AR226-0011

(2)

3M Environmental Laboratory

Final Report- Analytical Study

Single-dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-130

Study Number: AMDT-020895.1
Test Substance: FC-95 (T-6049)

Name and Address of Sponsor:
3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/20/95
Completion Date

1.0 SUMMARY

Samples of rabbit liver at 28 days post dermal administration of 0, 0.003, 0.06, and 0.30 mg/kg FC-95 (T-6049) were analyzed by combustion for total organic fluorine. There was no detectable level of total organic in any of the treated groups above that observed in control rabbit liver. Since in a pharmacokinetic study (HWI#6329-159), it was established that perfluorooctanesulfonate is persistent in rabbit liver with a biological half-life in excess of one month and that perfluorooctanesulfonate will serve as a good marker for absorption of FC-95, it is concluded that detectable dermal absorption does not occur at these dose levels. These dose levels are very low and possibly too low to make an assessment of dermal absorption.

2.0 INTRODUCTION

FC-95 is the potassium salt of perfluorooctanesulfonic acid. In a previous pharmacokinetic study (HWI#6329-159), it was shown that the biological half-life is >1 month. It is persistent in serum and liver after an intravenous dose. From previous studies in rats, the molecule is not known to biotransform or conjugate. Thus, analysis by combustion with subsequent measurement of fluoride will estimate the amount of perfluorooctanesulfonate present. At later times, the concentration in liver is higher than the amount in serum on the average. For use as a marker to assess dermal absorption, the level of total organic fluorine in liver is therefore a more sensitive test at 28 days post dose.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company.

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor

3.4 Stability of Reference Substance: To be determined by Sponsor

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples, the storage is -20 ± 10^{0} C.

3.6 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

4.0 EXPERIMENTAL - Overview

Samples of tissue and serum were available from HWI#6329-130 for analysis for total organic fluorine. The samples were combusted and analyzed for total organic fluorine. The data were assessed for extent of dermal absorption of FC-95.

5.0 EXPERIMENTAL - Methods

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

6.0 DATA ANALYSIS

The data from combustion analysis at 28 days post dermal administration of FC-95 (T-6049) are attached. The dosing material T-6049 is a 0.06% solution of FC-95. Doses in this analysis are expressed as the FC-95 equivalents. The liver was combusted and subsequently analyzed for total organic fluoride. There is no evidence of dermal absorption at any dose level for the groups of rabbits in the control, 0.003 mg/kg, 0.06 mg/kg, and 0.30 mg/kg groups. In all cases the amount of organic fluorine was below the practical quantitation levels and apparently below the detection limits.

Electrospray mass spectrometry did detect perfluorooctanesulfonate anion, but again the results are nebulous and below the practical quantitation limits.
There does not appear to be dermal absorption of FC-95 at these dose levels.

6.1 Circumstances That May Have Affected the Quality of the Data: No problems with this analysis are recognized that could affect the conclusion. However, the doses administrated are so low that even if all the dose had been absorbed it would be difficult to quantitate. In the higher dose group, 2 kg rabbits would receive only 600 ug total. For dermal dosing with analysis at 28 days and a half life on the order of a month, there would be less than 300 ug present in liver even if all the absorbed perfluorooctanesulfonate had gone to the liver.

7.0 CONCLUSION

FC-95 is not dermally absorbed at 0.30 mg/kg dose. This is a very low dose and possibly too low to make an assessment of dermal absorption.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-130 "Single Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits (Protocol type TP-3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-020895.1 including methods.

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-020895.1

9.10 Instrument Settings: See methods

9.11 Data: See attached:

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.1.1 Protocol and Final Report: HWI#6329-130 "Single Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

June 27, 1995

Performing Laboratory:

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Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-130

000007

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HWI 6329-130

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/21/94	12/21/94	Protocol Review	12/22/94	01/10/95
01/10/95	01/10/95	Animal Observation	01/10/95	02/10/95
01/24/95	01/24/95	Protocol Amendment	01/24/95	02/10/95
04/03/95	04/13/95	Data/Report Review	04/14/95	05/10/95
04/03/95	04/13/95	Data Review	04/14/95	05/10/95
06/27/95	06/27/95	Report Rereview	06/27/95	07/10/95

Randy Lentz
Representative, Quality Assurance Unit

6.27.95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity
Study of T-6049 in Rabbits

Test Material	T-6049
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Study Initiation Date	December 30, 1994
Experimental (In-life) Start Date	January 7, 1995
In-life End Date	February 10, 1995
Experimental Termination Date	June 27, 1995
Study Completion Date	June 27, 1995

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
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Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
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Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6049 when applied to the skin of rabbits.

The study was conducted using three male and three female acclimated rabbits of the Hra:(NZW)SPF strain for each treatment group.

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6049	5	3	3*
3	T-6049	100	3	3*
4	T-6049	500	3	3

a Administered at a dose volume of 2.0 mL/kg.
* One animal sacrificed on Day 2 due to possible broken back and replaced with another female animal.

The back of each rabbit was clipped free of hair and a single dose of the respective material at the indicated dose level was administered to the skin of the rabbits. The treatment sites remained intact. The area of application was covered with a gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing for a 24-hour exposure period.

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks were conducted daily thereafter for 28 days. The only exceptions to this were on Days 4 and 6 when the afternoon mortality check was inadvertently not conducted for the two replacement animals. Body weights were determined on Day -4 for randomization purposes, before test or control material administration (Day 1), and at in-life termination (Day 29). The initial dermal irritation reading was made before test or control material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. Blood samples were collected from a marginal ear vein of the animals before in-life initiation (Day 1), approximately 24-hours postdose (Day 2), on Days 4, 9, 16, and 23. Replacement animals were bled on Days 1, 2, 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from each animal. All samples were centrifuged and separated into serum and cellular fractions. All animals were euthanized at termination of the in-life phase and necropsied. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy

and weighed (volume only determined for bile). The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6049 did not result in any test material-related changes in body weight gain. All animals appeared clinically normal throughout the study with the exception of one Group 2 and one Group 3 female animals that were sacrificed on Day 2 due to injury (possible broken backs). These animals were replaced in the study and the replacement animals appeared normal throughout the study. No dermal irritation was observed at the dermal scoring intervals as a result of the application of distilled water or T-6049 at any of the dose levels. There were no test material-related lesions observed at necropsy.

OBJECTIVE

The objective of this study was to assess the systemic toxicity/absorption and relative skin irritancy of a test material when applied to the skin of rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6049 and described as a clear, colorless liquid. The control material was distilled water and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). Analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted or requested by the Sponsor. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test and control materials were stored at room temperature. A reserve sample of each test and control material was taken and will be retained in a freezer set to maintain a temperature of -20°C ±10° for 10 years in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any unused test material was returned to the Sponsor after completion of the in-life phase according to HWI SOP. Any remaining control material is retained for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc., Denver, Pennsylvania on December 28, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -4. The weight variation of the animals for each group of each sex selected for the study did not exceed ±2 standard deviations of the mean weight, and the mean body weights for each group of each sex were not statistically different at the 5% probability level. Two female animals (Nos. F53398 and F53355) were replaced after they were treated due to possible broken backs. These animals were replaced with two other female animals (Nos. F52999 and F53351) which were treated in the same manner.

Study Design

Animals weighing from 2,018 to 2,625 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6049	5	3	3*
3	T-6049	100	3	3*
4	T-6049	500	3	3

a Administered at a dose volume of 2.0 mL/kg.
* One animal sacrificed on Day 2 due to possible broken back and replaced with another female animal. This animal weighed 2,625 g at initiation.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Preparation of Exposure Area

On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair. The clipped area made up approximately 20% of the total body surface area. The intact skin of the test sites was inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped as needed throughout the study to aid in visualizing the application sites.

Dose Administration

All animals received a single administration of the respective test or control material. The day of treatment was designated as Day 1.

Group 1. An individual dose (2.0 mL/kg) was calculated and measured based on each animal's body weight on the day of treatment. The control material (distilled water) was applied evenly to the test site at a rate of approximately 0.05 mL/cm^2.

Groups 2, 3, and 4. For the Group-2 animals (5 mg/kg), the test material (T-6049) was mixed with distilled water to a concentration of 500 mg/mL and applied at a dose volume of 0.01 mL/kg. The mixture was stored at room temperature until administered. The test material was administered undiluted to the test sites of the Groups 3 and 4 animals (100 or 500 mg/kg, respectively) using the average bulk density of 0.99 g/mL to determine the dose volume for each dose level (0.10 and 0.51 mL/kg, respectively). The area of exposure for the 5, 100, and 500 mg/kg dose levels was 4, 25 (16 for replacement animal), and 100 cm^2, respectively. The approximate rate of application ranged from 0.006 to 0.014 mL/cm^2. An individual dose of the respective test material or test material mixture was calculated for each animal based on its body weight on the day of treatment.

Each area of application was covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. Collars were used to restrain the animals during the 24-hour exposure period.

Approximately 24 hours after test or control material application, the restraining collars and bandages were removed and any residual test material was removed with tap water and disposable paper towels.

Reason for Route of Administration

The dermal route is a potential route of exposure in humans.

Observations of Animals

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks (morning and afternoon) were conducted daily thereafter for 28 days. The only exceptions to this were on Days 4 and 6 when the afternoon mortality check was inadvertently not conducted for the two replacement animals.

Body weights were determined for randomization purposes on Day -4, before test material administration (Day 1), and at in-life termination (Day 29).

The initial dermal irritation reading was made before test or control material administration according to the Draize[1] technique (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8.

Sample Collections

Blood samples (approximately 4 mL) were collected from a marginal ear vein of all animals before experimental initiation (Day 1). Subsequent collection of blood was conducted approximately 24-hours postdose (Day 2), and on Days 4, 9, 16, and 23. Replacement animals were bled on Days 1, 2, 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were centrifuged and separated into serum and cellular fractions. These samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (Day 29), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated, and necropsied in random order. The sites of test and control material application were washed with lukewarm tap water before the necropsy procedure. All animals were subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples for all animals, with the exception of the animal sacrificed and necropsied on Day 2, were weighed (volume only determined for bile) and immediately placed on dry ice, then placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals were discarded.

Shipment of Blood, Bile, and Tissues

After experimental termination, the blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106), along with the collected corresponding weights or volumes. The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the tissue samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual and mean body weights are in Table 1. All animals exhibited body weight gains from Day 1 to Day 29.

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study with the following exceptions:

- One Group 2 female (No. F53398) treated with T-6049 at 5 mg/kg appeared to have injured its back at the time of the 24-hour blood collection. This animal was sacrificed, necropsied, and replaced with No. F52999. The replacement animal appeared normal throughout the study.
- One Group 3 female (No. F53355) treated with T-6049 at 100 mg/kg was found prior to completion of the exposure period with its leg caught in the cage floor, appeared to have injured its back, and was sacrificed. Since this animal did not receive its full test material exposure, the animal was discarded without a gross necropsy examination. The animal was replaced with No. F53351. The replacement animal appeared normal throughout the study.

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6049 at any of the dose levels.

Pathology

Individual animal pathology comments are presented in Table 4. Individual animal tissue weights and bile volumes are in Table 5. The treated skin in two animals dosed at 0 mg/kg, two animals dosed at 5 mg/kg, and one animal dosed at 500 mg/kg was diffusely red or had multiple red areas of variable size. These findings are not considered to be test material-related. There were no lesions observed in any of the remaining animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6049 were evaluated in male and female albino rabbits when administered as a single dermal application. There were no test material-related changes in body weight gain or in-life clinical findings at any of the dose levels. No test material-related dermal irritation was observed during the study. There were no test material-related lesions observed at necropsy.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

6-27-95
Date

REFERENCE

1. Draize, J. H., "Acute Dermal Toxicity (Single Exposure)," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 54-56 (1959).

PATHOLOGY REPORT

There were six rabbits (three males and three females) each from four dose levels of 0, 5, 100, and 500 mg/kg of body weight euthanized and necropsied at the termination of the study. One female (Animal No. F53398) dosed at 5 mg/kg of body weight was sacrificed on Day 2 because of an apparent broken back. This animal was replaced with another female (Animal No. F52999). The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, the treated skin in some animals was diffusely red or had multiple red areas of variable size. This included two animals dosed at 0 mg/kg, two animals dosed at 5 mg/kg, and one animal dosed at 500 mg/kg. There were no visible lesions in any of the remaining animals. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from each of these animals, and both kidneys from one male and one female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. The tissues from the female dosed at 5 mg/kg and sacrificed on Day 2 were collected but were not weighed. After necropsy, the animals were discarded.

The animal sacrificed on Day 2 had multiple dark red areas of variable size within the skeletal muscle of the lumbar-sacral region of the spinal column. These findings correlated with the clinical observation of an apparent broken back.

Thomas E. Palmer, PhD
Pathologist

6-27-95
Date

(6329-130.slh)
03/22/95

Table 1
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -4	Day 1	Day 29	Animal Number	Randomization Day -4	Day 1	Day 29
Group 1 (Control) - Distilled Water (0 mg/kg)							
F53358	2,066	2,231	2,816	F53404	2,200	2,286	2,997
F53359	2,030	2,093	2,670	F53405	2,426	2,474	2,911
F53353	2,136	2,312	3,012	F53356	2,110	2,224	2,685
Mean	2,077	2,212	2,833		2,245	2,328	2,864
Group 2 - T-6049 (5 mg/kg)							
F53402	2,288	2,382	2,938	F53399	2,095	2,122	2,580
F53364	2,244	2,364	2,923	F53349	2,376	2,492	2,919
F53352	2,102	2,018	2,622	F53398[a]	2,331	2,419	-
				F52999	2,485	2,625	3,272
Mean	2,211	2,255	2,828		2,319	2,413	2,924
Group 3 - T-6049 (100 mg/kg)							
F53360	2,157	2,194	2,943	F53350	2,320	2,409	2,996
F53400	2,076	2,122	2,696	F53355[b]	2,280	2,297	-
F53346	2,115	2,211	2,694	F53397	2,145	2,245	2,706
				F53351	2,059	2,253	2,606
Mean	2,116	2,176	2,778		2,175	2,302	2,769
Group 4 - T-6049 (500 mg/kg)							
F53365	2,126	2,276	2,951	F53403	2,170	2,270	2,860
F53354	2,325	2,478	3,008	F53000	2,241	2,268	2,914
F53347	2,103	2,152	2,646	F53345	2,306	2,341	2,951
Mean	2,185	2,302	2,868		2,239	2,293	2,908

a Animal No. F53398 was originally selected by the randomization program for use in the study and was treated. This animal was sacrificed after completion of the exposure period due to a possible broken back and was replaced with No. F52999. The body weights for No. F53398 are not included in the group means.

b Animal No. F53355 was originally selected by the randomization program for use in the study and was treated. This animal was sacrificed before completion of the exposure period due to a possible broken back and was replaced with No. F53351. The body weights for No. F53355 are not included in the group means.

- Not applicable.

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2	Day 3 through 29
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F53358	Appeared normal	✓	✓	✓
	F53359	Appeared normal	✓	✓	✓
	F53353	Appeared normal	✓	✓	✓
Female	F53404	Appeared normal	✓	✓	✓
	F53405	Appeared normal	✓	✓	✓
	F53356	Appeared normal	✓	✓	✓
		Group 2 - T-6049 (5 mg/kg)			
Male	F53402	Appeared normal	✓	✓	✓
	F53364	Appeared normal	✓	✓	✓
	F53352	Appeared normal	✓	✓	✓
Female	F53399	Appeared normal	✓	✓	✓
	F53349	Appeared normal	✓	✓	✓
	F53398	Appeared normal	✓	-	
		Possible broken back (at time of 24-hour bleeding interval)	-	✓	
		Moribund sacrifice	-	✓	
	F52999[a]	Appeared normal	✓	✓	✓

✓ Condition existed.
- Condition not evident.
a Animal No. F52999 replaced Animal No. F53398.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2	Day 3 through 29
		Group 3 - T-6049 (100 mg/kg)			
Male	F53360	Appeared normal	✓	✓	✓
	F53400	Appeared normal	✓	✓	✓
	F53346	Appeared normal	✓	✓	✓
Female	F53350	Appeared normal	✓	✓	✓
	F53355	Appeared normal	✓	-	
		Leg caught in cage floor (possible broken back)	-	✓	
		Moribund sacrifice	-	✓	
	F53397	Appeared normal	✓	✓	✓
	F53351[a]	Appeared normal	✓	✓	✓
		Group 4 - T-6049 (500 mg/kg)			
Male	F53365	Appeared normal	✓	✓	✓
	F53354	Appeared normal	✓	✓	✓
	F53347	Appeared normal	✓	✓	✓
Female	F53403	Appeared normal	✓	✓	✓
	F53000	Appeared normal	✓	✓	✓
	F53345	Appeared normal	✓	✓	✓

✓ Condition existed.
- Condition not evident.
a Animal No. F53351 replaced Animal No. F53355.

Table 3
Individual Dermal Irritation Scores

Group 1 (Control) - Distilled Water (0 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53358				Animal No. F53404			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53359				Animal No. F53405			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53353				Animal No. F53356			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6049 (5 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53402				Animal No. F53399			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53364				Animal No. F53349			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53352				Animal No. F53398[a]			
Erythema	0	0	0	0	0	0	-	-
Edema	0	0	0	0	0	0	-	-
Atonia	0	0	0	0	0	0	-	-
Desquamation	0	0	0	0	0	0	-	-
Coriaceousness	0	0	0	0	0	0	-	-
Fissuring	0	0	0	0	0	0	-	-
					Animal No. F52999[b]			
Erythema					0	0	0	0
Edema					0	0	0	0
Atonia					0	0	0	0
Desquamation					0	0	0	0
Coriaceousness					0	0	0	0
Fissuring					0	0	0	0

a Animal replaced with No. F52999.
b Replacement animal for No. F53398.
- Not applicable.

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 3 - T-6049 (100 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53360				Animal No. F53350			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53400				Animal No. F53355[a]			
Erythema	0	0	0	0	0	-	-	-
Edema	0	0	0	0	0	-	-	-
Atonia	0	0	0	0	0	-	-	-
Desquamation	0	0	0	0	0	-	-	-
Coriaceousness	0	0	0	0	0	-	-	-
Fissuring	0	0	0	0	0	-	-	-
	Animal No. F53346				Animal No. F53397			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
					Animal No. F53351[b]			
Erythema					0	0	0	0
Edema					0	0	0	0
Atonia					0	0	0	0
Desquamation					0	0	0	0
Coriaceousness					0	0	0	0
Fissuring					0	0	0	0

a Animal replaced with No. F53351.
b Replacement animal for No. F53355.
- Not applicable.

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6049 (500 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53365				Animal No. F53403			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53354				Animal No. F53000			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53347				Animal No. F53345			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 1 (Control) - Distilled Water (0 mg/kg)		
F53358	M	-	29	No visible lesions.
F53359	M	-	29	No visible lesions.
F53353	M	-	29	The skin has multiple red areas within the dermal application site, up to 3 cm x 2 cm. Collected with routine section.
F53404	F	-	29	No visible lesions.
F53405	F	-	29	No visible lesions.
F53356	F	-	29	The treated skin area appears diffusely dark red. Collected with routine section.
		Group 2 - T-6049 (5 mg/kg)		
F53402	M	-	29	No visible lesions.
F53364	M	-	29	No visible lesions.
F53352	M	-	29	No visible lesions.
F53399	F	-	29	The skin has diffusely dark red areas along the entire dorsal lumbar and thoracic region. Collected with routine section.
F53349	F	-	29	The skin has multiple red areas on the application site up to 1 cm in diameter. Collected with routine section.
F53398[a]	F	-	2	There are multiple dark red areas within the skeletal muscle of the lumbar sacral region surrounding the spinal column, up to 1.5 x 2.0 cm.
F52999[b]	F	-	29	No visible lesions.

- Not applicable.
a Animal replaced with No. F52999.
b Replacement animal for No. F53398.

Table 4 (Continued)
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
			Group 3 - T-6049 (100 mg/kg)	
F53360	M	-	29	No visible lesions.
F53400	M	-	29	No visible lesions.
F53346	M	-	29	No visible lesions.
F53350	F	-	29	No visible lesions.
F53397	F	-	29	No visible lesions.
F53351	F	-	29	No visible lesions.
			Group 4 - T-6049 (500 mg/kg)	
F53365	M	-	29	No visible lesions.
F53354	M	-	29	No visible lesions.
F53347	M	-	29	No visible lesions.
F53403	F	-	29	No visible lesions.
F53000	F	-	29	The skin has multiple red areas on the application site, up to 1 cm x 3 cm. Collected with routine section.
F53345	F	-	29	No visible lesions.

- Not applicable.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F53358	81.381	-	0.783	1.4
	F53359	73.857	-	0.761	0.8
	F53353	77.411	15.761	0.644	0.6
Female	F53404	70.277	12.585	0.620	1.8
	F53405	78.270	-	0.775	1.1
	F53356	74.687	-	0.915	0.5
		Group 2 - T-6049 (5 mg/kg)			
Male	F53402	78.731	14.611	0.468	2.3
	F53364	84.099	-	0.641	1.8
	F53352	67.613	-	0.706	1.8
Female	F53399	65.818	-	0.546	0.8
	F53349	77.596	14.211	0.489	0.9
	F53398[a]	-	-	-	-
	F52999[b]	87.510	-	0.660	0.5

- Not applicable.
a Moribund sacrifice on Day 2 (01/08/95) due to possible broken back. Tissues were collected, however, the corresponding weight or volume was not taken since this requirement became effective on 01/24/95. Animal replaced with No. F52999.
b Replacement animal for No. F53398.

Table 5 (Continued)
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 3 - T-6049 (100 mg/kg)			
Male	F53360	76.347	15.873	0.802	0.9
	F53400	74.958	-	0.395	1.6
	F53346	75.414	-	0.570	1.3
Female	F53350	74.050	15.120	0.784	1.4
	F53397	74.882	-	0.801	0.9
	F53351	80.686	-	0.279	0.5
		Group 4 - T-6049 (500 mg/kg)			
Male	F53365	82.598	-	0.441	1.8
	F53354	70.968	15.234	0.540	1.3
	F53347	83.696	-	0.716	2.0
Female	F53403	70.624	15.427	0.910	1.4
	F53000	79.617	-	0.714	0.8
	F53345	81.341	-	0.633	1.8

- Not applicable.

APPENDIX A

Protocol Deviations
Protocol TP3016.AB
Protocol Amendment No. 1

Protocol Deviations

Protocol	Actual Procedure
Page 7, 7. Experimental Design, C. Observation of Animals, (1) Clinical Observations, First Sentence. For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and Daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days.	On Day 4 and 6, the afternoon mortality check was inadvertently not conducted for the two replacement animals (Group 2, No. F52999, and Group 3, No. F53351).
Page 7, 7. Experimental Design, C. Observation of Animals, (3) Body Weights. For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day).	The body weight of the Group 2 animal that was sacrificed on Day 2 was inadvertently not documented.

These deviations are not considered to have had an adverse effect on the outcome of the study.

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of
T-6049 in Rabbits

Date:

December 30, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-130

Phone 608 241-4471
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704
Fax 608 241-7227

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of
T-6049 in Rabbits

HWI No.	6329-130
Test Material	T-6049
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN. 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	January 7, 1995
Experimental Termination Date	February 4, 1995
Draft Report Date	March 18, 1995

1. Study
Single-Dose Dermal Absorption/Toxicity Study in Rabbits

2. Purpose
To assess the systemic absorption and toxicity and relative skin irritancy of a test material when applied to the skin of rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

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

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6049

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test and control materials will be taken for this study.

The test and control material reserve samples will be stored at HWI in a freezer set to maintain a temperature of -20°C ±10°C for 10 years per HWI SOP. The Sponsor will be contacted after 10 years for disposition in accordance with the appropriate regulatory Good Laboratory Practices.

F. Retention
Any unused test material will be returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Distilled water

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this manufactured material is considered to be adequate for the purposes of this study.

D. Storage Conditions
Room temperature

E. Reserve Samples
See Section 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.0 kg

(5) Number and Sex
12 males and 12 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The animals will be placed into study groups using a stratified body weight randomization program within nine days of study initiation.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0*	3	3
2	T-6049	5**	3	3
3	T-6049	100	3	3
4	T-6049	500	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered at a dose volume of .01 mL/kg

(2) Preparation of Exposure Area
On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit will be clipped free of hair. The shaved area will constitute approximately 20% of the total body surface area. The treatment sites (intact skin) will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals will be clipped as needed throughout the study.

(3) Dose Administration
All animals will receive a single administration of the respective test or control material. The day of treatment will be designated as Day 1. The dose for each animal in Group 2 will be diluted with distilled water and applied at a dose volume of .01 mL/kg. The respective dose for each animal in Groups 3 and 4 will be applied undiluted. All doses in Groups 1-4 will be based on the animal's body weight just before administration and will be spread onto the area of exposure in a thin and uniform a layer. The area of application (Groups 1-4) will be covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. The rabbits will be collared during the 24-hour application period.

(4) Reason for Route of Administration
The dermal route is a potential route of exposure in humans.

(5) Removal of Test Material
Approximately 24 hours after test or control material application the bandages and collars will be removed and the residual test material will be removed using water or an appropriate solvent, if necessary.

C. Observation of Animals

(1) Clinical Observations
For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days. Observations may be extended when directed by the study director.

(2) Reading of Dermal Irritation
Before test or control material administration the initial dermal irritation reading will be made and recorded as the Day 1 reading (Attachment 1). Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8. Individual dermal irritation records will be maintained for each animal.

(3) Body Weights
For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day)

(4) Sample Collections

(a) Frequency
Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 9, 16, 23, and at experimental termination (Day 29)

(b) Number of Animals
All

(c) Method of Collection

Blood samples (approximately 4 mL) will be collected from the marginal ear vein of either ear on Days 1, 2, 4, 9, 16, and 23. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal sacrificed in a moribund condition or sacrificed at the time of necropsy (Day 29). The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain -20°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson or alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

D. Pathology

(1) Unscheduled Sacrifices and Deaths

Any animal dying during the study or sacrificed in a moribund condition will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section D. Pathology, (3) Sample Collection, will be collected. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifice

At termination of the experimental phase (Day 29), surviving animals will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, exsanguinated, and subjected to an abbreviated gross necropsy examination. The animals will be necropsied in random order and all abnormalities will be recorded.

(3) Sample Collection
The sites of test and control material application will be washed with lukewarm tap water prior to the necropsy procedure. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necopsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals will be discarded.

The tissues (liver, bile, dermal application site, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.C.(4).(c). The Sponsor is responsible for the retention and disposition of the samples.

E. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Tabulation of mortality data by sex and dose level
Description of any toxic effects/dermal irritation
Tabulation of mean body weights by sex and dose level
Gross pathology findings/gross pathology report

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Anatomical pathology records
Sample collection records
Shipping records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

1-5-95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

12-30-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12/30/94
Date

(6329-130.protdsk2)

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema

0 - None
1 - Slight
2 - Moderate
3 - Severe

Edema

0 - None
1 - Slight (barely perceptible to well defined by definite raising)
2 - Moderate (raised approximately 1 mm)
3 - Severe (raised more than 1 mm)

Atonia

0 - None
1 - Slight (slight impairment of elasticity)
2 - Moderate (slow return to normal)
3 - Marked (no elasticity)

Desquamation

0 - None
1 - Slight (slight scaling)
2 - Moderate (scales and flakes)
3 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1 - Slight (decrease in pliability)
2 - Moderate (leathery texture)
3 - Marked (tough and brittle)

Fissuring

0 - None
1 - Slight (definite cracks in epidermis)
2 - Moderate (cracks in dermis)
3 - Marked (cracks with bleeding)

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study
of T-6049 in Rabbits

HWI 6329-130

Sponsor	Contractor
3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220	Hazleton Wisconsin, Inc 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
John L. Butenhoff, PhD	Steven M. Glaza

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective January 10, 1995

1. Page 6, 7. Experimental Design; B. Dose Administration; (3) Dose Administration. Animal Nos. F53355 (Group 3 female) and F53398 (Group 2 female) were sacrificed on Day 2 due to injury (apparent broken backs). These animals will be replaced in this study. Replacement animals will be dosed, bled, and necropsied (includes tissue collection) at the same time as the animals in another 3M sponsored study (6329-137). Add the following as the second paragraph to this section:

> Due to the sacrifice on Day 2 of one Group 2 female (Animal No. F53398) and one Group 3 female (Animal No. F53355), replacement animals will be treated at the respective dose levels in the same manner as for the other animals in the study. The observations (clinical observations, reading of dermal irritation, and body weights) and the pathology of the animals (unscheduled sacrifices and deaths, scheduled sacrifice, and sample collection) will be conducted in the same manner as for the other animals in the study. The method of blood sample collection will be in the same manner as for the other animals in the study. The frequency of blood collection will be as follows:
>
> > Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 8, 15, 22, and at experimental termination (Day 29).

Phone 608-241-4471
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704
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Amendment No. 1

Effective January 24, 1995

At the request of the Sponsor, the weights of tissues collected and the volume of bile collected will be documented in the raw data. These weights and volumes will be included with the sample shipment. Modify the following sections of the protocol to include these additions.

2. Page 9, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the first and second paragraphs of this section with the following underlined additions:

> The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necropsied in each group will be collected, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C.
>
> The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.C.(4).(c).

3. Page 9, 8. Report. Add the following to this section:

> Individual animal tissue weights and bile volumes

PROTOCOL AMENDMENT APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

2/9/95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

2-1-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

2-02-95
Date

(6329-130.Aml.dsk2)

9.1.2 Analytical protocol AMDT-020895.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-130

Study Number: AMDT-020895.1
Test Substance: FC-95 (T-6049)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25, 1995

Method Numbers and Revisions:

AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochenmicals Using Electrospray Mass Spectrometry

Author: James D. Johnson

Approved By:

James D. Johnson 10/30/95
James D. Johnson Date
Study Director

John L. Butenhoff 10/30/95
John Butenhoff, PhD Date
Sponsor Representative

1.0 PURPOSE

The purpose of this study is to assess the dermal absorption of FC-95 (T-6049) in rabbits after a single dermal dose of FC-95.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.
2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum
2.1.3 Analytical Control Substance: None
2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4 liver), Sigma Chemical Company (2.1.2, 2.1.4 serum)

2.3 Number of Test and Control Samples: Tissues and fluids from 18 test animals and 6 control animals. One animal was replaced on day 2 (animal F53398 in the 5 mg/kg dose group replaced by animal F52999). Tissues and fluids include liver, kidney, serum, cellular fraction, dermal application site and bile. Analysis of these tissues will be at the discretion of the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), -20 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required studies longer than 28 days.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

Rabbits were dosed with T-6049 in a control group, 5 mg/kg, 100 mg/kg, and 500 mg/kg dermally. (See HWI#6329-130 for dosing, animal weights, tissue collection, etc.) Tissues and serum are available from these animals for analysis of total organic fluorine content. At the discretion of the Study Director samples will be analyzed to the extent necessary to provide information to assess the extent of dermal absorption.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochenmicals Using Electrospray Mass Spectrometry

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 per tissue or fluid. Statistics used, at the discretion of the Study Director, may include serum averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT Archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochenmicals Using Electrospray Mass Spectrometry

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

James D Johnson 10/3/95
Group Leader Date

Carl Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 10-31-95

Quality Assurance — Date: 10-31-95

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.
1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.
1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Syringe, capable of measuring 100 µL
4.1.2 Eppendorf type or disposable pipets
4.1.3 Gloves
4.1.4 Plastic grinding tubes
4.1.5 Plastic centrifuge tubes, 15 mL
4.1.6 Labels
4.1.7 Nitrogen
4.1.8 Timer
4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
4.1.10 Analytical pipets: glass volumetric pipets.
4.1.11 Disposable plastic 3 cc syringes.
4.1.12 Crimp cap autovials.

4.2 Reagents

4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
4.2.4 Ethyl Acetate
4.2.5 Methanol
4.2.6 Milli-Q water
4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluoroctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step **9.12** of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{\text{171 mg liver}^{*} / \text{1 ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 Calibration

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 Storage Conditions for Standards

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in **8.3**.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 Quality Control - not applicable
10.2 Precision and Accuracy- not applicable
10.3 Other Validation Parameters- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W_____	FC-95 approx 1 ppm actual ppm #W_____	FC-95 approx. 5 ppm actual ppm #W_____	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
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		-	-	-	

[1] study number where the original worksheet is located and place a copy.

Liver Extraction Process:	Date & Initials
Pipet 1 mL of Liver Solution	
Pipet 100 uL of 12 ppm Internal Standard Std. #	
Vortex 15 sec.	
Pipet 1 mL of 250 ppm Ammonium Acetate Std. #	
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	
Pipet 5 mL of Ethyl Acetate	
Vortex 20-30 sec.	
Shake 20 min.	
Centrifuge 20-25 min.	
Remove a 4 mL aliquot of organic layer	
Blow down to near dryness (<0.25 mL) with N_2	
Add 1 mL of 1:1 Acetonitrile/H_2O TN#	
Vortex 15 sec.	
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial.	

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 6/6/95

Quality Assurance — Date: 6/6/95

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 **Scope:** This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES^-.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES^-, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 **Using the Autosampler**

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

9.3.3.1 Push the sample button

9.3.3.2 Set sample loop size = 100 uL

9.3.3.3 Set inject/sample = 2

9.3.3.4 Set Cycle time = 0

9.3.3.5 Name the file: Livers

9.3.3.6 Identify the tray used

9.3.3.7 Add the samples to Queue by pressing "Enter"

9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 **Quality Control**

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 **Precision and Accuracy**

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 **Other Validation Parameters**

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor Original to: Study Director Copies to: QAU Files

Study Title: **Single-dose Dermal Absorption/Toxicity Study of T-6049 in Rabbits**

Study Number: AMDT-020895.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/13/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-19-95

QAU Auditor Date

000428

000077

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:
Jim Johnson
Dave Christenson

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-020895.1, HWI 6329-130
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total μg Fluoride in Whole Liver
Mean per Dose Group*

	μg	Std. Dev.
Control Group	22.4	± 5.7
5.0 mg/kg dose (T6049) (0.003 mg/kg)**	17.6	± 4.9
100 mg/kg dose (T6049) (0.06 mg/kg)**	15.8	± 2.6
500 mg/kg dose (T6049) (0.30 mg/kg)**	22.3	± 2.2

*Calculated as the mean of triplicate samples from each of three male and three female rabbits.
**Test material is a 0.06% solution of T6049, actual dose in parenthesis.

FC95 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
Liver blnk-1		0.835		0.1138			
Liver blnk-2		0.287		0.1347			
Liver spk-1	**106%**	1.140		0.1403			
Liver spk-2	**99%**	1.200		0.1248			
F53405-1		0.474		0.1141			
F53405-2		0.281	0.353	0.1270	78.27	27.65	0
F53405-3		0.305		0.1322			
F53356-1		0.212		0.1431			
F53356-2		0.249	0.220	0.1458	74.69	16.40	0
F53356-3		0.198		0.1367			
F53404-1		0.684		0.1279			
F53404-2		0.244	0.394	0.1186	70.28	27.68	0
F53404-3		0.254		0.1230			
F53353-1		0.232		0.1441			
F53353-2		0.205	0.206	0.1096	77.41	15.93	0
F53353-3		0.181		0.1355			
Liver blk 1		0.272		0.1165			
Liver blk 2		0.129		0.1550			
Liver spk 1	**91%**	1.111		0.1237			
Liver spk 2	**102%**	1.096		0.1408			
F53358-1		0.402		0.1346			
F53358-2		0.320	0.335	0.1164	81.38	27.27	0
F53358-3		0.283		0.1370			
F53359-1		0.336		0.1143			
F53359-2		0.209	0.247	0.1174	78.86	19.52	0
F53359-3		0.197		0.1142			
F53402-1		0.149		0.1518			
F53402-2		0.136	0.147	0.1645	78.73	11.61	5
F53402-3		0.157		0.1434			
F53364-1		0.275		0.1097			
F53364-2		0.285	0.298	0.1140	84.10	25.09	5
F53364-3		0.335		0.1085			
F53352-1		0.257		0.1685			
F53352-2		0.241	0.255	0.1418	67.61	17.26	5
F53352-3		0.267		0.1401			
F53349-1		0.180		0.1390			
F53349-2		0.242	0.234	0.1010	77.60	18.15	5
F53349-3		0.280		0.1437			
F53399-1		0.238		0.1014			
F53399-2		0.209	0.200	0.1021	65.82	13.20	5
F53399-3		0.155		0.1290			
F53346-1		0.167		0.1340			
F53346-2		0.172	0.222	0.1396	75.41	16.75	100
F53346-3		0.328		0.1283			
F53350-1		0.284		0.1296			
F53350-2		0.266	0.241	0.1477	74.05	17.87	100
F53350-3		0.174		0.1375			
F53397-1		0.238		0.1273			
F53397-2		0.291	0.253	0.1367	74.88	18.93	100
F53397-3		0.230		0.1128			

000434

000083

FC95 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
F53400-1		0.157		0.1418			
F53400-2		0.191	0.171	0.1233	74.96	12.80	100
F53400-3		0.165		0.1356			
F53360-1		0.144		0.1420			
F53360-2		0.209	0.168	0.1057	76.35	12.86	100
F53360-3		0.151		0.1309			
Liver blk 1		0.432		0.1137			
Liver blk 2		0.366		0.1019			
Liver spk 1	**95%**	1.389		0.1036			
Liver spk 2	**95%**	1.266		0.1132			
F53347-1		0.344		0.1221			
F53347-2		0.268	0.269	0.1073	83.70	22.50	500
F53347-3		0.195		0.1415			
F53365-1		0.206		0.1301			
F53365-2		0.289	0.216	0.1212	82.60	17.88	500
F53365-3		0.154		0.1486			
F53345-1		0.261		0.1438			
F53345-2		0.251	0.282	0.1499	81.34	22.96	500
F53345-3		0.335		0.1352			
F53354-1		0.420		0.1120			
F53354-2		0.364	0.333	0.1091	70.97	23.66	500
F53354-3		0.216		0.1272			
F53403-1		0.323		0.1126			
F53403-2		0.344	0.336	0.1375	70.62	23.71	500
F53403-3		0.340		0.1378			
F53000-1		0.307		0.1407			
F53000-2		0.272	0.293	0.1384	79.62	23.36	500
F53000-3		0.301		0.1257			
F52999-1		0.243		0.1369			
F52999-2		0.236	0.230	0.1040	87.51	20.13	5
F52999-3		0.211		0.1218			
F53351-1		0.193		0.1419			
F53351-2		0.188	0.195	0.1449	80.69	15.71	100
F53351-3		0.203		0.1210			
liver spk-3	**94%**	1.079		0.1312			
liver spk-4	**89%**	0.997		0.1355			
liver spk-5	**71%**	3.467		0.1253			
liver spk-6	**119%**	5.930		0.1215			
liver spk-7	**100%**	4.330		0.1401			

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

HWI# 6329-130

David Christenson
10-31-95

Study: Single-Dose Dermal Absorption
Protocol Number: TP3016.AB
Test Material: T-6049 in Rabbits (FC 95)
Matrix: Liver
R Squared Value: 0.9955
Response Factor Amount: 1.27E-05
Analyst: DLC
Date: 4/3/95
Method: AMDT-M-4
Instrument: Fisons VG 2000 Electrospray MS
LABBASE File: 040395A/B

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g **	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: 0 mg/kg Distilled Water	F53404	N/D	1.0422	1	N/D	69.156	N/D
	F53356	N/D	1.1273	1	N/D	73.403	N/D
	F53359	N/D	1.2009	1	N/D	72.193	N/D
	F53358	6571	1.0779	1	0.0618	69.308	0.004
	F53405	N/D	1.2166	1	N/D	77.164	N/D
	F53353	N/D	1.0734	1	N/D	75.915	N/D
Group 2: 5 mg /kg *	F53402	6645	1.1472	1	0.0587	76.184	0.004
	F53352	58373	1.1648	1	0.5080	81.324	0.041
	F53399	N/D	1.1776	1	N/D	63.494	N/D
	F53349	N/D	1.0143	1	N/D	76.006	N/D
	F53364	22296	1.0259	1	0.2203	80.666	0.018
Group 3: 100 mg/kg *	F53400	12849	1.3817	1	0.0943	73.828	0.007
	F53360	11111	1.054	1	0.1069	74.574	0.008
	F53350	18253	1.3119	1	0.1410	72.534	0.010
	F53346	N/D	1.3537	1	N/D	73.666	N/D
	F53397	N/D	1.2903	1	N/D	73.063	N/D
Group 4: 500 mg/kg	F53403	59686	1.0511	1	0.5756	69.012	0.040
	F53347	N/D	1.0447	1	N/D	83.100	N/D
	F53000	34395	1.1242	1	0.3101	77.255	0.024
	F53345	4623	1.2143	1	0.0386	77.250	0.003
	F53365	N/D	1.0281	1	N/D	66.252	N/D
	F53354	N/D	1.194	1	N/D	80.146	N/D

* Rabbits F52999 (G2) and F53351 (G3) were not analyzed

** The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

Replot

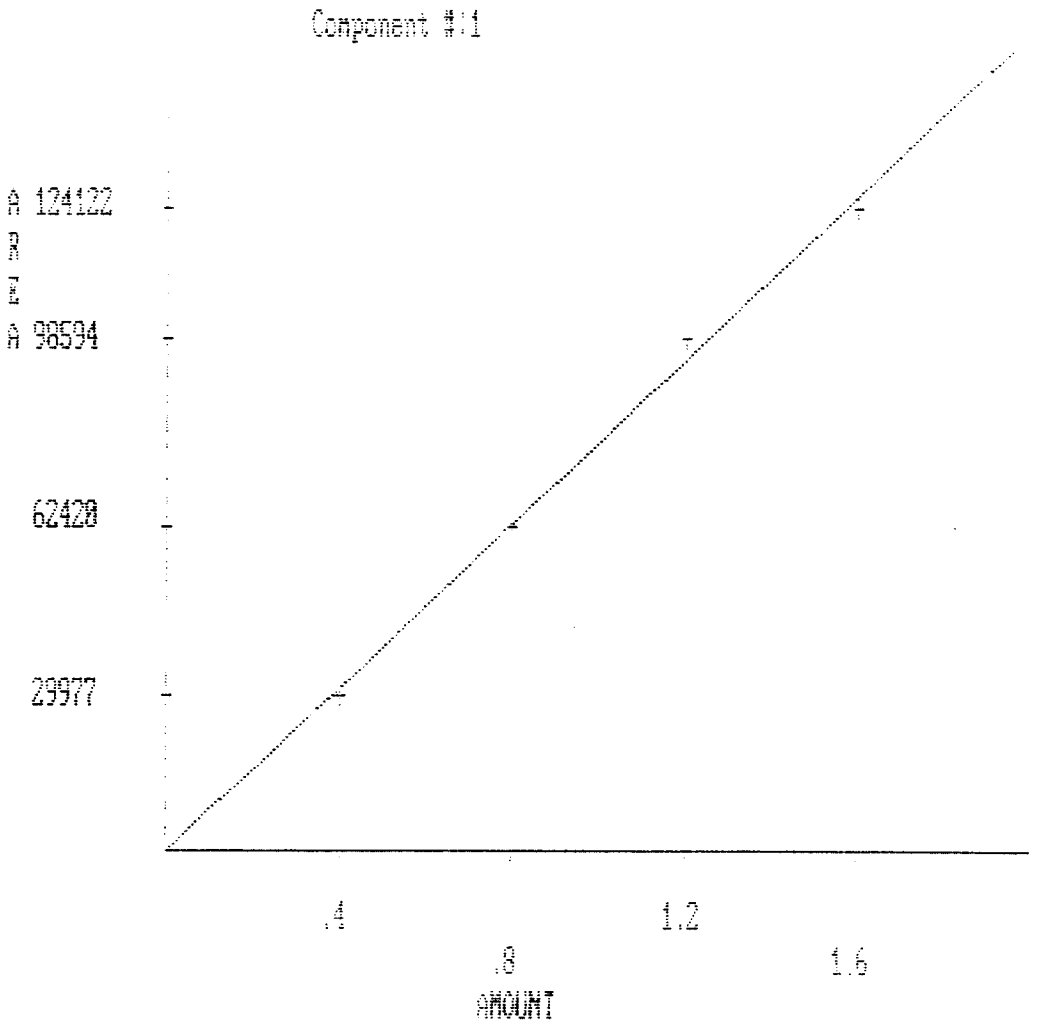
A-2

FILE: 040395A

4WI # 6329-130

FC-95

Method DLCLIV
Sample DLCLIV
Operator DLC
Run date 05-09-1995 06:46:52 Version: 11
Printed on 05-09-1995 AT 06:47:07
Straight Line Fit forced through Origin.



Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.4000	29977
2	0.8000	62420
3	1.2000	98594
4	1.6000	124122

Y = SLOPE * X + INTERCEPT

Area = 7.8924E-04 * Amount + 0.0000E+00
Amount = 1.2670E-05 * Area + 0.0000E+00
R squared = 0.9955

HWI # 6329-130 Electrospray MS Analysis

FC-95

A-3

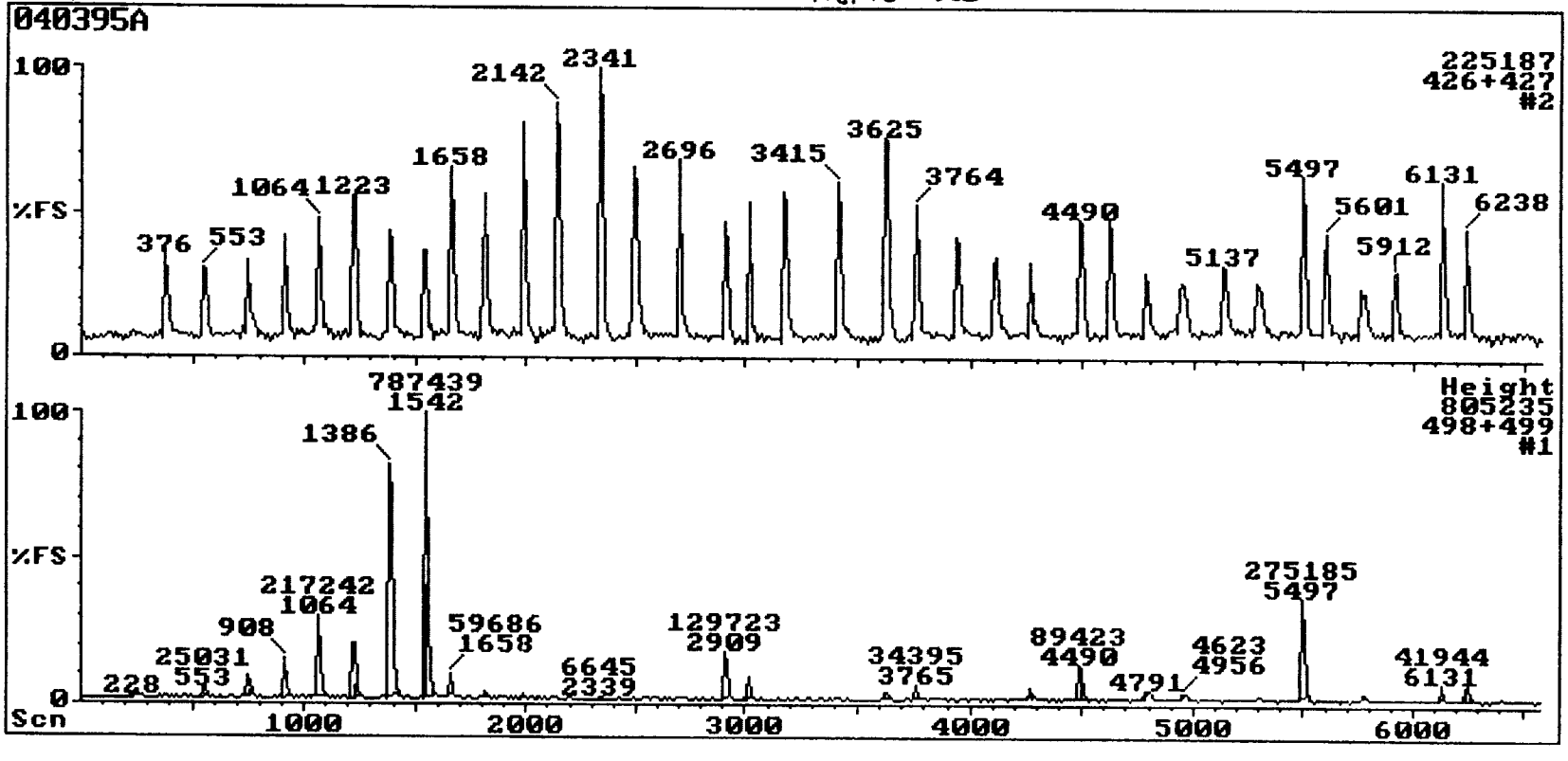
One sample misidentified (scan # 2696, F53357) actual animal number unknown.
10/18/95 DLC

Contains pages A-3 through A-10

000439

000454

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06
Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC



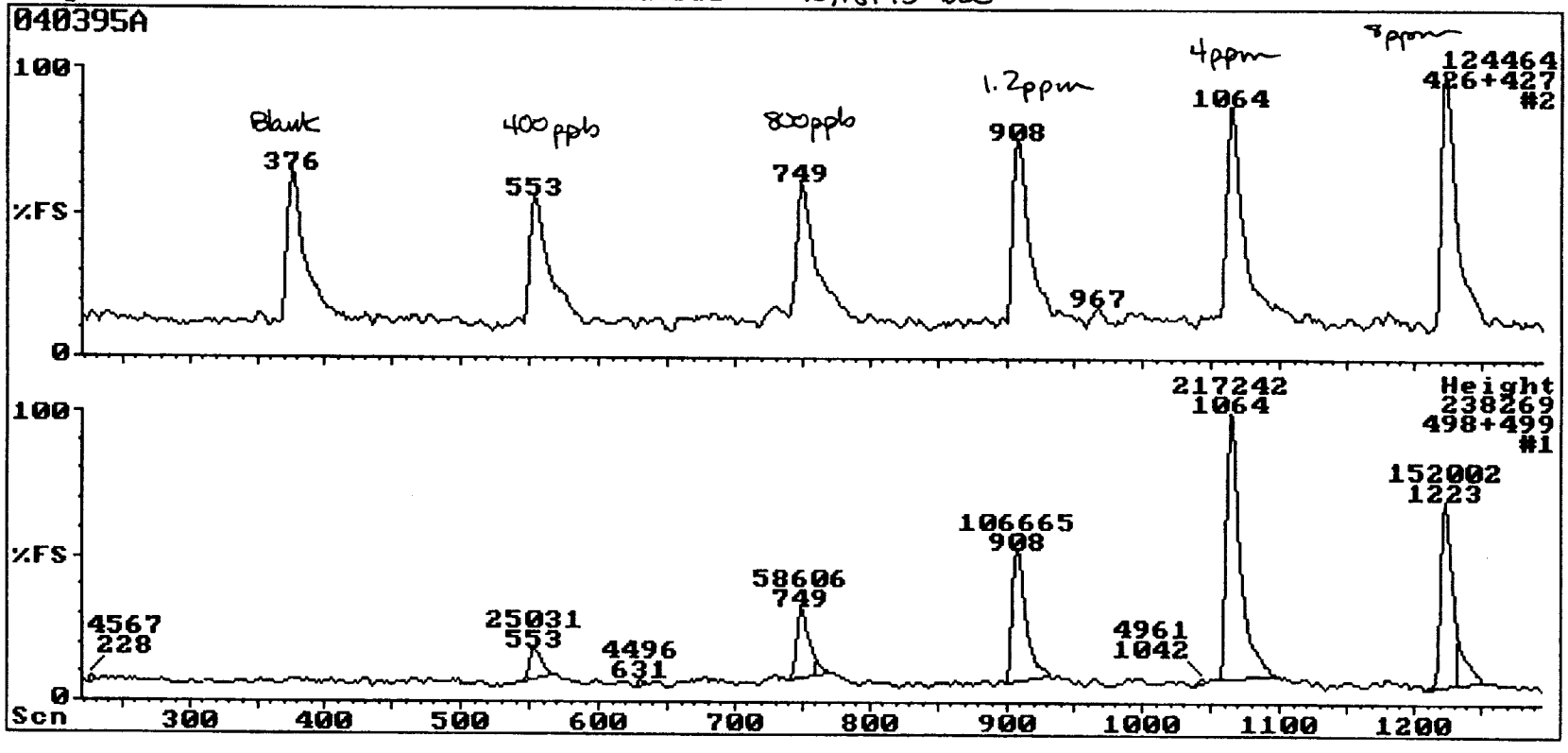
A-4

000440

Beef liver standards

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC



A-5

000441

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95

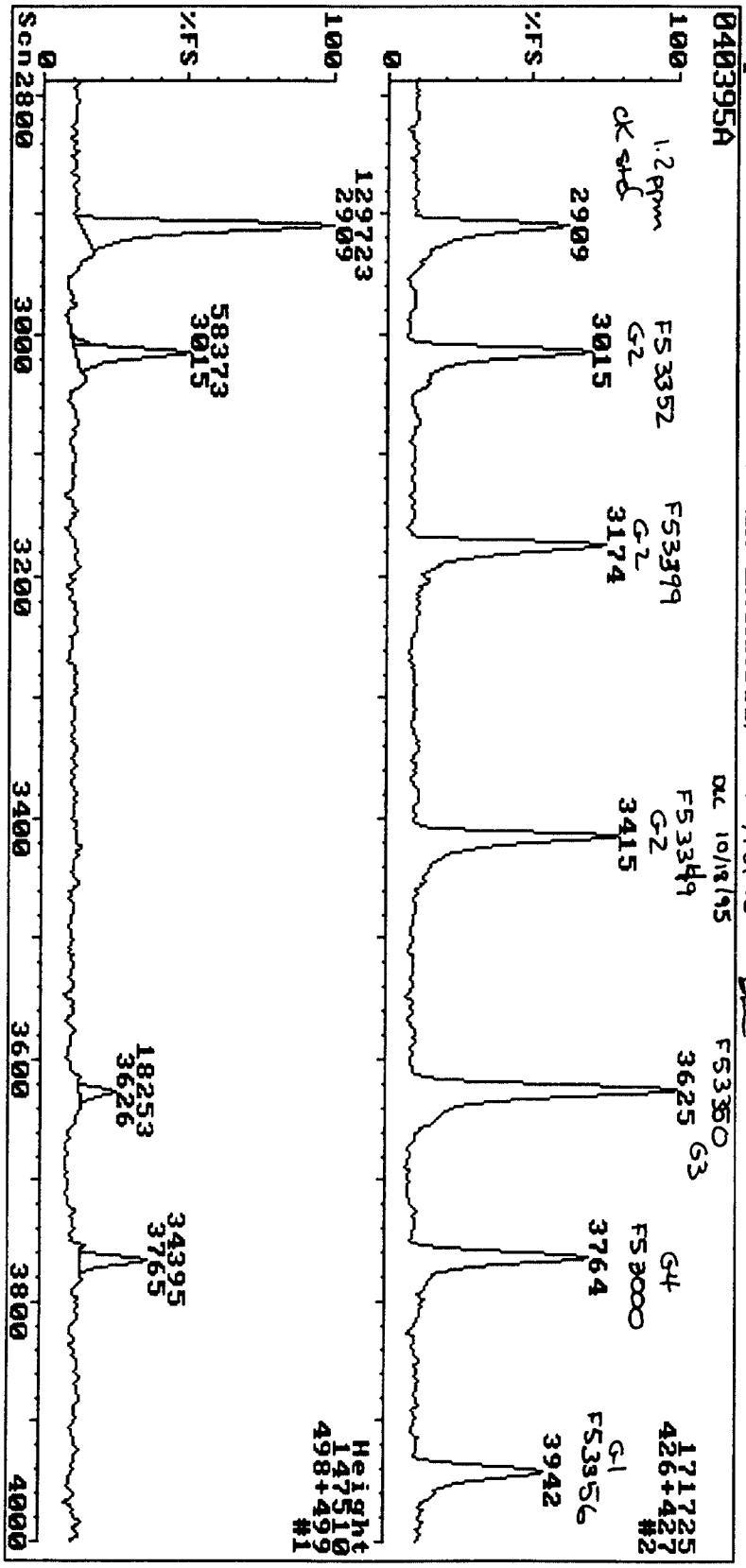
A-6

000091

000442

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC


File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06
Sample:CARBOPOL RABBIT LIVER EXTRACTS, 10/18/95 DLC
040395A
100
%FS
0
1.2 ppm
OK sub
2909
FS 3352
G2
3015
FS3379
G2
3174
DLC 10/18/95
FS3349
G2
3415
FS3360
3625 G3
G4
FS 2000
3764
G1
FS3356
3942
171725
426+427
#2
129723
2909
58373
3015
18253
3626
34395
3765
Height
147510
498+499
#1
Scn 2800 3000 3200 3400 3600 3800 4000

4-8

0000933

0004444

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample: ~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC

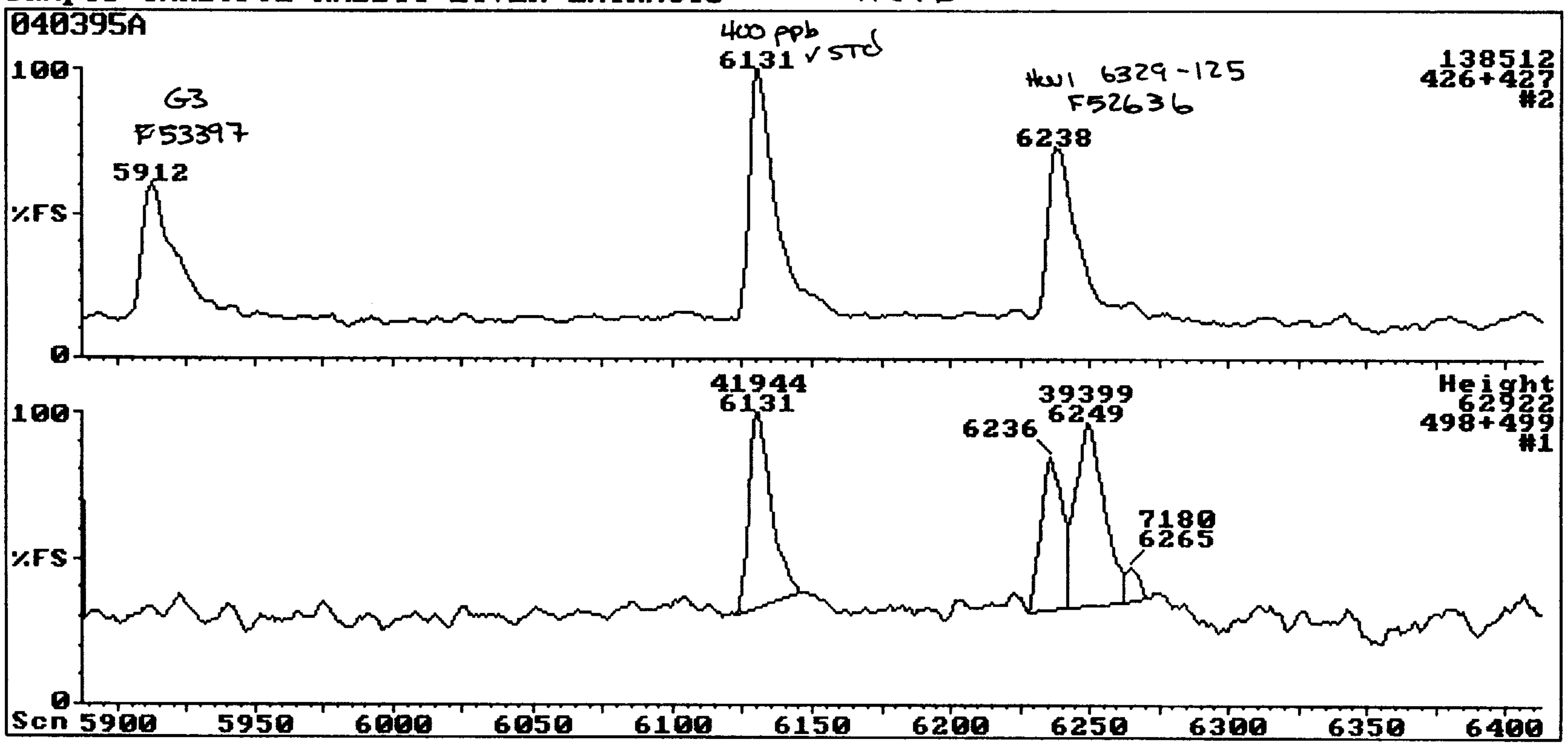
A-9

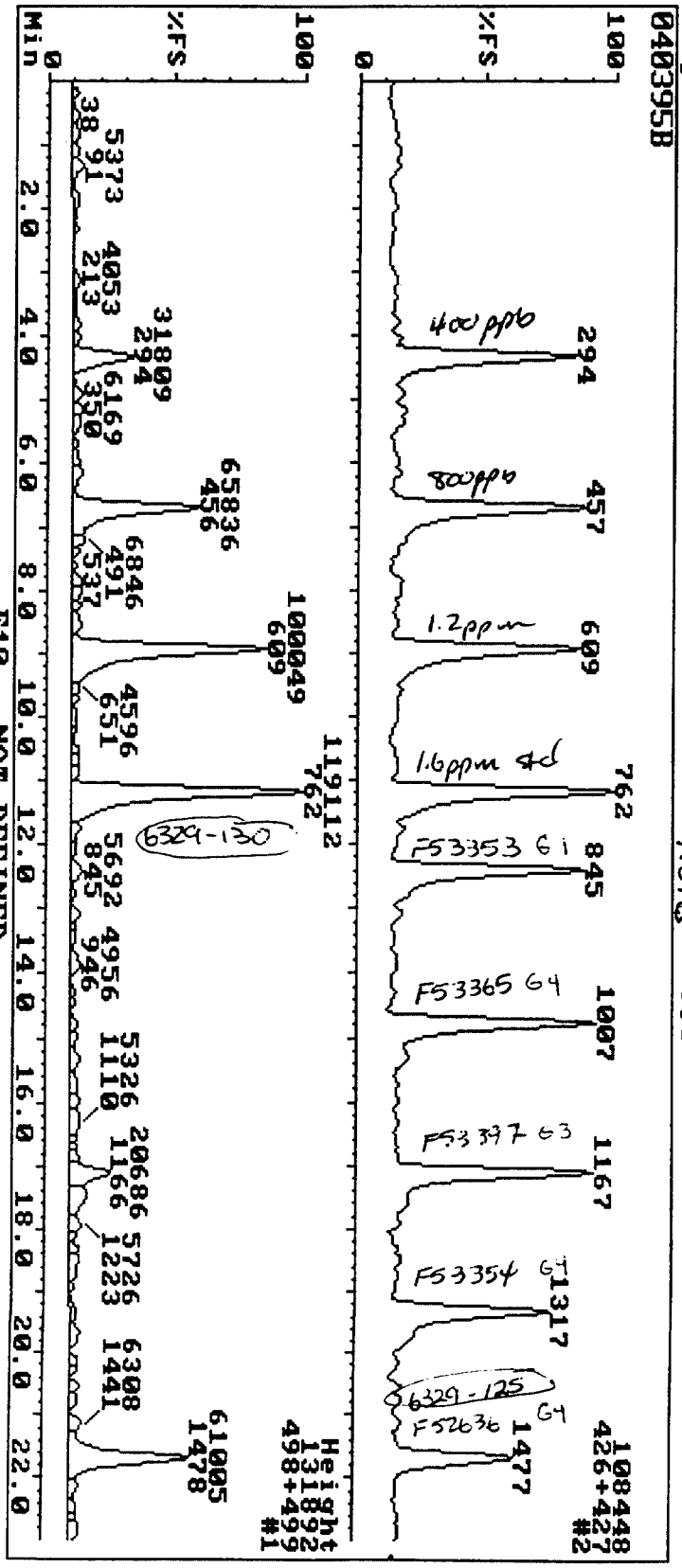
000093

000445

File:040395A LAB-BASE - The MS Data System 03/04/1995 09:06

Sample:~~CARBOPOL RABBIT LIVER EXTRACTS~~ 10/18/95 DLC




File:040395B LAB-BASE - The MS Data System 03/04/1995 10:53
Sample: [redacted] 10/18/95 DLC
040395B
400 ppb
800ppb
1.2ppm
1.6ppm std
F53353 61
F53365 64
F53397 63
F53354 64
6329-125
F52636 64
6329-130
F10 NOT DEFINED