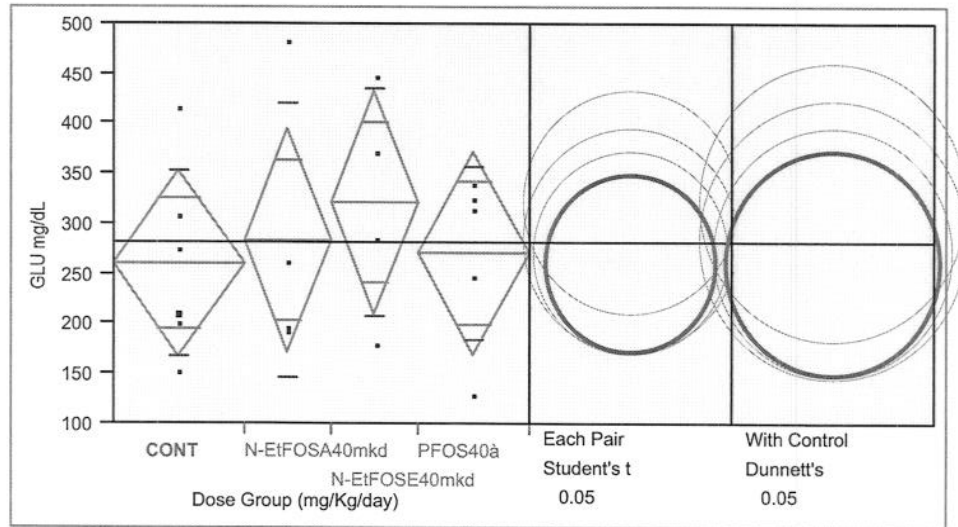
|d|

2.62898

Abs(Dif)-LSD	CONT
PFOS40mkd	-3.65919
CONT	-3.61602
N-EtFOSA40mkd	-3.87617
N-EtFOSE40mkd	-3.37617

Positive values show pairs of means that are significantly different.

GLU mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.053125
RSquare Adj	-0.13625
Root Mean Square Error	106.4679
Mean of Response	282.0526
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	9539.66	3179.9	0.2805
Error	15	170031.28	11335.4	Prob>F
C Total	18	179570.95	9976.2	0.8386

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	261.333	43.465
N-EtFOSA40mkd	4	285.000	53.234
N-EtFOSE40mkd	4	322.250	53.234
PFOS40mkd	5	272.400	47.614

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	261.333	93.863	38.319
N-EtFOSA40mkd	4	285.000	137.033	68.516
N-EtFOSE40mkd	4	322.250	114.782	57.391
PFOS40mkd	5	272.400	86.777	38.808

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd	CONT
N-EtFOSE40mkd	0.0000	37.2500	49.8500	60.9167
N-EtFOSA40mkd	-37.2500	0.0000	12.6000	23.6667
PFOS40mkd	-49.8500	-12.6000	0.0000	11.0667
CONT	-60.9167	-23.6667	-11.0667	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.13144

Abs(Dif)-LSD	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd	CONT
N-EtFOSE40mkd	-160.464	-123.214	-102.379	-85.566

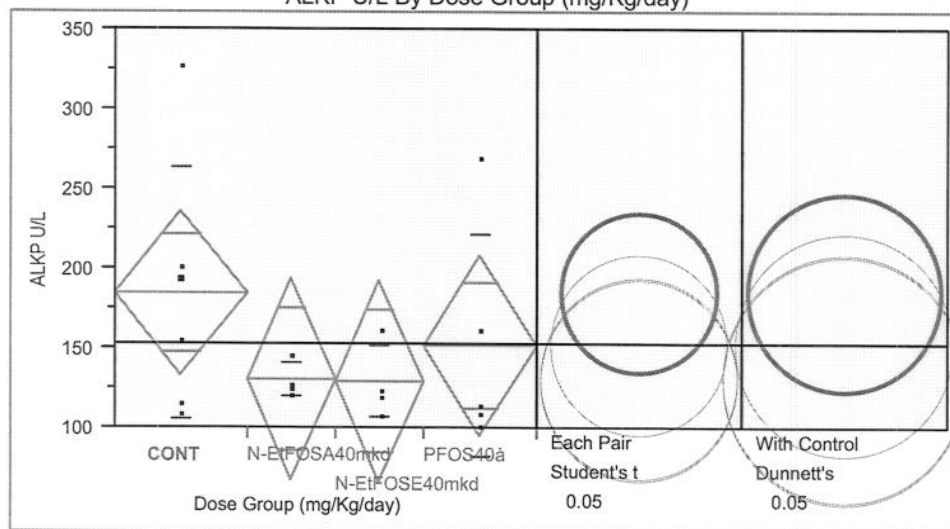
N-EtFOSA40mkd	-123.214	-160.464	-139.629	-122.816
PFOS40mkd	-102.379	-139.629	-143.523	-126.346
CONT	-85.566	-122.816	-126.346	-131.018

Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
N-EtFOSE40mkd	-119.760
N-EtFOSA40mkd	-157.010
PFOS40mkd	-158.423
CONT	-161.602

Positive values show pairs of means that are significantly different.

ALKP U/L By Dose Group (mg/Kg/day)



Oneway Anova Summary of Fit

RSquare	0.159836
RSquare Adj	-0.0082
Root Mean Square Error	59.956
Mean of Response	153.0526
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	10258.114	3419.37	0.9512
Error	15	53920.833	3594.72	Prob>F
C Total	18	64178.947	3565.50	0.4409

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	184.667	24.477
N-EtFOSA40mkd	4	130.750	29.978
N-EtFOSE40mkd	4	129.250	29.978
PFOS40mkd	5	152.000	26.813

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	184.667	80.2438	32.759

N-EtFOSA40mkd	4	130.750	11.1168	5.558
N-EtFOSE40mkd	4	129.250	23.6414	11.821
PFOS40mkd	5	152.000	70.1391	31.367

Dif=Mean[i]-Mean[j]	Means Comparisons			
	CONT	PFOS40mkd	N-EtFOSA40mkd	N-EtFOSE40mkd
CONT	0.0000	32.6667	53.9167	55.4167
PFOS40mkd	-32.6667	0.0000	21.2500	22.7500
N-EtFOSA40mkd	-53.9167	-21.2500	0.0000	1.5000
N-EtFOSE40mkd	-55.4167	-22.7500	-1.5000	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

t				
2.13144				
Abs(Dif)-LSD	CONT	PFOS40mkd	N-EtFOSA40mkd	N-EtFOSE40mkd
CONT	-73.7812	-44.7157	-28.5732	-27.0732
PFOS40mkd	-44.7157	-80.8232	-64.4760	-62.9760
N-EtFOSA40mkd	-28.5732	-64.4760	-90.3631	-88.8631
N-EtFOSE40mkd	-27.0732	-62.9760	-88.8631	-90.3631

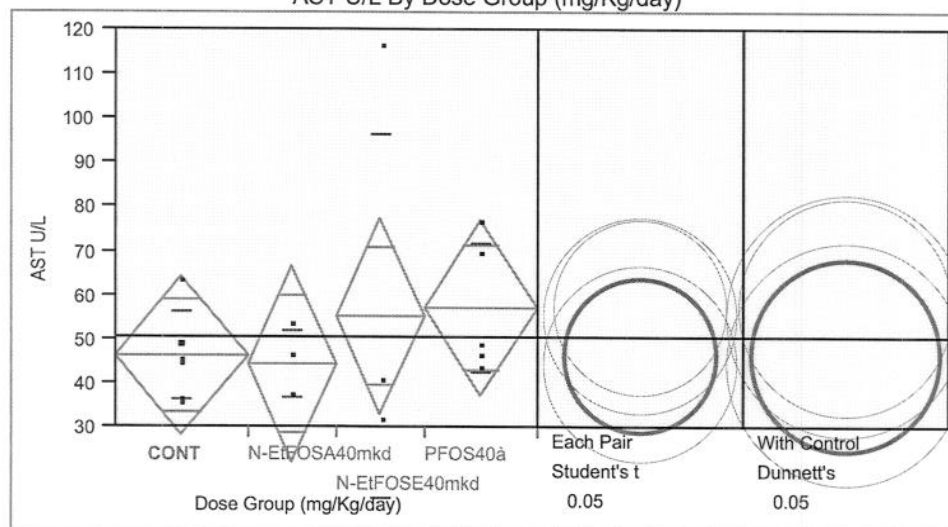
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

Abs(Dif)-LSD		d	
		CONT	
CONT	-91.0039		
PFOS40mkd	-62.7790		
N-EtFOSA40mkd	-47.8288		
N-EtFOSE40mkd	-46.3288		

Positive values show pairs of means that are significantly different.

AST U/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.083057
RSquare Adj	-0.10033
Root Mean Square Error	21.12468
Mean of Response	50.68421
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	606.3219	202.107	0.4529
Error	15	6693.7833	446.252	Prob>F
C Total	18	7300.1053	405.561	0.7191

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	46.1667	8.624
N-EtFOSA40mkd	4	44.2500	10.562
N-EtFOSE40mkd	4	55.5000	10.562
PFOS40mkd	5	57.4000	9.447

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	46.1667	10.1472	4.143
N-EtFOSA40mkd	4	44.2500	7.7621	3.881
N-EtFOSE40mkd	4	55.5000	41.2189	20.609
PFOS40mkd	5	57.4000	15.0100	6.713

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSE40mkd	CONT	N-EtFOSA40mkd
PFOS40mkd	0.0000	1.9000	11.2333	13.1500
N-EtFOSE40mkd	-1.9000	0.0000	9.3333	11.2500
CONT	-11.2333	-9.3333	0.0000	1.9167
N-EtFOSA40mkd	-13.1500	-11.2500	-1.9167	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

t

2.13144

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSE40mkd	CONT	N-EtFOSA40mkd
PFOS40mkd	-28.4770	-28.3044	-16.0313	-17.0544
N-EtFOSE40mkd	-28.3044	-31.8382	-19.7308	-20.5882
CONT	-16.0313	-19.7308	-25.9958	-27.1475
N-EtFOSA40mkd	-17.0544	-20.5882	-27.1475	-31.8382

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

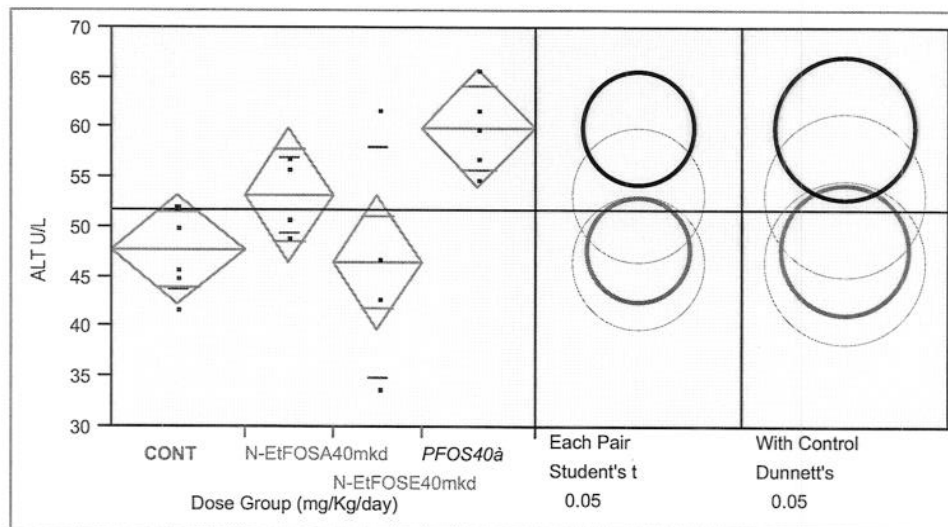
|d|

2.62898

Abs(Dif)-LSD	CONT
PFOS40mkd	-22.3956
N-EtFOSE40mkd	-26.5153
CONT	-32.0640
N-EtFOSA40mkd	-33.9319

Positive values show pairs of means that are significantly different.

ALT U/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.47363
RSquare Adj	0.368357
Root Mean Square Error	6.390531
Mean of Response	51.89474
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Model	3	551.2061	183.735	4.4990	
Error	15	612.5833	40.839		
C Total	18	1163.7895	64.655		0.0192

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	47.8333	2.6089
N-EtFOSA40mkd	4	53.2500	3.1953
N-EtFOSE40mkd	4	46.5000	3.1953
PFOS40mkd	5	60.0000	2.8579

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	47.8333	4.1191	1.6816
N-EtFOSA40mkd	4	53.2500	3.8622	1.9311
N-EtFOSE40mkd	4	46.5000	11.6762	5.8381
PFOS40mkd	5	60.0000	4.3012	1.9235

Means Comparisons

Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd
PFOS40mkd	0.0000	6.7500	12.1667	13.5000
N-EtFOSA40mkd	-6.7500	0.0000	5.4167	6.7500
CONT	-12.1667	-5.4167	0.0000	1.3333
N-EtFOSE40mkd	-13.5000	-6.7500	-1.3333	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

Abs(Dif)-LSD	PFOS40mkd	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd
PFOS40mkd	-8.61470	-2.38727	3.91872	4.36273

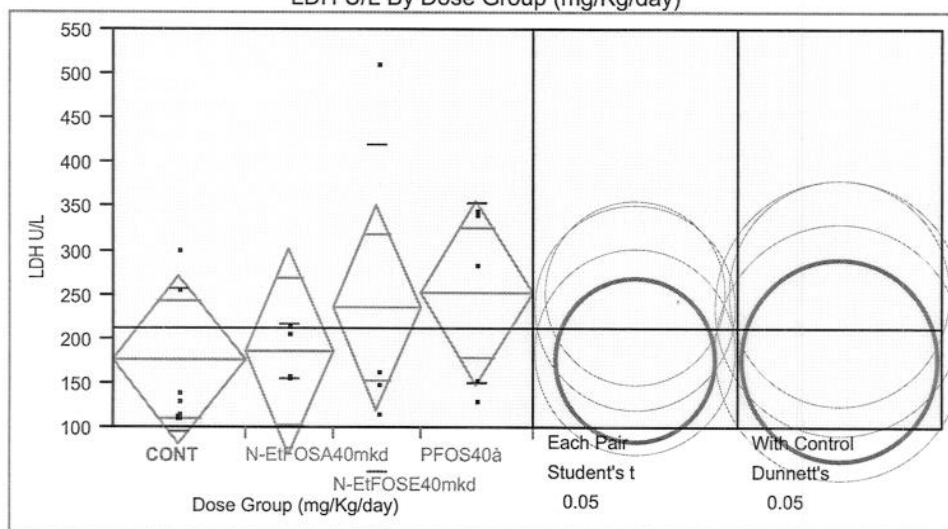
N-EtFOSA40mkd	-2.38727	-9.63153	-3.37568	-2.88153
CONT	3.91872	-3.37568	-7.86411	-7.45901
N-EtFOSE40mkd	4.36273	-2.88153	-7.45901	-9.63153

Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
PFOS40mkd	1.99340
N-EtFOSA40mkd	-5.42807
CONT	-9.69983
N-EtFOSE40mkd	-9.51141

Positive values show pairs of means that are significantly different.

LDH U/L By Dose Group (mg/Kg/day)



Oneway Anova Summary of Fit

RSquare	0.10172
RSquare Adj	-0.07794
Root Mean Square Error	110.3027
Mean of Response	211.6316
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	20666.12	6888.7	0.5662
Error	15	182500.30	12166.7	Prob>F
C Total	18	203166.42	11287.0	0.6457

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	177.500	45.031
N-EtFOSA40mkd	4	186.000	55.151
N-EtFOSE40mkd	4	237.000	55.151
PFOS40mkd	5	252.800	49.329

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	177.500	81.343	33.208

N-EtFOSA40mkd	4	186.000	31.454	15.727
N-EtFOSE40mkd	4	237.000	185.801	92.900
PFOS40mkd	5	252.800	103.541	46.305

Means Comparisons				
Dif=Mean[i]-Mean[j]	PFOS40mkd	N-EtFOSE40mkd	N-EtFOSA40mkd	CONT
PFOS40mkd	0.0000	15.8000	66.8000	75.3000
N-EtFOSE40mkd	-15.8000	0.0000	51.0000	59.5000
N-EtFOSA40mkd	-66.8000	-51.0000	0.0000	8.5000
CONT	-75.3000	-59.5000	-8.5000	0.0000

Alpha=

0.05

Comparisons for each pair using Student's t

t				
2.13144				
Abs(Dif)-LSD	PFOS40mkd	N-EtFOSE40mkd	N-EtFOSA40mkd	CONT
PFOS40mkd	-148.693	-141.912	-90.912	-67.062
N-EtFOSE40mkd	-141.912	-166.243	-115.243	-92.259
N-EtFOSA40mkd	-90.912	-115.243	-166.243	-143.259
CONT	-67.062	-92.259	-143.259	-135.737

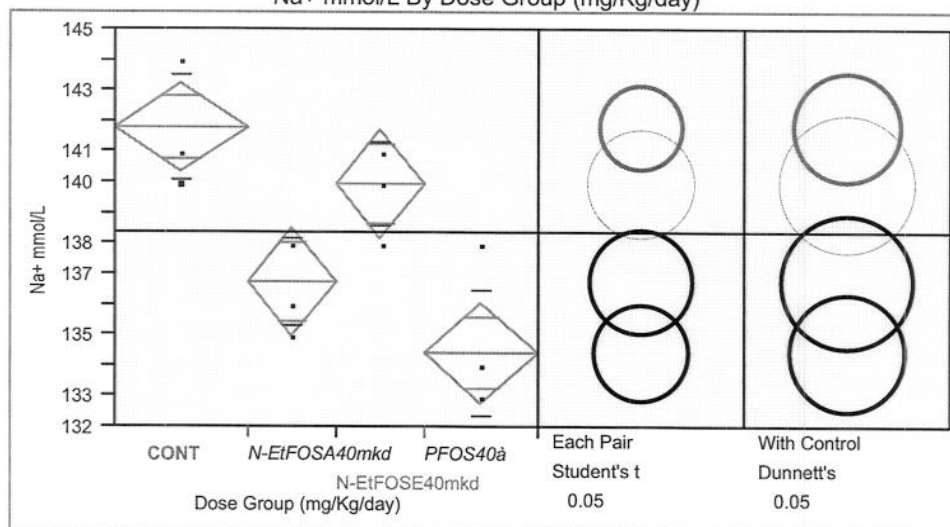
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
PFOS40mkd	-100.294
N-EtFOSE40mkd	-127.684
N-EtFOSA40mkd	-178.684
CONT	-167.422

Positive values show pairs of means that are significantly different.

Na⁺ mmol/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.793274
RSquare Adj	0.751929
Root Mean Square Error	1.727876
Mean of Response	138.4211
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	171.84825	57.2827	19.1866
Error	15	44.78333	2.9856	Prob>F
C Total	18	216.63158	12.0351	<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	141.833	0.70540
N-EtFOSA40mkd	4	136.750	0.86394
N-EtFOSE40mkd	4	140.000	0.86394
PFOS40mkd	5	134.400	0.77273

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	141.833	1.72240	0.70317
N-EtFOSA40mkd	4	136.750	1.50000	0.75000
N-EtFOSE40mkd	4	140.000	1.41421	0.70711
PFOS40mkd	5	134.400	2.07364	0.92736

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	0.00000	1.83333	5.08333	7.43333
N-EtFOSE40mkd	-1.83333	0.00000	3.25000	5.60000
N-EtFOSA40mkd	-5.08333	-3.25000	0.00000	2.35000
PFOS40mkd	-7.43333	-5.60000	-2.35000	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

	t			
	2.13144			
Abs(Dif)-LSD	CONT	N-EtFOSE40mkd	N-EtFOSA40mkd	PFOS40mkd
CONT	-2.12630	-0.54395	2.70605	5.20325
N-EtFOSE40mkd	-0.54395	-2.60418	0.64582	3.12946
N-EtFOSA40mkd	2.70605	0.64582	-2.60418	-0.12054
PFOS40mkd	5.20325	3.12946	-0.12054	-2.32925

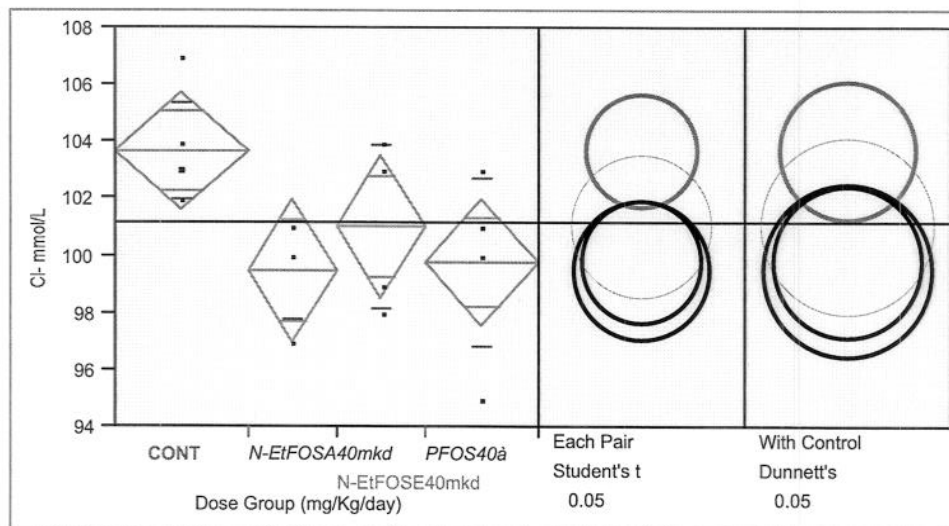
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

	d
	2.62898
Abs(Dif)-LSD	CONT
CONT	-2.62265
N-EtFOSE40mkd	-1.09887
N-EtFOSA40mkd	2.15113
PFOS40mkd	4.68268

Positive values show pairs of means that are significantly different.

CI- mmol/L By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.405319
RSquare Adj	0.286382
Root Mean Square Error	2.382342
Mean of Response	101.2105
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	58.02456	19.3415	3.4079
Error	15	85.13333	5.6756	Prob>F
C Total	18	143.15789	7.9532	0.0452

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	103.667	0.9726
N-EtFOSA40mkd	4	99.500	1.1912
N-EtFOSE40mkd	4	101.000	1.1912
PFOS40mkd	5	99.800	1.0654

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	103.667	1.75119	0.7149
N-EtFOSA40mkd	4	99.500	1.73205	0.8660
N-EtFOSE40mkd	4	101.000	2.94392	1.4720
PFOS40mkd	5	99.800	2.94958	1.3191

Means Comparisons

Dif=Mean[i]-Mean[j]	CONT	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSA40mkd
CONT	0.00000	2.66667	3.86667	4.16667
N-EtFOSE40mkd	-2.66667	0.00000	1.20000	1.50000
PFOS40mkd	-3.86667	-1.20000	0.00000	0.30000
N-EtFOSA40mkd	-4.16667	-1.50000	-0.30000	0.00000

Alpha=

0.05

Comparisons for each pair using Student's t

	CONT	N-EtFOSE40mkd	PFOS40mkd	N-EtFOSA40mkd
Abs(Dif)-LSD				
CONT	-2.93168	-0.61105	0.79189	0.88895

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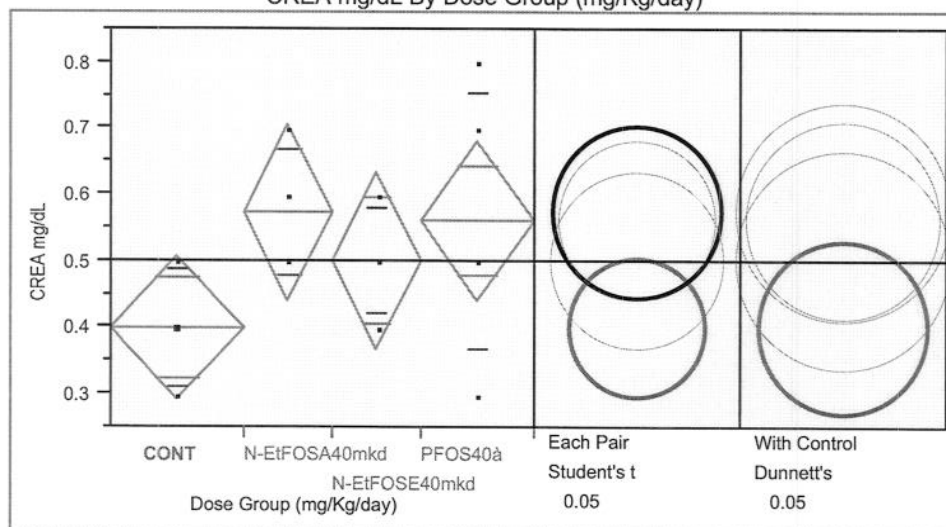
N-EtFOSE40mkd	-0.61105	-3.59056	-2.20631	-2.09056
PFOS40mkd	0.79189	-2.20631	-3.21150	-3.10631
N-EtFOSA40mkd	0.88895	-2.09056	-3.10631	-3.59056

Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
CONT	-3.61602
N-EtFOSE40mkd	-1.37617
PFOS40mkd	0.07415
N-EtFOSA40mkd	0.12383

Positive values show pairs of means that are significantly different.

CREA mg/dL By Dose Group (mg/Kg/day)



Oneway Anova Summary of Fit

RSquare	0.295588
RSquare Adj	0.154706
Root Mean Square Error	0.126359
Mean of Response	0.5
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.10050000	0.033500	2.0981
Error	15	0.23950000	0.015967	Prob>F
C Total	18	0.34000000	0.018889	0.1434

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	0.400000	0.05159
N-EtFOSA40mkd	4	0.575000	0.06318
N-EtFOSE40mkd	4	0.500000	0.06318
PFOS40mkd	5	0.560000	0.05651

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	0.400000	0.089443	0.03651

N-EtFOSA40mkd	4	0.575000	0.095743	0.04787
N-EtFOSE40mkd	4	0.500000	0.081650	0.04082
PFOS40mkd	5	0.560000	0.194936	0.08718

Means Comparisons				
Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSA40mkd	0.000000	0.015000	0.075000	0.175000
PFOS40mkd	-0.015	0.000000	0.060000	0.160000
N-EtFOSE40mkd	-0.075	-0.06	0.000000	0.100000
CONT	-0.175	-0.16	-0.1	0.000000

Alpha=

0.05

Comparisons for each pair using Student's t

t				
2.13144				
Abs(Dif)-LSD	N-EtFOSA40mkd	PFOS40mkd	N-EtFOSE40mkd	CONT
N-EtFOSA40mkd	-0.19044	-0.16567	-0.11544	0.001150
PFOS40mkd	-0.16567	-0.17034	-0.12067	-0.00309
N-EtFOSE40mkd	-0.11544	-0.12067	-0.19044	-0.07385
CONT	0.001150	-0.00309	-0.07385	-0.1555

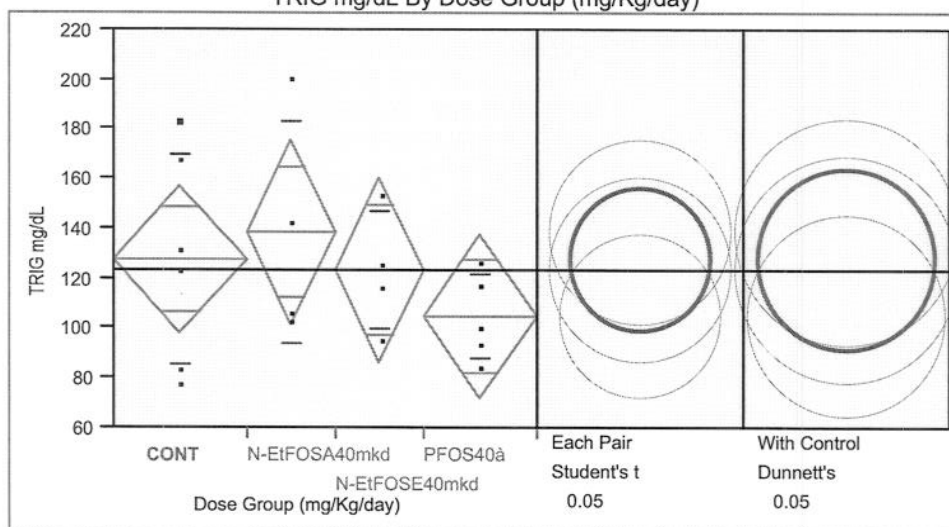
Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

d	
2.62898	
Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-0.03943
PFOS40mkd	-0.04115
N-EtFOSE40mkd	-0.11443
CONT	-0.19179

Positive values show pairs of means that are significantly different.

TRIG mg/dL By Dose Group (mg/Kg/day)



Oneway Anova
Summary of Fit

RSquare	0.130895
RSquare Adj	-0.04293
Root Mean Square Error	34.82966
Mean of Response	123.2105
Observations (or Sum Wgts)	19

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	2740.575	913.52	0.7530
Error	15	18196.583	1213.11	Prob>F
C Total	18	20937.158	1163.18	0.5375

Means for Oneway Anova

Level	Number	Mean	Std Error
CONT	6	128.167	14.219
N-EtFOSA40mkd	4	138.500	17.415
N-EtFOSE40mkd	4	123.250	17.415
PFOS40mkd	5	105.000	15.576

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
CONT	6	128.167	42.6447	17.410
N-EtFOSA40mkd	4	138.500	45.3836	22.692
N-EtFOSE40mkd	4	123.250	24.0468	12.023
PFOS40mkd	5	105.000	17.2482	7.714

Means Comparisons

Dif=Mean[i]-Mean[j]	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	PFOS40mkd
N-EtFOSA40mkd	0.0000	10.3333	15.2500	33.5000
CONT	-10.3333	0.0000	4.9167	23.1667
N-EtFOSE40mkd	-15.2500	-4.9167	0.0000	18.2500
PFOS40mkd	-33.5000	-23.1667	-18.2500	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

t
2.13144

Abs(Dif)-LSD	N-EtFOSA40mkd	CONT	N-EtFOSE40mkd	PFOS40mkd
N-EtFOSA40mkd	-52.4938	-37.5867	-37.2438	-16.3000
CONT	-37.5867	-42.8610	-43.0034	-21.7863
N-EtFOSE40mkd	-37.2438	-43.0034	-52.4938	-31.5500
PFOS40mkd	-16.3000	-21.7863	-31.5500	-46.9518

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|

2.62898

Abs(Dif)-LSD	CONT
N-EtFOSA40mkd	-48.7727
CONT	-52.8660
N-EtFOSE40mkd	-54.1893
PFOS40mkd	-32.2797

Positive values show pairs of means that are significantly different.

Above is for the male and female guinea pigs combined clinical chemistry statistics.

Appendix 8. Hepatic Palmitoyl Co-A oxidase activity

SPECIES	SEX	DOSE GRO	ID	PCOAO U
GP	F	CONT	8G01485	2.00
			8G01486	1.00
			9G00051	3.00
			9G00052	3.00
			N	4
			Mean	2.250
			SD	0.957
		N-EtFOSA40mkd	8G01488	3.00
			8G01490	1.00
			N	2
			Mean	2.000
			SD	1.414
		N-EtFOSE160mkd	9G00053	3.00
			9G00054	3.00
			N	2
			Mean	3.000
			SD	0.000
		N-EtFOSE40mkd	8G01487	2.00
			8G01489	2.00
			N	2
			Mean	2.000
			SD	0.000
		PFOS40mkd	8G01491	2.00
			8G01492	Missing
			9G00055	3.00
			9G00056	6.00
			N	3
			Mean	3.667
			SD	2.082
	M	CONT	8G01477	1.00
			8G01478	1.00
			9G00045	2.00
			9G00046	2.00
			N	4
			Mean	1.500
			SD	0.577
		N-EtFOSA40mkd	8G01481	1.00
			8G01482	1.00
			N	2
			Mean	1.000
			SD	0.000
		N-EtFOSE160mkd	9G00047	2.00
			9G00048	Missing
			N	1
			Mean	2.000
			SD	Missing
		N-EtFOSE40mkd	8G01479	1.00
			8G01480	1.00

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SPECIES	SEX	DOSE_GRO	ID	PCOAO_U_
			N	2
			Mean	1.000
			SD	0.000
		PFOS40mkd	8G01483	1.00
			8G01484	2.00
			9G00049	3.00
			9G00050	3.00
			N	4
			Mean	2.250
			SD	0.957
R	F	CONT	8R04040	Missing
			8R04041	4.00
			9R00469	6.00
			9R00470	7.00
			N	3
			Mean	5.667
			SD	1.528
		N-EtFOSA40mkd	8R04044	Missing
			8R04045	Missing
			N	0
			Mean	Missing
			SD	Missing
		N-EtFOSE160mkd	9R00471	5.00
			9R00472	8.00
			N	2
			Mean	6.500
			SD	2.121
		N-EtFOSE40mkd	8R04042	Missing
			8R04043	Missing
			N	0
			Mean	Missing
			SD	Missing
		PFOS40mkd	8R04046	Missing
			8R04047	Missing
			9R00473	10.00
			9R00474	18.00
			N	2
			Mean	14.000
			SD	5.657
	M	M556-160mkd	1R00748	Missing
			1R00749	Missing
			1R00750	Missing
			N	0
			Mean	Missing
			SD	Missing
SPECIES	SEX	DOSE_GRO	ID	PCOAO_U_
		CONT	1R00742	Missing
			1R00743	Missing
			1R00744	Missing
			8R04032	0.00
			8R04033	4.00
			9R00463	9.00

	9R00464	9.00
	N	4
	Mean	5.500
	SD	4.359
FOSA40mkg	1R00745	Missing
	1R00746	Missing
	1R00747	Missing
	N	0
	Mean	Missing
	SD	Missing
N-EtFOSA40mkd	8R04036	Missing
	8R04037	Missing
	N	0
	Mean	Missing
	SD	Missing
N-EtFOSE160mkd	9R00465	19.00
	9R00466	20.00
	N	2
	Mean	19.500
	SD	0.707
N-EtFOSE40mkd	8R04034	Missing
	8R04035	Missing
	N	0
	Mean	Missing
	SD	Missing
PFOS40mkd	8R04038	Missing
	8R04039	11.00
	9R00467	21.00
	9R00468	20.00
	N	3
	Mean	17.333
	SD	5.508

T-7071.1 FR
DT15

Appendix 9. Effect of acute FC administration on catalase and acylCoA oxidase expression

The following report summarizes the effects of *in vivo* administration of N-EtFOSE or PFOS on catalase and acylCoA oxidase gene expression and enzyme activity in liver tissue from exposed rats and guinea pigs, and was presented as a poster at the 2001 Society of Toxicology meeting (Wallace *et al.* 2001). Tissues analyzed in this study were derived from approximately 1 g of frozen liver from each of two male and two female rats and guinea pigs that received either 40 mg/kg/day PFOS or 160 mg/kg/day 2-(N-ethylperfluorooctanesulfonamido)-ethyl alcohol (N-EtFOSE) orally for 4 days, or an equivalent volume of 2% tween 80 (vehicle control) from March 1, 1999 to March 4, 1999. All animals were killed on March 5, one day after the last consecutive dose. Portions of the respective livers were quick frozen in liquid nitrogen and shipped on dry ice to Duluth for analysis.

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 (Table 1). 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 ($E^{240}=43.6 \text{ mM}^{-1}\text{cm}^{-1}$) 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 [³²P] 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.

RESULTS:

Rats – Both male and female rats express LCoAO and catalase activities, and there is no substantial difference in enzyme activities between the sexes. The same is true for the constitutive expression of mRNA for both enzymes, with the exception that unexposed male rats do not express the message for PCoAO.

Acute exposure to N-Et-FOSE causes a doubling of the specific activity of LCoAO in liver from both male and female rats, but catalase activity is unchanged. Associated with this is a proportionate 2-fold increase in the concentration of mRNA encoding for PCoAO, but no change in catalase mRNA, in livers from both sexes. Again, there is no remarkable sex difference in response to N-Et-FOSE exposure, at-least with this limited number of animals tested (n=2).

Exposure of rats to PFOS elicits a similar doubling of LCoAO activity for both sexes, and possibly a slight increase in catalase activity in liver from male rats. Hepatic catalase activity in female rats does not appear to be affected by PFOS exposure. Unlike N-Et-FOSE, which caused a doubling of mRNA band intensity for pCoAO, acute exposure to PFOS caused a 3-6 fold increase in PCoAO mRNA expression. This enhanced expression of message was more pronounced in female compared to male rats, and in both sexes resulted in no greater LCoAO enzyme activity than was observed at half the mRNA level for rats exposed to N-Et-FOSE.

Guinea Pigs - The response to guinea pigs to exposure to N-Et-FOSE and PFOS was dramatically different from that observed for rats. The constitutive activity of LCoAO in unexposed guinea pigs of either sex was very low (near LOD values) whereas catalase activity was 1.5-3 times higher. However, the activity of neither enzyme was stimulated following acute exposure to N-Et-FOSE or PFOS *in vivo*. Perhaps the most dramatic difference between species is the fact that guinea pig mRNA encoding for PCoAO was undetectable, even following exposure to N-Et-FOSE or PFOS. Part of the explanation for this observation may be that we were using hybridization probes developed against

the sequence for rat liver ACoAO because the sequence of the guinea pig enzyme is not known at this time. It may be that the guinea pig sequence is sufficiently different that the PCR product lacks complementarity for adequate hybridization to the probe for the rat sequence. Although this may indeed be an artifact, the absence of constitutively expressed message and the inability to stimulate expression of PCoAO mRNA are consistent with the low and uninducible enzyme activity in guinea pigs.

CONCLUSIONS: 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. We were not given the data for organ weights or necropsy findings and thus cannot comment on whether exposure to either agent caused hepatomegaly or other indications that are associated with peroxisome proliferation. However, based on our data, we suspect that the evidence will reveal the classical signs of peroxisome proliferation in rats, but not guinea pigs, caused by these acute exposures.

Summary of Screen for Hepatic Peroxisome Proliferation

Animal	Species	Sex	Treatment	Body wt (g)	Liver wt (g)	Liver Protein (mg/g)	P 450 content (nmol/mg)	LaurylCoA Oxidase Activity (nmol/min/mg)	Catalase activity (Units/mg protein)	ug RNA/u 1	pCoAO mRNA (% Control)	Catalase mRNA (% Control)
9R00463	Rat	M	Veh	298	12	151.8	0.086	4	219.9	2.9	nd	126.7
9R00464	Rat	M	Veh	313	13.1	145.6	0.067	5.3	158.5	2.65	nd	73.3
9R00469	Rat	F	Veh	210	7.6	188.3	0.154	9.2	141.1	2.19	100	91.5
9R00470	Rat	F	Veh	201	7.1	153.3	0.055	10.2	204.7	3.38	100	108.5
9R00465	Rat	M	N-Et-FOSE	272	13	157.5	0.331	12	219.5	3.5	290	140.1
9R00466	Rat	M	N-Et-FOSE	231	11.1	134.9	0.284	11.7	204.3	2.6	292	97.8
9R00471	Rat	F	N-Et-FOSE	174	7.5	165.8	0.04	17.8	186.1	1.93	253	117.8
9R00472	Rat	F	N-Et-FOSE	175	7.3	215.8	0.188	14.2	171.9	1.74	320	147.3
9R00467	Rat	M	PFOS	257	11.9	115.4	0.423	13.9	252	3.9	482	155.1
9R00468	Rat	M	PFOS	229	12.5	153	0.241	12	236.9	2.08	277	115.7
9R00473	Rat	F	PFOS	175	8.3	143.1	0.077	13.7	204.1	1.98	630	180.4
9R00474	Rat	F	PFOS	153	7.3	238.6	0.099	19.9	155.6	2	404	147.3
9G00045	Gpig	M	Veh	296	11.3	175.2	0.123	1.1	314.7	1.19	nd	103
9G00046	Gpig	M	Veh	298	11.9	252.7	0.089	1.3	390.7	1.63	nd	97
9G00051	Gpig	F	Veh	274	11.9	180.3	0.188	1.2	550.6	1.65	nd	91
9G00052	Gpig	F	Veh	289	14.2	222.1	0.17	0.9	488	2.3	nd	109
9G00047	Gpig	M	N-Et-FOSE	240	8.7	212.7	-	-	349.3	-	nd	-
9G00048	Gpig	M	N-Et-FOSE	226	8.5	269.1	0.013	0.7	379.3	1.71	nd	92
9G00053	Gpig	F	N-Et-FOSE	230	9.6	176.4	0.126	0.7	416.8	1.77	nd	105
9G00054	Gpig	F	N-Et-FOSE	236	8.9	218.3	0.195	1.5	478.1	1.28	nd	104
9G00049	Gpig	M	PFOS	215	7.2	153.2	-	-	413	-	nd	-
9G00050	Gpig	M	PFOS	240	10	222.1	0.074	0.9	460.8	1.27	nd	97
9G00055	Gpig	F	PFOS	221	8.4	127.8	0.031	0.9	672.9	1.65	nd	103
9G00056	Gpig	F	PFOS	237	7.4	171.6	0.016	1.4	531.6	1.52	nd	96

(-) insufficient sample for analysis
(nd) not detectable

Appendix 10: Final Report for FOSA (T-7132.1).

Title:

Comparative Molecular Biology of Perfluorooctanesulfonamide (FOSA, T-7132) in Rats following four consecutive days of dosing.

Final Report

February 18, 2004

Study Number: DT15

Protocol Amendment Number*: 2.

3M Medical Department Study Number: T-7132.1

Study Director: Andrew Seacat Ph.D.

Analytical laboratories:

1. Kendall B. Wallace, Ph.D. D.A.B.T.
Professor, Dept of Biochemistry and Molecular Biology
School of Medicine
10 University Drive
Duluth, MN 55812-2496
2. Dr. Xin Lu
Department of Pharmacology and Physiology
601 Elmwood Ave, box 711
Rochester, New York 14642

Study Protocol Title: Comparative Molecular Biology of Peroxisome Proliferation in Rats and Guinea Pigs

Study Initiated:

In-Life Start Date: November 16, 1998

In-Life End Date: December 21, 1998

Purpose:

The purpose of this study was to treat three male rats treated with either no compound (vehicle control) or 40 mg/kg/day perfluorooctanesulfonamide: C₈F₁₇SO₂NH₂ (FOSA, PFOSA, FOSAmide, T-7132). The study was designed to evaluate the metabolism and certain toxicological effects, such as hepatic peroxisome proliferation, of this compound and to compare these effects to other compounds dosed at the same concentration..

Methods

Test Materials:

Perfluorooctanesulfonamide (FOSA, PFOSA, FOSAmide, C₈F₁₇SO₂NH₂ NB # 120067-108, G. Moore 10/28/00, T-7132) was investigated. The vehicle control was propylene glycol.

Dose Groups:

Three male rats received propylene glycol as the vehicle control by oral gavage at a volume of 5 ml/kg body weight.

Three male rats received a dose of 40 mg FOSA/Kg body weight, via oral gavage on days one through four of the study, and were euthanized on day five. A suspension of 8 mg/ml of FOSA in propylene glycol was prepared, and a volume of 5 ml/kg was administered. This dosing regiment achieved a cumulative dose of 160 mg/kg after four successive days of dosing. This dose was the same as the dose of PFOS administered under amendment 1 of this protocol and is comparable to effective dose levels for hepatic peroxisome proliferation in rats of PFOS found in the literature. The LD₅₀ for FOSA is not known, but the cumulative dose of FOSA administered under this protocol is below the LD₅₀ for PFOS 251 mg/kg for PFOS in corn oil, as a point of reference.

Method of Specimen Collection:

The liver was removed as rapidly as possible after euthanasia. The livers were divided into ~ 1 g pieces and flash-frozen directly in liquid nitrogen in tared polypropylene (Nalgene) containers. The containers were moved to dry ice and weighed after all liquid nitrogen had evaporated. The tissue was stored at -70° C and shipped on dry ice.

Sera:

Up to 10 ml of blood was collected from each animal into glass serum tubes. Following clotting, the blood was centrifuged for 10 minutes at 1100 x g at 4° C. the sera was transferred to new tubes and be centrifuged again for 10 minutes at 1100 x g at 4° C.

Two aliquots of the serum sample (~ 0.75 ml) were saved for possible metabolite analysis.

Specimen Handling:

A 1-2 gram aliquot of the liver samples that were flash frozen in liquid nitrogen were shipped in dry ice to the analytical laboratories listed according to the livers sample identification chart below to:

Kendall B. Wallace, Ph.D. D.A.B.T.
Professor, Dept of Biochemistry and Molecular Biology
School of Medicine University of Minnesota
10 University Drive
Duluth, MN 55812-2496

Liver samples were analyzed 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.

Another 1-2 gram aliquot of the liver sample was sent to:

Dr. Michael Wempe
Laboratory of M. W. Anders
Department of Pharmacology and Physiology
601 Elmwood Ave, box 711
Rochester, New York 14642

Dr. Lin Xu performed the analysis of these samples and provided a brief summary of the analysis of liver samples from rats given a range of fluorocarbons. The parent and metabolites of the fluorocarbons were determined in liver samples by LC-MS/MS using previously published methods (Hansen *et al.* 2001).

Liver Sample Identification Chart for T-7132.1

Sample #	Animal #	Species	Sex	Dose Group
1	1R00742	Rat	M	control
2	1R00743	Rat	M	control
3	1R00744	Rat	M	control
4	1R00745	Rat	M	40 mg/kg/day FOSA
5	1R00746	Rat	M	40 mg/kg/day FOSA

T-7071.1 FR
DT15

6	1R00747	Rat	M	40 mg/kg/day FOSA
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Results and Discussion

Average body weights of the FOSA treatment group were significantly lower than the control group on days four and five (Table 1). Liver weights were not significantly different between the treated and control groups, but the liver weight as a percentage of body weight was significantly increased in the FOSA treatment group.

The results of P450 content (Figure 1) and Acyl CoA oxidase activity (Figure 2) for the liver samples indicated that FOSA induced the expression of these proteins and FOSA is therefore a hepatic peroxisome proliferator in rats.

The concentrations of the parent compound and metabolites in livers were measured (Table 2). The data show that PFOS was the major metabolite found in the livers of rats given FOSA and FOSA *N*-glucuronide was identified as a minor metabolite. Approximately 0.3 percent of the cumulative dose was present in the liver as either the parent compound, FOSA, or as the metabolite PFOS, one day after the last dose (Table 3). The low percentage of FOSA in the liver and the apparent low conversion of FOSA to PFOS has also been noted in in-vitro microsomal and liver slice metabolism studies (Xu *et al.* 2003) which observed that FOSA was converted to PFOS *in-vitro* by liver slices at a low rate, but not by microsomes or cytosol.

It is noteworthy that the control animals (1R00742, 1R00743, and 1R00744) contained significant concentrations of FOSA. Furthermore, no PFOS or FOSA *N*-glucuronide were found in the control samples with a high background of FOSA. A parallel analysis of livers from Fischer 344 rats maintained in the University of Rochester Vivarium did not show detectable concentrations of FOSA. These results suggest that the control liver samples for this study were contaminated at some point time ex-vivo by trace quantities of FOSA, as no metabolism to PFOS or FOSA *N*-glucuronide had occurred in the control samples.

Signatures:

Report prepared by,

Andrew M. Seacat, PhD, DABT
Study Director

Date

Reviewed by,

Daniel C. Hakes
Sponsor representative

Date

References:

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- Hansen, K. J., Clemen, L. A., Ellefson, M. E., and Johnson, H. O. (2001). Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environmental Science and Technology* **35**, 766-770.
- Lazarow, P. B. (1981). Assay of peroxisomal beta-oxidation of fatty acids. *Methods Enzymol* **72**, 315-9.
- Poosch, M. S., and Yamazaki, R. K. (1986). Determination of peroxisomal fatty acyl-CoA oxidase activity using a lauroyl-CoA-based fluorometric assay. *Biochim Biophys Acta* **884**, 585-93.
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- Xu, L., Seacat, A. M., Butenhoff, J. L., and Anders, M. W. (2003). Biotransformation of N-Ethyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide (N-EtFOSE) by rat liver microsomes, cytosol and slices. In *Toxicological Sciences, Suppl.*, Vol. 72, p. 314.

Tables

Table 1 Biological Parameters

DT15, Amendment #2										
Comparative Molecular Biology of Perfluorooctanesulfonamide (FOSA, T-7132) in Rats following four consecutive days of dosing.										
DT15 Body Weight (BW), Liver Weight (LW), Thyroid Weight (TW)										
			Date							
			2/19/01	2/19/01	2/21/01	2/22/01	2/23/01	2/23/01		
Sample #	Animal #	Dose Group	BW day 1 (g)	BW day 2 (g)	BW day 3 (g)	BW day 4 (g)	BW day 5 (g)	LW (g)	LW/BW (%)	TW (g)
1	1R0074	control	250	259	260	264	272	10.73	3.9%	10.62
2	1R0074	control	269	278	285	292	295	10.82	3.7%	10.87
3	1R0074	control	256	260	267	268	276	10.70	3.9%	10.65
Avg			258	266	271	275	281	10.75	3.8%	10.71
SD			10	11	13	15	12	0.06	0.14%	0.14
4	1R0074	40 mg/kg/day FOSA	250	254	250	239	227	10.77	4.7%	10.78
5	1R0074	40 mg/kg/day FOSA	249	245	243	233	225	10.81	4.8%	10.66
6	1R0074	40 mg/kg/day FOSA	259	265	263	255	254	10.64	4.2%	10.84
Avg			253	255	252	242	235	10.74	4.6%	10.76
SD			6	10	10	11	16	0.09	0.34%	0.09
P-value T-Test*			0.21	0.13	0.06	0.02	0.01	0.44	0.01	0.32

*T-Test (unpaired, one tailed, equal variance). A P-value of ≤ 0.05 was considered significantly different from control.

Table 2 Liver Fluorocarbon Concentration

Hepatic Concentrations of Fluorocarbons and Fluorocarbon Metabolites in Livers of Rats Given FOSA (40

Sample #	Animal #	Sex	Treatment	Weight of liver sample (g)	PFOS (ppm)	FOSA (ppm)	FOSAA (ppm)	FOSA <i>N</i> - glucuronide (ppm)
1	1R00742	M	Control	0.4600	0.0	154.1	0.0	0.00
2	1R00743	M	Control	0.9236	0.0	113.2	0.0	0.00
3	1R00744	M	Control	1.1287	0.0	273.2	0.0	0.00
4	1R00745	M	FOSA	0.2402	163.2	195.7	n.m.	0.44
5	1R00746	M	FOSA	0.7905	214.3	174.1	n.m.	0.39
6	1R00747	M	FOSA	0.8305	202.8	163.9	n.m.	0.34

mg/kg/day)

Note: n.m. = not measured.

Table 3 Percent Fluorocarbon Dose in Liver

Hepatic Percent of Dosed Fluorocarbons and Fluorocarbon Metabolites in Livers of Rats Given FOSA (40 mg/kg/day) for Four Days

Percent Dose in Liver										
Sample #	Animal #	Dose Group	Total Dose (mg)	PFOS in dose**	Liver PFOS (ppm)	Liver PFOS (mg)	% dose as PFOS in liver (%)	Liver FOSA (ppm)	Liver FOSA (mg)	% dose as FOSA in liver (%)
1	1R00742	control	0.00		0			154.1		
2	1R00743	control	0.00		0			113.2		
3	1R00744	control	0.00		0			273.2		
Avg								180.17		
SD								83.12		
4	1R00745	FOSA	644.8	644.8	163.2	1.76	0.27	195.7	2.1	0.33
5	1R00746	FOSA	660.2	660.2	214.3	2.32	0.35	174.1	1.9	0.29
6	1R00747	FOSA	614.3	614.3	202.8	2.16	0.35	163.9	1.7	0.28
Avg					193.43	2.08	0.32	177.90	1.91	0.30
SD					26.81	0.29	0.05	16.24	0.18	0.02

Figures

Figure 1 Cytochrome P450 content in Liver

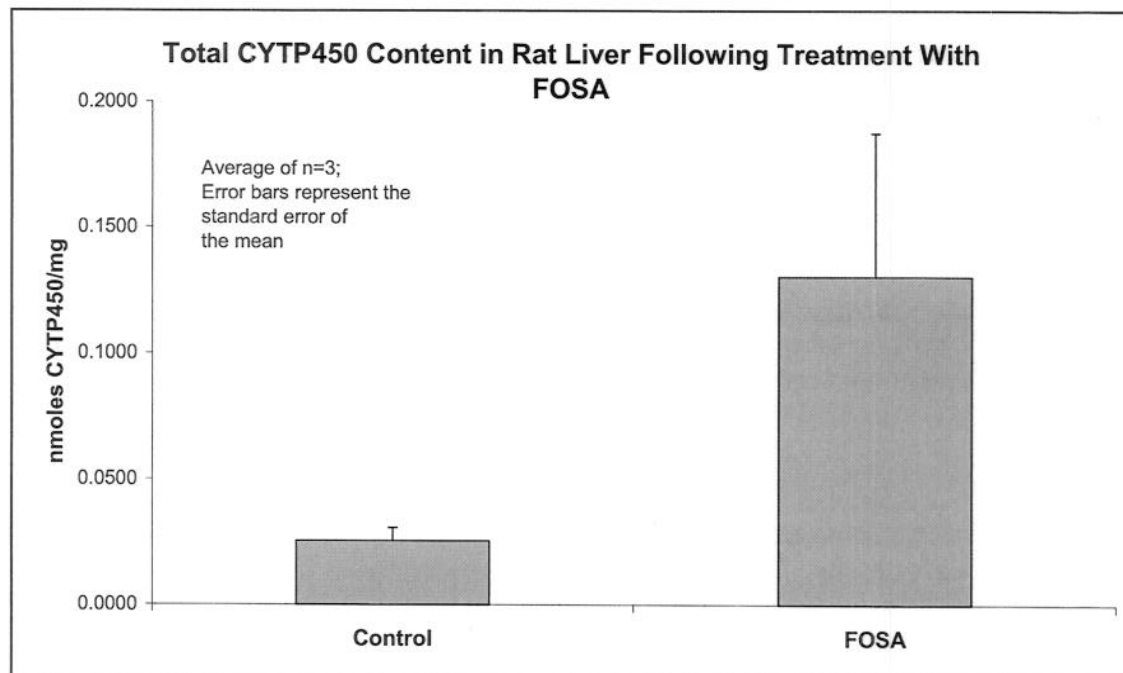
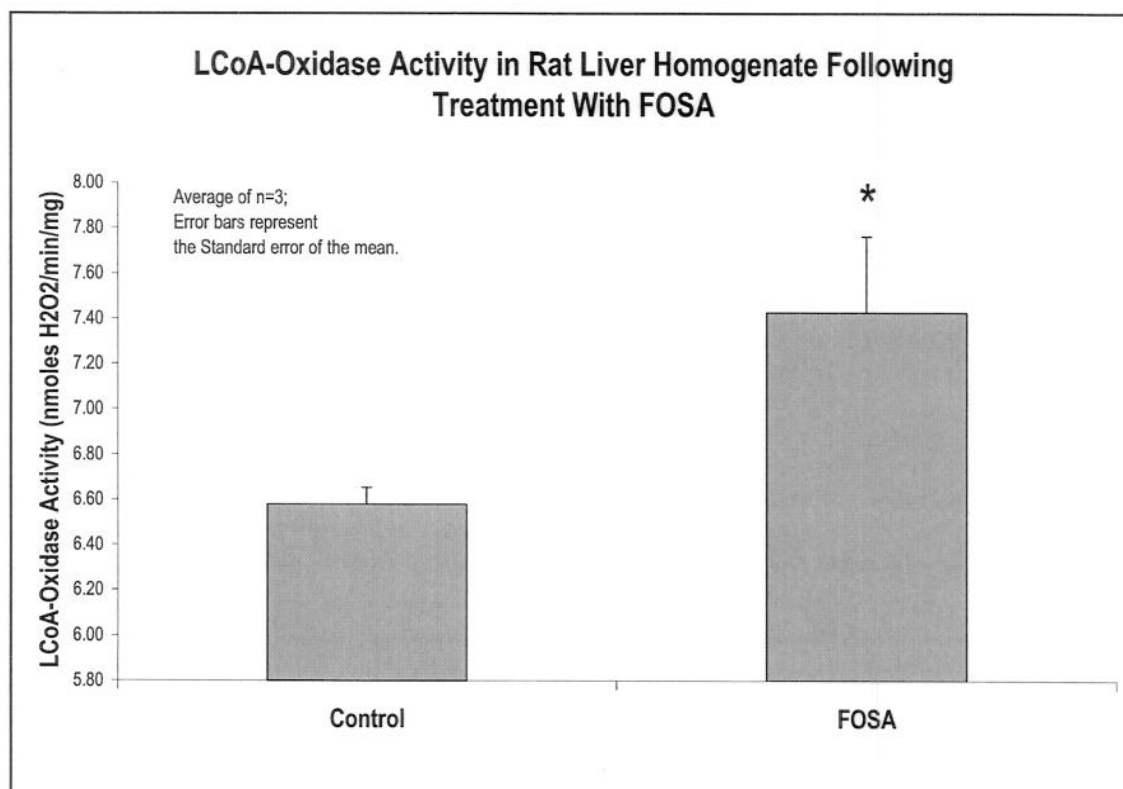


Figure 2: Lauroyl CoA oxidase activity in liver

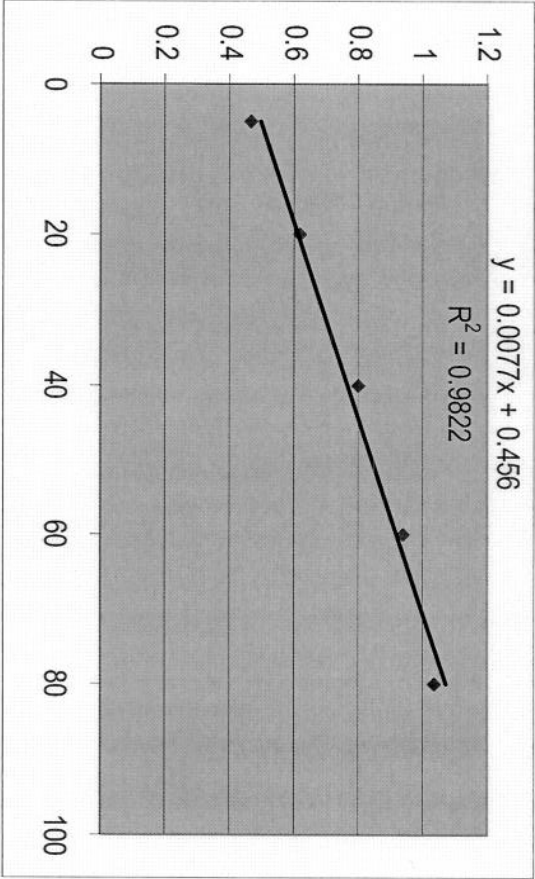


Appendix 1 Cytochrome P450 content

		Absorbance Values										
		450	500	protein conc. (ug/ul)	mg protein added	450 - 500	uM CYP 450	volume (ml)	total nmol	nmoles CYP 450/mg		
Sample #	Treatment											
1	Control	-0.0366	-0.0312	8.20	5	0.0054	0.059	3	0.178	0.0356		
2	Control	0.0254	0.0220	8.79	5	0.00333	0.037	3	0.110	0.0220		
3	Control	-0.0144	-0.0172	9.25	5	0.0028	0.031	3	0.092	0.0185		
4	FOSA	-0.0404	-0.0456	9.79	5	0.0052	0.057	3	0.171	0.0343		
5	FOSA	0.1360	0.1008	10.48	5	0.03518	0.387	3	1.160	0.2320		
6	FOSA	0.0059	-0.013	9.86	5	0.01894	0.208	3	0.624	0.1249		
					Statistical CS							
				Control	FOSA							
			n=1	0.0356	0.0343							
			n=2	0.0220	0.2320							
			n=3	0.0185	0.1249							
			Average	0.0253	0.1304							
			Stdev	0.0091	0.0989							
			Sterror	0.0052	0.0571							
			Varriance	0.0001	0.0098							
			ttest	1	0.02							
	Ttest (unpaired, one tailed, unequal variance)		p value	0.50	0.10							

Protein Determination

Standard Curve					
ul BSA	ul Lysis Buffer	Absorbance			
5	95	0.4645			
20	80	0.6167			
40	60	0.7992			
60	40	0.9413			
80	20	1.0379			



sample	Treatment	ul	Absorbance	ug protein	mg/ml	average	slope	intercep t				
1A	Control	5	0.7799	42.064935	8.412987	8.2	0.0077	0.456				
1B	Control	5	0.7635	39.935065	7.987013		0.0077	0.456				
2A	Control	5	0.8004	44.727273	8.945455	8.790909	0.0077	0.456				
2B	Control	5	0.7885	43.181818	8.636364		0.0077	0.456				
3A	Control	5	0.8155	46.688312	9.337662	9.248052	0.0077	0.456				
3B	Control	5	0.8086	45.792208	9.158442		0.0077	0.456				
4A	FOSA	5	0.844	50.38961	10.07792	9.788312	0.0077	0.456				
4B	FOSA	5	0.8217	47.493506	9.498701		0.0077	0.456	sample	mg/ml	ul	neede

[illegible]

[illegible]

[illegible]

	Protein Standard Curve									
ul BSA	ul Lysis Buffer	Absorbance								
5	95	0.4464								
20	80	0.6769								
40	60	0.854								
60	40	1.0135								
80	20	1.037								
sample	ul	Absorbance	ug protein	ug/ul	average	slope	intercept			
1	5	0.7814	34.435644	6.887129	7.054455	0.0101	0.4336			
1	5	0.7983	36.108911	7.221782		0.0101	0.4336	sample	ug/ul protein	ul needed to get 20 ug
2	5	0.7956	35.841584	7.168317	7.617822	0.0101	0.4336	1	7.05	2.84
2	5	0.841	40.336634	8.067327		0.0101	0.4336	2	7.62	2.63
3	5	0.7963	35.910891	7.182178	7.369307	0.0101	0.4336	3	7.37	2.71
3	5	0.8152	37.782178	7.556436		0.0101	0.4336	4	7.88	2.54
4	5	0.8225	38.50495	7.70099	7.882178	0.0101	0.4336	5	8.38	2.39
4	5	0.8408	40.316832	8.063366		0.0101	0.4336	6	8.61	2.32
5	5	0.8592	42.138614	8.427723	8.379208	0.0101	0.4336			
5	5	0.8543	41.653465	8.330693		0.0101	0.4336			
6	5	0.8501	41.237624	8.247525	8.610891	0.0101	0.4336			
6	5	0.8868	44.871287	8.974257		0.0101	0.4336			

Appendix 11: Final Report for FOSAA (T-7071.1)

Final Report

Title:

Comparative Molecular Biology of Peroxisome Proliferation in Rats
following four consecutive days of Perfluorooctanesulfonamidoacetate:
(FOSAA, T-7071)

Study Number: DT15

Protocol Amendment Number:* 2.

3M Medical Department Study Number: T-7071.1

Study Director: Andrew M. Seacat Ph.D., DABT

Analytical laboratories:

1. Kendall B. Wallace, Ph.D. D.A.B.T.
Professor, Dept of Biochemistry and Molecular Biology
School of Medicine
10 University Drive
Duluth, MN 55812-2496
2. Dr. Xin Lu
Department of Pharmacology and Physiology
601 Elmwood Ave, box 711
Rochester, New York 14642

Study Protocol Title: Comparative Molecular Biology of Peroxisome Proliferation in
Rats and Guinea Pigs

Study Initiated:

In-Life Start Date: November 16, 1998

In-Life End Date: December 21, 1998

Purpose

The purpose of this study was to treat three male rats treated with either no compound (vehicle control) or 160 mg/kg/day perfluorooctanesulfonamidoacetate: C8F17SO2NHCH2COO- (FOSAA, M556, T-7071). The study was designed to evaluate the metabolism and certain toxicological effects, such as hepatic peroxisome proliferation, of this compound and to compare these effects to other compounds dosed at the same concentration..

Test Materials

Perfluorooctanesulfonamidoacetate: (FOSAA, M556, C8F17SO2NHCH2COO- NB# 12837-84/30, from G. Moore 12/2000. 3M Medical department # T-7071) was investigated. The vehicle control was propylene glycol.

Dose Groups

Three male rats received propylene glycol as the vehicle control by oral gavage at a volume of 5 ml/kg body weight.

Three male rats received a dose of 160 mg FOSAA/Kg/day, via oral gavage on days one through four of the study, and were euthanized on day five. A suspension of 32 mg/ml M556 in propylene glycol was prepared, and a volume of 5 ml/kg was administered. This dosing regimen achieved a cumulative dose of 640 mg/kg M556 after four successive days of dosing. This dose was comparable to the cumulative dose of N-EtFOSE which induced peroxisomal palmitoyl Co-A oxidase (PCOAO) activity in the rat in a 4 week feeding study with 300 ppm N-EtFOSE.

Method of Specimen Collection

The liver was removed as rapidly as possible after euthanasia. The livers were divided into ~ 1 g pieces and flash-frozen directly in liquid nitrogen in tared polypropylene (Nalgene) containers. The containers were moved to dry ice and weighed after all liquid nitrogen had evaporated. The tissue was stored at -70° C and shipped on dry ice.

Sera:

Up to 10 ml of blood was collected from each animal into glass serum tubes. Following clotting, the blood was centrifuged for 10 minutes at 1100 x g at 4° C. the sera was

transferred to new tubes and be centrifuged again for 10 minutes at 1100 x g at 4° C. Two aliquots of the serum sample (~ 0.75 ml) were saved for possible metabolite analysis.

Specimen Handling

A 1-2 gram aliquot of the liver samples that were flash frozen in liquid nitrogen were shipped in dry ice to the analytical laboratories listed according to the livers sample identification chart below to:

Kendall B. Wallace, Ph.D. D.A.B.T.
Professor, Dept of Biochemistry and Molecular Biology
School of Medicine University of Minnesota
10 University Drive
Duluth, MN 55812-2496

Liver samples were analyzed 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.

Another 1-2 gram aliquot of the liver sample was sent to:

Dr. Michael Wempe
Laboratory of M. W. Anders
Department of Pharmacology and Physiology
601 Elmwood Ave, box 711
Rochester, New York 14642

Dr. Lin Xu performed the analysis of these samples and provided a brief summary of the analysis of liver samples from rats given a range of fluorocarbons. The parent and metabolites of the fluorocarbons were determined in liver samples by LC-MS/MS using previously published methods (Hansen *et al.* 2001).

Results and Discussion

Average body weights of the FOSAA treatment group were significantly lower than the control group on day five (Table 1). Liver weights were not significantly different between the treated and control groups, but the liver weight as a percentage of body weight was significantly increased in the FOSAA treatment group

The results of P450 content (Figure 1) and Acoyl CoA oxidase activity (Figure 2) for the liver samples indicated that FOSAA induced the expression of these genes and is therefore a good peroxisome proliferator in rats.

The concentrations of parent compound and metabolites in livers were measured (Table 1). The concentration of the parent compound FOSAA in the liver was approximately 555 ppm, which is equivalent to about 0.24 % of the cumulative dose of FOSAA (Table 3). The data show that PFOs and FOSA (apparent) were the major metabolites found in the livers of rats given FOSAA. The amount of PFOs and FOSA in the liver represented approximately 0.07 and 0.14 percent of the cumulative dose, respectively. If one subtracts the background levels of FOSA in the control livers from the FOSA levels found in the FOSAA treatment group, the concentrations are decreased by approximately 50%. Therefore a quantitative interpretation of the amount of metabolism of the parent compound to FOSA cannot be provided however the data qualitatively suggest that such metabolism occurred.

It is noteworthy that the control animals (1R00742, 1R00743, and 1R00744) contained significant concentrations of FOSA. No PFOs was found in the control samples with a high background of FOSA. These results suggest that the control liver samples were contaminated at some point time ex-vivo by trace quantities of FOSA, as no metabolism to PFOs had occurred in the control samples. A parallel analysis of livers from Fischer 344 rats maintained in the University of Rochester Vivarium did not show detectable concentrations of FOSA

Signatures:

Report prepared by,

Andrew M. Seacat, PhD, DABT
Study Director

Date

Reviewed by,

Daniel C. Hakes
Sponsor representative

Date

Tables

Table 1 Biological Parameters

DT15 Body Weight (BW), Liver Weight (LW)									
		Date							
Sample #	Animal #	Dose Group	BW day 1 (g)	BW day 2 (g)	BW day 3 (g)	BW day 4 (g)	BW day 5 (g)	LW (g)	LW/BW (%)
1	1R0074	control	250	259	260	264	272	10.73	3.9%
2	1R0074	control	269	278	285	292	295	10.82	3.7%
3	1R0074	control	256	260	267	268	276	10.70	3.9%
Avg			258	266	271	275	281	10.75	3.8%
SD			10	11	13	15	12	0.06	0.14%
7	1R0074	160 mg/kg/day	254	263	264	258	250	10.74	4.3%
8	1R0074	160 mg/kg/day	254	262	265	263	262	10.77	4.1%
9	1R0075	160 mg/kg/day	267	270	267	247	227	10.79	4.8%
Avg			258	265	265	256	246	10.77	4.4%
SD			8	4	2	8	18	0.03	0.33%
T-Test*			0.50	0.46	0.26	0.07	0.02	0.35	0.03

*T-T-Test (unpaired, one tailed, equal variance, $P \leq 0.05$ considered significantly different from control).

Table 2 Liver Fluorocarbon Concentration

Hepatic Concentrations of Fluorocarbons and Fluorocarbon Metabolites in Livers of Rats Given FOSAA
(160 mg/kg/day)

Sample #	Animal #	Sex	Treatment	Weight of liver sample (g)	PFOA (ppm)	FOSA (ppm)	FOSAA (ppm)
1	1R00742	M	Control	0.4600	0.0	154.1	0.0
2	1R00743	M	Control	0.9236	0.0	113.2	0.0
3	1R00744	M	Control	1.1287	0.0	273.2	0.0
7	1R00748	M	FOSAA	0.5520	238.4	447.3	614.4
8	1R00749	M	FOSAA	0.9373	81.9	235.7	424.0
9	1R00750	M	FOSAA	0.5320	100.7	267.4	627.1

Table 3 Percent Fluorocarbon Dose in Liver
Hepatic Percent of Dosed Fluorocarbons and Fluorocarbon Metabolites in Livers of Rats Given FOSAA
(160 mg/kg/day) for Four Days

Sample #	Animal #	Dose Group	Total Dose (mg)	PFOS in dose* (mg)	Liver PFOS (ppm)	Liver PFOS (mg)	% dose as PFOS in liver (%)	Liver FOSA (ppm)	Liver FOSA (mg)	% dose as FOSA in liver (%)	FOSAA (ppm)	Liver FOSAA (mg)	% dose as FOSAA in liver (%)
	1	1R0074 control	0.00		0			154.1			0		
	2	1R0074 control	0.00		0			113.2			0		
	3	1R0074 control	0.00		0			273.2			0		
	4												
Avg								180.2					
SD								83.1					
	7	1R0074 FOSAA	2465	2212	238.4	2.56	0.12	447.3	4.8	0.19	614.4	6.6	0.27
	8	1R0074 FOSAA	2453	2201	81.9	0.88	0.04	235.7	2.5	0.10	424.0	4.6	0.19
	9	1R0075 FOSAA	2439	2189	100.7	1.09	0.05	267.4	2.9	0.12	627.1	6.8	0.28
	0												
Avg			2452	2201	140.3	1.51	0.07	316.8	3.4	0.14	555.2	6.0	0.24
SD			13	12	85.4	0.92	0.04	114.1	1.2	0.05	113.8	1.2	0.05

** Mole fraction PFOS, e.g. for M556, Dose (mg) M556 * (499 MW of PFOS/ MW M556) = 2464.5 * (499/556) = 2212 mg PFOS in dose.

Figures

Figure 1 Cytochrome P450 content in Liver

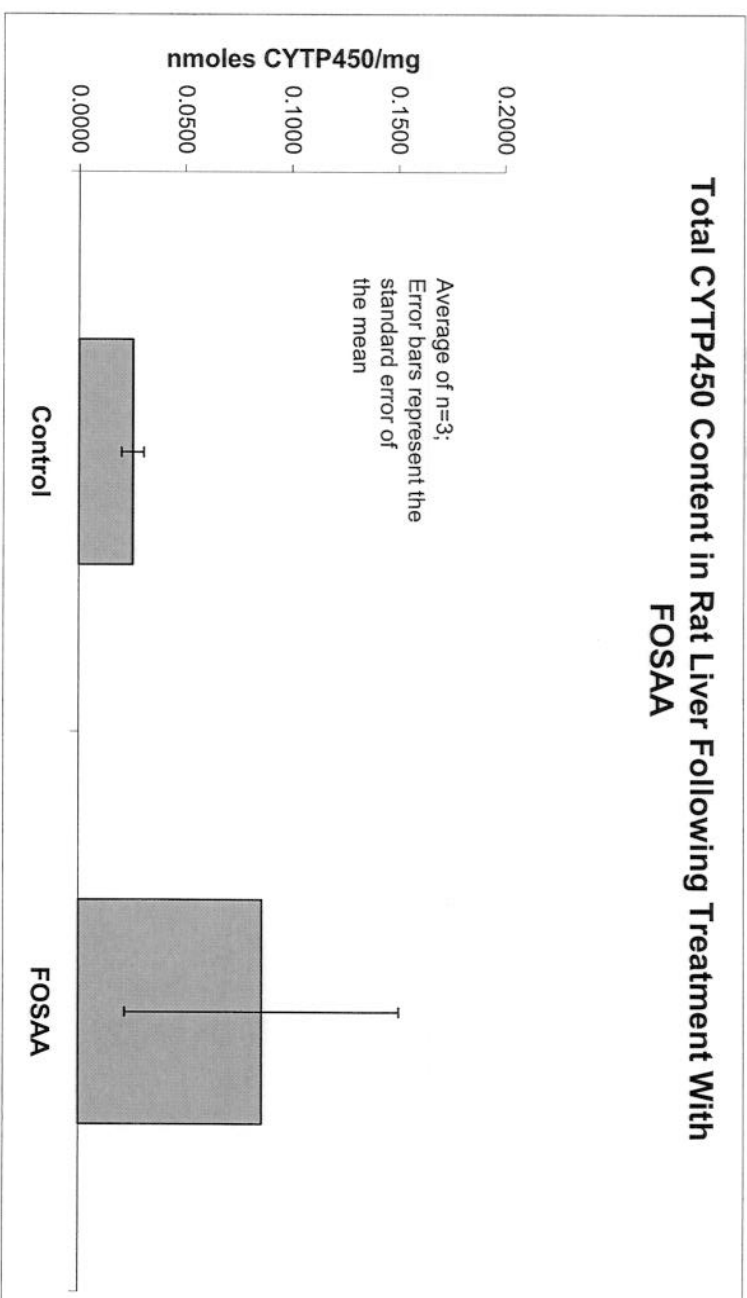
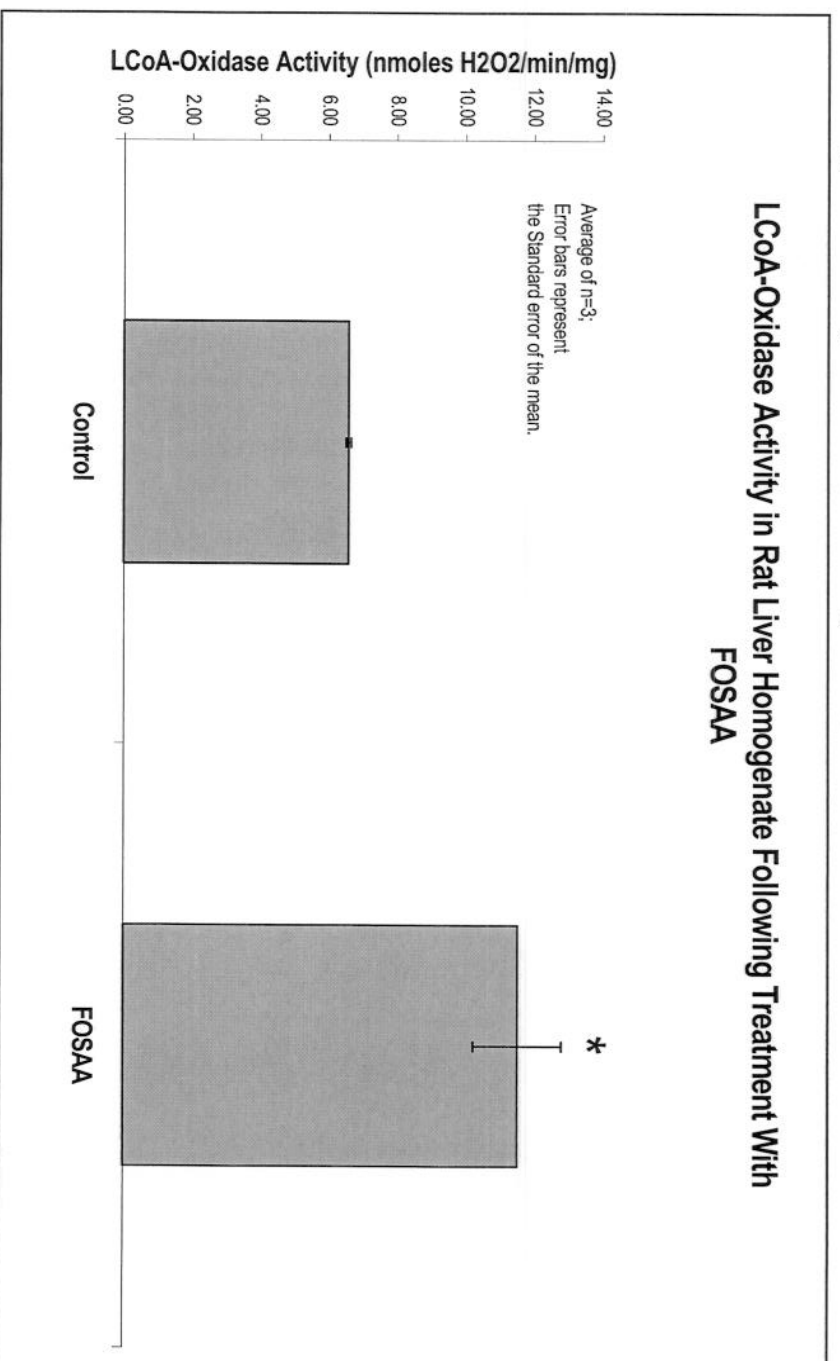


Figure 2: Lauroyl CoA oxidase activity in liver



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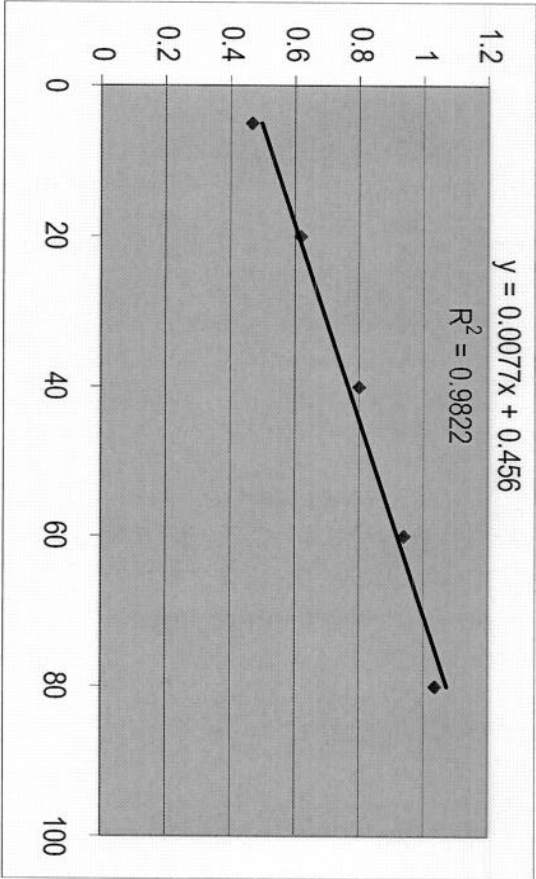
Appendix 1 Cytochrome P450 content

[illegible]

		Stdev	0.0091			0.1114			
		Sterror	0.0052			0.0643			
		Variance	0.0001			0.0124			
		f-test	1			0.01			
		Test (unpaired, one tailed, unequal variance)							
		p value	0.50			0.22			

Protein Determination

Standard Curve					
ul BSA	ul Lysis Buffer	Absorbance			
5	95	0.4645			
20	80	0.6167			
40	60	0.7992			
60	40	0.9413			
80	20	1.0379			



sample	ul	Absorbance	ug protein	mg/ml	average	slope	intercept	sample	mg/ml	ul needed to get 5 mg
1A	5	0.7799	42.064935	8.412987	8.2	0.0077	0.456	1	8.20	609.8
1B	5	0.7635	39.935065	7.987013		0.0077	0.456	2	8.79	568.8
2A	5	0.8004	44.727273	8.945455	8.790909	0.0077	0.456	3	9.25	540.7
2B	5	0.7885	43.181818	8.636364		0.0077	0.456	7	10.96	456.4
3A	5	0.8155	46.688312	9.337662	9.248052	0.0077	0.456	8	10.59	472.0
3B	5	0.8086	45.792208	9.158442		0.0077	0.456	9	9.58	521.8
7A	5	0.8816	55.272727	11.05455	10.95584	0.0077	0.456			
7B	5	0.874	54.285714	10.85714		0.0077	0.456			
8A	5	0.8679	53.493506	10.6987	10.59221	0.0077	0.456			
8B	5	0.8597	52.428571	10.48571		0.0077	0.456			
9A	5	0.8187	47.103896	9.420779	9.581818	0.0077	0.456			
9B	5	0.8311	48.714286	9.742857		0.0077	0.456			

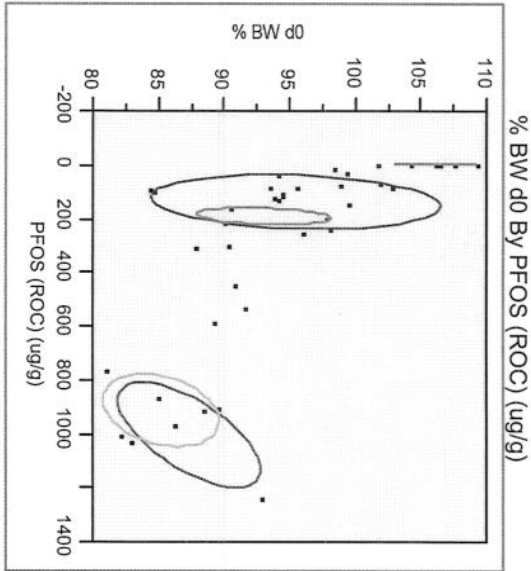
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Protein Standard Curve									
ul BSA	ul Lysis Buffer	Absorbance							
5	95	0.4464							
20	80	0.6769							
40	60	0.854							
60	40	1.0135							
80	20	1.037							
sample	ul	Absorbance	ug protein	ug/ul	average	slope	intercept		
1	5	0.7814	34.435644	6.887129	7.054455	0.0101	0.4336		
1	5	0.7983	36.108911	7.221782		0.0101	0.4336	sample	
								ug/ul protein	ul needed to get 20 ug
2	5	0.7956	35.841584	7.168317	7.617822	0.0101	0.4336	1	7.05
2	5	0.841	40.336634	8.067327		0.0101	0.4336	2	7.62
3	5	0.7963	35.910891	7.182178	7.369307	0.0101	0.4336	3	7.37
3	5	0.8152	37.782178	7.556436		0.0101	0.4336	7	8.75
7	5	0.8708	43.287129	8.657426	8.748515	0.0101	0.4336	8	7.88
7	5	0.88	44.19802	8.839604		0.0101	0.4336	9	8.54
8	5	0.8226	38.514851	7.70297	7.878218	0.0101	0.4336		
8	5	0.8403	40.267327	8.053465		0.0101	0.4336		
9	5	0.8562	41.841584	8.368317	8.542574	0.0101	0.4336		
9	5	0.8738	43.584158	8.716832		0.0101	0.4336		

A. Correlation of effects by different treatment groups to liver PFOS in rats.

Correlation of effects to liver PFOS (ug/g) in Rats



- Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=M556-160mkd
- Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=CONT
- Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=FOSA40mkg
- Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=N-EtFOSE160mkd
- Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=PFOS40mkd

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOs (ROC) (ug/g)	140.3333	85.44685	0.158821	0.8985	3
% BW d0	95.53118	9.405515			

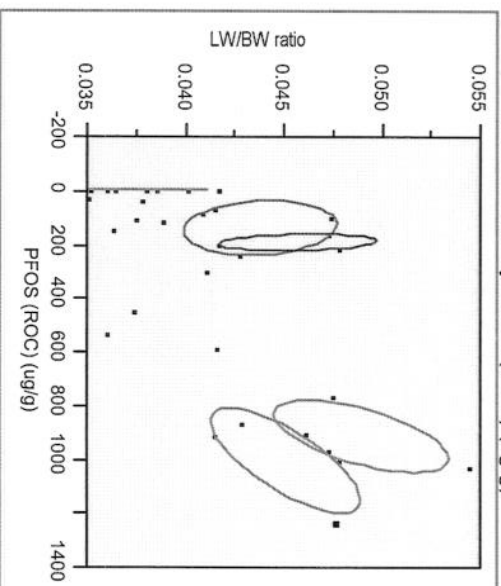
Bivariate Dose Group (mg/Kg/da=CONT					
Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOs (ROC) (ug/g)	0	0	0	1.0000	6
% BW d0	106.2098	2.660886			

Bivariate Dose Group (mg/Kg/da=FOSA40mkg					
Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOs (ROC) (ug/g)	193.4333	26.80678	0.253937	0.8365	3
% BW d0	93.07698	4.329203			

Bivariate Dose Group (mg/Kg/da=N-EtFOSE160mkg					
Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOs (ROC) (ug/g)	1008.075	166.3236	0.662735	0.3373	4
% BW d0	87.44432	4.602872			

Bivariate Dose Group (mg/Kg/da=PFOs40mkg					
Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOs (ROC) (ug/g)	918.15	113.933	0.3006	0.6994	4
% BW d0	85.28738	3.742806			

Correllation of BW to liver PFOs concentrations.



— Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=M556-160mkd)
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=CONT)
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=FOSA40mkg)
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=N-ElFPOSE160mkd)
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=PFOS40mkd)

Bivariate Dose Group (mg/Kg/da=M556-160mkd)			
Variable	Mean	Std Dev	Correlation
PFOS (ROC) (ug/g)	140.3333	85.44685	-0.12908
LW/BW ratio	0.043867	0.003308	
			Signif. Prob
			0.9176
			Number
			3

Bivariate Dose Group (mg/Kg/da=CONT)			
Variable	Mean	Std Dev	Correlation
PFOS (ROC) (ug/g)	0	0	0
LW/BW ratio	0.03818	0.002552	
			Signif. Prob
			1.0000
			Number
			6

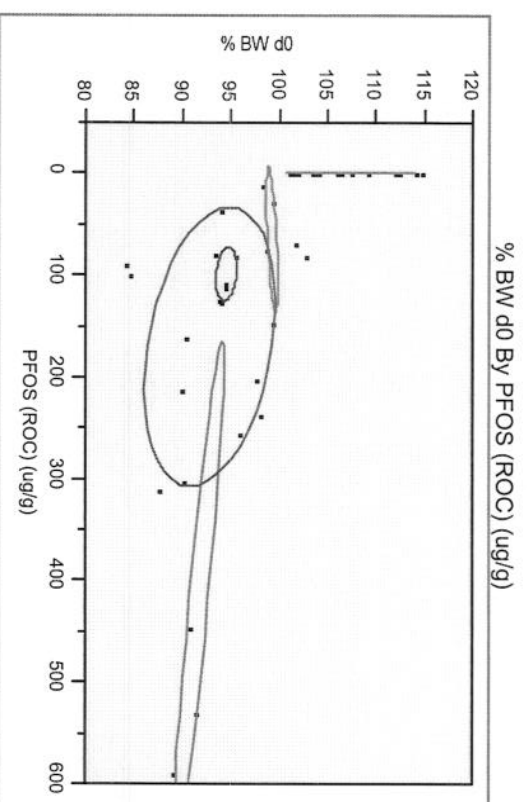
Bivariate Dose Group (mg/Kg/da=FOSA40mkg)			
Variable	Mean	Std Dev	Correlation
PFOS (ROC) (ug/g)	193.4333	26.80678	-0.21723
LW/BW ratio	0.045793	0.003394	
			Signif. Prob
			0.8606
			Number
			3

Bivariate Dose Group (mg/Kg/da=N-ElFPOSE160mkd)			
Variable	Mean	Std Dev	Correlation
PFOS (ROC) (ug/g)	1008.075	166.3236	0.751977
LW/BW ratio	0.045166	0.003235	
			Signif. Prob
			0.2480
			Number
			4

Bivariate Dose Group (mg/Kg/da=PFOS40mkd)			
Variable	Mean	Std Dev	Correlation
PFOS (ROC) (ug/g)	918.15	113.933	0.609934
LW/BW ratio	0.049007	0.003768	
			Signif. Prob
			0.3901
			Number
			4

Pigs, males and female combined.

Correlation of effects to liver PFOS (ug/g) in Guinea Pigs



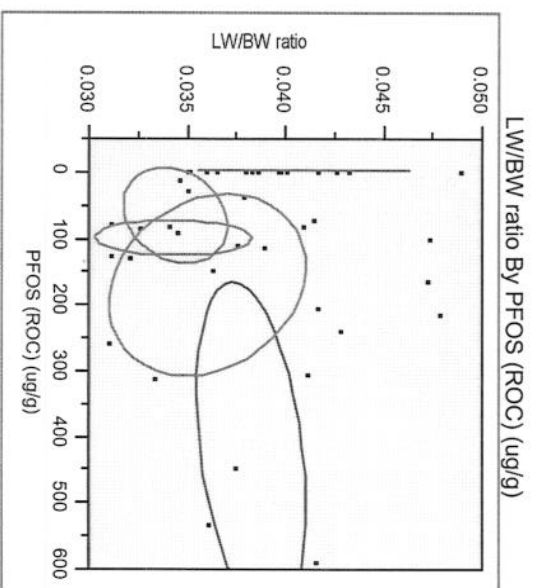
Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	0.014286	0.037796	0.592756	0.1607	7
% BW d0	107.5171	5.69689			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	100.4	22.36172	-0.20567	0.7943	4
% BW d0	94.66277	0.909529			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	419.35	214.6788	-0.92559	0.0744	4
% BW d0	91.83908	2.210568			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	66.55	60.59266	0.63895	0.3611	4
% BW d0	99.38514	0.513448			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	171.4857	116.5397	-0.30285	0.5091	7
% BW d0	92.95562	5.748654			



Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=CONT			
Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=N-ElFOSA40mkd			
Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=N-ElFOSE160mkd			
Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=N-ElFOSE400mkd			
Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=PFOS40mkd			

Bivariate Dose Group (mg/Kg/da=CONT				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	0.014286	0.037796	-0.27546	0.5499
LW/BW ratio	0.041022	0.004556		
Number				7

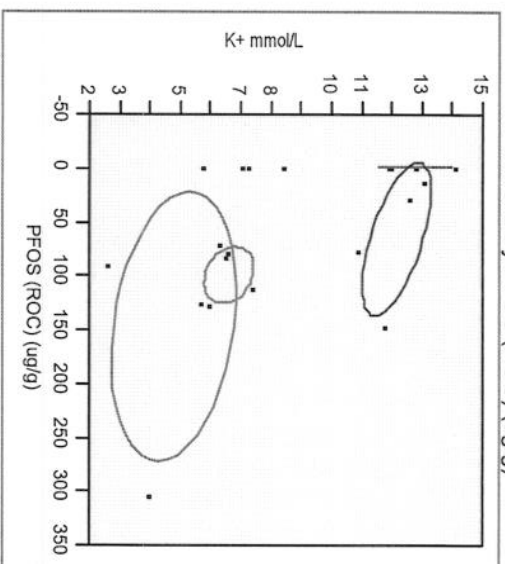
Bivariate Dose Group (mg/Kg/da=N-ElFOSA40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	100.4	22.36172	0.032225	0.9678
LW/BW ratio	0.034365	0.003385		
Number				4

Bivariate Dose Group (mg/Kg/da=N-ElFOSE160mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	419.35	214.6788	0.343072	0.6569
LW/BW ratio	0.038328	0.00237		
Number				4

Bivariate Dose Group (mg/Kg/da=N-ElFOSE400mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	66.55	60.59266	0.214886	0.7851
LW/BW ratio	0.03445	0.002243		
Number				4

Bivariate Dose Group (mg/Kg/da=PFOS40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	171.4857	116.5397	-0.21072	0.6502
LW/BW ratio	0.036088	0.004271		
Number				7

K+ mmol/L By PFOS (ROC) (ug/g)



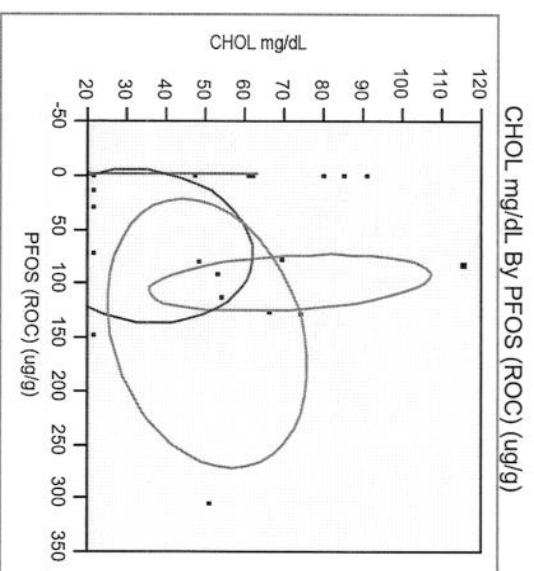
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 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=N-ElFOSE40mkd)
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da=PFOS40mkd)

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	0.025	0.05	0.918156	0.0818	4
K+ mmol/L	12.8	1.01653			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	100.4	22.36172	-0.24593	0.7541	4
K+ mmol/L	6.65	0.695222			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	66.55	60.59266	-0.62394	0.3761	4
K+ mmol/L	12.2	0.962635			

Variable	Mean	Std Dev	Correlation	Signif. Prob	Number
PFOS (ROC) (ug/g)	148.2	106.5116	-0.25046	0.7495	4
K+ mmol/L	4.825	1.746186			



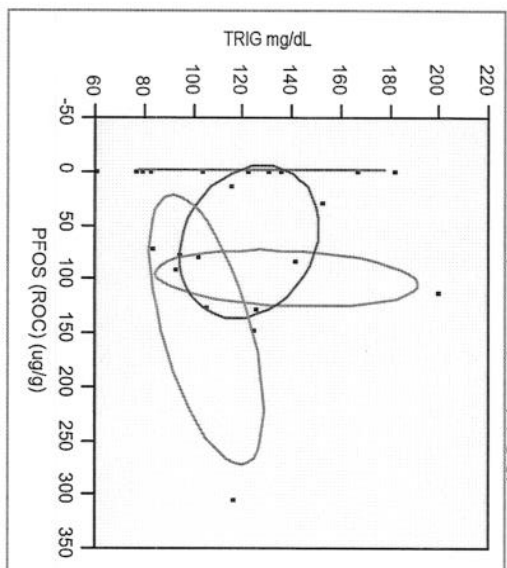
Bivariate Dose Group (mg/Kg)da=CONT				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	0.016667	0.040825	-0.43116	0.3933
CHOL mg/dL	40.08333	19.97853		

Bivariate Dose Group (mg/Kg)da=N-EtFOSA40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	100.4	22.36172	-0.30166	0.6983
CHOL mg/dL	71.75	30.43436		

Bivariate Dose Group (mg/Kg)da=N-EtFOSE40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	66.55	60.59266	0.111675	0.8883
CHOL mg/dL	34.375	23.75		

Bivariate Dose Group (mg/Kg)da=PFOS40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	148.2	106.5116	0.24662	0.7534
CHOL mg/dL	50.875	21.58848		

TRIG mg/dL By PFOS (ROC) (ug/g)



— Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da)=CONT
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da)=N-EtFOSA40mkd
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da)=N-EtFOSA40mkd
 — Bivariate Normal Ellipse P=0.500 Dose Group (mg/Kg/da)=PFOS40mkd

Bivariate Dose Group (mg/Kg/da)=CONT				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	0.016667	0.040825	-0.50738	0.3042
TRIG mg/dL	128.1667	42.64466		

Bivariate Dose Group (mg/Kg/da)=N-EtFOSA40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	100.4	22.36172	0.198189	0.8018
TRIG mg/dL	138.5	45.38355		

Bivariate Dose Group (mg/Kg/da)=N-EtFOSA40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	66.55	60.59266	-0.19932	0.8007
TRIG mg/dL	123.25	24.04683		

Bivariate Dose Group (mg/Kg/da)=PFOS40mkd				
Variable	Mean	Std Dev	Correlation	Signif. Prob
PFOS (ROC) (ug/g)	148.2	106.5116	0.595121	0.4049
TRIG mg/dL	106	19.74842		