AR226-1902

SRPT T-6295.8, T-6889.1, T-6316.4
DT15-A

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY

Title: Inter-Species Comparison of Mechanisms Fluorochemical Toxicity following Intraperitoneal dosing of perfluorooctanesulfonate (PFOS, T-6295) or ammonium perfluorooctanoate (APFO, T-6889) in Rats and Guinea Pigs and oral dosing of N-ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE, T-6316) in Rats.

Final Report
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Study Numbers. T-6295.8, T-6889.1, 6316.4
Strategic Toxicology Study Number: DT15-A

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

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

Study Director: Andrew M. Seacat Ph.D., DABT
Toxicology Specialist
3M Medical Dept. Corporate Toxicology and Regulatory Services
3M Center Building 220-2E-02

Study Toxicologist: Deanna Luebker M.S
Senior Toxicologist
3M Medical Dept. Corporate Toxicology and Regulatory Services

In-Life Start Dates	**Part A:**	**10/23/1997 and 11/17/1997**
In-Life End Dates	**Part A:**	**11/04/1997 and 12/1/1997**
In-Life Start Dates	**Part B:**	**02/10/1998 and 03/11/1998**
In-Life End Dates	**Part B:**	**03/10/1998 and 03/13/1997**
In-Life Start Date	**Part C:**	**09/16/1998**
In-Life End Date	**Part C:**	**09/18/1998**

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Introduction

The research presented in this report was designed to elucidate the mechanism(s) that initiate peroxisomal proliferation in rats, but not necessarily in guinea pigs or primates, by some 3M fluorochemicals. The research objectives were to determine if the fluorochemicals disrupt the function of liver fatty acid binding proteins (L-FABPs) in both rats and guinea pigs and if so, to what extent. The intermediate objectives were to investigate the hypothesis that FC compounds alter L-FABP levels and/or functionality and that it is the disruption of fatty acid processing leading to higher levels of unassociated long chain fatty acids in the cytoplasm, and that rats and guinea pigs will respond differently. Further objectives were to investigate and compare the molecular mechanisms of peroxisome proliferation in rats and guinea pigs. Three fluorochemicals; perfluorooctane sulfonate (PFOS, T-6295), *N*-ethyl perfluorooctanesulfonamido ethanol (*N*-EtFOSE, T-6316) and perfluorooctanoate (APFO, T-6889) were tested in this study that were either previously known (Sohlenius *et al.* 1994; Sohlenius *et al.* 1993) or suspected to cause peroxisome proliferation in the rat. The hypothesis tested was that these fluorochemicals would induce peroxisome proliferation in the rat, but not the guinea pig, and the data supported that hypothesis. The effect of these compounds on peroxisome proliferation in the rat, but not the guinea pig were presented in an abstract and poster presented at the Society of Toxicology meeting in 2001 (Wallace *et al.* 2001). Other investigators using these fluorochemicals have also subsequently shown peroxisome proliferation with some of these compounds in vivo in rats or or in-vitro (Maloney and Waxman 1999; Yang *et al.* 2002; Berthiaume and Wallace 2002).

L-FABPs isolated from certain rats and guinea pigs dosed in the current study were used to investigate the effect of those fluorochemicals on the binding of a fluorescently labeled probe to L-LFABP. The detailed methods, results and conclusions of the L-FABP analyses were published elsewhere (Nabbefeld 1998; Luebker *et al.* 2002), and are only briefly mentioned in the results and discussion section of this report.

Guinea pigs may serve as better surrogate models for human risk assessment than do other rodents because, like humans, they are resistant to peroxisome proliferation. However, the molecular and biochemical mechanisms that differentiate the response of these species to peroxisome proliferators in general and for the perfluorosulfonamides in particular is unclear. Therefore, the specific aims of this study were to:

1. Characterize the peroxisomal enzyme and fatty acid binding proteins response in the liver to these compounds
2. Perform toxicity tests such as serum clinical chemistry and to liver histology that may indicate an explanation for any the species differences in response to these compounds.
3. Correlate any observed alterations of the above functions to liver and serum levels of perfluorosulfonamides and their metabolites.

Methods

This study, (DT-15A) was conducted in three segments, parts A, B and C under a broadly written protocol dated 9/18/1997 that had been approved by the 3M institutional animal use

committee (3M IACUC Animal use application # 2088). Part A consisted of a study designed to compare the toxicity and peroxisomal proliferation in male rats and male guinea pigs dosed via intraperitoneal (i.p.) injection with PFOS or APFO. The results of Part A led to further testing under part B, which consisted of a dose-response and time-course for the observed effects of PFOS in both male and female rats and guinea pigs dosed via intraperitoneal (i.p.) injection. The results of parts A and B led to the conclusion that i.p. dosing was not the best route of administration, therefore an exploratory oral dosing procedure in rats was performed with N-EtFOSE in Part C

Test Materials

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 and the ones generally used in this report for each compound are in bold:

1. Vehicle control (2% Tween 80).

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-6889: Ammonium perfluorooctanoate (PFOA, **APFO**, FC-143, Formula: $C_7F_{15}COO^-NH_4^+$, MW = 431.1 g/mole).

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

5. 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.

The protocol stated that T-6868, N-ethyl perfluorooctane sulfonamide (FX-12, Sulfuramide ™), Formula $C_8F_{17}SO_2NHC_2H_5$) would be tested, however, T-6868 was not tested in DT15-A.

Procedures

<u>Part A: Procedures for a single intraperitoneal dosing of PFOS (T-6295) and APFO (T-6889) in male rats and guinea pigs.</u>

For part A, a total of 15 male rats and 13 male guinea pigs, 6-8 weeks in age and weighing between 150 and 250 grams were purchased from Charles River. Three to four male rats or guinea pigs per treatment group were used. Following an adaptation period of not less than one week after arrival at 3M, animals were administered treatments by intraperitoneal (i.p.) injection,

at equimolar doses that were less than 40% of the rat oral LD 50 for PFOS or the rat i.p. 24 - hour LD 50 for APFO.

There were solubility problems in corn oil for the compounds. Therefore, doses of each test material at 32 mM in 2% Tween-80 and using a dose volume of 5 mL/Kg were used. The doses were calculated to be to the highest dose that could be delivered at less than 40% of the LD 50 for each test material. The selection of these dose levels is discussed further in the protocol. Fresh solutions of test substances were prepared for each dosing. The dose groups and final number of animals in each dose group were as follows:

1. Control groups. The vehicle control group consisted of 2 male rats and three male guinea pigs. Each animal in the vehicle control group received a single i.p. injection 2% Tween-80. In addition, a no treatment control group consisted of two male rats that received no treatment were included in the study to check for any pronounced effects of the vehicle control on the subtle endpoints of gene induction planned for this study. The biological and clinical chemistry data from the vehicle control group and the no treatment control rats were combined.
2. PFOS. A single i.p. injection of 32 mM PFOS in 2% Tween-80 (final concentration =17.4 mg PFOS/mL) was given at a volume of 5 mL/Kg (final dose ~ 87.15 mg/Kg) to a total three male rats and four male guinea pigs.
3. APFO. A single i.p. injection of 32 mM APFO in 2% Tween-80 (final concentration =13.8 mg APFO/mL) was given at a volume of 5 mL/Kg (final dose ~ 70 mg/Kg) to a total four male rats and three male guinea pigs.
4. Wyeth-14643 (WY). A single i.p. injection of WY in corn oil (final concentration = 52.45 mg/mL WY) was given at a volume of 1 mL/Kg (final dose ~ 52.3 mg/Kg) to a total four male rats and three male guinea pigs.

Example dose calculation for 32 mM PFOS i.p. at 5 ml/Kg, assuming an average weight of 350 g for the guinea pig this came to: (0.35 kg)(0.005 L/kg)(538.1 g/mol)(0.032 mol/L) = 30.1 mg/guinea pig, or approximately 86 mg/Kg PFOS.

All animals were observed for mortality and signs of toxicity during the first four hours after dosing, at 24 hours and daily thereafter for the duration of the study. Body weights were recorded immediately prior to dosing, daily thereafter, and at necropsy. Following the first exposure and up to four times throughout the in-life phase of the study, the animals were housed individually in metabolic cages overnight, and urine and feces were collected. Animals were sacrificed by CO_2 and gross necropsy performed on either day 12 or day 14 post-dose. Organ tissues (liver, kidney, testes and pancreas) and urine and feces were stored frozen at -80°C for biochemical analysis. Sections of liver kidney, testes and pancreas were placed in 10% buffered formaldehyde and submitted for histological analyses by light microscopy. Some livers were perfused with gluteraldehyde and submitted for histological analysis by electron microscopy. Blood samples were collected at necropsy by heart puncture to analyze for test compound, metabolite formation and to monitor for changes in blood chemistry.

Part B: Procedures for a Time-Course of PFOS (T-6295) toxicity in male and female rats and guinea pigs following a single i.p. injection of PFOS at two doses.

Based on the results of Part A (discussed below) the doses, dose volumes, and the time points chosen for sample analyses were modified. A time course of 3, 6, 12 and 28 days was conducted to measure peroxisome proliferation induction in the liver of male and female rats and guinea pigs treated with either 16 mg/Kg or 100 mg/Kg PFOS.

A total of 22 rats and 20 guinea pigs, 12 male and 10 female rats, and 10 male and 10 female guinea pigs, 6-8 weeks in age and weighing between 150 and 250 grams were purchased from Charles River. Following an adaptation period of not less than one week after arrival at 3M, animals were administered treatments by intraperitoneal (i.p.) injection.

Eight rats and eight guinea pigs (4/sex) were treated in each fluorochemical dose group. The dose groups were 16 mg/Kg PFOS and 100 mg/Kg PFOS. To limit the volume of the i.p. injection, a 100 mg/mL suspension of PFOS was made by dissolving 200 mg PFOS in 0.5 mL corn oil then adding 1.5 ml 2% Tween 80 and creating an emulsion in a glass tissue grinder. A single i.p. injection of the 100 mg/mL PFOS suspension was given at a volume of 1 mL/Kg to the 100 mg/Kg dose group animals, and at a volume of 0.16 mL/Kg to the animals in the 16 mg/Kg dose group. One animal per sex per species per fluorochemical dose group were sacrificed on days 3, 6, 12 and 28 post-dose.

Two animals per sex per species received the vehicle control (25% corn oil in 2% Tween 80), one vehicle control animal each at a volume of volume of 1 mL/Kg, corresponding to the 100 mg/Kg dose group animals, and at 0.16 mL/Kg, corresponding to the 16 mg/Kg dose group. One animal per sex per species per dose group were sacrificed on days 3, 6, 12 and 28 post-dose.

Two male rats received WY as a positive control group. One of the positive control rats received a single i.p. injection of a 100 mg/mL suspension of WY at a volume of 1 mL/Kg to for a total dose or 100 mg/Kg WY. The other positive control rat received a single i.p. injection of a 100 mg/mL suspension of WY at a volume of 0.16 mL/Kg for a total dose of 16 mg/Kg dose group WY. The positive control group rats were sacrificed on day 3 post-dose.

Part C Procedures for hepatic peroxisomal gene induction following oral dosing of N-EtFOSE in rats.

The results of parts A and B led to the conclusion that i.p. dosing was not the best route of administration, therefore an exploratory oral dosing procedure in rats was performed with N-EtFOSE in Part C. The purpose was to generate samples for peroxisomal gene induction assays in liver, and for N-EtFOSE metabolite analysis in liver and serum. A dose of 20 mg/Kg/day N-EtFOSE for two days was chosen as an initial screening dose. This dose was based on a dietary study with 300 ppm N-EtFOSE that caused liver effects and induced palmitoyl Co A oxidase activity in liver over a 4-week period in which the average N-EtFOSE consumption in the dietary study was calculated to be approximately 23 mg/Kg/day for the second week of the study (Ref. 3M Medical Dept. T-6316.1). Three male rats were dosed with 20 mg/Kg/day N-EtFOSE for two days. A 4 mg/mL suspension of N-EtFOSE in 2% Tween 80 was prepared in a glass

tissue grinder and delivered to the rats by oral gavage at a dose volume of 5 mL/Kg for two consecutive days. Two rats were dosed with the vehicle control, 2% Tween 80at a dose volume of 5 mL/Kg for two consecutive days. The animals were euthanized and necropsied on day 3 post-dose. Livers were weighed and chunks of liver were frozen in liquid nitrogen then put in polypropylene vials, placed on dry ice and then stored at -80 . Serum was obtained by centrifugation from blood collected by heart puncture at necropsy and stored at -80 The ~ 2g liver samples that were flash frozen in liquid nitrogen were sent on dry ice for analysis of gene induction to:

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

Results and Discussion

Part A Results on the Toxicity of PFOS and APFO in rats and guinea pigs.

Results: Rats Part A
In rats, there were no statistically significant changes in body weight. Liver weight and liver-to-body weight ratios were increased following treatment with all three compounds, particularly APFO; however, these changes were not statistically significant (Table 1; Appendix 1).

The serum clinical chemistry analysis revealed that cholesterol was lowered to below the limit of detection of 45 mg/dL in rats treated with PFOS. Glucose was significantly lowered in rats treated with PFOS and WY. No statistically significant changes occurred in the clinical chemistry parameters of rats treated with APFO.

The histological results for 3 out of four rats treated i.p. with 52 mg/Kg WY (Animal numbers 7R4845 7R4847 and 7R4837) showed macroscopic masses of white spots on the liver surface covered in thin scar tissue and evidence of mononuclear infiltration. Multifocal microscopic subchronic granulomas were noted.

The histological results for rats treated i.p. with APFO (animal numbers 7R04851-53) showed enlarged livers and kidneys. Macroscopic lesions of mottled black spots on one liver were noted (7R04851). Microscopically, this animal had a pad of scar tissue with an imbedded granuloma on the liver surface.

The histological results for one rat treated with PFOS (7R04838) showed signs of liver damage repair. All other rats, including the control group rats, had no significant change present in liver, kidney, or pancreas.

Results: Guinea pigs Part A

One guinea pig (Animal number 7G0253) that was treated with 100 mg/Kg APFO died overnight following treatment. No tissues were collected from that animal and the initial body weight (358 g) of that animal is not included in the tables or statistical analyses. The cause of death is unknown, but may have been compound related. The guinea pigs treated with PFOS had significantly lower weight gains compared to control. The guinea pigs treated with APFO had significantly greater liver to body weight ratios compared to control.

One of four guinea pigs treated with 17.4 mg/Kg i.p. PFOS (7G02254) showed signs of liver damage repair. No significant changes were present in the liver, kidney, testes, or pancreas of any of the other guinea pigs examined microscopically (7G2004 through 7G2013). Tissues from two out of three control guinea pigs (7G2255 & 7G2256) were never analyzed and were disposed of.

Liver samples submitted for L-FABP analysis from rats and guinea pigs treated in Part A.

Certain liver samples from control and PFOS treated rats and guinea pigs were used for the isolation of liver fatty acid binding protein at the University of San Francisco (Animals 7G02004-control guinea pig, 7G02005-PFOS guinea pig, 7R04844-control rat and 7R04848-PFOS rat). The goal of the LFABP study was to assess the effect of FCs on L-FABP function as evaluated by the ability of the fluorescent fatty acid analogue 11 - (5-dimethylaminonapthalenesulphonyl) - undecanoic acid (DAUDA) to bind to L-FABP isolated from rats and guinea pigs treated and not treated with PFOS *in vivo*. Results showed a decreased maximum binding capacity of L-FABP from PFOS treated rats without an increase in Kd. The complete methods and the results of those analyses are reported elsewhere (Luebker *et al.* 2002; Nabbefeld 1998).

Conclusions from Part A:

The histological results of liver damage with evidence of scar tissue on the surface of the livers of some animals 12 or 14 days after treatment with WY, APFO, and/or PFOS led us to consider that the doses, dose volumes, route of administration and the time points chosen for part A were not necessarily the best model for the objectives of the study. Further analyses of tissues for total organic fluorine, electron microscopy, or peroxisome proliferation from part A were aborted and Part B was planned.

Part B: Results of Time-Course of PFOS toxicity at two doses in male and female rats and guinea pigs.

Results: Rats Part B

All rats treated with either 16 mg/Kg or 100 mg/Kg PFOS gained weight following dosing and maintained body weights that were slightly less than control values (Table 3, Figures 1 and 2). Statistical analyses of body weight suggested that there were no significant differences between treated and control, but had very little power because of the low N (statistics not shown). Liver weights were increased in both male and female rats treated with 100 mg/Kg dose groups (Appendix 3). The liver to body weight ratios in rats were significantly increased in combined

male and female liver to body weight ratio values from the 100 mg/Kg dose group versus control values (Table 5, Appendix 3). The female rats had more of an increase in liver to body weight ratios than did the male rats. Hypertrophy and white pigmentation were noted by gross observation at necropsy in some of the high dose group rats (Appendix 3). Kidney weights and kidney to BW ratios were not significantly affected in rats (Appendix 3).

Results: Guinea pigs Part B

PFOS was more toxic to guinea pigs than rats, and apparently more toxic to female guinea pigs than male guinea pigs. Three of the four female guinea pigs, and one male guinea pig treated with 100 mg/Kg PFOS i.p. died on day 2 of the study. The remaining female guinea pig lost 12% of its initial body weight by day 6 and was humanely euthanized on day 6. All other guinea pigs gained weight and maintained body weights that were slightly lower than control values throughout the time course (Table 4, Figure 4).The guinea pig kidney to body weight ratios from all animals, males and females combined, were significantly increased by treatment with 100 mg/Kg PFOS i.p. (Appendix III).

Female guinea pigs in particular were found to be very sensitive to PFOS toxicity, evidenced by the fact that three out of four female guinea pigs died within the first 24 hours after dosing with a 100 mg/Kg dose of PFOS in 25% corn oil and 75% Tween 80 and the remaining female guinea pig lost weight up to day 6 post-dose. In contrast, one out of four male guinea pig died after a i.p. dose of 100 mg/Kg dose of PFOS in 25% corn oil and 75% Tween 80 at that dose, and the remaining 3 male guinea pigs survived and gained weight following treatment.

The administration of the 100 mg/Kg PFOS dose mixed with 25% corn oil may have contributed to the observed toxicity of PFOS to guinea pigs dosed in part B. None of the male guinea pigs died following i.p. administration of a 100 mg/Kg PFOS dose in 2% Tween 80 at a volume of 5 mL/Kg, whereas one male and three female guinea pigs died when the same dose was delivered in 25% corn oil at a volume of 1mL/ Kg. There is a precedent for delivery of PFOS in corn oil by oral gavage to be more toxic than when delivered in an aqueous solution. The use of corn oil in the dose preparation has previously led to a clear difference in the oral LD 50 of PFOS in rats. The rat 24 hour oral LD-50 for PFOS in a 20:80 acetone: corn oil suspension was determined to be 251 mg/kg (Dean *et al.* 1978). In contrast, the rat oral LD-50 for PFOS in an aqueous suspension was determined to be 1.25 - 2.5 g/kg (Gabriel 1976). No deaths or gross lesions were observed 48 hours after dosing male and female rats with a single oral gavage dose of 250 mg/kg aqueous suspensions of PFOS (Goldenthal *et al.* 1979). Thus, the use of a mixture of 25% corn oil in an aqueous suspension of 2% Tween 80 as the vehicle to deliver PFOS may have contributed to the observed increased toxicity of PFOS to guinea pigs when compared to an 2% Tween 80 vehicle alone.

Conclusions for Part B:

Intraperitoneal administration of PFOS led to the formation of scar tissue on the liver. The findings in this study led to the decision that i.p. dosing was not the appropriate route of administration, and that subsequent studies should be conducted following oral administration of these compounds.

Part C: Results Oral dosing of rats with 20 mg/Kg N-EtFOSE

There was no apparent effect of treatment on body weight or liver weight (Appendix IV). No signs of toxicity or liver effects were apparent by gross observations at necropsy. The rats used were of different ages and initial body weights, therefore, statistics were not performed on these data. The liver specimens were analyzed for induction of mRNA for peroxisome proliferation specific genes. There was no apparent indication of gene induction by N-EtFOSE by Northern Blot analysis, however the total mRNA was partially degraded in all samples, therefore, a quantitative analysis of the results was not performed. Further analysis of these samples was aborted, and the tissues were disposed of.

Conclusions

Standard toxicity tests of serum clinical chemistry and microscopic examination of the liver obtained in Parts A of this study indicated that male guinea pigs were more sensitive to the toxic effects of PFOS and APFO than were male rats. In Part B, three out of four female guinea pigs and one out of four male guinea pigs died overnight, and the remaining female guinea pig was sacrificed on day 6 due to excessive weight loss, following i.p treatment with 100 mg/Kg PFOS. In contrast, none of the male or female rats died following a100 mg/Kg i.p. dose of PFOS. These data showed that the guinea pigs, females in particular, were very sensitive to PFOS toxicity relative to rats. The findings in this study led to the conclusion that intraperitoneal dosing was not the appropriate route of administration, and that subsequent studies should be conducted following oral administration of these compounds to test the stated hypotheses. An initial oral dose study with 20 mg/Kg N-EtFOSE in male rats showed no apparent effects by gross observations therefore the doses were considered to be low as there were no apparent liver effects, and the gene induction studies were aborted. Therefore, Oral dosing and higher doses of N-EtFOSE were deemed necessary for future experiments.

Signatures

Prepared by:

Andrew M. Seacat 5/25/2004

Andrew M. Seacat, Ph.D., DABT. Date
Toxicology Specialist & Study Director

Part A. Histopathology Report.
Elden Lamprecht 3M Lab Animal Research Services:
Rats
7R4845 Macroscopic mass imbedded in liver surface covered in thin scar tissue. Its substance has been dissolved leaving a foamy matrix of protein and mononuclear cells. The interface of the mass and liver consists of foamy mononuclear cells.

7R4847 Macrospopic mass protruding from liver surface covered by thin layer of scar tissue. Its substance has been dissolved leaving a foamy matrix of protein and mononuclear cells. The interface of the mass and liver consists of foamy mononuclear cells.

7R4851 A pad of scar tissue with an imbedded granuloma on the liver surface.

7R4837 Multifocal microscopic subchronic granulomas are present in liver.

7R4838 Parietal pleura of liver is markedly thickened with repair tissue. A generalized mild periportal vacuolation of hepatocytes is present. Some degredation of hepatocytes may be present.
All other rats had no significant change present in liver, kidney or pancreas.

Guinea pigs

7G02254 -A macroscopic pad of granulomatous inflammation present on the surface. It has a foamy appearance with pore contents being dissolved leaving. The matrix which remains is composed of mononuclear cells, some of which are engorged or vacuolated. The pad is covered by an increasingly thick layer of scar tissue. Scar tissue interfaces with the liver parenchyma. No change in liver parenchyma. A second smaller pad is present on another location of the liver.

7G2255 & 7G2256 - never analyzed and disposed of.

7G2004 - 7G2013 - No significant changes were present in the liver, kidney, testes or pancreas.

Tables:

Table 1. Part A. Summary of Toxicity of PFOS and APFO in male rats

Table 1. Rats	Control		PFOS			APFO			WY		
Parameter	Avg	SD	avg	SD	%control	avg	SD	%control	avg	SD	%control
Body wt (g) day1	332	122	258	12.5	77.7	311	125.9	93.6	300	75.7	90.2
Body wt (g) day12/14	369	79	341	9.8	92.4	413	95.8	111.9	398	56.0	107.9
weight gain	72	4	83	11.5	115.3	102	41.7	142.0	99	20.3	137.2
Liver wt (g)	17	5.47	18.90	1.41	111.2	26.63	6.43	156.6	20.58	3.4	121.1
liver/body wt	0.033	0.023	0.04	0.033	112.7	0.060	0.010	181.8	0.05	0.0	151.5
Clinical Chemistry											
Chol mg/dL	54	8.19	<45[1]	0.00	NA[2]	60	12.8	111.1	60	5.3	111.6
Ca mg/dL	11.6	0.10	11.0	0.20	94.8	11.65	0.44	100.4	11.43	0.80	98.5
Phos mg/dL	12.6	0.85	11.8	0.90	93.6	12.55	1.62	99.9	11.78	0.32	93.7
TBIL mg/dL	0.25	0.30	0.10	0.00	40.0	0.13	0.05	50.0	0.10	0.00	40.0
Alb mg/dL	3.50	0.20	3.43	0.21	98.1	3.73	0.39	106.4	3.43	0.10	97.9
TP mg/dL	6.23	0.15	6.13	0.21	98.4	6.48	0.48	103.9	6.28	0.24	100.7
BUN mg/dL	15.3	3.3	15	1.5	100.5	14.5	1.29	95.1	14.75	2.63	96.7
GLU mg/dL	263	29.5	149†	26	56.8	254	160	96.7	175	39.9†	66.5
ALKP U/L	335	81.0	337	87	100.7	374	62.8	111.9	316	67	94.3
AST U/L	255	310	91	8.2	35.7	88	9.9	34.5	138	173	54.3
ALT U/L	79.7	10.8	80	10	100.7	87	6.58	109.1	67	12.5	84.0
LDH U/L	391	144	335	55.2	85.5	334	90.1	85.2	717	993	183.2
Na+ mmol/L	142	1.15	144	3.1	101.6	145	2.1	102	151	9.56	106.1
K+ mmol/L	8.53	0.83	6.6	1.7	77.0	8.50	1.00	99.6	7.13	0.42	83.5
Cl- mmol/L	101	1.5	99	1.0	97.7	101	0.82	99.7	98.5	3.00	97.2
CREA mg/dL	0.57	0.12	0.40	0.0	70.6	0.43	0.05	75.0	0.48	0.10	83.8
GGT U/L	8.0	0.00	8.00	0.00	100.0	8.0	0.0	100.0	8.0	0.0	100.0
TRIG mg/dL	171	130	77	34	45.4	141	53	82.7	175	32	102.5

1. The limit of Detection for Cholesterol (CHOL) was 45 mg/dL.
2. NA Not applicable

† Significantly different from control values by Student's T-test

Table 2. Part A. Summary of Toxicity of PFOS and APFO in male guinea pigs

Table 2 Guinea Pigs	Control		PFOS			APFO			WYETH		
Parameter	Avg	SD	Avg	SD	%control	avg	SD	%control	Avg	SD	%control
Body wt (g) d1	333.00	17.09	335.50	20.9	100.8	316.67	18.72	95.1	322.00	11.36	96.7
Body wt (g) d12/14	422.00	17.62	385.00	20.3	91.2	377.67	44.56	89.5	401.67	10.02	95.2
weight gain	89.00	2.89	49.50†	17.9	55.6†	61.00	25.98	68.5	79.67	1.53	89.5
Liver (g)	17.00	0.50	17.45	3.43	102.6	17.57	1.16	103.3	18.10	1.77	106.5
liver/body wt d12/14	0.04	0.00	0.05	0.010	125.0	0.05†	0.00	116.7	0.05	0.00	112.6
Clinical Chemistry											
Chol mg/dL	55.00	10.00	51.25	7.46	93.2	47.33	4.04	86.1	47.67	4.62	86.7
Ca mg/dL	11.70	0.56	10.83	0.36	92.5	11.07	0.57	94.6	11.27	0.45	96.3
Phos mg/dL	11.70	2.25	8.53	1.49	72.9	8.87	0.45	75.8	8.43	0.55	72.1
TBIL mg/dL	0.47	0.64	0.10	0.00	21.4	0.10	0.00	21.4	0.10	0.00	21.4
Alb mg/dL	2.47	0.31	2.25	0.17	91.2	2.37	0.21	95.9	2.37	0.06	95.9
TP mg/dL	5.00	0.46	4.73	0.26	94.5	4.83	0.25	96.7	4.67	0.15	93.3
BUN mg/dL	18.33	3.79	16.25	2.87	88.6	15.33	0.58	83.6	18.00	2.65	98.2
GLU mg/dL	266.67	178.75	148.50	49.29	55.7	130.33	8.62	48.9	151.67	52.25	56.9
ALKP U/L	349.00	107.48	283.50	39.14	81.2	326.67	38.63	93.6	328.00	75.11	94.0
AST U/L	151.00	132.18	53.50	6.45	35.4	65.67	20.11	43.5	70.67	20.40	46.8
ALT U/L	63.33	7.51	52.75	5.19	83.3	51.00	4.00	80.5	38.00	15.59	60.0
LDH U/L	891.33	1050.23	192.50	50.40	21.6	264.00	68.61	29.6	238.00	22.54	26.7
Na+ mmol/L	139.67	3.51	141.00	1.83	101.0	142.00	2.83	101.7	#DIV/0!	#DIV/0!	#DIV/0!
K+ mmol/L	9.90	2.55	5.30	1.50	53.5	7.70	0.57	77.8	#DIV/0!	#DIV/0!	#DIV/0!
Cl- mmol/L	103.67	0.58	103.00	3.56	99.4	102.00	0.00	98.4	#DIV/0!	#DIV/0!	#DIV/0!
CREA mg/dL	0.40	0.10	0.38	0.05	93.8	0.33	0.06	83.3	0.33	0.06	83.3
GGT U/L	10.45	13.51	12.50	1.29	119.6	12.67	2.08	121.2	11.33	0.58	108.5
TRIG mg/dL	268.33	108.15	117.75	54.71	43.9	140.33	37.81	52.3	128.67	13.58	48.0

1. The limit of Detection for Cholesterol (CHOL) was 45 mg/dL.
2. NA Not applicable

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

Table 3. Part B. Summary of Body Weights in Rats, Means and Std Deviations

Gender	Dose PFOS (mg/Kg)	Day Post-Dose	Mean Body Weight	Std Dev	N
F	Cont	1	194	10	2
F	Cont	3	NR	NA	0
F	Cont	6	211	7	2
F	Cont	12	228	NA	1
F	Cont	28	250	NA	1
F	16	1	183	6	4
F	16	3	192	NA	1
F	16	6	207	7	3
F	16	12	215	7	2
F	16	28	223	NA	1
F	100	1	192	4	4
F	100	3	185	NA	1
F	100	6	195	6	3
F	100	12	221	2	2
F	100	28	239	NA	1
M	Cont	1	244	9	2
M	Cont	3	NR	NA	0
M	Cont	6	290	11	2
M	Cont	12	337	NA	1
M	Cont	28	413	NA	1
M	16	1	241	11	4
M	16	3	278	NA	1
M	16	6	292	17	3
M	16	12	327	21	2
M	16	28	419	NA	1
M	100	1	251	9	4
M	100	3	247	NA	1
M	100	6	279	12	3
M	100	12	334	21	2
M	100	28	386	NA	1
M	Cont	1	244	9	2
M	Cont	3	NR	NA	0
M	Cont	6	290	11	2
M	Cont	12	337	NA	1
M	Cont	28	413	NA	1

Table 4. Part B. Summary of Body Weights in Guinea Pigs, Means and Std Deviations

Gender	Dose PFOS (mg/Kg)	Day Post-Dose	Mean BW (g)	Std Dev	N
F	Cont	1	365	49	2
F	Cont	3	NR	NA	0
F	Cont	6	398	70	2
F	Cont	12	500	NA	1
F	Cont	28	573	NA	1
F	16	1	359	23	4
F	16	3	349	NA	1
F	16	6	393	17	3
F	16	12	442	26	2
F	16	28	464	NA	1
F	100	1	355	26	4
F	100	3	NR	NA	0
F	100	6	317	NA	1
F	100	12	NA	NA	0
F	100	28	NA	NA	0
M	Cont	1	370	7	2
M	Cont	3	NR	NA	0
M	Cont	6	429	10	2
M	Cont	12	511	NA	1
M	Cont	28	626	NA	1
M	16	1	377	18	4
M	16	3	371	NA	1
M	16	6	415	25	3
M	16	12	478	24	2
M	16	28	610	?	1
M	100	1	376	7	4
M	100	3	359	NA	1
M	100	6	373	35	2
M	100	12	456	NA	1
M	100	28	NR	NA	0

Table 5. Part B Liver to Body Weight ratios in rats

Dose Group	Species	Gender	N	Mean	Std Dev
Cont	Rat	M & F	4	0.05	0.01
16 mg/Kg PFOS	Rat	M & F	8	0.05	0.01
100 mg/Kg PFOS	Rat	M & F	8	0.06†	0.01
Conttrol	GP		4	0.04	0.003
16 mg/Kg	GP		8	0.04	0.002
100 mg/Kg	GP		4	0.04	0.001

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

Figures

Figure 1: Part B: Female Rat Body Weight Vs Time.

Time-Course of PFOS toxicity at two doses, 16 mg/Kg and 100 mg/Kg.

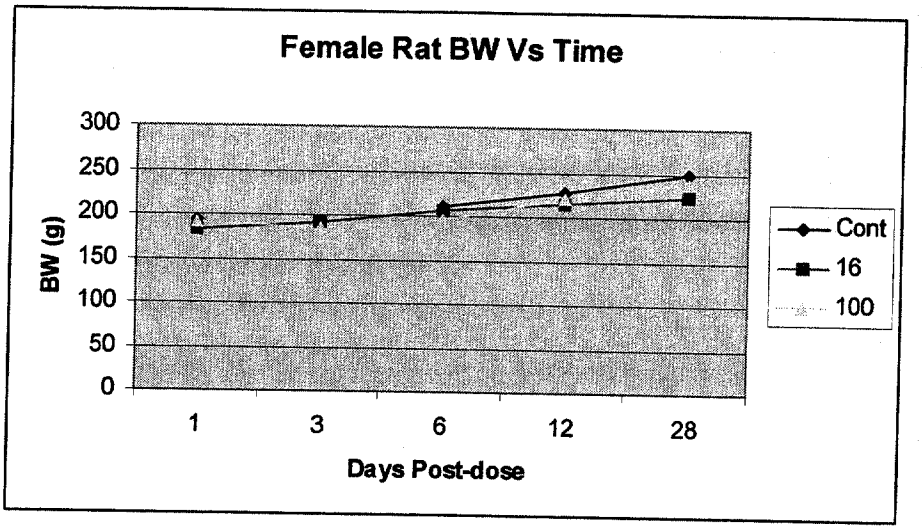


Figure 2: Part B: Male Rat Body Weight Vs Time.

Time-Course of PFOS toxicity at two doses, 16 mg/Kg and 100 mg/Kg.

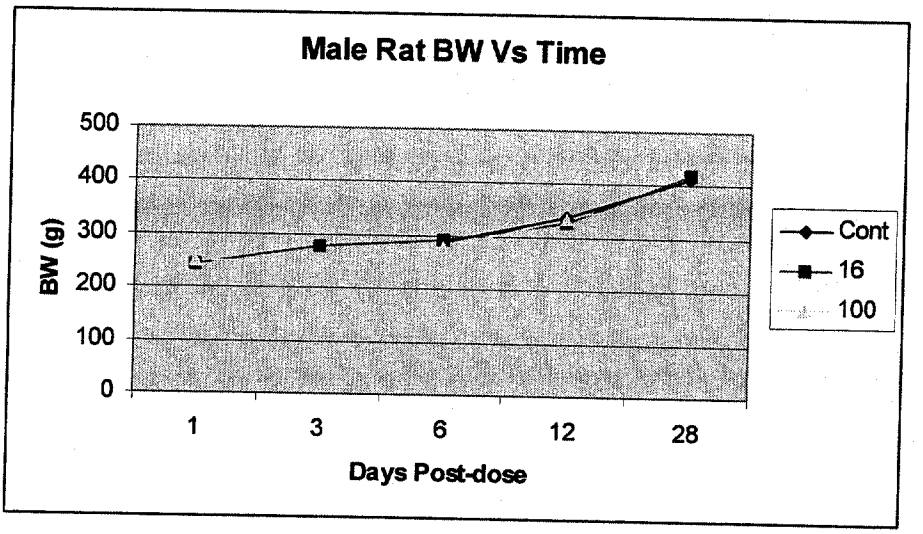


Figure 3: Part B: Female Guinea Pig Body Weight Vs Time.

Time-Course of PFOS toxicity at two doses, 16 mg/Kg and 100 mg/Kg.

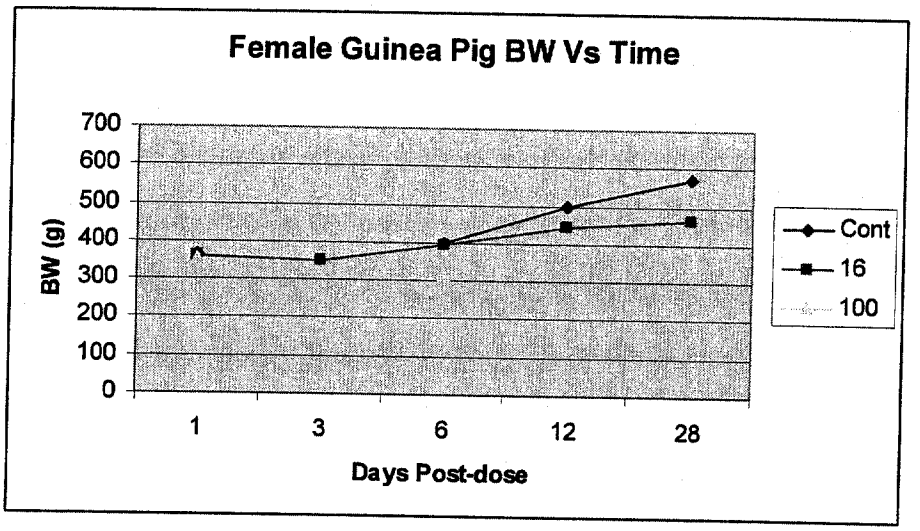
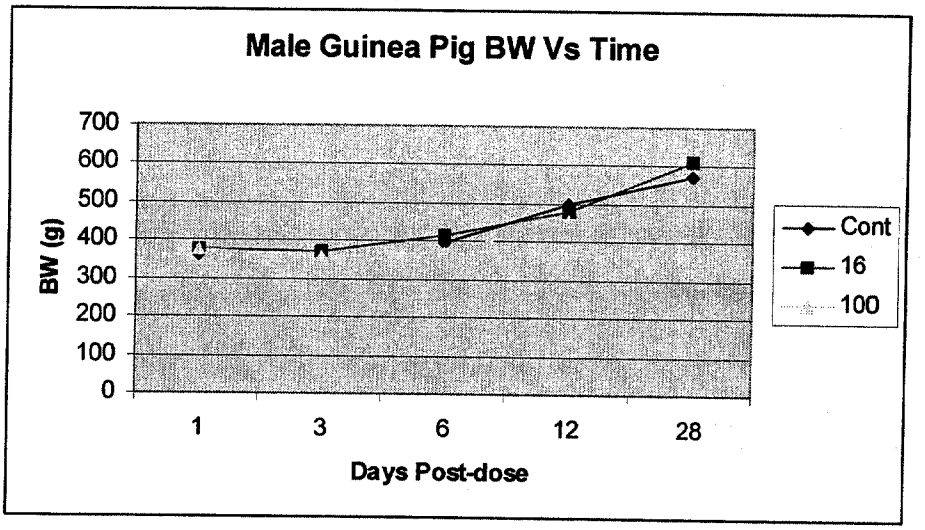


Figure 4: Part B: Male Guinea Pig Body Weight Vs Time.

Time-Course of PFOS toxicity at two doses, 16 mg/Kg and 100 mg/Kg.



Appendix 1. Deviations to the Protocol:

1. The protocol states that there would be Five male and five female rats and guinea pigs per treatment group, with 5 treatment groups for a total of 50 rats and 50 guinea pigs.

In Part A, only male animals were used. Three to four animals per treatment group were used and a total of 15 male rats and 13 male guinea pigs were used in Part A.

In Part B, a total of 20 rats and 20 guinea pigs, 10 males and 10 females per species were used.

Thus, in both parts A and B, a total of 35 rats and 33 guinea pigs were used.

2. The protocol stated that N-ethyl perfluorooctane sulfonamide (FX-12) and *N*-ethylperfluorooctane sulfonamido ethanol (FC-10, N-EtFOSE, T-6316) would be dosed. These two dose groups were removed from the protocol, and a positive control, Wyeth-14643 (WY), a model persoxisome proliferators, was added as a dose group.

3. The protocol stated that all treatments would be administered in corn oil.

PFOS (T-6295) and APFO (T-6889) were administered in 2% Tween 80.
Wyeth-14643 (WY) was administered in 25% corn oil.

4. The protocol stated that Dunnett's t-test would be performed on all data.

Student's t-test were performed instead where noted.

5. The protocol stated that all treatments would be given by i.p. dosing. Dosing by oral gavage was performed in Part C.

Appendix II. Part A: Toxicity of PFOS and APFO Individual and Summary Data

Part A: Rat data	CONTROL						PFOS				
	Nvh.	Nvh.	Vh	Vh							
	*1	*2	*3	^*4	Avg	SD	*1	*2	*3	Avg	SD
animal # 7r)-xxxx	C1	C2	4844	4836			4848	4849	7850		
Parameter											
Body wt (g) d1			245	418	332	122	272	254	248	258	12.5
Body wt (g) d12		328	320	487	369	79.4	344	349	330	341.0	9.8
wt gain			74.7	69	72	4.0	72	95	82	83.0	11.5
wt gain %	#DIV/0!	#DIV/0!	30%	17%	23%	0.1	26%	37%	33%	0.3	0.1
Liver (g)	12.5	15.9		23.2	17	5.5		17.9	19.9	18.9	1.4
liver/body wt	0.04	0.05	0.00	0.05	0.03	0.02	0.00	0.05	0.06	0.04	0.03
Kidney (g)	3.2	2.9	3.2	4.9	3.6	0.9	3.3	2.9	3.2	3.1	0.2
Testis (g)	3.3	3.4	2.9	3.9	3.4	0.4	3.3	3.3	3.1	3.2	0.1
Clinical Chemistry											
Chol mg/dL	52	63	47		54.0	8.2	<45	<45	<45	NA	NA
Ca mg/dL	11.5	11.7	11.6		11.6	0.1	10.8	11.2	11	11.0	0.2
Phos mg/dL	13.4	11.7	12.6		12.6	0.9	10.9	12.7	11.7	11.8	0.9
TBIL mg/dL	0.1	0.1	0.1	0.7	0.3	0.3	0.1	0.1	0.1	0.1	0.0
Alb mg/dL	3.5	3.7	3.3		3.5	0.2	3.5	3.6	3.2	3.4	0.2
TP mg/dL	6.2	6.4	6.1		6.2	0.2	6.3	6.2	5.9	6.1	0.2
BUN mg/dL	13	15	13	20	15.3	3.3	14	17	15	15.3	1.5
GLU mg/dL	244	248	297		263	29.5	119	166	163	149.3	26.3
ALKP U/L	271	326	451	290	334	80.9	276	436	299	337.0	86.5
AST U/L	98	89	114	719	255	309	84	100	89	91.0	8.2
ALT U/L	67	77	82	93	79	10	70	90	81	80	10
LDH U/L	332	286	556		391	144	277	387	340	334	55.2
Na+ mmol/L	141	143	143		142	1.2	148	142	144	144	3.1
K+ mmol/L	9.2	8.8	7.6		8.5	0.8	5.3	8.5	5.9	6.6	1.7
Cl- mmol/L	101	100	103		101	1.5	98	99	100	99.0	1.0
CREA mg/dL		0.5	0.5		0.6	0.1	0.4	0.4	0.4	0.4	0.0
GGT U/L	8	8	8		8.0	0.0	8	8	8	8.0	0.0
TRIG mg/dL	70	131	120	361	170	129	57	117	58	77.3	34.4

Part A: Rat data (cont)	APFO						WY					
	*1	*2	*3	^*4	avg	SD	*1	2	*3	^*4	Avg	SD
animal # 7R0-xxxx	4851	4852	4853	4838			4845	4846	4847	4837		
Parameter												
Body wt (g) d1	227	253	265	498	310	125	264	258	263	413.0	299.5	75.7
Body wt (g) d12	385	349	363	555	413	95	367	368	376	482	398.3	56.0
wt gain	158	96	98	57.0	102	41.7	103	110	113	69	98.8	20.3
wt gain %	70%	38%	37%	11%	39%	0.2	39%	43%	43%	17%	35%	0.1
Liver (g)	28.9	19.6	23.6	34.4	26.6	6.4	19.6	18.6	18.5	25.6	20.5	3.4
liver/body wt	0.08	0.06	0.07	0.06	0.06	0.0	0.05	0.05	0.05	0.05	0.05	0.0
Kidney (g)	3.9	3.5	3.4	4.8	3.9	0.6	3.7	3.2	3.4	4.3	3.7	0.5
Testis (g)	3.6	3.0	3.4	3.6	3.4	0.3	3.1	3.4	3.2	3.7	3.4	0.3
Clinical Chemistry												
Chol mg/dL	53.0	56.0	52.0	79.0	60.0	12	65.0	63.0	60.0	53.0	60.3	5.3
Ca mg/dL	11.5	11.4	11.4	12.3	11.7	0.4	12.6	11.1	11.2	10.8	11.4	0.8
Phos mg/dL	11.2	12.0	12.1	14.9	12.6	1.6	12.1	12.0	11.5	11.5	11.8	0.3
TBIL mg/dL	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0
Alb mg/dL	3.4	3.4	3.9	4.2	3.7	0.4	3.4	3.5	3.3	3.5	3.4	0.1
TP mg/dL	6.1	6.1	6.6	7.1	6.5	0.5	6.1	6.3	6.1	6.6	6.3	0.2
BUN mg/dL	14.0	13.0	16.0	15.0	14.5	1.3	17.0	12.0	13.0	17.0	14.8	2.6
GLU mg/dL	206	175	145	491	254	160	210	142	139	209	175	40
ALKP U/L	419	284	379	415	374	63	332	401	284	245	316	67
AST U/L	94	84.0	98.0	76.0	88.0	9.9	76.0	8.0	76.0	394	139	173
ALT U/L	88	95	86	79	87	6	52	74	62	80	67	12
LDH U/L	340	234	309	451	334	90	262	233	167	2205	717	993
Na+ mmol/L	145	142	145	147	145	2	165	146	144	149	151	10
K+ mmol/L	7.0	8.9	9.1	9.0	8.5	1.0	7.3	7.4	6.5	7.3	7.1	0.4
Cl- mmol/L	101	100	101	102	101	1	100	96	96	102	99	3
CREA mg/dL		0.4	0.4	0.5	0.4	0.0	0.5	0.4	0.4	0.6	0.5	0.1
GGT U/L	8.0	8.0	8.0	8.0	8.0	0.0	8.0	8.0	8.0	8.0	8.0	0.0
TRIG mg/dL	107	118	119	220	141	53	196	130	175	198	175	32

* = Histology
missing data
^ = sacrificed on day 14 rather than 12
Limit of detection for Cholesterol = 45 mg/dl

Part A: Guinea Pig data	Vehicle					PFOS					
	*1	^*2	^*3	Avg	SD	*1	*2	*3	^*4	Avg	SD
animal # 7GO-xxxx	2004	2256	2255			2005	2006	2007	2254		
Parameter											
Body wt (g) d1	317	331	351	333	17	313	329	337	363	336	21
Body wt (g) d12	408	417	442	422	18	387	371	369	413	385	20
wt gain	91	86	91	89	3	74	42	32	50	50	18
Liver (g)	17.2	16.7	17.7	17.2	0.5	13.1	16.4	20.7	19.6	17.5	3.4
liver/body wt	0.04	0.04	0.04	0.04	0.00	0.03	0.04	0.06	0.05	0.05	0.01
Kidney (g)	3.9	3.4	4.1	3.8	0.4	3.5	3.9	4.4	4.1	4.0	0.4
Testis (g)	1.2	1.6	1.5	1.4	0.2	1.0	0.8	0.9	1.6	1.1	0.4
Clinical Chemistry											
Chol mg/dL	55	65	45	55	10	50	45	48	62	51	7
Ca mg/dL	12	12	11	12	1	11	11	11	11	11	0
Phos mg/dL	10	14	11	12	2	8	7	8	11	9	1
TBIL mg/dL	0.1	1.2	0.1	0.5	0.6	0.1	0.1	0.1	0.1	0.1	0.0
Alb mg/dL	2.2	2.8	2.4	2.5	0.3	2.1	2.2	2.2	2.5	2.3	0.2
TP mg/dL	4.6	5.5	4.9	5.0	0.5	4.5	4.6	4.7	5.1	4.7	0.3
BUN mg/dL	14	21	20	18	4	14	14	20	17	16	3
GLU mg/dL	118	465	217	267	179	117	130	125	222	149	49
ALKP U/L		425	273	349	107	278	340	264	252	284	39
AST U/L	87	303	63	151	132	58	47	49	60	54	6
alt U/L	56	71	63	63	8	49	59	55	48	53	5
LDH U/L	277	2104	293	891	1050	183	149	173	265	193	50
Na+ mmol/L	143	136	140	140	4	142	139	140	143	141	2
K+ mmol/L	8.1		11.7	9.9	2.5	5.0	4.3	4.4	7.5	5.3	1.5
Cl- mmol/L	104	103	104	104	1	103	101	100	108	103	4
CREA mg/dL	0.3	0.5	0.4	0	0.10	0.3	0.4	0.4	0.4	0.38	0.05
GGT U/L		20	1	10	14	12	14	13	11	13	1
TRIG mg/dL	157	373	275	268	108	98	94	80	199	118	55

Part A: Guinea Pig data (cont)	APFO					WYETH (in corn oil)				
	*1	*2	*3	avg	SD	*1	*2	*3	Avg	SD
animal # 7GO-xxxx	2008	2009	2010			2011	2012	2013		
Parameter										
Body wt (g) d1	309	338	303	317	19	317	335	314	322	11
Body wt (g) d12	355	429	349	378	45	398	413	394	402	10
wt gain	46	91	46	61	26	81	78	80	80	2
Liver (g)	16.8	18.9	17.0	17.6	1.2	16.2	19.7	18.4	18.1	1.8
liver/body wt	0.05	0.04	0.05	0.05	0.00	0.04	0.05	0.05	0.05	0.00
Kidney (g)	4.2	3.9	3.8	4.0	0.2	4.1	4.8	4.6	4.5	0.4
Testis (g)	1.1	0.9	1.0	1.0	0.1	0.9	1.8	0.8	1.2	0.6
Clinical Chemistry										
Chol mg/dL	<45	<45	52	52	NA	53	45	45	48	5
Ca mg/dL	11	11	12	11	1	11	11	12	11	0
Phos mg/dL	9	8	9	9	0	9	8	8	8	1
TBIL mg/dL	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0
Alb mg/dL	2.3	2.2	2.6	2.4	0.2	2.4	2.3	2.4	2.4	0.1
TP mg/dL	4.8	4.6	5.1	4.8	0.3	4.7	4.5	4.8	4.7	0.2
BUN mg/dL	15	16	15	15	1	19	20	15	18	3
GLU mg/dL	132	121	138	130	9	121	122	212	152	52
ALKP U/L	323	290	367	327	39	408	259	317	328	75
AST U/L	88	49	60	66	20	66	93	53	71	20
alt U/L	51	47	55	51	4	47	20	47	38	16
LDH U/L	342	213	237	264	69	250	252	212	238	23
Na+ mmol/L	144	140		142	3				NA	NA
K+ mmol/L	7.3	8.1		7.7	0.6				NA	NA
Cl- mmol/L	102	102		102	0				NA	NA
CREA mg/dL	0.3	0.3	0.4	0.33	0.06	0.4	0.3	0.3	0.33	0.06
GGT U/L	11	15	12	13	2	11	11	12	11	1
TRIG mg/dL	127	111	183	140	38	127	143	116	129	14

* = Histology
missing data
^ = sacrificed on day 14 rather than 12

Appendix III. Part B: Time course with PFOS. Individual Data

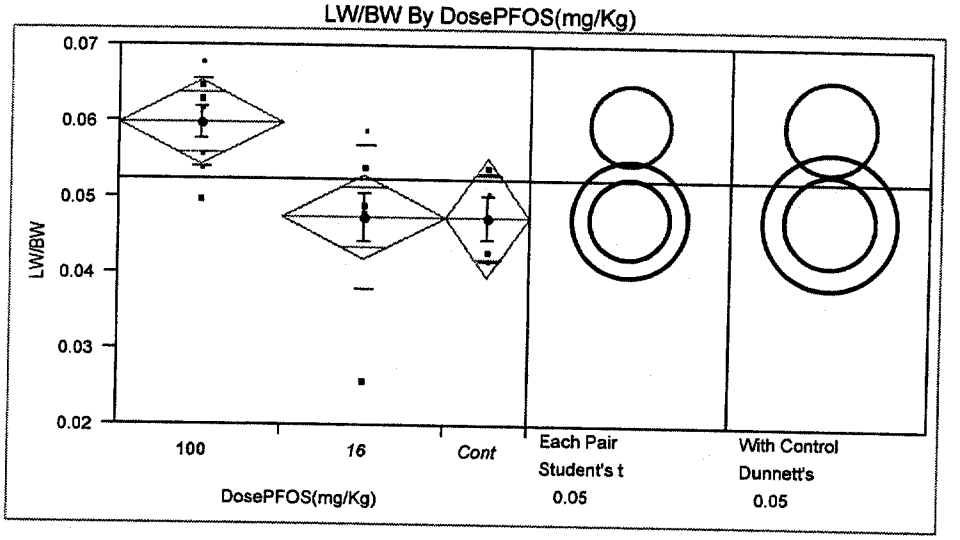
Part B. Individual Rat data Time course with PFOS.

Animal #	Gender	Dose PFOS (mg/Kg)	Species	BW (g) d1	BW (g) d3	BW (g) d6	BW (g) d12	BW (g) d28	LW (g)	LW/BW	KW (g)	KW/BW	Sac day
8R00344	F	100	Rat	188	185	?	?	?	10.06	0.054	0.88	0.005	3
8R00345	F	100	Rat	188	?	188	?	?	12.7	0.068	0.9	0.005	6
8R00346	F	100	Rat	195	?	198	222	?	13.8	0.062	0.88	0.004	13
8R00347	F	100	Rat	196	?	199	219	239	13.4	0.056	0.97	0.004	28
8R00340	F	16	Rat	189	192	?	?	?	9.31	0.048	0.98	0.005	3
8R00341	F	16	Rat	187	?	215	?	?	12.7	0.059	0.87	0.004	6
8R00342	F	16	Rat	180	?	202	220	?	10.6	0.048	0.87	0.004	13
8R00343	F	16	Rat	177	?	203	210	223	10.8	0.048	1.06	0.005	28
8R00339	F	V Cont1[1]	Rat	201	?	216	?	?	11.1	0.051	1	0.005	6
8R00348	F	V Cont2[2]	Rat	187	?	206	228	250	10.4	0.042	1.01	0.004	28
8R00334	M	100	Rat	244	247	?	?	?	14.8	0.06	1.18	0.005	3
8R00335	M	100	Rat	247	?	279	?	?	18	0.065	1.08	0.004	6
8R00336	M	100	Rat	265	?	291	348	?	21.8	0.063	1.58	0.004	13
8R00337	M	100	Rat	248	?	267	319	386	19.4	0.05	1.5	0.004	28
8R00330	M	16	Rat	253	278	?	?	?	13.5	0.049	1.3	0.005	3
8R00331	M	16	Rat	240	?	288	?	?	15.5	0.054	1.3	0.005	6
8R00332	M	16	Rat	227	?	277	312	?	15	0.048	1.32	0.004	13
8R00333	M	16	Rat	245	?	310	341	419	10.8	0.026	1.06	0.003	28
8R00329	M	V Cont1[1]	Rat	250	?	297	?	?	12.7	0.043	1.17	0.004	6
8R00338	M	V Cont2[2]	Rat	237	?	282	337	413	22.1	0.054	1.57	0.004	28
8R0004	M	Wyeth 1[3]	Rat	520	513								3
8R0003	M	Wyeth 2[4]	Rat	488	485								3

Notes:
1. V cont 1 = 0.16 mL/Kg dose volume of the vehicle, 25% corn oil in 80% Tween 80.
2. V cont 2 = 1.0 mL/Kg dose volume of the vehicle, 25% corn oil in 80% Tween 80.
3. Wyeth 1 = Positive control high dose 100 mg/Kg Wyeth 14643. Wyeth animal data was excluded from further analysis.
4. Wyeth 2 = Positive control low dose 16 mg/Kg Wyeth 14643. Wyeth animal data was excluded from further analysis.

Gross observations:
Animal number 8R0336 Male rat 100 mg/Kg dose group, necropsied on day 6 post-dose, had white edges on liver.
Animal number 8R0336 Male rat 100 mg/Kg dose group, necropsied on day 12 post-dose, had a hypertrophic liver with white edges.
Animal number 8R0337 Male rat 100 mg/Kg dose group, necropsied on day 28 post-dose, had an abnormal, thickened liver with white edges.

Part B. Liver Weight to Body Weight ratio in male and female rats combined data. SAS analysis



Oneway Anova
Summary of Fit

RSquare	0.419071
RSquare Adj	0.350727
Root Mean Square Error	0.007664
Mean of Response	0.0524
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00072030	0.000360	6.1317
Error	17	0.00099850	0.000059	Prob>F
C Total	19	0.00171880	0.000090	0.0099

Means for Oneway Anova

Level	Number	Mean	Std Error
100	8	0.059750	0.00271
16	8	0.047500	0.00271
Cont	4	0.047500	0.00383

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	8	0.059750	0.006018	0.00213
16	8	0.047500	0.009562	0.00338
Cont	4	0.047500	0.005916	0.00296

Means Comparisons

Dif=Mean[i]-Mean[j]	100	16	Cont
100	0.000000	0.012250	0.012250
16	-0.01225	0.000000	0.000000
Cont	-0.01225	0.000000	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

t
2.10980

Abs(Dif)-LSD	100	16	Cont

100	-0.00808	0.004165	0.002348
16	0.004165	-0.00808	-0.0099
Cont	0.002348	-0.0099	-0.01143

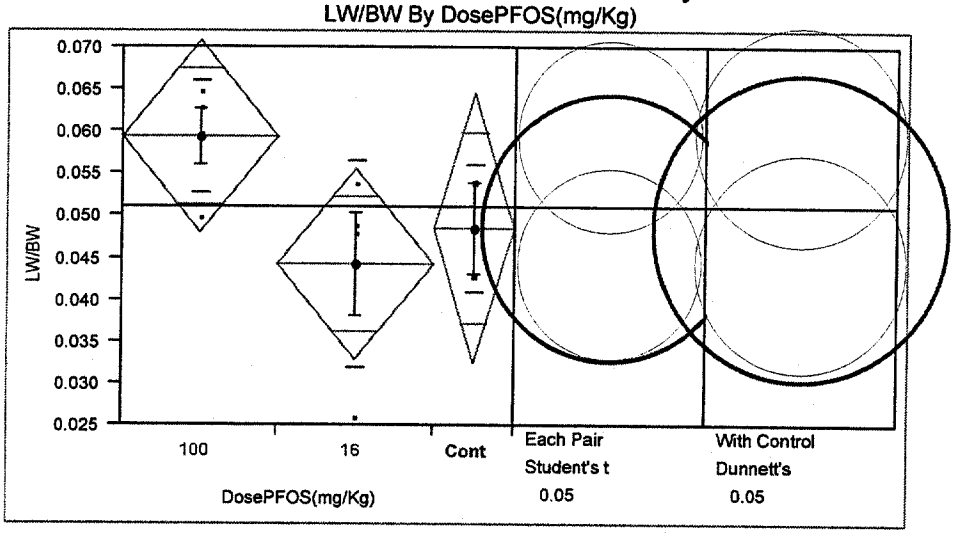
Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

|d|
2.42255

Abs(Dif)-LSD	100
100	-0.00928
16	0.002967
Cont	0.000881

Positive values show pairs of means that are significantly different.

Part B. Male rat LW/BW ratio summary



Oneway Anova

Summary of Fit

RSquare	0.423397
RSquare Adj	0.258653
Root Mean Square Error	0.009697
Mean of Response	0.0512
Observations (or Sum Wgts)	10

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00048335	0.000242	2.5700
Error	7	0.00065825	0.000094	Prob>F
C Total	9	0.00114160	0.000127	0.1456

Means for Oneway Anova

Level	Number	Mean	Std Error
100	4	0.059500	0.00485
16	4	0.044250	0.00485
Cont	2	0.048500	0.00686

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	4	0.059500	0.006658	0.00333
16	4	0.044250	0.012447	0.00622
Cont	2	0.048500	0.007778	0.00550

Means Comparisons

Dif=Mean[i]-Mean[j]	100	Cont	16
100	0.000000	0.011000	0.015250
Cont	-0.011	0.000000	0.004250
16	-0.01525	-0.00425	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

t

2.36464

Abs(Dif)-LSD	100	Cont	16
100	-0.01621	-0.00886	-0.00096

Cont	-0.00886	-0.02293	-0.01561
16	-0.00096	-0.01561	-0.01621

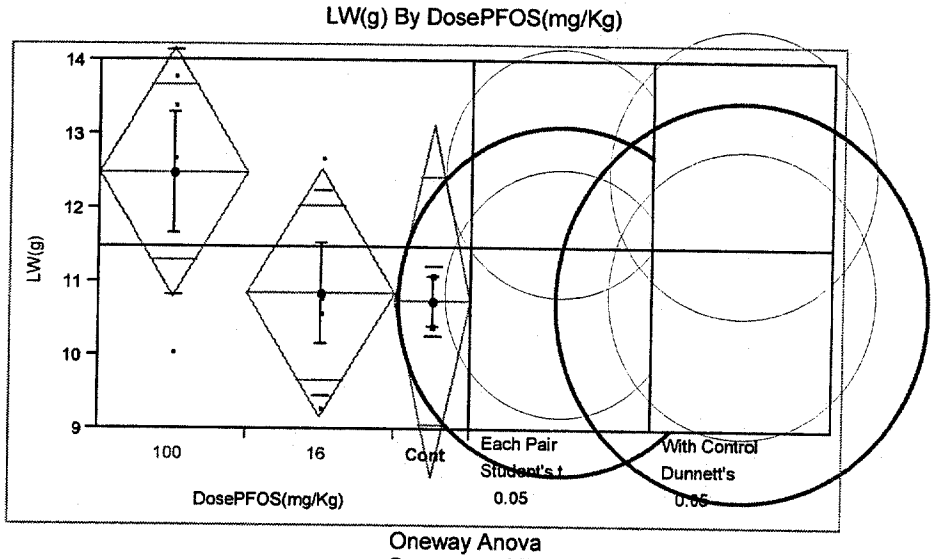
Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

|d|
2.70602

Abs(Dif)-LSD	Cont
100	-0.01173
Cont	-0.02624
16	-0.01848

Positive values show pairs of means that are significantly different.

Part B. Female rat Liver weight summary



Oneway Anova

Summary of Fit

RSquare	0.315261
RSquare Adj	0.119621
Root Mean Square Error	1.444065
Mean of Response	11.487
Observations (or Sum Wgts)	10

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	6.720735	3.36037	1.6114
Error	7	14.597275	2.08532	Prob>F
C Total	9	21.318010	2.36867	0.2657

Means for Oneway Anova

Level	Number	Mean	Std Error
100	4	12.4900	0.7220
16	4	10.8525	0.7220
Cont	2	10.7500	1.0211

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	4	12.4900	1.68258	0.84129
16	4	10.8525	1.39751	0.69875
Cont	2	10.7500	0.49497	0.35000

Means Comparisons

Dif=Mean[i]-Mean[j]	100	16	Cont
100	0.00000	1.63750	1.74000
16	-1.63750	0.00000	0.10250
Cont	-1.74000	-0.10250	0.00000

Alpha= 0.05

Comparisons for each pair using Student's t

t

2.36464

Abs(Dif)-LSD	100	16	Cont
100	-2.41456	-0.77706	-1.21722

16	-0.77706	-2.41456	-2.85472
Cont	-1.21722	-2.85472	-3.41470

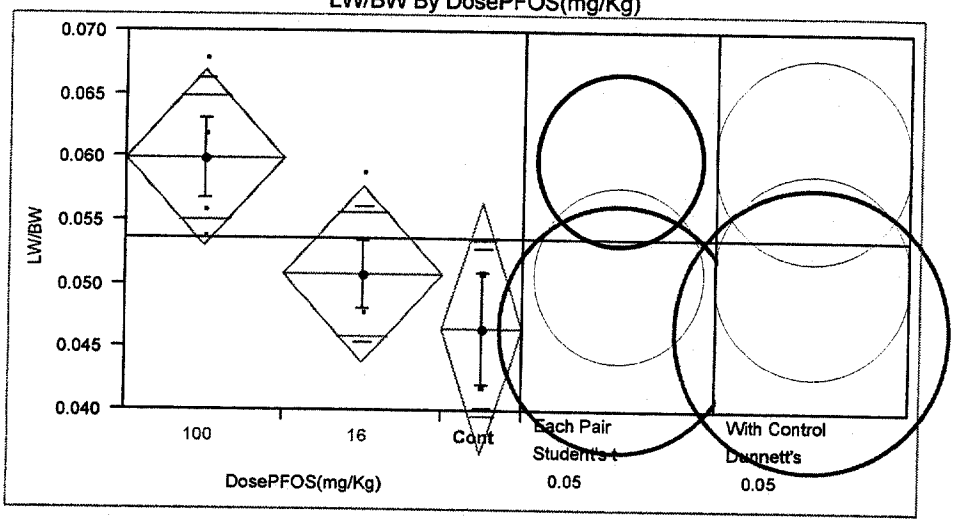
Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

|d|
2.70602

Abs(Dif)-LSD	Cont
100	-1.64415
16	-3.28165
Cont	-3.90768

Positive values show pairs of means that are significantly different.

Part B. Female rat LW/BW ratio Summary



Oneway Anova

Summary of Fit

RSquare	0.541849
RSquare Adj	0.410949
Root Mean Square Error	0.005991
Mean of Response	0.0536
Observations (or Sum Wgts)	10

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00029715	0.000149	4.1394
Error	7	0.00025125	0.000036	Prob>F
C Total	9	0.00054840	0.000061	0.0651

Means for Oneway Anova

Level	Number	Mean	Std Error
100	4	0.060000	0.00300
16	4	0.050750	0.00300
Cont	2	0.046500	0.00424

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	4	0.060000	0.006325	0.00316
16	4	0.050750	0.005500	0.00275
Cont	2	0.046500	0.006364	0.00450

Means Comparisons

Dif=Mean[i]-Mean[j]	100	16	Cont
100	0.000000	0.009250	0.013500
16	-0.00925	0.000000	0.004250
Cont	-0.0135	-0.00425	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

t

2.36464

Abs(Dif)-LSD	100	16	Cont
100	-0.01002	-0.00077	0.001231

16	-0.00077	-0.01002	-0.00802
Cont	0.001231	-0.00802	-0.01417

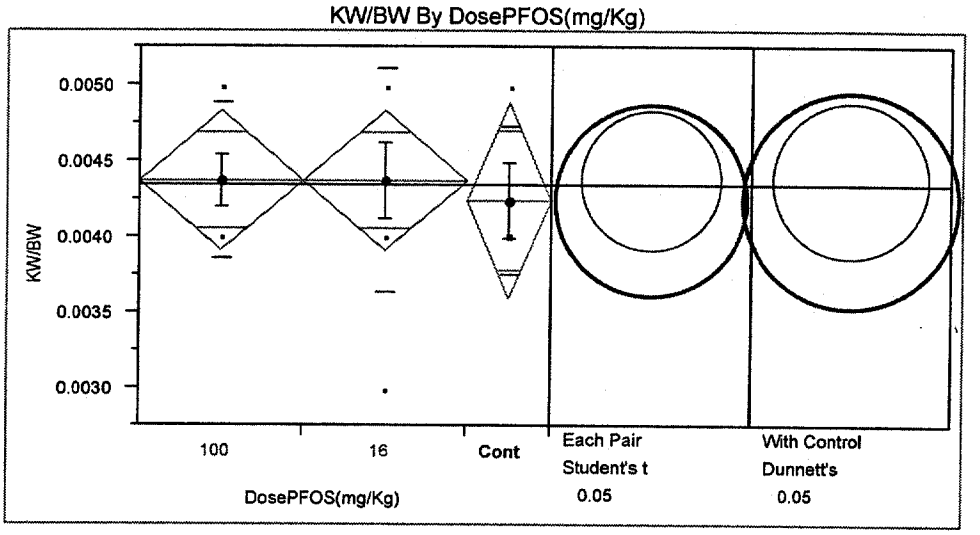
Positive values show pairs of means that are significantly different.
Comparisons with a control using Dunnett's Method

|d|
2.70602

Abs(Dif)-LSD	Cont
100	-0.00054
16	-0.00979
Cont	-0.01621

Positive values show pairs of means that are significantly different.

Part B. Kidney Weight to Body Weight ratio in male and female rats combined data. SAS analysis.



Oneway Anova
Summary of Fit

RSquare	0.007634
RSquare Adj	-0.10912
Root Mean Square Error	0.000618
Mean of Response	0.00435
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00000005	2.5e-8	0.0654
Error	17	0.0000065	3.824e-7	Prob>F
C Total	19	0.00000655	3.447e-7	0.9369

Means for Oneway Anova

Level	Number	Mean	Std Error
100	8	0.004375	0.00022
16	8	0.004375	0.00022
Cont	4	0.004250	0.00031

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	8	0.004375	0.000518	0.00018
16	8	0.004375	0.000744	0.00026
Cont	4	0.004250	0.000500	0.00025

Means Comparisons

Dif=Mean[i]-Mean[j]	100	16	Cont
100	0.000000	0.000000	0.000125
16	0.000000	0.000000	0.000125
Cont	-0.00013	-0.00013	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

t
2.10980

Abs(Dif)-LSD	100	16	Cont
100	-0.00065	-0.00065	-0.00067
16	-0.00065	-0.00065	-0.00067
Cont	-0.00067	-0.00067	-0.00092

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|
2.37772

Abs(Dif)-LSD	Cont
100	-0.00078
16	-0.00078
Cont	-0.00104

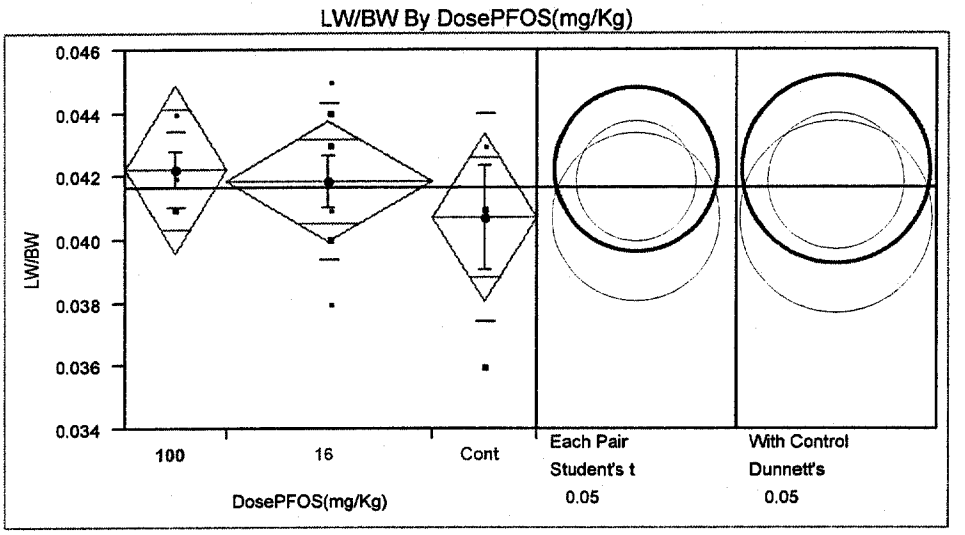
Positive values show pairs of means that are significantly different.

Part B. Guinea Pig: Individual Data

Guinea Pig data 2-10-98

Animal #	Gender	Dose PFOS (mg/Kg)	Species	BW (g) d1	BW (g) d3	BW (g) d6	BW (g) d12	BW (g) d28	LW (g)	LW/ BW ratio	KW (g)	KW/ BW ratio	Sac day	Notes
8G00283	F	100	GP	360	?	317	?	?	12.9	0.041	1.46	0.005	6	-12% BWi day 6
8G00281	F	100	GP	383	?	?	?	?	?	?	?	.		Dead day 2
8G00282	F	100	GP	355	?	?	?	?	?	?	?	.		Dead day 2
8G00284	F	100	GP	321	?	?	?	?	?	?	?	.		Dead day 2
8G00276	F	16	GP	332	349	?	?	?	14	0.04	?	.	3	
8G00277	F	16	GP	356	?	392	?	?	15.8	0.04	1.67	0.004	6	
8G00278	F	16	GP	387	?	410	460	?	20.4	0.044	1.85	0.004	13	
8G00279	F	16	GP	360	?	377	423	464	19.9	0.043	1.75	0.004	28	
8G00275	F	Cont	GP	399	?	447	500	573	23.5	0.041	2.01	0.004	28	
8G00280	F	Cont	GP	330	?	348	?	?	12.7	0.036	1.56	0.004	6	
8G00271	M	100	GP	365	359	?	?	?	15.9	0.044	1.98	0.006	3	
8G00272	M	100	GP	381	?	348	?	?	14.7	0.042	1.83	0.005	6	
8G00273	M	100	GP	380	?	397	456	?	19.1	0.042	2.13	0.005	13	
8G00274	M	100	GP	377	?	?	?	?	?	?	?	.		Dead day 2
8G00266	M	16	GP	375	371	?	?	?	16.8	0.045	1.84	0.005	3	
8G00267	M	16	GP	364	?	394	?	?	16.1	0.041	1.74	0.004	6	
8G00268	M	16	GP	366	?	409	461	?	17.3	0.038	1.84	0.004	13	
8G00269	M	16	GP	402	?	442	495	610	27	0.044	2.7	0.004	28	
8G00265	M	Cont	GP	365	?	436	511	626	27	0.043	2.65	0.004	28	
8G00270	M	Cont	GP	375	?	422	?	?	18.0	0.043	1.98	0.005	6	

Part B. Guinea Pig Liver to Body Weight Ratio Summary Statistics:



Oneway Anova
Summary of Fit

RSquare	0.059254
RSquare Adj	-0.08548
Root Mean Square Error	0.002487
Mean of Response	0.041687
Observations (or Sum Wgts)	16

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00000506	0.000003	0.4094
Error	13	0.00008038	0.000006	Prob>F
C Total	15	0.00008544	0.000006	0.6723

Means for Oneway Anova

Level	Number	Mean	Std Error
100	4	0.042250	0.00124
16	8	0.041875	0.00088
Cont	4	0.040750	0.00124

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	4	0.042250	0.001258	0.00063
16	8	0.041875	0.002475	0.00088
Cont	4	0.040750	0.003304	0.00165

Means Comparisons

Dif=Mean[i]-Mean[j]	100	16	Cont
100	0.000000	0.000375	0.001500
16	-0.00038	0.000000	0.001125
Cont	-0.0015	-0.00112	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

t
2.16037

Abs(Dif)-LSD	100	16	Cont
100	-0.0038	-0.00291	-0.0023
16	-0.00291	-0.00269	-0.00216
Cont	-0.0023	-0.00216	-0.0038

Positive values show pairs of means that are significantly different.

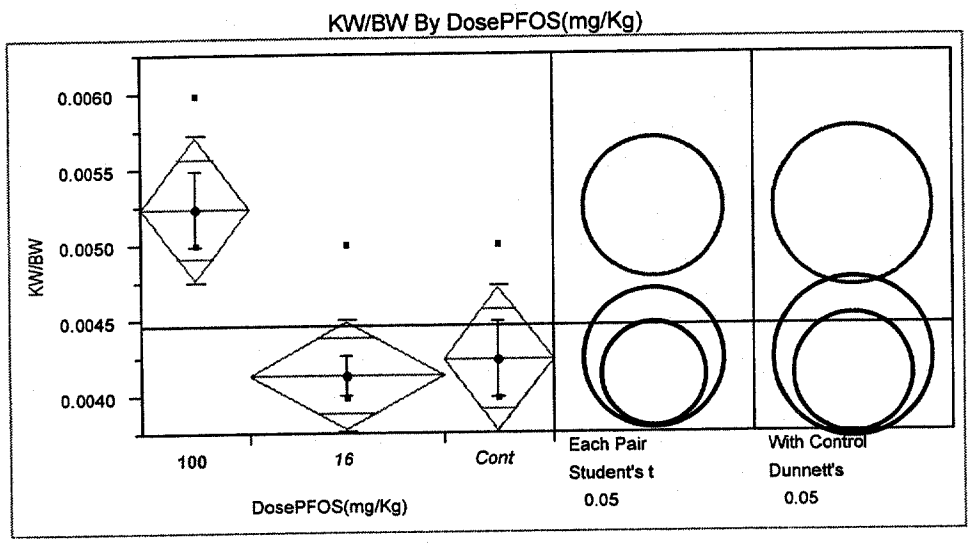
Comparisons with a control using Dunnett's Method

|d|
2.46358

Abs(Dif)-LSD	100
100	-0.00433
16	-0.00338
Cont	-0.00283

Positive values show pairs of means that are significantly different.

Part B. Kidney Weight to Body Weight ratio in male and female guinea pigs. SAS analysis



Oneway Anova
Summary of Fit

RSquare	0.58887
RSquare Adj	0.520349
Root Mean Square Error	0.000443
Mean of Response	0.004467
Observations (or Sum Wgts)	15

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.00000338	0.000002	8.5939
Error	12	0.00000236	1.964e-7	Prob>F
C Total	14	0.00000573	4.095e-7	0.0048

Means for Oneway Anova

Level	Number	Mean	Std Error
100	4	0.005250	0.00022
16	7	0.004143	0.00017
Cont	4	0.004250	0.00022

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
100	4	0.005250	0.000500	0.00025
16	7	0.004143	0.000378	0.00014
Cont	4	0.004250	0.000500	0.00025

Means Comparisons

Dif=Mean[i]-Mean[j]	100	Cont	16
100	0.000000	0.001000	0.001107
Cont	-0.001	0.000000	0.000107
16	-0.00111	-0.00011	0.000000

Alpha= 0.05

Comparisons for each pair using Student's t

t
2.17882

Abs(Dif)-LSD	100	Cont	16
100	-0.00068	0.000317	0.000502
Cont	0.000317	-0.00068	-0.0005
16	0.000502	-0.0005	-0.00052

Positive values show pairs of means that are significantly different.

Comparisons with a control using Dunnett's Method

|d|
2.49055

Abs(Dif)-LSD	100
100	-0.00078
Cont	0.000219
16	0.000415

Positive values show pairs of means that are significantly different.

Appendix IV. Part C Oral dosing with N-EtFOSE in rats. Individual data.

Animal#	Gender	Dose N-EtFOSE (mg/Kg/day) for two days.	Species	BW (g) d1	BW (g) d3	LW(g)	LW/BW	Sacrifice day post dose	Necropsy Date
8R02179	M	Control	Rat	384.6	388.7	17.3	0.045	3	9/18/98
8R02466	M	Control	Rat	416.3	420.4	20.1	0.048	3	9/18/98
8R02467	M	Control	Rat	453.7	461.2	21.9	0.047	3	9/18/98
8R02176	M		20 Rat	523.7	539	30.2	0.056	3	9/18/98
8R02177	M		20 Rat	485.6	488.4	23.8	0.049	3	9/18/98
8R02178	M		20 Rat	497.3	503	23.8	0.047	3	9/18/98

Note, 20 mg/Kg is approximately 300 ppm in the diet.
Rats were dosed on two consecutive days, 9/16/1998, and 9/17/1998 with 20 mg/Kg/day N-EtFOSE, then sacrificed on 9/18/1998.

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