3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY

Protocol for Study No. T-6316.15; ST–30

N-EtFOSE Bile Method Development in Rats

***Study Objective*:**
The objective of this study is to collect urine and bile specimens from control and N-EtFOSE treated rats for method development. This study will support the N-EtFOSE ADME study in Cynomologus monkeys currently underway at Covance Madison.

Research Client: 3M Specialty Chemicals Division
Biodegradable Surfactants Team
3M Center, Building 236
Saint Paul, MN 55133-3220

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

Study Location: 3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55133-3220

Study Director: Andrew M. Seacat, Ph.D.
Sr. Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55133-3220
Ph.: 651-575-3161 FAX: 651-733-1773

***Study Toxicologist*:** Deanna Luebker, MS
Advanced Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55133-3220
Ph: 651-737-1374 FAX: 651-733-1773

Proposed Study Timeline:

In-Life Start Date: 8/12/1999
In-Life End Date: 8/20/1999

Regulatory Compliance:
This study will be performed in the 3M Strategic Toxicology Laboratory under a defined protocol and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:

Dan Hakes, Product Responsibility Liaison 3M Chemicals Division, will furnish high-purity N-EtFOSE.

Identification:

Name: Narrow Range N-Ethyl perfluorooctanesulfonamido ethanol, FM-3923.

Molecular Formula: C8F17SO2N(CH2CH3)CH2CH2OH

Lot Number: Lots 30035, 30037, 30039 mixed and analyzed as one sample.

Purity:

98.14 %

Stability:

Documentation will be kept on file.

Storage Conditions:

Upon receipt, test material will be stored tightly sealed at room temperature.

Characteristics:

Information on synthesis methods, composition or other characteristics that define the test material will be kept on file.

Animals:

Species: Rat
Strain: Sprague Dawley
Source: Harlan
Specifications: Bile Cannulated – Free End
Age at initiation of treatment: 6-8 weeks
Weight at initiation of treatment: approximately 150-250g
Number and sex: 20 males, 20 females

Table 1 - Dose Groups

Group	Dose	N	Sex
1	0 mg/kg	5	Male
2	20 mg/kg	5	Male
3	0 mg/kg	5	Female
4	20 mg/kg	5	Female

Identification: ear tag with animal number or unique tail mark.
AUA Number: 2246

Husbandry:

Housing:

Upon arrival at 3M all animals will be individually housed in standard cages. All rats from groups 2 and 4 and 1 each from groups 1 and 3 will be transferred to individual metabolism cages following dosing and housed

there through day 4-post dose. All other rats will remain individually housed in standard cages throughout the study.

Diet/Water:

Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet, supplied by Harlan Teklad, Madison, WI, and tap water will be provided to all rats *ad libitum* throughout the study.

Environment:

Environmental controls for the animal room will be set to maintain a temperature of 72 ± 3°F, humidity of 30-70%, a minimum of 10 exchanges of room air per hour and a 12 hour light/dark cycle.

Dose and Dosing Procedures:

Method of administration/Dose preparation:

A single 20mg/kg dose of N-Et FOSE will be administered via oral gavage to rats in groups 2 and 4 on day zero of the study. The dosing solution will be prepared as a 0.04% (4mg/ml) uniform solution in 2% Tween 80 using a 15 ml tissue grinder. A volume of 5 ml suspension / kg body weight will be administered to each rat. Re-suspension of solids will be performed with 5 strokes of the tissue grinder pestel before each sample is drawn-up in the syringe for dosing. A single 5 ml / kg body weight dose of 2% Tween 80 will be administered via oral gavage to rats in groups 1 and 3 on day zero of the study (see table 2).

Observation of Animals:

Clinical Observations:

Each animal will be observed daily for mortality and morbidity and notable findings will be recorded. Additional findings will be recorded as they are observed.

Body Weights:

Each animal will be weighed immediately prior to dosing and immediately prior to euthanasia.

Specimen Collection:

Frequency (See table 2):

Bile will be collected daily upon arrival at 3M. (All bile collected prior to dosing will serve as control bile.) Urine collections will be made on days 1 - 4 post dose. Necropsies will be performed on day 4-post dose.

Table 2 - Schedule

Sun	Mon	Tues	Wed	Thurs	Fri	Sat
				Aug 12 Dy – 4 Arrival @ 3M Bile coll.	Aug 13 Dy –3 Bile coll.	Aug 14 Dy –2 Bile coll.
Aug 15 Dy –1 Bile coll	Aug 16 Dy 0 DOSE Bile coll.	Aug 17 Dy 1 postdose Bile coll. Urine coll.	Aug 18 Dy 2 postdose Bile coll. Urine coll.	Aug 19 Dy 3 postdose Bile coll. Urine coll.	Aug 20 Dy 4 postdose Bile coll. Urine coll. Necropsy	

Method of Specimen Collection:

Bile will be collected from each rat daily upon arrival at 3M. The exteriorized portion of the catheter will be removed from the pouch of the protective jacket and the wire plug in the Free End catheter will be removed. A newly opened syringe will be used to withdrawn bile from the catheter of each rat. The bile from each rat will be equally split and placed into 2 labeled tubes. The wire plug will be inserted back into the catheter following bile collection and the exteriorized portion of the catheter place in the pouch of the protective jacket to prevent catheter chewing. Each tube of bile will be frozen (-70 °C) until analysis. Urine will be collected from each metabolism cage on days 1-4 post dose. For each urine specimen, the initial volume will be recorded and the sides of the urine collection apparatus will be washed with 10-20 ml deionized water. The urine and wash from each cage will be collected in a 50 ml tube and the final volume will be brought to 45 ml with additional deionized water. Each urine specimen will be frozen (-70 °C) until analysis. On day 4 post dose animals will be euthanized by CO_2 and gross necropsy performed. During necropsy, blood (≈ 6 ml) will be collected via the abdominal aorta and transferred to blood collection tubes without anticoagulant. Blood samples will be allowed to clot for a period of 15 to 30 minutes at room temperature, and the clot will be spun down in a centrifuge at 1100 x g for 5 minutes. The serum will be transferred to labeled 1.5 ml microfuge tubes and centrifuged again at 2000 x g to remove any remaining red blood cells. Each sera sample will then be transferred to a separate labeled polypropylene microfuge tube and (-70 °C) until analysis. Each liver will be removed, rinsed in saline, weighed, placed into a labeled sterile sample bag and frozen (-70 °C) until analysis.

Specimen Handling and Analysis:

Specimens will temporarily be stored in a freezer set to maintain -60 to -80°C in the Strategic Toxicology Laboratory.

For metabolite analysis, urine, liver and sera will be packed in dry ice and shipped to:

Kris Hansen, Ph.D.
3M Environmental Technology and Safety Services
935 Bush Avenue
St. Paul, MN 55133-3331
Ph: 612-778-6081, FAX: 612-778-6176.

For analytical method development purposes, bile will be packed in dry iced and shipped to:

Karsten Levsen
Fraunhofer Institut fuer Toxikologie und Aerosolforschung
Nikolai Fuchsstr 1. D-30625
Hannover
GERMANY

The Strategic Toxicology laboratory will retain one half of all the bile specimens collected for in-house method development, verification analysis and/or as a retain for possible future analysis.

All results will be provided for inclusion in the final report.

The number, type and date of collection of specimens to be generated for analysis are as follows:

Table 3 - Specimens

Specimen	day -4	day -3	day -2	day -1	day 0	day 1 PD	day 2 PD	day 3 PD	day 4 PD	Tota
Bile for Fraunhofer	20	20	20	20	20	20	20	20	20	180
Bile for in-house analysis	20	20	20	20	20	20	20	20	20	180
Urine	-	-	-	-	-	12	12	12	12	48
Serum	-	-	-	-	-	-	-	-	20	20
Liver	-	-	-	-	-	-	-	-	20	20

Data Analysis:
Data collected on N-EtFOSE metabolite concentrations in serum, liver and urine using validated methods will be analyzed for toxicokinetic parameters and for statistically significant differences between groups using Students T-test and/or ANOVA. Data collected during the method development and validation phases of bile and urine metabolite analysis will be analyzed for precision and accuracy as necessary to develop a validated method. Provided that enough of the retained bile and urine samples are

available following the development of validated method they may then be analyzed for N-EtFOSE metabolite levels under the validated methods to generate data for use in a definitive report.

Responsibilities:

- Deanna Luebker and Andrew Seacat will be responsible for dosing the animals, collecting in-life specimens, performing the necropsies, collecting and sending specimens for analysis and may perform additional experiments on the collected tissues for method development or verification.
- Kris Hansen, 3M Environmental, will be responsible for analysis of serum and liver tissues, and the urine samples following development of a validated method for urinary metabolites of N-Et FOSE if possible.
- Karsten Levsen, Fraunhofer Institut fuer Toxikologie und Aerosolforschung will be responsible for the development of a validated method for biliary metabolites and the subsequent analysis of the retained bile samples, provided there is a sufficient supply.
- Andrew Seacat and Deanna Luebker will draft a final report and ensure the report receives appropriate 3M review before a final report is issued.

Signatures:

Andrew M. Seacat 8/13/99

Dr. Andrew Seacat
Senior Research Toxicologist
Study Director

Date

Deanna Luebker 8/13/99

Deanna Luebker, MS
Advanced Toxicologist
Study Toxicologist

Date

SCL
NA- T-6316.2 NetFOSE ADME in cynomolgus monkey

Sponsor Representative

Date