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Study Title:

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA; 3M T-7091.1) in Rats

Study Number: TRC 1132-100

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Date:

Original Final Report: August 9, 2000
Amended Final Report: November 8, 2004

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STUDY REPORT

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA; 3M T-7091.1) in Rats

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STUDY TIMELINE:

Initiation: January 12, 1999
Completion of in-life phase: April 6, 1999
Final Report: August 9, 2000
Amended Final Report: November 8, 2004

REGULATORY COMPLIANCE

This study was conducted in the spirit of Good Laboratory Practice (GLP) regulations.

PURPOSE

The objective of this study was to assess cell proliferation and peroxisome proliferation in rats administered test material in the diet.

INTRODUCTION

This study was designed to assess cell proliferation and peroxisome proliferation in rats administered N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA; 3M T-7091.1). In addition, rats were administered Wy-14,643 as a positive control for cell proliferation and peroxisome proliferation. The endpoints evaluated for test article effect are listed in Text Table 1. Because this study was a multidisciplinary team effort, the results are summarized in Sections I, II and III of this report, and individual studies are reported in the Appendix.

Text Table 1. Experimental Endpoints Examined

Endpoint Examined	Performing Laboratory	Location of Report Herewithin
Cell proliferation	Pathology Associates	Sections I, II, III
Histopathology	Pathology Associates	Sections I, II, III
Clinical chemistry	LabCorp	Sections I, II, III
Body and liver weights	TherImmune	Sections I, II, IV - Appendix 1
Electron microscopy	Pathology Associates	Sections I, II, V - Appendix 2
Palmitoyl CoA oxidase	Covance	Sections I, II, VI - Appendix 3

FINAL REPORT AMENDEMENTS

The study report was originally finalized August 9, 2000. The present study report (amended final report dated November 8, 2004) has been revised to reflect a correction in the test article abbreviation for N-ethyl perfluorooctanesulfonamide from "PFOSA" to N-EtFOSA, and in the concentration of Wy-14,643 from "1000 ppm" to 100 ppm. These corrections have been made to all Sections of this report, except for Section IV (Appendix 1), which is the report from the testing facility (TherImmune Research Corporation; presently, Gene Logic) that was only obtainable in pdf format and therefore, unable to be revised. Additionally, due to the inability to obtain signatures from all participating investigators involved in this study, this amended final report is signed only by the principal investigator, Dr. Sandra Eldridge, on behalf of original signatures from Dr. Carolyn Moyer (study pathologist, Pathology Associates; originally signed August 9, 2000), Dr. James Nold (EM pathologist, Pathology Associates; originally signed October 4, 1999), Dr. Gary Wolfe (study director, TherImmune; originally signed May 2, 2000), and Dr. Ron Markevitch (Palmitoyl CoA Oxidase activity, Covance; originally signed December 1, 1999). The study protocol (original dated January 12, 1999) and all protocol amendments have also been revised to reflect the corrections noted above, and are included in this amended final study report unsigned.

MATERIALS AND METHODS

Experimental Design

The experimental design was as follows in Text Table 2.

Text Table 2. Group Designations, Dietary Levels and Scheduled Sacrifice Time Points

Group Number (Time Point)	Number of Male Rats							
	Control (0 ppm)	N-EtFOSE 300	100	30 ppm	PFOS 20 ppm	N-EtFOSA 100 ppm	Wy-14,643 100 ppm	Total No. of Animals
1 (48 hrs)	10	10	10	10	10	10	5	65
2 (7 days)	10	5	5	5	5	5	5	40
3 (14 days)	10	5	5	5	5	5	5	40
4 (1 wk recovery)	10	5	5	5	5	5	5	40
5 (4 wk recovery)	10	5	5	5	5	5	5	40
Total No. of Animals	50	30	30	30	30	30	25	225

In-life Portion of Study

The in-life portion of this study was conducted at TherImmune Research Corporation. Methods for the in-life portion of this study, including test article formulation and administration, test animals and husbandry, observations, termination and tissue collection are reported in Section IV, Appendix 1.

Cell Proliferation Staining and Evaluation

Representative samples of the left lateral lobe of the liver and any macroscopic lesions were collected and preserved in formalin. After fixation, each sample of liver was processed and stained proliferation cell nuclear antigen (PCNA). In addition, liver sections prepared from the same tissue blocks were stained with hematoxylin and eosin (H&E) and examined microscopically.

Sections of paraffin-embedded tissues were cut at approximately 5 μm and placed on positively charged slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA) to ensure adhesion during processing for PCNA. Standard immunohistochemical methods for PCNA were used to stain tissues. Briefly, tissue sections were incubated with a monoclonal antibody to PCNA (DAKO,

Carpinteria, CA) and reagents required for the avidin-biotin peroxidase (ABC Kit, Vectastain, Burlingame, CA) method for the detection of the antigen-antibody complex. PCNA expression was localized by the chromagen 3,3'-diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO). Tissue sections were counterstained with hematoxylin.

The percentage of hepatocytes in S-phase (labeling index, LI) was determined by scoring at least 3000 hepatocytes in 10 fields of liver. A negative control slide was included in the staining run and consisted of study tissue that was not incubated with the primary antibody.

For cell proliferation evaluations, slides were first perused at low magnification (100X) to judge quality of staining, processing and sectioning, potential patterns of cellular proliferation, and histomorphologic changes. Cell proliferation was then quantified at higher magnification (200X) as described above. Histomorphology was further assessed by evaluating the H&E slide prepared from the same tissue block for each animal evaluated for cell proliferation.

Clinical Chemistry

Animals were fasted overnight before animal's scheduled necropsy; blood was collected from a jugular vein into an EDTA-coated tube. Serum enzyme levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol and triglycerides were determined by LabCorp, and reported separately to Pathology Associates.

Tissue Collection for Electron Microscopic Evaluation

Sections of liver from all animals were collected, minced to approximately one millimeter cubes, placed in McDowell-Trump fixative, and submitted to Pathology Associates's North Carolina laboratory for processing, sectioning and evaluation. Electron microscopy was performed on select animals as described in Section V, Appendix 2.

Palmitoyl-CoA Oxidase Tissue Collection and Analyses

A sample (approximately 500 mg) of the right lateral lobe of the liver was collected from select animals and flash-frozen in liquid nitrogen. The liver tissue was stored in a freezer set to maintain -60 to -80° C until analyzed by Covance for palmitoyl-CoA Oxidase activity. The liver samples analyzed included all study animals, except for the Wy-14,643 animals and all animals from the 4-week recovery groups. In addition to this study, samples from a previous 3M study will be analyzed for palmitoyl-CoA Oxidase activity and reported separately; these samples consist of liver samples from 35 rats and 35 guinea pigs.

Remaining Liver Tissue

The remaining liver tissue was frozen and is being stored at -60 to -80° C for possible future analysis.

Statistical Analysis

The Student's *t*-test (two-sided, unequal variance) was used to test for statistical significance in LI and clinical chemistry between control and treatment groups using Microsoft Excel version 5.0. A *P* value of less than 0.05 was judged to be statistically significant. Analysis of variance using Dunnett's procedure was used to analyze body and organ weight data with a *P* value of less than 0.05 judged to be statistically significant.

Record Retention

All raw data, documentation, records, protocol, specimens, and final report generated as a result of this study will be archived in the storage facilities of Pathology Associates for a period of 1 year following submission of the final report to the Sponsor. One year after submission of the final report, all of the aforementioned materials will be sent to the Sponsor and a return fee will be charged. All raw data stored on magnetic media will be retained by Pathology Associates.

RESULTS

Cell Proliferation and Histopathology

Group mean PCNA labeling indices are presented in Section II, Table 1. The severity and incidence of cell proliferative responses are presented in Section II, Table 2. The severity is defined as the fold increase in PCNA LI compared to controls. The incidence represents the number of animals within a treatment group with a LI greater than the highest concurrent control value. Individual animal cell proliferation data are presented in Section III, Table 1.

Statistically significant increases in cell proliferation were revealed in only one treatment group: 100 ppm N-EtFOSA at the 7 day time point. Although statistically significant, the labeling indices for individual animals were within the range seen in the control group; therefore, this response was not biologically significant. Biologically significant increases in cell proliferation, as determined by a severity of at least 2-fold and an incidence of at least one animal in the treatment group, were identified only in the positive control group (100 ppm Wy-14,643) during the treatment period at 48 hours and 7 days. During the recovery period, biologically significant increases in cell proliferation were seen in 300 and 30 ppm N-EtFOSE, 20 ppm PFOS, and 100 ppm N-EtFOSA, but not in 100 ppm N-EtFOSE or 100 ppm Wy-14,643, at the 4 week recovery period.

Histologic findings are presented in Section III, Table 3. Examination of the H&E slides revealed no significant changes in the liver of rats sacrificed at 48 hours or 7 days. At 14 days, and 1 and 4 week recovery time points, lipid vacuolization was observed in animals from all groups, including controls. In addition, livers from Wy-14,643 treated animals exhibited mild Kupffer cell hypertrophy and multifocal, minimal hepatocellular necrosis at 14 days. At the 1 and 4 week recovery time points, no significant treatment related findings were noted, except for

the mild Kupffer cell hypertrophy seen in Wy-14,643 treated animals which was absent 4 weeks post-dosing.

Clinical Chemistry

Group mean clinical chemistry parameters are presented in Section II, Table 3. Individual animal clinical chemistry data are presented in Section III, Table 2

Statistical differences in ALT, ALP and AST were seen sporadically among treatment groups and time points, but were within the normal range for each parameter. Triglycerides were significantly decreased as well as outside the normal range in the following treatment groups: 300 ppm N-EtFOSE at 7 days and 1 week recovery, and 100 ppm N-EtFOSA at the same time points. Cholesterol was significantly decreased as well as outside the normal range in all treatment groups at one or more time points during the dosing period. At the 1 week recovery period, but not the 4 week recovery period, cholesterol remained decreased in all groups except 20 ppm PFOS and 100 ppm Wy-14,643.

Body and Liver Weight

Group mean body weights, liver weights and liver to body weight ratios are presented in Section II, Table 4. Individual animal body and organ weight data are presented in Section IV, Appendix 1.

Body weights were not affected during the dosing period. Mean body weight was reduced only at the 1 week recovery period in groups 300 ppm N-EtFOSE, 100 ppm N-EtFOSA and 100 ppm Wy-14,643. Liver weights were significantly elevated at one or more time points during the dosing period in all treatment groups except 30 ppm N-EtFOSE and 20 ppm PFOS. At the 48 hour time point, liver weights were elevated in groups 100 ppm N-EtFOSE and 100 ppm Wy-14,643. At the 7 day time point, liver weights were elevated in groups 100 ppm N-ETFOSA and 100 ppm Wy-14,643. At the 14 day time point, liver weights were elevated in group 300 ppm N-EtFOSE. At both the 1 and 4 week recovery periods, liver weight was elevated in the 300 ppm N-EtFOSE group.

Liver to body weight ratios were significantly increased at one or more time points during the dosing period in all treatment groups except 20 ppm PFOS. At both the 1 and 4 week recovery time points, liver to body weight ratios were significantly increased in treatment groups 300 ppm N-EtFOSE and 100 ppm N-EtFOSA, whereas Wy-14,643 was elevated at the 1 week recovery period, but not the 4 week.

Peroxisome Proliferation

Electron microscopy (EM) and analysis of palmitoyl CoA oxidase activity were used to assess peroxisome proliferation. Palmitoyl CoA oxidase group summary data are presented in Section

II, Table 5. The EM report is presented in Section V, Appendix 2, and individual animal palmitoyl CoA oxidase data are presented in Section VI, Appendix 3.

At 48 hours, Wy-14,643 induced a doubling in the number of peroxisomes compared to controls. None of the other treatment groups examined (100 ppm N-EtFOSE, 20 ppm PFOS or 100 ppm N-EtFOSA) revealed an increase in mean number of peroxisomes per hepatocyte.

Palmitoyl CoA oxidase activity did not differ remarkably among controls and all treatment groups at all time points examined.

DISCUSSION

In the present study, multiple endpoints were examined to assess cell proliferation and peroxisome proliferation in the liver of rats given N-EtFOSE, PFOS, N-EtFOSA, or Wy-14,643 as a positive control test material. A summary of the findings during the dosing period and recovery period are presented in Text Tables 3 and 4, respectively.

As expected, the positive control test material, 100 ppm Wy-14,643 produced the anticipated increase in hepatocellular proliferation and liver weight without a concomitant increase in liver-associated serum enzymes. Cholesterol was lowered as expected. These changes were reversed upon cessation of dosing.

In contrast, none of the test materials induced cell proliferation during the dosing period, whereas, during the recovery period, hepatocellular proliferation was increased in the N-EtFOSE, PFOS and N-EtFOSA groups. This apparent response may have been an aberration due to the low control group mean labeling index seen at the week 4 recovery time point. These animals, which were 13 weeks of age at this time, exhibited a control LI of only 0.016%. A PCNA labeling index of 0.17% has been reported for control rats of the same age (Eldridge and Goldsworthy, 1996). Thus, the control labeling index value seen in this study at the week 4 recovery time point is approximately 10-fold below that which has been previously reported. Furthermore, the response was not dose-related in the N-EtFOSE treatment groups.

Like Wy-14,643, the test materials did not affect liver-associated serum enzyme levels, but did cause a decrease in cholesterol. N-EtFOSE (300 ppm) and N-EtFOSA (100 ppm) also caused a decrease in triglycerides that was reversible within 4 weeks following cessation of dosing. It is interesting to note that Wy-14,643 only lowered triglycerides at 7 days of dosing, but not after 14 days of dosing.

Palmitoyl CoA oxidase activity and peroxisomal proliferation were not elevated in any test materials evaluated besides the positive control Wy-14,643.

Taken together, these findings do not support N-EtFOSE, PFOS or N-EtFOSA to be peroxisome proliferators in rats. Although these test materials produced an apparent cell proliferative response in the liver of rats, the proliferative response did not occur upon initial dosing as seen

with Wy-14,643, rather it appeared during the recovery period following 14 days of dosing and was not associated with overt cytotoxicity. The cell proliferative response seen at the week 4 recovery time point may have been an aberration due to the abnormally low control mean labeling index.

Like Wy-14,643, these materials decreased cholesterol in rats, but the lowering effect was reversible up to 4 weeks post-dosing as it was with Wy-14,643.

Text Table 3. Summary of Findings to Assess Hepatocellular Proliferation and Peroxisome Proliferation During the Dosing Period[a]

Endpoint	Controls	N-EtFOSE 300 ppm	N-EtFOSE 100 ppm	N-EtFOSE 30 ppm	PFOS 20 ppm	N-EtFOSA 100 ppm	Wy-14,643 100 ppm
Cell proliferation	-	-	-	-	-	-	Increase
Body weight	-	-	-	-	-	-	-
Liver weight	-	Increase	Increase	-	-	Increase	Increase
Liver/body wt.	-	Increase	Increase	Increase	-	Increase	Increase
ALT	-	-	-	-	-	-	-
ALP	-	-	-	-	-	-	-
AST	-	-	-	-	-	-	-
Triglycerides	-	Decrease	-	-	-	Decrease	-
Cholesterol	-	Decrease	Decrease	Decrease	Decrease	Decrease	Decrease
Palmitoyl CoA oxidase	-	-	-	-	-	-	ND
Peroxisomes	-	-	-	-	-	-	-

[a]An increase or decrease identified statistically and/or descriptively (judged to be biologically relevant) at any time during the 14 day dosing period.
ND, not determined

Text Table 4. Summary of Findings to Assess Hepatocellular Proliferation and Peroxisome Proliferation During the Recovery Period[a]

Endpoint	Controls	N-EtFOSE 300 ppm	N-EtFOSE 100 ppm	N-EtFOSE 30 ppm	PFOS 20 ppm	N-EtFOSA 100 ppm	Wy-14,643 100 ppm
Cell proliferation	-	Increase	-	Increase	Increase	Increase	-
Body weight	-	Decrease	-	-	-	Decrease	Decrease
Liver weight	-	Increase	-	-	-	-	-
Liver/body wt.	-	Increase	-	-	-	Increase	Increase
ALT	-	-	-	-	-	-	-
ALP	-	-	-	-	-	-	-
AST	-	-	-	-	-	-	-
Triglycerides	-	Decrease	-	-	-	Decrease	-
Cholesterol	-	Decrease	Decrease	Decrease	-	Decrease	-
Palmitoyl CoA oxidase	-	-	-	-	-	-	ND
Peroxisomes	ND	ND	ND	ND	ND	ND	ND

[a]An increase or decrease identified statistically and/or descriptively (judged to be biologically relevant) at any time during the recovery period.
ND, not determined

SUMMARY

N-EtFOSE, PFOS and N-EtFOSA are not peroxisome proliferators, do not have overt hepatocellular proliferative effects, but do exhibit a cholesterol lowering effect in rats that is reversible within 4 weeks following cessation of exposure.

LITERATURE CITED

Eldridge, S. R., and Goldsworthy, S. M. Cell proliferation rates in common cancer target tissues of B6C3F1 mice and F344 rats: effects of age, gender, and choice of marker. Fund. Appl. Toxicol. 32: 159-167, 1996.

II. SUMMARY TABLES

Table II-1. Group Summary of Cell Proliferation Data

Treatment Group	48 Hr. PCNA Labeling Index Mean	48 Hr. PCNA Labeling Index SEM	7 Day PCNA Labeling Index Mean	7 Day PCNA Labeling Index SEM	14 Day PCNA Labeling Index Mean	14 Day PCNA Labeling Index SEM
Controls	0.080%	0.012%	0.046%	0.013%	0.118%	0.028%
N-EtFOSE 300 ppm	0.052%	0.006%	0.031%	0.013%	0.058%	0.016%
N-EtFOSE 100 ppm	0.093%	0.030%	0.026%	0.008%	0.040%	0.012%
N-EtFOSE 30 ppm	0.047%	0.014%	0.030%	0.004%	0.113%	0.038%
PFOS 20 ppm	0.066%	0.013%	0.038%	0.012%	0.060%	0.014%
N-EtFOSA 100 ppm	0.063%	0.010%	0.105%*	0.012%	0.054%	0.011%
Wy-14,643 100 ppm	0.536%	0.256%	0.102%	0.036%	0.110%	0.048%

Treatment Group	1 Week Recovery PCNA Labeling Index Mean	1 Week Recovery PCNA Labeling Index SEM	4 Week Recovery PCNA Labeling Index Mean	4 Week Recovery PCNA Labeling Index SEM
Controls	0.050%	0.017%	0.016%	0.007%
N-EtFOSE 300 ppm	0.047%	0.007%	0.078%	0.046%
N-EtFOSE 100 ppm	0.092%	0.022%	0.023%	0.006%
N-EtFOSE 30 ppm	0.065%	0.025%	0.064%	0.030%
PFOS 20 ppm	0.052%	0.032%	0.050%	0.028%
N-EtFOSA 100 ppm	0.063%	0.011%	0.049%	0.016%
Wy-14,643 100 ppm	0.042%	0.025%	0.030%	0.024%

*Statistically greater than controls, P<0.05

Table II-2. Severity and Incidence of Proliferative Responses in Rat Liver

Treatment Group	48 Hr. PCNA Labeling Index Severity[a]	48 Hr. PCNA Labeling Index Incidence[b]	7 Day PCNA Labeling Index Severity	7 Day PCNA Labeling Index Incidence	14 Day PCNA Labeling Index Severity	14 Day PCNA Labeling Index Incidence
Controls	-	-	-	-	-	-
N-EtFOSE 300 ppm	0.7	0/9	0.7	0/5	0.5	0/5
N-EtFOSE 100 ppm	1.2	2/10	0.6	0/5	0.3	0/5
N-EtFOSE 30 ppm	0.6	1/10	0.7	0/5	1.0	0/5
PFOS 20 ppm	0.8	1/10	0.8	0/5	0.5	0/5
N-EtFOSA 100 ppm	0.8	0/10	2.3	0/5	0.5	0/5
Wy-14,643 100 ppm	6.7	4/5	2.2	1/3	0.9	0/5

Treatment Group	1 Week Recovery PCNA Labeling Index Severity	1 Week Recovery PCNA Labeling Index Incidence	4 Week Recovery PCNA Labeling Index Severity	4 Week Recovery PCNA Labeling Index Incidence
Controls	-	-	-	-
N-EtFOSE 300 ppm	0.9	0/5	4.9	2/5
N-EtFOSE 100 ppm	1.8	0/5	1.4	0/5
N-EtFOSE 30 ppm	1.3	1/5	4.0	2/5
PFOS 20 ppm	1.0	1/5	3.1	2/5
N-EtFOSA 100 ppm	1.3	0/5	2.9	3/5
Wy-14,643 100 ppm	0.8	0/5	1.9	1/5

[a]Fold increase over controls

[b]Number of animals with a PCNA labeling index greater than the highest control value

Table II-3. Group Summary of Clinical Chemistry Values

ALANINE AMINOTRANSFERASE (Normal Range: 20 - 60)

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	31.8	34.8	38.0	36.4	40.3
N-EtFOSE 300 ppm	32.8	43.0	42.6	38.8	49.6
N-EtFOSE 100 ppm	32.1	34.2	44.4	44.4	50.6*
N-EtFOSE 30 ppm	31.0	34.4	38.8	38.4	41.6
PFOS 20 ppm	32.7	33.2	46.4*	41.0	42.4
N-EtFOSA 100 ppm	30.2	31.8	44.4	47.6	77.4
Wy-14,643 100 ppm	36.3	33.2	60.6*	93.2	38.8

ALKALINE PHOSPHATASE (Normal Range: 150 - 380)

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	296.8	287.6	208.3	184.1	137.4
N-EtFOSE 300 ppm	263.9	249.8	193.0	199.0	140.8
N-EtFOSE 100 ppm	281.3	239.8	198.4	151.6	130.4
N-EtFOSE 30 ppm	297.4	244.4	227.0	233.4*	117.8
PFOS 20 ppm	286.3	251.6	265.0	195.6	129.8
N-EtFOSA 100 ppm	316.5	294.4	273.2	176.4	136.8
Wy-14,643 100 ppm	306.0	287.2	326.0*	171.0	118.2

ASPARTATE AMINOTRANSFERASE (Normal Range: 70 - 125)

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	144.8	155.6	131.3	120.4	154.4
N-EtFOSE 300 ppm	123.6	135.4	133.6	112.4	159.2
N-EtFOSE 100 ppm	127.3	127.6	139.2	139.4	178.0
N-EtFOSE 30 ppm	145.8	132.6	141.8	123.4	187.0
PFOS 20 ppm	136.9	134.6	152.2	115.0	176.8
N-EtFOSA 100 ppm	130.9	132.6	121.4	129.4	185.2
Wy-14,643 100 ppm	150.0	119.4*	173.2	164.8	133.0

Table II-3, continued

TRIGLYCERIDES (Normal Range: 25 - 120)

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	41.6	50.8	37.0	46.5	36.1
N-EtFOSE 300 ppm	30.3	18.2*	23.4	24.0*	34.6
N-EtFOSE 100 ppm	37.5	29.6*	30.0	33.6	48.6
N-EtFOSE 30 ppm	47.4	37.4	37.2	30.4*	45.0
PFOS 20 ppm	34.4	36.4	31.0	27.6*	38.8
N-EtFOSA 100 ppm	30.6	35.4	19.8*	23.6*	34.2
Wy-14,643 100 ppm	45.4	35.2*	43.6	41.0	70.0

CHOLESTEROL (Normal Range: 50 - 150)

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	67.3	68.3	59.8	54.5	48.3
N-EtFOSE 300 ppm	56.1	37.0*	40.0*	30.4*	49.4
N-EtFOSE 100 ppm	67.3	39.4*	41.4*	37.8*	47.4
N-EtFOSE 30 ppm	60.3	48.2*	50.6	43.0*	52.6
PFOS 20 ppm	61.1	58.4*	42.0*	47.2	50.2
N-EtFOSA 100 ppm	58.4	53.6*	34.8*	38.6*	48.6
Wy-14,643 100 ppm	36.8*	61.0	87.2*	82.8*	66.6

*Statistically different from controls, $P<0.05$

Table II-4. Group Summary of Body and Liver Weights

MEAN BODY WEIGHT

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	207.6 g	248.1 g	297.4 g	339.3 g	407.3 g
N-EtFOSE 300 ppm	208.7 g	235.1 g	287.7 g	293.3 g *	395.5 g
N-EtFOSE 100 ppm	221.1 g	258.6 g	284.4 g	318.0 g	385.0 g
N-EtFOSE 30 ppm	207.9 g	244.2 g	300.0 g	321.9 g	393.6 g
PFOS 20 ppm	208.9 g	243.4 g	285.8 g	320.5 g	401.9 g
N-EtFOSA 100 ppm	213.7 g	257.5 g	268.1 g	309.8 g *	382.7 g
Wy-14,643 100 ppm	217.2 g	253.6 g	283.1 g	289.2 g *	392.7 g

MEAN LIVER WEIGHT

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	7.514 g	8.475 g	9.795 g	11.321 g	12.800 g
N-EtFOSE 300 ppm	7.878 g	9.642 g	14.148 g *	14.118 g *	17.146 g *
N-EtFOSE 100 ppm	8.515 g*	9.665 g	11.272 g	11.966 g	12.785 g
N-EtFOSE 30 ppm	7.788 g	8.541 g	11.053 g	10.549 g	13.103 g
PFOS 20 ppm	7.748 g	8.830 g	10.002 g	11.095 g	13.457 g
N-EtFOSA 100 ppm	7.982 g	10.193 g *	10.766 g	12.681 g	15.152 g
Wy-14,643 100 ppm	9.555 g *	16.087 g *	19.975 g *	11.991 g	13.480 g

MEAN LIVER WEIGHT TO BODY WEIGHT RATIO

Treatment Group	48 hr	7 days	14 days	1 week recovery	4 week recovery
Control	3.620%	3.409%	3.290%	3.320%	3.115%
N-EtFOSE 300 ppm	3.769%	4.105%*	4.903%*	4.794%*	4.333%*
N-EtFOSE 100 ppm	3.850%	3.740%	3.967%*	3.764%	3.316%
N-EtFOSE 30 ppm	3.749%	3.496%	3.684%*	3.278%	3.346%
PFOS 20 ppm	3.703%	3.630%	3.495%	3.460%	3.351%
N-EtFOSA 100 ppm	3.731%	3.949%*	4.013%*	4.094%*	3.956%*
Wy-14,643 100 ppm	4.400%*	6.349%*	7.058%*	4.142%*	3.417%

*Statistically different from controls, $P<0.05$

Table II-5. Group Summary of Palimitoyl CoA Oxidase Activity

Treatment Group	48 Hr. PCOAO[a] Mean	48 Hr. PCOAO S.D.	48 Hr. PCOAO Range	48 Hr. Sample Size	7 Day PCOAO Mean	7 Day PCOAO S.D.	7 Day PCOAO Range	7 Day Sample Size
Controls	8	1.8	5 - 11	10	5	1.3	4 - 8	10
N-EtFOSE 300 ppm	9	2.9	6 - 16	10	6	2.5	3 - 10	5
N-EtFOSE 100 ppm	8	2.3	4 - 12	10	7	1.9	4 - 9	5
N-EtFOSE 30 ppm	9	2.1	6 - 13	10	6	1.9	4 - 9	5
PFOS 20 ppm	8	1.9	6 - 11	10	7	1.5	5 - 9	5
N-EtFOSA 100 ppm	7	1.9	5 - 11	10	6	2.1	3 - 8	5

Treatment Group	14 Day PCOAO Mean	14 Day PCOAO S.D.	14 Day PCOAO Range	14 Day Sample Size	1 Week Recovery PCOAO Mean	1 Week Recovery PCOAO S.D.	1 Week Recovery PCOAO Range	1 Week Recovery Sample Size
Controls	6	1.4	5 - 9	10	7	2.3	3 - 10	10
N-EtFOSE 300 ppm	3	0.8	2 - 4	5	4	1.3	3 - 6	5
N-EtFOSE 100 ppm	3	1.6	1 - 5	5	5	0.8	4 - 6	5
N-EtFOSE 30 ppm	5	0.8	4 - 6	5	6	1.9	4 - 8	5
PFOS 20 ppm	4	0.4	4 - 5	5	6	1.8	3 - 8	5
N-EtFOSA 100 ppm	3	1.1	2 - 4	5	6	0.4	6 - 7	5

[a]Palmitoyl CoA Oxidase activity, IU/g

III. INDIVIDUAL ANIMAL DATA TABLES

Table III-1. Individual Animal Cell Proliferation Data
48 Hour, 7 Day and 14 Day Sacrifices

	48 Hour Interim Sacrifice		7 Day Interim Sacrifice		14 Day Interim Sacrifice	
Treatment Group	Animal Number	PCNA Labeling Index	Animal Number	PCNA Labeling Index	Animal Number	PCNA Labeling Index
Controls	R10381	0.145%	R10446	0.071%	R10486	0.106%
	R10382	0.052%	R10447	0.058%	R10487	0.190%
	R10383	0.081%	R10448	0.030%	R10488	0.332%
	R10384	0.078%	R10449	0.000%	R10489	0.056%
	R10385	0.113%	R10450	0.027%	R10490	0.082%
	R10386	0.086%	R10451	0.136%	R10491	0.133%
	R10387	0.105%	R10452	0.058%	R10492	0.056%
	R10388	0.080%	R10453	0.050%	R10493	0.086%
	R10389	0.027%	R10454	0.027%	R10494	0.026%
	R10390	0.029%	R10455	0.000%	R10495	0.117%
N-EtFOSE 300 ppm	R10391	0.053%	R10456	0.000%	R10496	0.119%
	R10392	0.052%	R10457	0.077%	R10497	0.029%
	R10393	0.057%	R10458	0.027%	R10498	0.056%
	R10394	0.058%	R10459	0.024%	R10499	0.031%
	R10395	ND	R10460	0.027%	R10500	0.055%
	R10396	0.059%				
	R10397	0.026%				
	R10398	0.085%				
	R10399	0.053%				
	R10400	0.026%				
N-EtFOSE 100 ppm	R10401	0.056%	R10461	0.024%	R10501	0.028%
	R10402	0.087%	R10462	0.026%	R10502	0.058%
	R10403	0.190%	R10463	0.053%	R10503	0.059%
	R10404	0.323%	R10464	0.000%	R10504	0.000%
	R10405	0.029%	R10465	0.025%	R10505	0.057%
	R10406	0.103%				
	R10407	0.000%				
	R10408	0.055%				
	R10409	0.055%				
	R10410	0.029%				
N-EtFOSE 30 ppm	R10411	0.152%	R10466	0.026%	R10506	0.152%
	R10412	0.000%	R10467	0.025%	R10507	0.240%
	R10413	0.084%	R10468	0.047%	R10508	0.088%
	R10414	0.055%	R10469	0.024%	R10509	0.057%
	R10415	0.059%	R10470	0.026%	R10510	0.029%
	R10416	0.030%				
	R10417	0.000%				
	R10418	0.029%				
	R10419	0.030%				
	R10420	0.028%				
PFOS 20 ppm	R10421	0.153%	R10471	0.029%	R10511	0.090%
	R10422	0.053%	R10472	0.085%	R10512	0.030%
	R10423	0.052%	R10473	0.026%	R10513	0.093%
	R10424	0.086%	R10474	0.026%	R10514	0.030%
	R10425	0.027%	R10475	0.026%	R10515	0.059%
	R10426	0.053%				
	R10427	0.027%				
	R10428	0.027%				
	R10429	0.077%				
	R10430	0.109%				
N-EtFOSA 100 ppm	R10431	0.089%	R10476	0.130%	R10516	0.031%
	R10432	0.059%	R10477	0.076%	R10517	0.031%
	R10433	0.084%	R10478	0.134%	R10518	0.057%
	R10434	0.054%	R10479	0.077%	R10519	0.090%
	R10435	0.000%	R10480	0.106%	R10520	0.059%
	R10436	0.029%				
	R10437	0.085%				
	R10438	0.059%				
	R10439	0.114%				
	R10440	0.058%				
Wy-14,643 100 ppm	R10441	1.543%	R10481	ND	R10521	0.290%
	R10442	0.274%	R10482	ND	R10522	0.029%
	R10443	0.326%	R10483	0.078%	R10523	0.029%
	R10444	0.117%	R10484	0.173%	R10524	0.115%
	R10445	0.422%	R10485	0.054%	R10525	0.089%

ND, not determined due to lack of tissues on slide or suboptimal staining

Table III-1. Individual Animal Cell Proliferation Data 1 and 4 Week Recovery

	1 Week Recovery Sacrifice		4 Week Recovery Sacrifice	
Treatment Group	Animal Number	PCNA Labeling Index	Animal Number	PCNA Labeling Index
Controls	R10526	0.152%	R10566	0.058%
	R10527	0.026%	R10567	0.000%
	R10528	0.028%	R10568	0.028%
	R10529	0.028%	R10569	0.000%
	R10530	0.027%	R10570	0.000%
	R10531	0.150%	R10571	ND
	R10532	0.029%	R10572	0.028%
	R10533	0.029%	R10573	0.000%
	R10534	0.000%	R10574	0.000%
	R10535	0.027%	R10575	0.027%
N-EtFOSE 300 ppm	R10536	0.059%	R10576	0.000%
	R10537	0.056%	R10577	0.000%
	R10538	0.058%	R10578	0.241%
	R10539	0.030%	R10579	0.030%
	R10540	0.031%	R10580	0.117%
N-EtFOSE 100 ppm	R10541	0.146%	R10581	0.028%
	R10542	0.089%	R10582	0.029%
	R10543	0.062%	R10583	0.030%
	R10544	0.137%	R10584	0.000%
	R10545	0.028%	R10585	0.029%
N-EtFOSE 30 ppm	R10546	0.154%	R10586	0.175%
	R10547	0.029%	R10587	0.030%
	R10548	0.087%	R10588	0.000%
	R10549	0.029%	R10589	0.059%
	R10550	0.028%	R10590	0.058%
PFOS 20 ppm	R10551	0.060%	R10591	0.077%
	R10552	0.000%	R10592	0.028%
	R10553	0.000%	R10593	0.146%
	R10554	0.173%	R10594	0.000%
	R10555	0.027%	R10595	0.000%
N-EtFOSA 100 ppm	R10556	0.028%	R10596	0.031%
	R10557	0.090%	R10597	0.060%
	R10558	0.059%	R10598	0.000%
	R10559	0.083%	R10599	0.061%
	R10560	0.057%	R10600	0.094%
Wy-14,643 100 ppm	R10561	0.000%	R10601	0.026%
	R10562	0.000%	R10602	0.000%
	R10563	0.054%	R10603	0.126%
	R10564	0.026%	R10604	0.000%
	R10565	0.132%	R10605	0.000%

ND, not determined due to lack of tissues on slide or suboptimal staining

Table III-2. Individual Animal Clinical Chemistry
48 Hour Interim Sacrifice

Treatment Group	Animal Number	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	TRIG (mg/dl)	CHOL (mg/dl)
Controls	R10381	33	259	138	22	85
	R10382	29	349	131	57	70
	R10383	38	250	148	25	65
	R10384	33	293	163	32	49
	R10385	30	266	130	60	68
	R10386	29	205	127	34	66
	R10387	37	316	218	65	56
	R10388	33	293	137	41	72
	R10389	20	346	148	30	85
	R10390	36	391	108	50	57
N-EtFOSE 300 ppm	R10391	33	399	126	22	43
	R10392	27	251	134	21	60
	R10393	30	318	134	12	43
	R10394	39	226	153	51	55
	R10395	33	166	115	43	73
	R10396	37	322	102	28	52
	R10397	29	244	90	27	67
	R10398	25	144	99	41	71
	R10399	33	322	132	17	38
	R10400	42	247	151	41	59
N-EtFOSE 100 ppm	R10401	35	212	120	27	58
	R10402	44	273	137	47	64
	R10403	37	277	143	37	75
	R10404	36	367	171	40	64
	R10405	32	275	169	39	75
	R10406	33	291	101	49	81
	R10407	31	342	126	35	51
	R10408	22	255	92	31	72
	R10409	28	294	117	40	67
	R10410	23	227	97	30	66
N-EtFOSE 30 ppm	R10411	31	297	153	39	71
	R10412	35	352	125	19	30
	R10413	51	301	241	51	62
	R10414	36	268	123	33	75
	R10415	23	373	98	98	64
	R10416	21	184	111	37	75
	R10417	30	306	182	21	61
	R10418	31	296	205	46	57
	R10419	26	337	112	72	57
	R10420	26	260	108	58	51
PFOS 20 ppm	R10421	36	338	144	33	68
	R10422	48	369	155	34	51
	R10423	30	256	103	31	38
	R10424	39	232	219	40	65
	R10425	31	189	99	22	68
	R10426	30	303	149	42	93
	R10427	29	245	141	17	41
	R10428	27	235	122	54	61
	R10429	35	208	127	48	84
	R10430	22	488	110	23	42
N-EtFOSA 100 ppm	R10431	34	421	123	23	57
	R10432	39	435	171	16	45
	R10433	30	194	114	27	75
	R10434	34	252	124	44	60
	R10435	36	397	123	30	43
	R10436	27	426	171	27	44
	R10437	26	210	120	49	81
	R10438	28	307	121	34	61
	R10439	25	334	146	23	41
	R10440	23	189	96	33	77
Wy-14,643 100 ppm	R10441	41	443	147	33	35
	R10442	45	254	187	43	33
	R10443	33	319	151	39	44
	R10444	37	256	133	37	36
	R10445	27	258	132	75	36

Table III-2. Individual Animal Clinical Chemistry
7 Day Interim Sacrifice

Treatment Group	Animal Number	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	TRIG (mg/dl)	CHOL (mg/dl)
Controls	R10446	41	315	216	67	71
	R10447	40	279	137	35	66
	R10448	29	214	152	34	71
	R10449	30	373	118	38	79
	R10450	32	237	138	32	55
	R10451	31	313	178	83	65
	R10452	31	370	144	59	61
	R10453	30	203	216	52	56
	R10454	37	289	134	61	72
	R10455	47	283	123	47	87
N-EtFOSE 300 ppm	R10456	30	181	121	13	36
	R10457	39	311	130	15	33
	R10458	43	353	121	18	36
	R10459	67	228	168	18	41
	R10460	36	176	137	27	39
N-EtFOSE 100 ppm	R10461	38	204	172	27	36
	R10462	34	245	132	19	34
	R10463	32	214	85	44	56
	R10464	33	335	145	17	34
	R10465	34	201	104	41	37
N-EtFOSE 30 ppm	R10466	31	208	125	47	60
	R10467	32	188	116	38	49
	R10468	34	261	103	45	48
	R10469	37	242	128	23	33
	R10470	38	323	191	34	51
PFOS 20 ppm	R10471	33	166	126	47	53
	R10472	35	216	137	19	60
	R10473	40	373	123	25	67
	R10474	29	188	138	48	60
	R10475	29	315	149	43	52
N-EtFOSA 100 ppm	R10476	39	297	156	29	55
	R10477	32	414	177	24	44
	R10478	27	180	77	76	69
	R10479	27	232	124	24	58
	R10480	34	349	129	24	42
Wy-14,643 100 ppm	R10481	35	254	108	21	49
	R10482	26	254	127	34	71
	R10483	32	275	131	39	65
	R10484	35	322	85	38	51
	R10485	38	331	146	44	69

Table III-2. Individual Animal Clinical Chemistry
14 Day Interim Sacrifice

Treatment Group	Animal Number	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	TRIG (mg/dl)	CHOL (mg/dl)
Controls	R10486	39	169	133	53	74
	R10487	45	166	132	34	77
	R10488	39	212	110	50	58
	R10489	32	226	158	25	49
	R10490	36	212	141	29	57
	R10491	34	229	164	48	72
	R10492	35	282	128	37	39
	R10493	37	196	88	37	66
	R10494	41	176	112	23	52
	R10495	42	215	136	34	54
N-EtFOSE 300 ppm	R10496	37	226	128	35	48
	R10497	33	134	97	19	31
	R10498	34	134	119	19	42
	R10499	57	208	167	17	35
	R10500	52	263	157	27	44
N-EtFOSE 100 ppm	R10501	51	284	118	19	27
	R10502	33	201	121	31	40
	R10503	62	154	174	18	39
	R10504	31	148	169	48	55
	R10505	45	205	114	34	46
N-EtFOSE 30 ppm	R10506	35	286	151	54	50
	R10507	51	149	206	41	50
	R10508	45	267	119	31	46
	R10509	29	219	121	43	54
	R10510	34	214	112	17	54
PFOS 20 ppm	R10511	50	206	113	36	54
	R10512	42	340	146	16	21
	R10513	50	230	197	34	39
	R10514	43	280	195	17	46
	R10515	47	269	110	52	50
N-EtFOSA 100 ppm	R10516	48	220	149	20	36
	R10517	63	249	148	22	36
	R10518	48	419	92	25	36
	R10519	34	238	114	15	27
	R10520	29	240	104	17	39
Wy-14,643 100 ppm	R10521	54	313	137	22	73
	R10522	62	234	161	35	107
	R10523	63	354	242	57	93
	R10524	49	375	160	55	87
	R10525	75	354	166	49	76

Table III-2. Individual Animal Clinical Chemistry
1 Week Recovery Sacrifice

Treatment Group	Animal Number	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	TRIG (mg/dl)	CHOL (mg/dl)
Controls	R10526	57	158	243	45	63
	R10527	34	274	114	81	61
	R10528	59	176	187	52	62
	R10529	27	150	105	39	67
	R10530	34	166	106	18	40
	R10531	27	162	96	27	42
	R10532	30	271	98	52	53
	R10533	32	118	71	74	50
	R10534	29	226	94	29	48
	R10535	35	140	90	48	59
N-EtFOSE 300 ppm	R10536	29	199	158	6	17
	R10537	46	197	123	31	23
	R10538	47	215	112	20	32
	R10539	29	191	78	24	33
	R10540	43	193	91	39	47
N-EtFOSE 100 ppm	R10541	65	123	269	51	49
	R10542	32	142	119	26	35
	R10543	50	159	94	23	28
	R10544	37	190	108	32	28
	R10545	38	144	107	36	49
N-EtFOSE 30 ppm	R10546	34	250	115	29	47
	R10547	32	228	154	19	39
	R10548	40	233	127	30	48
	R10549	43	252	118	37	32
	R10550	43	204	103	37	49
PFOS 20 ppm	R10551	48	191	125	23	33
	R10552	47	249	114	31	50
	R10553	35	135	145	21	53
	R10554	42	214	111	26	38
	R10555	33	189	80	37	62
N-EtFOSA 100 ppm	R10556	37	150	125	24	39
	R10557	39	178	138	20	32
	R10558	52	207	112	17	43
	R10559	40	186	138	22	31
	R10560	70	161	134	35	48
Wy-14,643 100 ppm	R10561	57	157	162	51	105
	R10562	112	141	148	55	98
	R10563	52	150	123	28	70
	R10564	70	186	150	44	78
	R10565	175	221	241	27	63

Table III-2. Individual Animal Clinical Chemistry
4 Week Recovery Sacrifice

Treatment Group	Animal Number	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	TRIG (mg/dl)	CHOL (mg/dl)
Controls	R10566	40	160	170	51	47
	R10567	38	108	171	32	62
	R10568	40	133	153	25	47
	R10569	58	125	155	39	53
	R10570	49	167	139	30	54
	R10571	ND	ND	ND	ND	ND
	R10572	34	137	125	37	41
	R10573	32	135	156	32	43
	R10574	37	135	202	51	49
	R10575	35	137	119	28	39
N-EtFOSE 300 ppm	R10576	51	133	187	36	63
	R10577	43	113	136	32	38
	R10578	47	140	161	33	58
	R10579	47	155	158	24	27
	R10580	60	163	154	48	61
N-EtFOSE 100 ppm	R10581	55	142	181	40	39
	R10582	46	138	222	33	38
	R10583	47	126	169	104	64
	R10584	57	152	164	31	44
	R10585	48	94	154	35	52
N-EtFOSE 30 ppm	R10586	54	120	207	21	54
	R10587	31	154	137	49	44
	R10588	40	84	206	28	47
	R10589	35	124	225	58	45
	R10590	48	107	160	69	73
PFOS 20 ppm	R10591	46	147	235	25	43
	R10592	36	127	183	39	55
	R10593	49	139	186	29	35
	R10594	38	115	148	36	45
	R10595	43	121	132	65	73
N-EtFOSA 100 ppm	R10596	51	133	143	43	48
	R10597	103	173	256	28	49
	R10598	125	137	242	35	38
	R10599	35	95	120	40	60
	R10600	73	146	165	25	48
Wy-14,643 100 ppm	R10601	42	144	168	51	60
	R10602	33	105	168	132	89
	R10603	38	123	104	51	50
	R10604	43	118	124	52	67
	R10605	38	101	101	64	67

Table III-3. Individual Animal Histopathology Findings
48 Hour Interim Sacrifice

Treatment Group	Animal Number	Histopathology Findings Liver
Controls	R10381	No significant findings
	R10382	No significant findings
	R10383	No significant findings
	R10384	No significant findings
	R10385	No significant findings
	R10386	No significant findings
	R10387	No significant findings
	R10388	No significant findings
	R10389	No significant findings
	R10390	No significant findings
N-EtFOSE 300 ppm	R10391	No significant findings
	R10392	No significant findings
	R10393	No significant findings
	R10394	No significant findings
	R10395	No significant findings
	R10396	No significant findings
	R10397	No significant findings
	R10398	No significant findings
	R10399	No significant findings
	R10400	No significant findings
N-EtFOSE 100 ppm	R10401	No significant findings
	R10402	No significant findings
	R10403	No significant findings
	R10404	No significant findings
	R10405	No significant findings
	R10406	No significant findings
	R10407	No significant findings
	R10408	No significant findings
	R10409	No significant findings
	R10410	No significant findings
N-EtFOSE 30 ppm	R10411	No significant findings
	R10412	No significant findings
	R10413	No significant findings
	R10414	No significant findings
	R10415	No significant findings
	R10416	No significant findings
	R10417	No significant findings
	R10418	No significant findings
	R10419	No significant findings
	R10420	No significant findings
PFOS 20 ppm	R10421	No significant findings
	R10422	No significant findings
	R10423	No significant findings
	R10424	No significant findings
	R10425	No significant findings
	R10426	No significant findings
	R10427	No significant findings
	R10428	No significant findings
	R10429	No significant findings
	R10430	No significant findings
N-EtFOSA 100 ppm	R10431	No significant findings
	R10432	No significant findings
	R10433	No significant findings
	R10434	No significant findings
	R10435	No significant findings
	R10436	No significant findings
	R10437	No significant findings
	R10438	No significant findings
	R10439	No significant findings
	R10440	No significant findings
Wy-14,643 100 ppm	R10441	No significant findings
	R10442	No significant findings
	R10443	No significant findings
	R10444	No significant findings
	R10445	No significant findings

Table III-3. Individual Animal Histopathology Findings
7 Day Interim Sacrifice

Treatment Group	Animal Number	Histopathology Findings Liver
Controls	R10446	No significant findings
	R10447	No significant findings
	R10448	No significant findings
	R10449	No significant findings
	R10450	No significant findings
	R10451	No significant findings
	R10452	No significant findings
	R10453	No significant findings
	R10454	No significant findings
	R10455	No significant findings
N-EtFOSE 300 ppm	R10456	No significant findings
	R10457	No significant findings
	R10458	No significant findings
	R10459	No significant findings
	R10460	No significant findings
N-EtFOSE 100 ppm	R10461	No significant findings
	R10462	No significant findings
	R10463	No significant findings
	R10464	No significant findings
	R10465	No significant findings
N-EtFOSE 30 ppm	R10466	No significant findings
	R10467	No significant findings
	R10468	No significant findings
	R10469	No significant findings
	R10470	No significant findings
PFOS 20 ppm	R10471	No significant findings
	R10472	No significant findings
	R10473	No significant findings
	R10474	No significant findings
	R10475	No significant findings
N-EtFOSA 100 ppm	R10476	No significant findings
	R10477	No significant findings
	R10478	No significant findings
	R10479	No significant findings
	R10480	No significant findings
Wy-14,643 100 ppm	R10481	No significant findings
	R10482	No significant findings
	R10483	No significant findings
	R10484	No significant findings
	R10485	No significant findings

Table III-3. Individual Animal Histopathology Findings
14 Day Interim Sacrifice

Treatment Group	Animal Number	Histopathology Findings Liver
Controls	R10486	lipid vacuolization, lipid, minimal
	R10487	lipid vacuolization, lipid, minimal
	R10488	No significant findings
	R10489	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10490	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10491	No Significant Findings
	R10492	lipid vacuolization, lipid, minimal
	R10493	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10494	lipid vacuolization, lipid, minimal
	R10495	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
N-EtFOSE 300 ppm	R10496	lipid vacuolization, lipid, mild
	R10497	lipid vacuolization, lipid, mild
	R10498	lipid vacuolization, lipid, mild
	R10499	lipid vacuolization, lipid, mild
	R10500	lipid vacuolization, lipid, mild
N-EtFOSE 100 ppm	R10501	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10502	lipid vacuolization, lipid, mild
	R10503	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10504	No significant findings
	R10505	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
N-EtFOSE 30 ppm	R10506	lipid vacuolization, lipid, mild
	R10507	lipid vacuolization, lipid, mild
	R10508	lipid vacuolization, lipid, mild
	R10509	lipid vacuolization, lipid, minimal
	R10510	lipid vacuolization, lipid, minimal
PFOS 20 ppm	R10511	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10512	No significant findings
	R10513	No significant findings
	R10514	No significant findings
	R10515	lipid vacuolization, lipid, minimal
N-EtFOSA 100 ppm	R10516	lipid vacuolization, lipid, mild
	R10517	lipid vacuolization, lipid, minimal
	R10518	lipid vacuolization, lipid, minimal
	R10519	lipid vacuolization, lipid, minimal
	R10520	lipid vacuolization, lipid, minimal
Wy-14,643 100 ppm	R10521	hypertrophy, Kupffer cell, mild; hepatocellular necrosis, multifocal, minimal; lipid vacuolization, lipid, minimal
	R10522	hypertrophy, Kupffer cell, mild; hepatocellular necrosis, multifocal, minimal; lipid vacuolization, lipid, minimal
	R10523	hypertrophy, Kupffer cell, mild; hepatocellular necrosis, multifocal, minimal; lipid vacuolization, lipid, minimal
	R10524	hypertrophy, Kupffer cell, mild; hepatocellular necrosis, multifocal, minimal; lipid vacuolization, lipid, minimal
	R10525	hypertrophy, Kupffer cell, mild; hepatocellular necrosis, multifocal, minimal; lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal

Table III-3. Individual Animal Histopathology Findings
1 Week Recovery Sacrifice

Treatment Group	Animal Number	Histopathology Findings Liver
Controls	R10526	No significant findings
	R10527	lipid vacuolization, lipid, mild
	R10528	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10529	lipid vacuolization, lipid, mild
	R10530	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10531	lipid vacuolization, lipid, minimal
	R10532	lipid vacuolization, lipid, minimal
	R10533	lipid vacuolization, lipid, minimal
	R10534	lipid vacuolization, lipid, minimal
	R10535	lipid vacuolization, lipid, mild
N-EtFOSE 300 ppm	R10536	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10537	lipid vacuolization, lipid, mild
	R10538	lipid vacuolization, lipid, mild
	R10539	lipid vacuolization, lipid, mild
	R10540	lipid vacuolization, lipid, minimal
N-EtFOSE 100 ppm	R10541	lipid vacuolization, lipid, minimal
	R10542	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10543	lipid vacuolization, lipid, mild
	R10544	lipid vacuolization, lipid, minimal
	R10545	lipid vacuolization, lipid, minimal
N-EtFOSE 30 ppm	R10546	lipid vacuolization, lipid, mild
	R10547	lipid vacuolization, lipid, minimal
	R10548	lipid vacuolization, lipid, minimal
	R10549	lipid vacuolization, lipid, minimal
	R10550	lipid vacuolization, lipid, minimal
PFOS 20 ppm	R10551	lipid vacuolization, lipid, minimal
	R10552	lipid vacuolization, lipid, minimal
	R10553	lipid vacuolization, lipid, minimal
	R10554	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10555	lipid vacuolization, lipid, minimal
N-EtFOSA 100 ppm	R10556	lipid vacuolization, lipid, mild
	R10557	No significant findings
	R10558	No significant findings
	R10559	lipid vacuolization, lipid, minimal
	R10560	lipid vacuolization, lipid, minimal
Wy-14,643 100 ppm	R10561	hypertrophy, Kupffer cell, mild; hepatocellular necrosis, multifocal, minimal; lipid vacuolization, lipid, minimal; spleen, follicular hyperplasia, moderate
	R10562	hypertrophy, Kupffer cell, mild; lipid vacuolization, lipid, minimal
	R10563	hypertrophy, Kupffer cell, mild; lipid vacuolization, lipid, minimal
	R10564	hypertrophy, Kupffer cell, mild; lipid vacuolization, lipid, minimal
	R10565	hypertrophy, Kupffer cell, mild; lipid vacuolization, lipid, minimal

Table III-3. Individual Animal Histopathology Findings
4 Week Recovery Sacrifice

Treatment Group	Animal Number	Histopathology Findings Liver
Controls	R10566	lipid vacuolization, lipid, mild
	R10567	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10568	lipid vacuolization, lipid, minimal
	R10569	lipid vacuolization, lipid, mild
	R10570	lipid vacuolization, lipid, minimal
	R10571	aggregates, mixed inflammatory cell, multifocal, minimal
	R10572	lipid vacuolization, lipid, minimal
	R10573	aggregates, mixed inflammatory cell, multifocal, minimal
	R10574	lipid vacuolization, lipid, minimal
	R10575	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
N-EtFOSE 300 ppm	R10576	lipid vacuolization, lipid, mild
	R10577	lipid vacuolization, lipid, mild
	R10578	lipid vacuolization, lipid, minimal
	R10579	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
	R10580	lipid vacuolization, lipid, minimal
N-EtFOSE 100 ppm	R10581	lipid vacuolization, lipid, mild
	R10582	lipid vacuolization, lipid, minimal
	R10583	No significant findings
	R10584	No significant findings
	R10585	lipid vacuolization, lipid, mild; aggregates, mixed inflammatory cell, multifocal, minimal
N-EtFOSE 30 ppm	R10586	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10587	No significant findings
	R10588	lipid vacuolization, lipid, minimal
	R10589	lipid vacuolization, lipid, minimal
	R10590	No significant findings
PFOS 20 ppm	R10591	aggregates, mixed inflammatory cell, multifocal, minimal
	R10592	lipid vacuolization, lipid, minimal
	R10593	lipid vacuolization, lipid, minimal
	R10594	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
	R10595	aggregates, mixed inflammatory cell, multifocal, minimal
N-EtFOSA 100 ppm	R10596	aggregates, mixed inflammatory cell, multifocal, minimal
	R10597	lipid vacuolization, lipid, minimal
	R10598	No significant findings
	R10599	No significant findings
	R10600	lipid vacuolization, lipid, minimal; aggregates, mixed inflammatory cell, multifocal, minimal
Wy-14,643 100 ppm	R10601	No significant findings
	R10602	No significant findings
	R10603	No significant findings
	R10604	lipid vacuolization, lipid, minimal; testes, sperm granuloms; epididymis, sperm granuloma
	R10605	No significant findings

IV. APPENDIX 1 – IN-LIFE REPORT

V. APPENDIX 2 – ELECTRON MICROSCOPY REPORT

ANCILLARY PATHOLOGY REPORT

ELECTRON MICROSCOPIC EVALUATION OF LIVER IN CRL:CD®(SD)IGS BR RATS)

CELL PROLIFERATION STUDY WITH N-ETHYL PERFLUOROOCTANESULFONAMIDO ETHANOL (N-EtFOSE; 3M T-6316.11), PERFLUOROOCTANE SULFONIC ACID POTASSIUM SALT (PFOS; 3M T-6295.16), AND N-ETHYL PERFLUOROOCTANESULFONAMIDE (N-EtFOSA 3M T-7091.1) IN RATS

TRC STUDY NUMBER 1132-100
PAI EM PROJECT NUMBER EM 99.62

SUMMMARY

An increase in the mean number of peroxisomes per hepatocyte was detected by electron microscopy in male Crl:CD®(CD) IGS BR rats given 100 ppm Wy-14,643 by diet after 48 hours of treatment. The mean number of peroxisomes was approximately doubled over control values. The mean numbers of peroxisomes per hepatocyte were not remarkably different for animals given 100 ppm N-EtFOSE, 20 ppm PFOS, or 100 ppm N-EtFOSA for 48 hours when compared with control values.

PROCEDURES

The purpose of this study was to examine by electron microscopy the livers from selected rats administered the test materials to assess peroxisome proliferation in hepatocytes.

Male Crl:CD®(CD) IGS BR rats were given the test material in the diet according to Text Table 1.

Text Table 1. Group Designations, Dietary Levels and Scheduled Sacrifice Time Points

Group Number[1] (Time Point)	Number of Male Rats							
	Control (0 ppm)	N-EtFOSE 300	N-EtFOSE 100	N-EtFOSE 30 ppm	PFOS 20 ppm	N-EtFOSA 100 ppm	Wy-14,643 100 ppm	Total No. of Animals
1 (48 hrs)	10	10	10	10	10	10	5	65
2 (7 days)	10	5	5	5	5	5	5	40
3 (14 days)	10	5	5	5	5	5	5	40
4 (1 wk recovery)	10	5	5	5	5	5	5	40
5 (4 wk recovery)	10	5	5	5	5	5	5	40
Total No. of Animals	50	30	30	30	30	30	25	225

[1] Animals in Groups 1 through 3 received the test diet for 48 hours, 7 days, and 14 days, respectively. Animals in Groups 4 and 5 received the test diet for 14 days followed by a 1 or 4 week recovery period, respectively.

After the prescribed dosing or recovery period, the animals were fasted overnight, bled for serum samples, anesthetized with CO_2, weighed, and exsanguinated. Postmortem procedures included weighing the liver and collecting samples of liver for cell proliferation studies, routine histopathology, palmitoyl-CoA Oxidase activity analysis, and electron microscopy. Liver samples for cell proliferation and routine histopathology were collected in zinc formalin and submitted to Pathology Associates International (PAI) Maryland for evaluation. Liver samples for palmitoyl-CoA Oxidase activity analysis were flash frozen in liquid nitrogen and submitted to Covance Laboratories for analyses. Liver samples for electron microscopy evaluation were thin-sliced and placed in McDowell-Trump fixative (McDowell EM, 1976), and submitted to this laboratory (PAI North Carolina) for electron microscopy processing and evaluation. Per sponsor request, only liver samples from selected rats were processed and evaluated ultrastructurally (Text Table 2). Only liver samples from animals dosed for 48 hours were examined by electron microscopy.

Text Table 2. Animals Selected for Electron Microscopic Evaluation

Treatment Group	Animal Number	Timepoint
Control	R10384 R10388	48 hour
N-EtFOSE 100 ppm	R10403 R10404	48 hour
PFOS 20 ppm	R10421 R10430	48 hour
N-EtFOSA 100 ppm	R10431 R10439	48 hour
Wy-14,643 100 ppm	R10441 R10443	48 hour

The selected livers were then processed into Spurr's resin for ultrastructural examination. Thick (1μ) sections were cut, stained with toluidine blue, and examined to select representative areas for further electron microscopy processing. Thin sections (approximately 90 nm) were cut, mounted on 200-mesh copper grids, stained with 5% methanolic uranyl acetate and Reynold's lead citrate, and examined on a Zeiss 900 transmission electron microscope. Centrilobular hepatocytes, where clearly identifiable in liver sections, were preferentially examined. Five representative electron photomicrographs of hepatocytes were taken and significant ultrastructural features were summarized for each photograph and animal on a designated transmission electron micrograph interpretation form. The number of peroxisomes in hepatocytes was manually counted for each photographed hepatocyte and recorded. The mean number of peroxisomes per hepatocyte was manually calculated by summing the number of peroxisomes counted in five hepatocytes per animal and dividing by five.

RESULTS AND DISCUSSION

Individual interpretations of electron micrographs for each animal selected for evaluation follow this report narrative. The original signed/dated raw data sheets and photomicrographs are maintained in the archived study file at Pathology Associates' North Carolina facility.

After 48 hours of treatment, the numbers of peroxisomes appeared significantly increased over control values only for animals given 100 ppm of Wy-14,643 (Text Table 3). The mean number of peroxisomes was approximately double the control values. The mean numbers of peroxisomes per hepatocyte, however, were not remarkably different for animals given 100 ppm N-EtFOSE, 20 ppm PFOS, or 100 ppm N-EtFOSA when compared with control values. Figures 1 through 4 show hepatocytes from control and non-affected treated animals. Some normal ultrastructural features are illustrated in these figures. Figure 5 shows a hepatocyte from an animal given 100 ppm Wy-14,642, illustrating the increased numbers of peroxisomes.

Text Table 3. Quantitation of Hepatocellular Peroxisomes

Treatment Group	Timepoint	Animal Number	Mean Number of Peroxisomes per Hepatocyte
Control	48 hour	R10384 R10388	11.4 13.6
N-EtFOSE 100 ppm	48 hour	R10403 R10404	16.6 11.4
PFOS 20 ppm	48 hour	R10421 R10430	15.2 3.8
N-EtFOSA 100 ppm	48 hour	R10431 R10439	17.8 5.0
Wy-14,643 100 ppm	48 hour	R10441 R10443	29.6 29.4

No other ultrastructural abnormalities were identified in the samples evaluated.

CONCLUSION

As evaluated by electron microscopy, hepatocellular peroxisomes were increased in male Crl:CD®(CD) IGS BR rats given 100 ppm Wy-14,643 by diet after 48 hours of treatment. The mean number of peroxisomes was approximately doubled over control values. The mean numbers of peroxisomes per hepatocyte were not remarkably different for animals given 100 ppm N-EtFOSE, 20 ppm PFOS, or 100 ppm N-EtFOSA for 48 hours when compared with control values.

REFERENCES

McDowell, EM and Trump, BF: Histologic fixative for routine diagnostic light and electron microscopy. *Arch. Pathol. Lab. Med.,* 100:405-414, 1976.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10384

Tissue: Liver

Treatment Group: Control

Block No(s).: 99.62-4

Sex: Male

Photo/Negative No(s).: Z17346 - Z17350

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): Sept. 29, 1999

Features: Z17346. Liver. Hepatocyte. 10,320X. This hepatocyte contains a central oval nucleus and is bordered by adjacent hepatocytes on two sides and sinusoids ventrally and dorsally. The lighter staining endothelial cell cytoplasm is evident. Mitochondria and rough endoplasmic reticulum (RER) profiles are frequent through the cytoplasm of the hepatocyte. The intervening cytosol appears finely granular and contains ribosomes, glycogen and smooth endoplasmic reticulum (SER). Bile canaliculi are present on the left and upper right margins. Peroxisomes are infrequent; four (5) peroxisomes counted.
Z17347. Liver. Hepatocyte. 10,320X. This hepatocyte is bordered by endothelial cells on both the right and the left. Mitochondria and rough endoplasmic reticulum (RER) profiles are frequent through the cytoplasm of the hepatocyte. A prominent microvillous border is evident at the top right margin of this cell. Nine (9) mitochondria counted.
Z17348. Liver. Hepatocyte. 10,320X. This hepatocyte is surrounded by two adjacent hepatocytes and a sinusoid along the right margin. The cell has abundant RER intermixed with mitochondria and other organelles. Several mitochondria are irregularly-shaped and contain cytoplasmic invagination. Eight (8) peroxisomes counted.
Z17349. Liver. Hepatocyte. 10,320X. This hepatocyte contains several lipid droplets, of which one contains a dark round membranous inclusion. An endothelial cell is present at the top right, and on the left some lamellar debris is present in the extracellular space. Thirteen (13) peroxisomes counted.
Z17350. Liver. Hepatocyte. 10,320X. Similar to Z17349, several medium-sized lipid droplets are present in this hepatocyte. There are abundant mitochondria and numerous RER profiles evident. Twenty-two (22) peroxisomes counted.

Conclusions: Normal hepatocyte. Mean number of peroxisomes per hepatocyte is 11.4.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10388

Tissue Liver

Treatment Group: Control

Block No(s) 99.62-8
Z17351 -

Sex: Male

Photo/Negative No(s).: Z17355

Significant Lesions (check one): _____ Yes X No

Interpreting Pathologist (signature and date): James B. Nold Sept. 29, 1999

Features: Z17351. Liver. Hepatocyte. 10,320X. This hepatocyte has a central round nucleus with two prominent nucleoli. The cytoplasm is finely granular and contains many mitochondria and RER profiles. There are also abundant lysosomes and some peroxisomes. Several bile canaliculi are present between this cell and adjacent hepatocytes. At the right margin is a dark-staining hepatocyte, and a sinusoidal erythrocyte is present in a sinusoid at the bottom of the photograph. Twelve (12) peroxisomes counted.

Z17352. Liver. Hepatocyte. 10,320X. Numerous mitochondria and RER profiles are present in this hepatocyte. Its nucleus is polygonal and centrally located. At the right margin are two darker hepatocytes that appear to have somewhat larger and more numerous mitochondria. Random dark peroxisomes and lysosomes are present in the cytoplasm. Fifteen (15) peroxisomes counted.

Z17353. Liver. Hepatocyte. 10,320X. On the left a thin border of endothelial cell lining a sinusoid is present. The hepatocyte has a central round nucleus and the cytoplasm contains many mitochondria, RER profiles, and several pale-staining lipid droplets. Some peroxisomes and lysosomes are also present. Five (5) peroxisomes counted.

Z17354. Liver. Hepatocyte. 10,320X. The central hepatocyte is bordered by hepatocytes on most margins, although a segment of sinusoid is present on the left (with erythrocyte) and bottom. Several bile canaliculi are present between the central and bordering hepatocytes. Several small lipid droplets are present. Numerous dark lysosomes and peroxisomes are present. Twenty-three (23) peroxisomes counted. Peroxisomes, lysosomes and some mitochondria were very difficult to differentiate in this photograph.

Z17355. Liver. Hepatocyte. 10,320X. This hepatocyte contains four small- to medium-sized lipid droplets. Sinusoids are present at the top and bottom of the photograph, lined by paler staining endothelial cells. Thirteen (13) peroxisomes counted.

Conclusions: Normal hepatocyte. Mean of 13.6 peroxisomes per hepatocyte counted.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No. R10403

Tissue: Liver

Treatment Group: N-EtFOSE 100ppm

Block No(s).: 99.62-23

Z17356 -

Sex: Male

Photo/Negative No(s).: Z17360

Significant Lesions (check one): _____ Yes ___X___ No

Interpreting Pathologist (signature and date): [signature] Sept. 29, 1999

Features: Z17356. Liver. Hepatocyte. 10,320X. This hepatocyte contains approximately ten small- to medium-sized lipid droplets. The cytoplasm has numerous mitochondria and RER profiles present. Bile canaliculi are present between this cell and adjacent hepatocytes on the left and the right. Erythrocytes in sinusoids are present on three margins. Some stain debris/artifact is present on the photograph. Eight (8) peroxisomes counted.
Z17357. Liver. Hepatocyte. 10,320X. This hepatocyte is binucleated. An endothelial cell is present at the top, but all other margins are adjacent hepatocytes. The cytoplasm contains numerous mitochondria and RER profiles. One distinct lipid droplet is present, and there are numerous small to medium-sized dark peroxisomes and/or lysosomes. Twenty-three (23) peroxisomes counted.
Z17358. Liver. Hepatocyte. 10,320X. A nucleus is not evident in this plane of section for this hepatocyte. Four medium-sized lipid droplets are present, and the other organelles appear very similar to Z17357. Some lysosomes contain clear vacuoles within them and many are irregularly shaped. Thirteen (13) peroxisomes counted.
Z17357. Liver. Hepatocyte. 10,320X. Hepatocyte contains one large lipid droplet. Twenty-three (23) peroxisomes counted. Some stain debris/artifact present on the photograph.
Z17360. Liver. Hepatocyte. 10,320X. Multiple small- to medium-sized lipid droplets are present. Sixteen (16) peroxisomes counted.

Conclusions: Normal hepatocyte. 16.6 average peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No. R10404

Tissue: Liver

Treatment Group: N-EtFOSE 100ppm

Block No(s).: 99.62-24

Sex: Male

Photo/Negative No(s).: Z17361 - Z17365

Significant Lesions (check one): Yes ___X___ No

Interpreting Pathologist (signature and date): John B. Arnold September 30, 1999

Features: Z17361, Liver. Hepatocyte. 10,320X. This central hepatocyte exhibits a microvillous border adjacent to sinusoids on three margins and borders with three adjacent hepatocytes on three other margins. Bile canaliculi are evident with two of the adjacent hepatocytes. The hepatocyte has a round central nucleus and quite a few small- to medium-sized lipid droplets. There are numerous mitochondria and RER profiles. The remaining cytosol is finely granular and contains scattered lysosomes and peroxisomes. Sixteen (16) peroxisomes counted.

Z17362, Liver. Hepatocyte. 10,320X. This hepatocyte appears somewhat diamond-shaped in this photograph, with a sinusoid at the top left and bottom. Part of a sinusoidal lining cell and its nucleus is evident at the top left of the photograph. The hepatocyte has a round central nucleus and abundant RER profiles and mitochondria. Also, numerous lysosomes and a few peroxisomes are present. Six (6) peroxisomes counted.

Z17363, Liver. Hepatocyte. 10,320X. This hepatocyte appears morphologically similar to those described above. Several small lipid droplets are also present. Eleven (11) peroxisomes counted.

Z17364, Liver. Hepatocyte. 10,320X. Most of two hepatocytes are present in this picture. A long profile of a bile canaliculus is present between the two cells. The complete hepatocyte on the right contains several medium-sized and one large lipid droplet. Eight (8) peroxisomes counted.

Z17365, Liver. Hepatocyte. 10,320X. This is a large hepatocyte next to a sinusoid at the top right of the photograph. Two erythrocytes are present in the sinusoid. The hepatocyte has a large round nucleus and numerous mitochondria and RER profiles. Also, there are multiple lysosomes and peroxisomes in the cytoplasm. Clear distinction between some peroxisomes and lysosomes is difficult. Sixteen (16) peroxisomes counted.

Conclusions: Normal hepatocyte. Mean of 11.4 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10421

Tissue: Liver

Treatment Group: PFOS 20ppm

Block No(s).: 99.62-41

Sex: Male

Photo/Negative No(s).: Z17366 - Z17370

Significant Lesions (check one): _____ Yes ___X___ No

Interpreting Pathologist (signature and date): Sept. 30, 1999

Features: Z17366. Liver. Hepatocyte. 10,320X. This hepatocyte has a centrally located polygonal nucleus surrounded by cytoplasm rich in mitochondria and RER profiles. Multiple small- to medium-sized lipid droplets are also present. The remaining cytoplasm appears finely granular and contains some lysosomes and peroxisomes. Twenty-seven (27) peroxisomes counted.
Z17377. Liver. Hepatocyte. 10,320X. This hepatocyte has adjacent hepatocytes both dorsally and ventrally. To the left is a sinusoid lined by an endothelial cell and containing an erythrocyte. An endothelial-lined sinusoid is also present along the right margin. Approximately nine small lipid droplets are present in the cytoplasm. Fifteen (15) peroxisomes counted.
Z17368. Liver. Hepatocyte. 10,320X. In this hepatocyte the mitochondria appear smaller and more condensed. Profiles of RER and SER are easily evident in the cytoplasm. Five (5) peroxisomes counted.
Z17369. Liver. Hepatocyte. 10,320X. Several small and one large lipid droplet are present in this hepatocyte. At the top right and bottom left margins of the photograph are erythrocytes within sinusoids. The hepatocyte's nucleus is not present in this plane of section. Numerous mitochondria and RER profiles are visible. The intervening cytosol is finely granular and contains SER and other organelles, including some peroxisomes and lysosomes. Some crystalloid structures appear very prominent in some peroxisomes. Fifteen (15) peroxisomes counted.
Z17370. Liver. Hepatocyte. 10,320X. Hepatocyte appears very similar to Z17369. A nucleus is not evident in the plane of section photographed. Several small- to medium-sized lipid droplets are present. Sixteen (16) peroxisomes counted.

Conclusions: Normal hepatocyte. Mean of 15.2 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10430

Tissue: Liver

Treatment Group: PFOS 20 ppm

Block No(s).: 99.62-50

Z17371 -

Sex: Male

Photo/Negative No(s).: Z17375

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): Sept. 30, 1999

Features: Z17371. Hepatocyte. 10,320X. This hepatocyte has an eccentrically located oval nucleus. It is bordered by an sinusoid at the top and right and another hepatocyte on the left. Numerous mitochondria, RER profiles, finely granular cytosol, and a few small lipid droplets are present in the cytoplasm. Some small lysosomes are scattered in the cytoplasm Five (5) peroxisomes counted.

Z17372. Liver. Hepatocyte. 10,320X. This hepatocyte is surrounded by hepatocytes on all sides except the top, which is a sinusoid. The cell has abundant RER profiles and mitochondria in the cytoplasm, as well as multiple irregularly-shaped small to medium-sized lipid droplets. Some lysosomes and a few peroxisomes are present. Four (4) peroxisomes counted.

Z17373. Liver. Hepatocyte. 10,320X. The hepatocyte appears essentially similar to previously described hepatocytes, containing a large round nucleus and numerous mitochondria and RER profiles. Some lipid droplets are present. Lysosomes and peroxisomes are rare. Two (2) probable peroxisomes counted.

Z17374. Liver. Hepatocyte. 10,320X. This hepatocyte is somewhat lighter staining than most of the previously examined cells were, and is located beneath a centrilobular vein along the right. Some collagen fibers are evident in the Space of Disse beneath the endothelial cell margin. Peroxisomes in the hepatocyte are better defined than in most other cells, and have prominent crystalloid structures. Eight (8) peroxisomes counted.

Z17375. Liver. Hepatocyte. 10,320X. This hepatocyte is bordered by a sinusoid along the top margin. The cell's microvillous border is next to the endothelial lined sinusoid, which contains several erythrocytes. Adjacent hepatocytes are on the right and left of the central hepatocyte. A nucleus is not present in this plane of section. The cytoplasm is finely granular and contains multiple mitochondria and RER profiles. Zero (0) peroxisomes are clearly discernible.

Conclusions: Normal hepatocyte. Average 3.8 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10431

Tissue: Liver

Treatment Group: N-EtFOSA 100ppm

Block No(s).: 99.62-51
Z17376 - Z17380

Sex: Male

Photo/Negative No(s).: Z17396

Significant Lesions (check one): _____ Yes ___X___ No

Interpreting Pathologist (signature and date): _____ Sept. 30, 1999

Features: Z17376. Liver. Hepatocyte. 10,320X. This light-staining hepatocyte has a central polygonal nucleus surrounded by cytoplasm containing numerous mitochondria. RER and SER profiles are present and there are greater than a dozen small- to medium-sized lipid droplets. Nine (9) peroxisomes counted.
Z17377. Liver. Hepatocyte. 10,320X. This hepatocyte stains somewhat darker than the preceding example. The cytoplasm is finely granular and mitochondria appear denser. Peroxisomes and some lysosomes are generally smaller and darker than the mitochondria, and are often difficult to differentiate from each other. Twenty-two (22) peroxisomes counted.
Z17378. Liver. Hepatocyte. 10,320X. The top margin of this cell shows a microvillous border adjacent to a sinusoid. The nucleus is not evident in this plane of section for this hepatocyte. The cytoplasm contains abundant SER between the mitochondria and other organelles, including some lysosomes and peroxisomes. Sixteen (16) peroxisomes counted.
Z17379. Liver. Hepatocyte. 10,320X. Mitochondria and RER profiles are prominent in this hepatocyte's cytoplasm. A single medium-size lipid droplet is present near the top border of this cell. A sinusoid lined by an endothelial cell is present along the left margin. Seventeen (17) peroxisomes counted.
Z17380. Overexposed negative; not printed.
Z17396. Liver. Hepatocyte. 10,320X. The centered hepatocyte is bordered by adjacent hepatocytes on the wide and bottom. At the top is a sinusoid lined by an endothelial cell and containing some granulocytes and erythrocytes. The hepatocyte has a central polygonal nucleus surrounded by numerous mitochondria and RER profiles. Scattered darker peroxisomes are present. Twenty-five (25) peroxisomes counted.

Conclusions: Normal hepatocyte. Average 17.8 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10439

Tissue: Liver

Treatment Group: N-EtFOSA 100ppm

Block No(s).: 99.62-59

Sex: Male

Photo/Negative No(s).: Z17381 - Z17385

Significant Lesions (check one): _____ Yes ___X___ No

Interpreting Pathologist (signature and date): _____ October 1, 1999

Features: Z17381. Liver. Hepatocyte. 10,320X. A medium-sized round nucleus is present in this hepatocyte. It is surrounded by numerous mitochondria and RER profiles. Five lipid droplets are evident in the cytoplasm. Some mitochondria have dark inclusions; this may be some stain precipitate. Several lysosomes are present, but zero (0) peroxisomes are clearly discernible.
Z17382. Liver. Hepatocyte. 10,320X. This hepatocyte is somewhat lighter staining than Z17381. The round nucleus is surrounded by numerous mitochondria and RER profiles. The cytoplasm contains several small- to medium-sized lipid droplets. Similarly, several lysosomes are present, but zero (0) peroxisomes are clearly discernible.
Z17383. Liver. Hepatocyte. 10,320X. This slightly darker-staining hepatocyte has a sinusoid lined by an endothelial cell at its upper right margin. The cytoplasm contains numerous mitochondria and RER profiles. Several lipid droplets are present. Nine (9) peroxisomes counted.
Z17384. Liver. Hepatocyte. 10,320X. Erythrocyte containing sinusoids are evident on three sides of this hepatocyte. Several medium- to large-sized lipid droplets are present in the cytoplasm of this hepatocyte. There are numerous mitochondria and RER profiles in the cytoplasm. Five (5) peroxisomes counted.
Z17385. Liver. Hepatocyte. 10,320X. The cytoplasm of this hepatocyte contains numerous RER profiles and mitochondria. Several lipid droplets, one with a lamellar body, are present. Some scattered lysosomes and peroxisomes are present, and are difficult to clearly differentiate from each other. Eleven (11) peroxisomes counted.

Conclusions: Normal hepatocyte. Average 5.0 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10441

Tissue: Liver

Treatment Group: Wy-14,643 100ppm

Block No(s).: 99.62-61

Sex: Male

Photo/Negative No(s).: Z17386 - Z17390

Significant Lesions (check one): X Yes _____ No

Interpreting Pathologist (signature and date): _____ Oct. 01, 1999

Features: Z17386. Liver. Hepatocyte. 10,320X. This plate of three hepatocytes has sinusoids on both the right and left margins. The central hepatocyte has an oval nucleus which is surrounded by numerous mitochondria and finely granular cytoplasm. Peroxisomes are the darker staining organelles. Twenty-two (22) peroxisomes counted.
Z17387. Liver. Hepatocyte. 10,320X. Four medium-sized lipid droplets are present in this hepatocyte. The cytoplasm is finely granular and contains numerous mitochondria and some RER profiles. Three erythrocytes are present in the sinusoid at the top of the photograph. Thirty-seven (37) peroxisomes counted.
Z17388. Liver. Hepatocyte. 10,320X. This hepatocyte appears ultrastructurally similar to those described above. It has a central round nucleus surrounded by cytoplasm containing numerous mitochondria, finely granular cytosol, RER profiles, and some lipid droplets. Additionally some variably-sized dark-staining peroxisomes are present. An erythrocyte in a sinusoid is present at the top right margin of the photograph. Twenty-two (22) peroxisomes counted.
Z17389. Liver. Hepatocyte. 10,320X. Hepatocyte appears ultrastructurally similar to Z17388. Forty-one (41) peroxisomes counted.
Z17390. Liver. Hepatocyte. 10,320X. Only a small segment of nucleus is present in this hepatocyte. This hepatocyte is bordered by adjacent hepatocyte on three sides and by an endothelial-lined sinusoid on the left. Its cytoplasm contains numerous mitochondria, RER profiles, scattered dark peroxisomes, and some lysosomes. Twenty-six (26) peroxisomes counted.

Conclusions: Increased peroxisomes. Average 29.6 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 1132-100 (EM99.62)

Species/Strain: Crl:CD®(SD) IGS BR Rat

Animal No.: R10443

Tissue: Liver

Treatment Group: Wy-14,643 100ppm

Block No(s).: 99.62-63

Sex: Male

Photo/Negative No(s).: Z17391 - Z17395

Significant Lesions (check one): X Yes _____ No

Interpreting Pathologist (signature and date): _____ Oct. 01, 1999

Features: Z17391. Liver. Hepatocyte. 10,320X. A polygonal-shaped hepatocyte is centered in the photograph. At the top is a sinusoid containing an endothelial cell and erythrocyte on the left and a perisinusoidal lipid-storing cell at the right. Another sinusoid is present at the bottom of the photograph. The hepatocyte contains numerous mitochondria and RER profiles. The intervening cytosol is finely granular and also contains scattered dark lysosomes and peroxisomes. Thirty (30) peroxisomes counted.
Z17392. Liver. Hepatocyte. 10,320X. This hepatocyte contains greater than a dozen small- to medium-sized lipid droplets. Numerous RER profiles and mitochondria are present in the cytoplasm. Twenty-six (26) peroxisomes counted.
Z17393. Liver. Hepatocyte. 10,320X. This somewhat lighter-staining hepatocyte is bordered by adjacent hepatocytes on all margins in this photograph. A nucleus is not evident in this plane of section. Several lipid droplets are present. Forty-one (41) peroxisomes counted.
Z17394. Liver. Hepatocyte. 10,320X. This hepatocyte appears morphologically similar to Z17393, except it has a large central polygonal nucleus. Thirty-eight (38) peroxisomes counted.
Z17395. Liver. Hepatocyte. 10,320X. This round hepatocyte appears essentially similar to Z17391. The hepatocyte contains numerous mitochondria and RER profiles. The intervening cytosol is finely granular and also contains scattered dark lysosomes and peroxisomes. Twelve (12) peroxisomes counted.

Conclusions: Increased peroxisomes. Average 29.4 peroxisomes per hepatocyte.

VI. APPENDIX 3 – PALMITOYL CoA OXIDASE REPORT

Table VI-1. Individual Animal Palmitoyl CoA Oxidase Activity

	48 Hour Interim Sacrifice		7 Day Interim Sacrifice		14 Day Interim Sacrifice		1 Week Recovery Sacrifice	
Treatment Group	**Animal Number**	**PCOAO* Activity**	**Animal Number**	**PCOAO* Activity**	**Animal Number**	**PCOAO* Activity**	**Animal Number**	**PCOAO* Activity**
Controls	R10381	9	R10446	8	R10486	5	R10526	6
	R10382	9	R10447	4	R10487	6	R10527	9
	R10383	6	R10448	5	R10488	5	R10528	7
	R10384	6	R10449	5	R10489	5	R10529	5
	R10385	5	R10450	5	R10490	8	R10530	10
	R10386	6	R10451	5	R10491	6	R10531	10
	R10387	8	R10452	4	R10492	7	R10532	7
	R10388	11	R10453	5	R10493	9	R10533	8
	R10389	7	R10454	5	R10494	5	R10534	3
	R10390	8	R10455	7	R10495	7	R10535	5
N-EtFOSE 300 ppm	R10391	7	R10456	6	R10496	2	R10536	3
	R10392	101	R10457	3	R10497	4	R10537	5
	R10393	10	R10458	6	R10498	2	R10538	5
	R10394	16	R10459	6	R10499	3	R10539	3
	R10395	6	R10460	10	R10500	3	R10540	6
	R10396	7						
	R10397	7						
	R10398	10						
	R10399	8						
	R10400	10						
N-EtFOSE 100 ppm	R10401	5	R10461	6	R10501	2	R10541	6
	R10402	8	R10462	7	R10502	5	R10542	4
	R10403	10	R10463	9	R10503	1	R10543	4
	R10404	9	R10464	4	R10504	3	R10544	5
	R10405	9	R10465	8	R10505	4	R10545	5
	R10406	4						
	R10407	7						
	R10408	8						
	R10409	12						
	R10410	9						
N-EtFOSE 30 ppm	R10411	10	R10466	5	R10506	5	R10546	5
	R10412	9	R10467	6	R10507	6	R10547	5
	R10413	13	R10468	4	R10508	4	R10548	8
	R10414	9	R10469	7	R10509	4	R10549	4
	R10415	6	R10470	9	R10510	5	R10550	8
	R10416	7						
	R10417	9						
	R10418	8						
	R10419	12						
	R10420	9						
PFOS 20 ppm	R10421	11	R10471	8	R10511	4	R10551	6
	R10422	8	R10472	7	R10512	4	R10552	6
	R10423	8	R10473	5	R10513	5	R10553	5
	R10424	6	R10474	8	R10514	4	R10554	8
	R10425	8	R10475	9	R10515	4	R10555	3
	R10426	6						
	R10427	6						
	R10428	10						
	R10429	11						
	R10430	8						
N-EtFOSA 100 ppm	R10431	6	R10476	7	R10516	2	R10556	6
	R10432	5	R10477	3	R10517	4	R10557	6
	R10433	9	R10478	8	R10518	2	R10558	6
	R10434	6	R10479	6	R10519	4	R10559	6
	R10435	6	R10480	4	R10520	2	R10560	7
	R10436	5						
	R10437	11						
	R10438	7						
	R10439	6						
	R10440	6						

*Palmitoyl CoA Oxidase activity, IU/g

VII. APPENDIX 4 – STUDY PROTOCOL AND AMENDMENTS

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Pathology Associates

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL

Study Title:

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA 3M T-7091.1) in Rats

Date:

January 12, 1999

Performing Laboratory

R.O.W. Sciences
15 Firstfield Road
Gaithersburg, Maryland 20878

Laboratory Study Identification:

Study Number: (1132-100)

PAI Project Number: (Histology number to be assigned by PAI by protocol amendment)

Study

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluoroctanesulfonamide (N-EtFOSA 3M T-7091.1) in Rats

Purpose

To assess cell proliferation and peroxisome proliferation in rats administered test material in the diet.

Sponsor

3M Corporate Toxicology
Building 220-2E-02, 3M Center
St. Paul, MN 55144-1000

Study Representative

Marvin T. Case, D.V.M, Ph.D.
3M Corporate Toxicology
Phone No.: 651 733-5180
Fax No.: 651 733-1773
Email: mtcase@mmm.com

Alternative Study Representative

Andrew M. Seacat, Ph.D.
3M Corporate Toxicology
Phone No.: 651 575-3161
Fax No.: 651 733-1773
Email: amseacat@mmm.com

Test Facility

TherImmune Research Corporation (formerly, R.O.W. Sciences)
15 Firstfield Road

Gaithersburg, Maryland 20878

Study Monitor

Sandra R. Eldridge, Ph.D.

Pathology Associates International

Phone No. 301 624-2036

Fax No. 301 663-8994

Email: srepaisaic@aol.com

Study Director

Gary W. Wolfe, Ph.D., D.A.B.T.

R.O.W. Sciences

Phone No.: 301 330-3723

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Principal Investigator

Sandra R. Eldridge, Ph.D.

Pathology Associates International

Phone No. 301 624-2036

Fax No. 301 663-8994

Email: SREPAISAIC@aol.com

Study Pathologist

Carolyn Moyer, D.V.M., Diplomate, A.C.V.P.

Pathology Associates International

Phone No. 301 624-2928

Fax No. 301 663-8994

Proposed Study Timetable

In-life Start Date: To be added by protocol amendment; Day 0

In life End Date: To be added by protocol amendment

Audited Draft Report Date: To be added by protocol amendment

Regulatory Compliance

This study will be conducted in the spirit of Good Laboratory Practice (GLP) regulations.

Animal Care and Use Statement

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations, 9 CFR 1-4. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

Quality Assurance

Not applicable.

Test Materials

Test Material:	N-EtFOSE (completed by 3M)	PFOS (completed by 3M)	N-EtFOSA (to be added by protocol amendment)	Wy-14,643 (to be added by protocol amendment)
Identification:	N-EtFOSE	PFOS	N-EtFOSA	Wy
Lot Number:	FM 3929 Lots 30035, 30037, 30039	217	Lot 541	
Purity:	99.2%	99%		
Stability:	> 5 years	> 5 years	> 5 years	
Storage Conditions:	room temp.	room temp.	room temp.	
Characteristics:	waxy solid	white powder	amber waxy solid	

Reserve (Archive) Samples

A reserve sample (approximately 5 g) of each lot will be taken and stored at room temperature. These samples will be transferred to the Sponsor after completion of the in-life phase to be retained in accordance with 40 CFR 792.195.

Disposition of Test Material

After authorization from the Sponsor, any remaining test material will be returned to:

Marvin Case, D.V.M., Ph.D.
3M Corporate Toxicology
Building 220-2E-02, 3M Center
St. Paul, Minnesota 55144-1000
Phone No.: 651-733-5180
Fax No.: 651-733-1773

Animals

Species:	Rat
Strain:	Crl:CD®(SD) IGS BR
Source:	Charles River Laboratories, Inc., Raleigh, NC
Age at Initiation of Treatment:	Preferable 6 weeks of age, but not more than 8 weeks of age
Weight at Initiation of Treatment:	150 to 300 g
Number and Gender:	males
Identification:	unique identification by individual car tags and cage cards

Husbandry

Housing:	Single housed in hanging stainless steel wire cages
Diet:	Teklad 7012 Certified Rodent Diet. Fresh food will be provided weekly. Feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinate hydrocarbons, organophosphates, and specified nutrients. Specified nutrients analyses are on file at R.O.W. Sciences.
Water:	Tap water, provided ad libitum via an automatic watering system or water bottles. The water is analyzed at least two times per year for contaminants and specific microbes. The results of these analyses are on file at R.O.W. Sciences.
Contaminants:	The study director and/or the Sponsor have considered possible interfering substances potentially present in animal feed and water, including the test material itself or possible structurally related materials as well as the items listed in (2) and (3) above. None of these contaminants are reasonably expected to be present in animal feed or water at levels sufficient to interfere with this study.
Environment:	The targeted temperatures are between 64 and 79°F with a relative humidity between 30% and 70%. Temperature and humidity are monitored continuously. A 12-hour light/12-hour dark cycle will be maintained. Ten or greater air changes/hour will be maintained.
Acclimation:	Animals will be acclimated to the facility for a minimum of 7 days prior to the start of dosing. Animals will be observed for general health and suitability for testing during this period. Animals that are diseased or unsuitable for testing will be removed from the study.
Randomization:	Using computer-generated random numbers wit assignment to groups, At the time of randomization, the weight variation of the animals of each sex used should not exceed ± 2 S.D. of the mean weight, and the mean body weights for each group of each sex will not be statistically different.
Justification:	Rats will be used because of the extensive historical data base, and the FDA requirements for a rodent species.

Group Designations, Dietary Levels and Scheduled Sacrifice Time Points

Group Number (Time Point)	Number of Male Rats							
	Control (0 ppm)	N-EtFOSE 300	100	30 ppm	PFOS 20 ppm	N-EtFOSA 100 ppm	Wy-14,643 100 ppm	Total No. of Animals
1 (48 hrs)	10	10	10	10	10	10	5	65
2 (7 days)	10	5	5	5	5	5	5	40
3 (14 days)	10	5	5	5	5	5	5	40
4 (1 wk recovery)	10	5	5	5	5	5	5	40
5 (4 wk recovery)	10	5	5	5	5	5	5	40
Total No. of Animals	50	30	30	30	30	30	25	225

Dosing Procedures

Method of Administration

Dietary. Animals in Groups 1 through 3 will receive test diet for 48 hours, 7 days, and 14 days, respectively.

Animals in Groups 4 and 5 will receive test diet for 14 days followed by a 1 or 4 week recovery period, respectively.

Reason for Dosing Route

The potential human exposure is by the oral route.

Dose Preparation

Before initiation of treatment, dose preparation of each test material will be mixed. All dose preparations will be stored at room temperature. Dose preparation will be documented and reported. See Attachment I for test diet preparation procedures.

Retention Sample

Samples (approximately 100 g) will be taken from the dose preparation and stored at room temperature. Unless used for analyses, these samples will be discarded at least 1 month after completion of the in-life phase.

Observation of Animals

Clinical Observations

Each animal will be observed twice daily (a.m. and p.m.) for mortality and moribundity; findings will be recorded as they are observed.

Body Weights

Prior to treatment (at randomization), weekly for Week 1 through 4 weeks of recovery.

Food Consumption

Weekly for Week 1 through 4 weeks of recovery.

Clinical Chemistry

Animals will be fasted overnight before animal's scheduled necropsy; blood will be collected from a jugular vein into an EDTA-coated tube. Serum enzyme levels of alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), cholesterol and triglycerides will be determined.

Termination

Unscheduled Sacrifices and Deaths

Necropsies will be done. Animals to be sacrificed will be anesthetized with CO_2, weighed, and exsanguinated.

Scheduled Sacrifices

Interim Sacrifices

At 48 hrs, 7 days, and 14 days, animals will be fasted overnight, bled for serum samples, anesthetized with CO_2, weighed, and exsanguinated.

NOTE: Two serum samples will be needed, (1) a 0.5 ml sample for clinical chemistry and (2) a 1.5 ml sample for compound level analysis.

The abdominal cavity of each animal will be opened, the liver will be removed and weighed, and liver samples will be collected. Animals will be discarded after liver collection.

Terminal Sacrifices

After 1 and 4 weeks of recovery, animals will be fasted overnight, bled for serum samples, anesthetized with CO_2, weighed, exsanguinated, and necropsied.

NOTE: Two serum samples will be needed, (1) a 0.5 ml sample for clinical chemistry and (2) a 1.5 ml sample for compound level analysis.

Postmortem Procedures

Necropsy

The necropsy will include an examination of the external features of the carcass; all external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Cell Proliferation Tissue Collection and Immunohistochemical Evaluation

Representative samples of the left lateral lobe of the liver and any macroscopic lesions of the liver will be collected and preserved in zinc formalin.

After fixation, each sample of liver will be delivered to:

Sandra R. Eldridge, Ph.D.
Pathology Associates International
15 Worman's Mill Court, Suite I
Frederick, Maryland 21701

Proliferation cell nuclear antigen (PCNA) evaluation will be done on the samples. In addition, liver sections prepared from the same tissue block will be stained with hematoxylin and eosin and examined microscopically.

Palmitoyl-CoA Oxidase Tissue Collection and Analyses

A sample (approximately 500 mg) of the right lateral lobe of the liver will also be collected from select animals and flash-frozen in liquid nitrogen. See Attachment II for procedure. The liver tissue will be stored in a freezer set to maintain -60 to -80° C until analyzed by Covance for palmitoyl-CoA Oxidase activity. The liver samples to be analyzed will include all study animals, EXCEPT for the Wy-14,643 animals and all animals from the 4-week recovery groups. In addition to this study, samples from a previous 3M study will be analyzed for palmitoyl-CoA Oxidase activity; these samples consist of liver samples from 35 rats and 35 guinea pigs.

Tissue Collection for Electron Microscopic Evaluation

Sections of liver from all animals will be collected, minced to approximately one millimeter cubes and placed in a fixative appropriate for electron microscopy. The containers and fixative will be provided by PAI. Electron microscopy will be performed on one animal per treatment group exhibiting the highest cell proliferative response as well as one control animal at the discretion of the Sponsor, from one time point as well as the 4-week recovery. Thus, EM will be performed on one animal from the control, N-EtFOSE (one dose only to be determined), PFOS, N-EtFOSA, and Wy groups at one of the time points, as well as the 4 week recovery, for a total of 10 animals.

Remaining Liver Tissue

The remaining liver tissue will be frozen and stored at -60 to -80° C for possible future analysis.

Organ Weights

At the scheduled sacrifices, the liver will be weighed.

Histopathology

Liver from each animal that is examined for cell proliferation will be stained with hematoxylin and eosin, and examined microscopically for histopathologic changes.

Reports

One copy of the draft report will be sent to the Sponsor. The report will include the following information:

Experimental Design and Methods

Results

dose analyses
mortality
clinical observations
body weights

body weight changes
food consumption
test material consumption
clinical pathology results
palmitoyl-CoA oxidase activities
macroscopic observations
microscopic observations
ultrastructural observations
cell proliferation assessments

Record Retention

All raw data, documentation, records, protocol, specimens, and final report generated as a result of this study will be archived in the storage facilities of PAI for a period of 1 year following submission of the final report to the Sponsor. One year after submission of the final report, all of the aforementioned materials will be sent to the Sponsor and a return fee will be charged. All raw data stored on magnetic media will be retain by PAI.

PROTOCOL APPROVAL

Marvin Case, D.V.M., Ph.D. Date
Study Representative
3M Corporate Toxicology

Gary W. Wolfe, Ph.D., D.A.B.T Date
Study Director
R.O.W. Sciences

Sandra R. Eldridge, Ph.D. Date
Principle Investigator
Pathology Associates International

Attachment: I

Test Diet Preparation Procedures

1) Determine the amount of test diet (feed) that is to be prepared and weigh out that amount of feed.
2) Calculate the amount of test article that is needed to prepare the test diet at the desired concentration.
3) Accurately weigh out the necessary amount of test article.
4) Transfer the weighed test article to a container and add a small volume of acetone to container. Manually mix to dissolve the test material adding acetone as necessary (typical ratio of test material to acetone is 1 g: 15-20 ml acetone). Visually inspect test material/acetone for solubility of test material.
5) Prepare a pre-mix by transferring the dissolved test material into 4 kg of feed in a Hobart mixing bowl. Mix for 10 minutes. Transfer the premix to a larger mixer, add remaining amount of weighed diet, mix for 30 minutes.

Attachment: II

Collection of Tissue Samples for Biochemical and Molecular Analysis

Because of the extreme instability of certain enzymes and biomolecules, it is essential that tissues be harvested as soon after death as possible and flash frozen immediately in liquid nitrogen. Failure to follow these procedures may lead to loss of the entire sample and all the energies and resources that were invested into generating the samples. Therefore, make every effort to comply with the following:

1) Harvest the tissue samples as soon as possible after death. Delays may allow for biodegradation and/or inactivation of the desired endpoint.
2) Immediately submerse the tissue sample directly into liquid nitrogen. Dry ice or other alternatives will not suffice. It is important that the tissue be immersed directly in liquid nitrogen; transferring it to a dry vessel (or sample container) suspended in liquid nitrogen will not suffice. The tissue may freeze to the vessel wall and will then be impossible to remove without completely destroying the vessel (or sample container).
3) Be absolutely sure to maintain the tissue frozen. It should be stored in a sealed container at -70□C and shipped or transferred on dry ice. If needed, the frozen sample can be fractured (broken into portions for different applications) by placing in a crucible which contains liquid nitrogen to keep the sample frozen while grinding/fracturing.

PROTOCOL AMENDMENT #1

Date: February 24, 1999

Study Number: 1132-100

Title: Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perflurooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA 3M T-7091.1) in Rats

Change/replace the following:

a) Title page: PAI Project Number: 99-1233

b) Page 3: In-life Start Date: 2/23/99
In-life End Date: 4/6/99
Draft Report Date: 5/18/99

c) Page 4: Test Materials

Test Material:	N-EtFOSA	Wy-14,643 (Cayman Chemical)
Identification:	N-EtFOSA	Wy
Lot Number:	541	11990
Purity:	Not provided	Not provided
Stability:	> 5 years	> 1 year
Storage Conditions:	room temp.	room temp.
Characteristics:	amber waxy solid	white powder

d) Page 5: Animal identification is by ear tag

e) Page 8: Observation of Animals; add,
Physical Examinations
Weekly at each weigh interval.

f) Entire protocol: Change R.O.W. Sciences to TherImmune Research Corporation

g) Page 8: Clinical Chemistry
...blood will be collected from a jugular vein into a serum-separator tube.

h) Page 9: Scheduled Sacrifices
Serum samples for clinical chemistry will be performed by PAI at Chevy Chase, MD.

Serum samples for compound level analysis will be stored in a freezer set to maintain -60 to -80°C and packed on dry ice and shipped to:

Dr. Kris J. Hansen
3M E.T. & S
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106
Telephone: 612 778-6018
Fax: 612 778-6176

For both Interim and Terminal Sacrifices:

NOTE: Two serum samples will be needed, (1) at least a 0.25 ml sample for clinical chemistry and (2) the remaining sample for compound level analysis.

i) Page 10: Palmitoyl-CoA Oxidase Tissue Collection and Analysis
Liver samples for palmitoyl-CoA oxidase activity will be shipped on dry ice to:

Dr. Ron Markevitch
Covance Laboratories Inc.
3301 Kingsman Boulevard
Madison, WI 53704
Telephone: 608 242-2712 ext. 2337
Fax: 608 241-7227

j) Page 10: Tissue Collection for Electron Microscopic Evaluation
Liver samples for EM should be shipped to:

Sharon Ambrose
PAI-NC
4915 D Prospectus Drive
Durham, NC 27713

k) Page 10: Remaining Liver Tissue
The remaining liver tissue will be frozen and stored at -60 to -80°C in PAI's long-term archive for possible future analysis.

l) Page 10: Cell Proliferation Tissue Collection and Immunohistochemical Evaluation
...liver will be collected and preserved in formalin.

m) Page 10: Tissue Collection for Electron Microscopic Evaluation
Sections of the left lateral lobe of the liver from all animals...

n) Page 7: Change dose of Wy-14,643 from 1000 ppm to 100 ppm
o) Page 13, Item #5: Prepare a pre-mix by transferring the dissolved test material into feed. Mix until homogeneous.....

Reason for Amendment:

a) Assigned after protocol approved
b) Dates determined after protocol signed; study is non-GLP, therefore report will not be audited
c) Information obtained after protocol signed
d) Spelling error
e) Physical exams should be conducted in addition to body weights
f) Testing facility changed name
g) No anticoagulant is used for clinical chemistry
h) Provide shipping instructions and specify minimum amount of serum needed for clinical chemistry
i) Provide shipping instructions
j) Provide shipping instructions
k) Provide storage instructions
m) Specify lobe of liver to be taken for EM
n) 100 ppm Wy, 14,643 is adequate for inducing a cell proliferative response, increase in liver weight and increase in peroxisomes
o) Accurate description of test diet preparation procedure

Approvals:

Marvin Case, D.V.M., Ph.D. Date
Study Representative
3M Corporate Toxicology

Gary W. Wolfe, Ph.D., D.A.B.T Date
Study Director
TherImmune Research Corporation

Sandra R. Eldridge, Ph.D. Date
Principle Investigator
Pathology Associates International

PROTOCOL AMENDMENT #2

Date: April 16, 1999

Study Number: 1132-100

Title: Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perflurooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA 3M T-7091.1) in Rats

Add the following:

a) Page 9: Necropsy
Gross lesions will be collected, processed and stained with H&E for microscopic evaluation.

Reason for Amendment:

a) For histopathologic examination of gross lesions.

Approvals:

Marvin Case, D.V.M., Ph.D. Date
Study Representative
3M Corporate Toxicology

Gary W. Wolfe, Ph.D., D.A.B.T Date
Study Director
TherImmune Research Corporation

Sandra R. Eldridge, Ph.D. Date
Principle Investigator
Pathology Associates International

PROTOCOL AMENDMENT #3

Date: August 9, 1999

3M Study Number: TRC 1131-100

Title: "Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perflurooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA 3M T-7091.1) in Rats"

Original:

Page 10: **Tissue Collection for Electron Microscopic Evaluation**

Sections of liver from all animals will be collected, minced to approximately one millimeter cubes and placed in a fixative appropriate for electron microscopy. The containers and fixative will be provided by PAI. Electron microscopy will be performed on one animal per treatment group exhibiting the highest cell proliferative response as well as one control animal at the discretion of the Sponsor, from one time point as well as the 4-week recovery. Thus, EM will be performed on one animal from the control, N-EtFOSE (one dose only to be determined), PFOS, N-EtFOSA, and Wy groups at one of the time points, as well as the 4 week recovery, for a total of 10 animals.

Change/replace the following:

Page 10: **Tissue Collection for Electron Microscopic Evaluation**

Sections of liver from all animals will be collected, minced to approximately one millimeter cubes and placed in a fixative appropriate for electron microscopy. The containers and fixative will be provided by PAI. Electron microscopy will be performed on two animals per treatment group from the 48-hour time point. Thus, EM will be performed on two animals from the control, N-EtFOSE (100 ppm dose group only), PFOS, N-EtFOSA, and Wy-14,643 groups at the 48-hour time point for a total of 10 animals.

Reason for Amendment:

Animals selected on the basis of cell proliferation results.

Approvals:

Marvin Case, D.V.M., Ph.D. Date
Study Representative
3M Corporate Toxicology

Gary W. Wolfe, Ph.D., D.A.B.T Date
Study Director
R.O.W. Sciences

Sandra R. Eldridge, Ph.D. Date
Principle Investigator
Pathology Associates International

PROTOCOL AMENDMENT #4

Date: May 30, 2000

Study Number: TRC 1132-100

Title: Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perflurooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA; 3M T-7091.1) in Rats

Change/replace the following:

a) Globally replace PFOSA with N-EtFOSA because the acronym for N-Ethyl Perfluorooctanesulfonamide that is currently accepted is N-EtFOSA, rather than PFOSA.
b) Page 4: Revise table because 3M provided the lot numbers, and purity information is changing with ongoing analysis at 3M.

Test Material:	N-EtFOSE (provided by 3M)	PFOS (provided by 3M)	PFOSA (provided by 3M)	Wy-14,643 (Cayman Chemical)
Identification:	N-EtFOSE	PFOS	PFOSA	Wy
Lot Number:	30035, 30037, 30039 combined	217	541	11990d
Purity:	Responsibility of 3M	Responsibility of 3M	Responsibility of 3M	98%
Stability:	> 5 years	> 5 years	> 5 years	> 2 years
Storage Conditions:	room temp.	room temp.	room temp.	room temp.
Characteristics:	waxy solid	white powder	amber waxy solid	white powder

Approvals:

Marvin Case, D.V.M., Ph.D. Date
Study Representative
3M Corporate Toxicology

Gary W. Wolfe, Ph.D., D.A.B.T Date
Study Director
TherImmune Research Corporation

Sandra R. Eldridge, Ph.D. Date
Principle Investigator
Pathology Associates International

VIII. SIGNATURE PAGE

Study Title:

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA 3M T-7091.1) in Rats

Study Number: TRC 1131-100

Sandra R. Eldridge, Ph.D. — Date: 11-8-04
Principle Investigator
Pathology Associates Division of Charles River Laboratories, Inc.