

3M Environmental Laboratory

Final Report- Analytical Study**Single-dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits****In-Vivo Study Reference Number:** HWI#6329-129**Study Number:** AMDT-112294.1**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

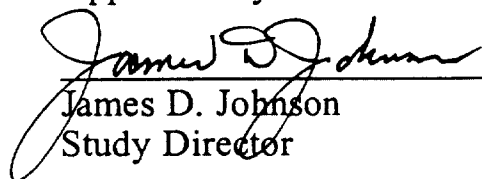
Method Numbers and Revisions:

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
AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified
Dohrmann DX2000 Organic Halide Analyzer-Serum

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:


James D. Johnson
Study Director

11/20/95

Completion Date

1.0 SUMMARY

Rabbit serum and liver were analyzed for fluorine content at various times after rabbits were dosed intravenously with a single dose of FC-95 (T-6049). The fluorine in liver was detected after a single dose of 100 mg/kg (60 ug/kg FC-95) or after 500 mg/kg (300 ug/kg FC-95). The liver fluoride ion concentration in the Group 5 animal (300 ug/kg), at 48 hours post dose was 1.0 ppm and for the rabbit dosed with 60 ug/kg the concentration was about 0.4 ppm. The presence of perfluorooctanesulfonate was confirmed with electrospray mass spectrometry.

The method would probably be able to detect a dose somewhere between 10 and 60 ug/kg. Analysis of fluorine at 28 days in liver and serum will likely provide a marker for dermal absorption for dermally applied fluorochemicals that are biotransformed to perfluorooctanesulfonate anion. The data agree with a second pharmacokinetic study of FC-95 which was carried out to 28 days with more animals (HWI#6329-159).

2.0 INTRODUCTION

This study was designed to provide information as to whether the perfluorooctanesulfonate anion does go to the liver and other tissues when the material is administered in an intravenous dose, and to ascertain the change in concentration with time after dose in serum and liver.

It is known from studies done previously with rats that the half-life of perfluorooctanesulfonate anion is quite long (>1 month). It was expected that the half-life in rabbits would also be long. Perfluorooctanesulfonate anion is a likely biotransformation product of several fluorochemicals that are to be tested for dermal absorption in other studies. The pharmacokinetics of the perfluorooctanesulfonate anion is very relevant to these dermal absorption studies.

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 $-20 \pm 10^{\circ}$ 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

Serum and tissues from animals dosed as described (HWI#6329-129), were available for analysis for fluorine compounds. Since perfluorooctanesulfonate anion is not biotransformed, the analysis was accomplished with combustion and subsequent analysis for fluorine. The fluorine data are related directly to perfluorooctanesulfonate concentration. The fluorine analysis of serum collected at different time intervals after dosing provides data which can be interpreted pharmacokinetically.

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

5.5 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

6.0 DATA ANALYSIS

The material T-6049 was a solution of FC-95 diluted to 0.06% in water. The doses of 5, 10, 100, and 500 mg/kg are thus 3, 6, 60, and 300 ug/kg. These are very low doses.

The liver fluoride ion concentration in the Group 5 animal (300 ug/kg), at 48 hours post dose was 1.0 ppm and for the rabbit dosed with 60 ug/kg the concentration was about 0.4 ppm. The control rabbit, the 5 mg/kg rabbit, and the 10 mg/kg rabbit concentrations were not above the practical limit of quantitation.

For livers of the two highest doses using analysis by electrospray mass spectrometry, the perfluorooctanesulfonate was detectable and the estimated amounts for the 100 and 500 mg/kg rabbits were 0.3 and 0.6 ppm, respectively.

These data show that perfluorooctanesulfonate will be a very sensitive marker for dermal absorption tests of FC-95 and for any fluorochemicals that are biotransformed to perfluorooctanesulfonate. The fact that the material can be observed in liver after a 60 ug/kg dose is suggestive that a very large portion of the dose will be in liver if the material is present systemically.

Other data was collected on serum samples using thermal extraction of fluoride by means of a modified Dohrmann organic halide analyzer and fluoride measurement by means of a Orion expandable ion analyzer (see appendices). This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances That May Affect the Quality of the Data: These values for concentration are from combustion analyses of the biological material. The recoveries are based on spiking the biological material with known amounts of FC-95. The fluorine is measured by selective ion electrode and the Orion meter is calibrated over a 5 point range. However, if there is a different response at different concentrations of FC-95 other than the point used as a calibration check, there could be a bias at those concentrations even though the amount of fluorine is being measured accurately. This can come from other variables in the method such as the efficiency of combustion. Thus, the values reported for concentration are not absolute values. The values are certainly sufficient to make the point that the perfluorooctanesulfonate anion is present at 48 hours in liver.

7.0 CONCLUSION

The method would probably be able to detect a dose somewhere between 10 and 60 ug/kg. Analysis of fluorine at 28 days in liver and serum will likely provide a marker for dermal absorption for dermally applied fluorochemicals that are biotransformed to perfluorooctanesulfonate anion. The data agree with a second pharmacokinetic study of FC-95 which was carried out to 28 days with more animals (HWI#6329-159).

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#6329-129 "Single Dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-112294.1 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-112294.1

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.

9.11.3 Summary and raw data; ppm F⁻ in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.1.1 Protocol and Final Report: HWI#6329-129
“Single Dose Intravenous Pharmacokinetic Study of
T-6049 in Rabbits” (Protocol type TP8084.PK for
dosing of animals, tissue collection, etc.)

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Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic
Study of T-6049 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

February 1, 1995

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-129

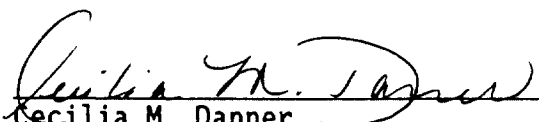
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QUALITY ASSURANCE STATEMENT

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.

<u>Inspection Dates</u>		<u>Phase</u>	<u>Date</u>	<u>Date to</u>
<u>From</u>	<u>To</u>		<u>Reported to</u>	<u>Management</u>
			<u>Study Director</u>	
11/06/94	11/06/94	Protocol Review	11/08/94	12/10/94
11/16/94	11/16/94	Animal Observation	11/16/94	12/10/94
01/10/95	01/10/95	Data/Report Review	01/10/95	02/10/95
01/30/95	01/30/95	Report Rereview	01/30/95	02/10/95


Cecilia M. Danner
Representative, Quality Assurance Unit

2/1/95
Date

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STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic
Study of T-6049 in Rabbits

Test Material	T-6049
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (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	
Experimental Start Date	November 15, 1994
Experimental Termination Date	November 17, 1994

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
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

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SUMMARY

This study was done to assess the level of systemic exposure of T-6049 when administered by intravenous injection to rabbits.

Female Hra:(NZW)SPF rabbits were assigned at random to five groups (one/group). On Day 0, the animals received a single intravenous injection of the vehicle (sterile water for injection) or 5, 10, 100, or 500 mg of T-6049/kg of body weight (Groups 1 through 5, respectively). The dose volume was 0.5 mL/kg for all groups.

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection. Body weights were determined just before test material administration (Day 0). A blood sample (approximately 4 mL) was collected from an auricular artery or marginal ear vein of the animals at 2-, 4-, 6-, 8-, 12-, and 24-hours post-injection. In addition, at the time of experimental termination (48-hours post-injection), approximately 20 mL of blood was obtained from each animal. All samples were centrifuged, separated into serum and cellular fractions, and sent to the Sponsor. Approximately 48 hours post-injection, the animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and sent frozen to the Sponsor after termination of the in-life phase.

The animals treated with sterile water for injection, 5 mg T-6049/kg, and 10 mg T-6049/kg appeared normal throughout the study. At the 0.5, 2, and 4 hour post-dose observation, the animal treated with 100 mg T-6049/kg appeared hypoactive and the animal treated with 500 mg T-6049/kg exhibited a staggered gait. In addition, the animal treated with 500 mg/kg showed a decrease in food consumption at the 48-hour post-dose observation.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6049, when administered as a single intravenous injection to 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 mixtures for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study were 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 Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), 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). A sample of the test material/vehicle mixtures for concentration, solubility, homogeneity, and stability analyses was not taken before administration as this was not requested by the Sponsor. The purity and stability of the USP grade control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test material was stored at room temperature. The control material was stored refrigerated. Any unused test material will be returned to the Sponsor after completion of all testing according to Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any remaining vehicle may be used 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 received from HRP, Inc., Kalamazoo, Michigan on October 19, 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 the required temperature and humidity conditions existed, they were documented and considered to have had no adverse effect on the study outcome. Animal husbandry and housing at HWI complied with standards outlined in the "Guide for the Care and Use of Laboratory Animals".¹

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 selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,587 to 2,857 g at initiation of treatment were placed into the following study groups:

<u>Group</u>	<u>Treatment</u>	<u>Dose Level (mg T-6049/kg)</u>	<u>Dose Volume (mL/kg)</u>	<u>Number of Animals</u>
1 (Control)	*	0	0.5	1
2	T-6049	5	0.5	1
3	T-6049	10	0.5	1
4	T-6049	100	0.5	1
5	T-6049	500	0.5	1

* Sterile Water for Injection, USP.

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

Dose Preparation and Administration

The test material was diluted with Sterile Water for Injection to achieve a specific concentration for each dose level in Groups 2 through 4. The test material was administered undiluted at the 500 mg/kg dose level, using the bulk density of 0.98 g/mL to determine the dose volume. An individual dose of each respective test solution or control was calculated for each animal based on its body weight on the day of treatment. The respective test solution was administered by intravenous injection into a marginal ear vein. The dose was given as a slow push (approximately 30 to 60 seconds in duration). The prepared test solutions were stored at room temperature until administered. After administration, any remaining test solutions were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection.

Body weights were determined just before test material administration (Day 0).

Sample Collection

A blood sample (approximately 4 mL) was collected from either ear via the catheterization of the auricular artery or from the marginal ear vein of all animals at 2, 4, 6, 8, 12, and 24 hours post-injection. At the time of necropsy (approximately 48-hours post-injection), approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were stored at room temperature until centrifuged and separated into serum and cellular fractions. The blood samples were then stored in a freezer set to maintain a temperature of $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ until shipped to the Sponsor.

Pathology

At termination of the experimental phase (approximately 48-hours post-injection), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not conducted, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$. After tissue/bile collection, the animals were discarded.

Shipment of Tissues

After completion of the in-life phase the blood samples, livers, bile, 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). The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the 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.

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RESULTS

Body Weights

Individual body weights at initiation are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. The animals treated with sterile water for injection, 5 mg T-6049/kg, and 10 mg T-6049/kg appeared normal throughout the study. At the 0.5, 2, and 4 hour post-dose observation, the animal treated with 100 mg T-6049/kg appeared hypoactive and the animal treated with 500 mg T-6049/kg exhibited a staggered gait. In addition, the animal treated with 500 mg/kg showed a decrease in food consumption at the 48-hour post-dose observation.

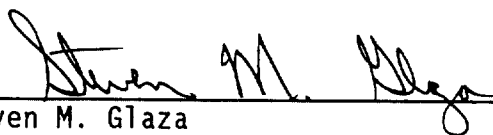
Pathology

All animals survived to termination of the experimental phase and were not examined grossly when sacrificed.

DISCUSSION

The level of systemic exposure of T-6049 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 5, 10, 100, and 500 mg/kg. Administration of this material resulted in hypoactivity at the 100 mg/kg dose level, and staggered gait and a decrease in food consumption at the 500 mg/kg dose level.

SIGNATURE



Steven M. Glaza
Study Director
Acute Toxicology

Date

2-1-95

REFERENCE

1. NIH Publication No. 86-23 (revised 1985).

Table 1
Individual Body Weights (g)

<u>Group</u>	<u>Dose Level (mg/kg)</u>	<u>Sex</u>	<u>Animal Number</u>	<u>Day 0</u>
1	0	Female	F52652	2,634
2	5	Female	F52653	2,587
3	10	Female	F52654	2,620
4	100	Female	F52665	2,857
5	500	Female	F52666	2,794

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Table 2
Individual Clinical Signs

<u>Group</u>	<u>Dose Level (mg/kg)</u>	<u>Sex</u>	<u>Animal Number</u>	<u>Observation</u>	<u>Hour</u>				
					<u>0.5</u>	<u>2</u>	<u>4</u>	<u>24</u>	<u>48</u>
1	0	Female	F52652	Appeared normal	✓	✓	✓	✓	✓
2	5	Female	F52653	Appeared normal	✓	✓	✓	✓	✓
3	10	Female	F52654	Appeared normal	✓	✓	✓	✓	✓
4	100	Female	F52665	Appeared normal	-	-	-	✓	✓
				Hypoactivity	✓	✓	✓	-	-
5	500	Female	F52666	Appeared normal	-	-	-	✓	-
				Staggered gait	✓	✓	✓	-	-
				Decreased food consumption	-	-	-	-	✓

✓ Indicates condition exists.
- Not applicable.

APPENDIX A
Protocol TP8084.PK

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a CORNING Company

BEST COPY AVAILABLE

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6049 in Rabbits

Date:

November 9, 1994

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-129

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STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-6049 in Rabbits

HWI No.	6329-129
Test Material	T-6049
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (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	Week of November 14, 1994
Experimental Termination Date	Week of November 14, 1994
Draft Report Date	Week of December 19, 1994

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1. Study
Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. Purpose
To assess the level of systemic exposure when the test material is administered as a single intravenous injection to rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines with the exception that analysis of the test material mixtures for concentration, solubility, homogeneity, and stability will not be conducted:

- ☐ Conduct as a Nonregulated Study
- ☒ 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). Samples of test material/vehicle mixture(s) for concentration, solubility, homogeneity, and stability analyses will be taken before administration if requested by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination for possible analysis.

- D. Storage
Room temperature
 - E. Reserve Samples
Reserve samples will not be required for this study.
 - F. Retention
Any unused test material will be discarded after issuance of the final report, unless directed otherwise by the Sponsor.
 - G. Safety Precautions
As required by HWI SOPs and policies
6. Control Material
- A. Identification
Sterile water for injection
 - B. Physical Description
Clear, colorless liquid
 - C. Purity and Stability
The purity and stability of this USP grade material is considered to be adequate for the purposes of this study.
 - D. Storage
Refrigerated
 - 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
2.5 to 3.5 kg
- (5) Number and Sex
5 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% $\pm$ 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.
- (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

<u>Group</u>	<u>Dose Level (mg/kg)^a</u>	<u>Number of Females</u>
1	0 (Control)	1
2	5	1
3	10	1
4	100	1
5	500	1

a The dose volume will be 0.5 ml/kg for Groups 1-4 and approximately 0.5 mL/kg of body weight (depending on the bulk density of the test material) for Group 5.

C. Dosing Procedures(1) Dosing Route

Intravenous injection into a marginal ear vein over approximately 30 to 60 seconds.

(2) Reason for Dosing Route

Intravenous injection is an acceptable route to assess systemic exposure.

(3) Dosing Duration

Single dose

(4) Dose Preparation

The test material will be diluted with sterile water for injection to achieve a specific concentration for each dose level in Groups 1-4. The test material will be administered undiluted at the 500 mg/kg dose level, using the bulk density to determine the dose volume. Individual doses will be calculated based on the animal's body weight taken just before test material administration. The prepared test mixtures will be stored at room temperature until administration.

D. Observation of Animals(1) Clinical Observations

The animals will be observed for clinical signs of toxicity at approximately 0.5, 2.0, 4.0, 24, and 48 hours after treatment.

(2) Body Weights
Just before test material administration.

(3) Sample Collections

(a) Frequency
2, 4, 6, 8, 12, 24, and 48 hours post-injection

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from either ear via the catheterization of the auricular artery or from the marginal ear vein at 2, 4, 6, 8, 12, and 24 hours post-injection. 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 at the time of necropsy (48 hours post-injection). Approximately 20 mL of blood will be collected from moribund animals during the study, also, if possible. 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 a temperature of $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$. The separated serum and cellular fractions will be sent frozen 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 will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

E. Termination

(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, bled via the vena cava, and exsanguinated.

(2) Scheduled Sacrifice

At approximately 48 hours post-injection, animals surviving to termination will be anesthetized with sodium pentobarbital, bled via the vena cava, and exsanguinated. An abbreviated gross necropsy examination will not be done, however, tissues will be collected.

(a) Sample Collection

The whole liver and bile from each animal dying during the study, sacrificed in a moribund condition, or surviving to termination will be collected. Both kidneys from each animal will also be collected. The tissues will be placed on dry ice immediately after collection and then placed in a freezer set to maintain a temperature of $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$.

The tissues (liver, bile, 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.D.(3).(c). The Sponsor is responsible for the retention and disposition of the samples.

F. 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

Description of any toxic effects

Gross pathology findings/gross pathology report (if applicable)

000029

000475

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
- Sample collection records
- Pathology 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
John L. Butenhoff, PhD
Sponsor's Representative
3M

11-18-94
Date

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

11-9-94
Date

Cecilia M. ...
Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

11/9/94
Date

(6329-129.protdisk1)

9.1.2 Analytical protocol AMDT-112294.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

In-Vivo Study Reference Number: HWI#6329-129

Study Number: AMDT-112294.1

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

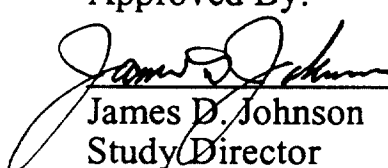
Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25, 1995

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 Fluorochemicals Using
Electrospray Mass Spectrometry
AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified
Dohrmann DX2000 Organic Halide Analyzer-Serum

Author: James D. Johnson

Approved By:

 11/16/95
James D. Johnson Date
Study Director

 11/20/95
John Butenhoff, PhD Date
Sponsor Representative

1.0 PURPOSE

This study is designed to provide information as to whether the perfluorooctanesulfonate anion does go to the rabbit liver and other tissues when the material is administered in an intravenous dose, and to ascertain the change in concentration with time after dose in serum and liver. These data will be used to interpret dermal absorption studies where perfluorooctanesulfonate is directly applied or is present as a biotransformation product. The persistence of perfluorooctanesulfonate as a marker in liver and serum or possibly other tissues is a critical part for the interpretation of these dermal studies.

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: Liver and serum from 4 test animals and 1 control animal. Other biological tissues (kidney, bile, cellular fraction) will be available for analysis if deemed appropriate by 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), $-20 \pm 10^{\circ}\text{C}$ (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 for studies longer than 28 days. This study is in parallel with a 28 day dermal absorption study so all tissues will be retained.

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

The tissues from animals dosed as described (HWI#6329-129), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (See Methods--next Section) is the appropriate analysis to present definitive data for fluorine in the liver. For these analyses, bovine liver appears to be a good approximation to rabbit liver for matrix spikes and recovery determinations. Since perfluorooctanesulfonate anion is not biotransformed, fluorine content will be an accurate estimate of the concentration of the compound.

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 Fluorochemicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

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. Half-life of disappearance from serum will be determined from the linear regression of the averages of the concentrations at different time points for the post distribution part of the serum concentration versus time curve.

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 Fluorochemicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

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

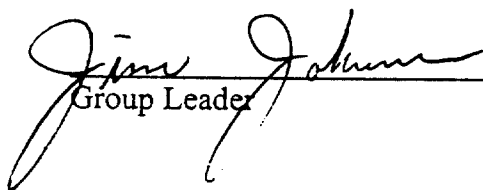
Adoption Date: 10-4-95

Revision Number: 0

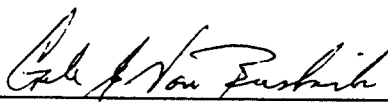
Revision Date: None

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 $\frac{(\text{Orion reading in ppm} - \text{intercept})}{(\text{Slope})}$

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

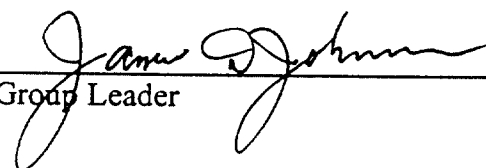
Adoption Date: 10-4-95

Revision Number: 0

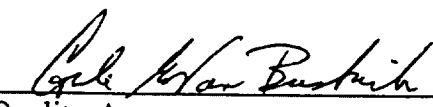
Revision Date: None

Author: Rich Youngblom

Approved By:


Group Leader

10/3/95
Date


Quality Assurance

10-4-95
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

<u>Revision Number</u>	<u>Reason for Change</u>	<u>Revision Date</u>
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3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4

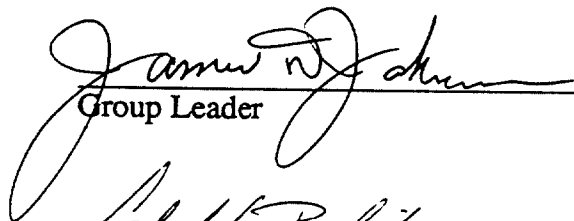
Adoption Date: 10-31-95

Revision Number: 0

Revision Date: None

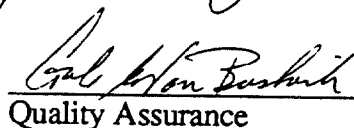
Author: Dave Christenson/Cynthia Weber

Approved By:


Group Leader

10-31-95

Date


Quality Assurance

10-31-95

Date

Software: MS Word, 6.0

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

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000048

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 μ L
- 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 μ m.
- 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), ($\text{Na}_2\text{CO}_3/\text{NaHCO}_3$) 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.

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

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:

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

*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 perfluorooctanesulfonate 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 μ m 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

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Worksheet AMDT-M-4

[illegible]

000499

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3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5

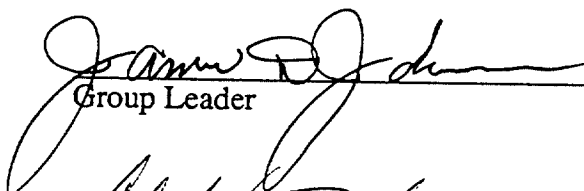
Adoption Date: 6-6-95

Revision Number: 0

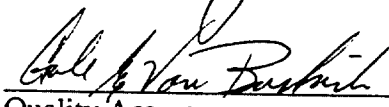
Revision Date: None

Author: Dave Christenson/Cynthia Weber

Approved By:


Group Leader

6/6/95
Date


Quality Assurance

6/6/95
Date

Software: MS Word, 6.0

Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

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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 spectrometer 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. Observe 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 μ L

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 ($\pm 50\%$) 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

3M Environmental Laboratory

Method

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

Method Identification Number: AMDT-M-14

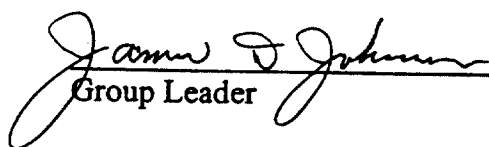
Adoption Date: 10-3-95

Revision Number: 0

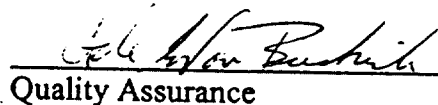
Revision Date: None

Author: Rich Youngblom

Approved by:


Group Leader

10/3/95
Date


Quality Assurance

9-27-95
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 fluids, particularly serum.

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 100 µl. This may vary from matrix to matrix.

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7.0 SAMPLE HANDLING

7.1 Samples are to be handled with plastic pipettes. A new pipette is to be used for each sample.

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 serum studies, use beef serum as the matrix.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked serum 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 serum as the matrix.

8.3.2.3 Put serum blank in Dohrmann boat. Combust 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.

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8.3.3 Standard Curve

8.3.3.1 If beef serum is frozen, thaw at least enough to complete the standard curve analysis for the day (≈ 30 mL).

8.3.3.2 Pipette 100 μ L of beef serum into 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 μ L of standard and eject it on or in the beef serum.

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 pipette 100 μ L of sample into 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-WATER 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 and/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 $\frac{(\text{Orion reading in ppm} - \text{intercept})}{(\text{Slope})}$

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.

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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

<u>Revision Number</u>	<u>Reason for Change</u>	<u>Revision Date</u>
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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

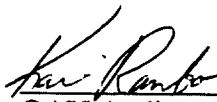
Study Title: Single-dose Intravenous Pharmacokinetic Study of T-6049 in Rabbits

Study Number: AMDT-112294.1

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		Phase	Date Inspection Reported to	
From	To		Management	Study Director
10-11-95	11-03-95	Final Report	11-03-95	11-03-95



QAU Auditor 11-3-95
Date

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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

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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-112294.1, HWI 6329-129
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group

ID	ug
Control group	23.37
5.0 mg/kg dose (T6049)	23.28
10.0 mg/kg dose (T6049)	16.01
100 mg/kg dose (T6049)	35.87
500 mg/kg dose (T6049)	92.03

FC95 PK		Actual	Average		Whole	Total F- in	
ID	%	ppm F-	ppm F-	liver	liver	whole	Dosage
	rcvry	in liver	in liver	burned	weight	liver	(mg/kg)
		(W/W)	(W/W)	(grams)	(grams)	(ug)	
BLNK LIVER 1		1.052		0.1216			
BLNK LIVER 2		0.423		0.1551			
BLNK LIVER 3		0.758		0.1135			
BLNK LIVER 4		0.473		0.1502			
BLNK LIVER 5		0.308		0.1473			
LIVER SPK 63-1	77%	0.895		0.1300			
LIVER SPK 63-2	86%	1.024		0.1274			
LIVER SPK 63-3	83%	1.045		0.1205			
LIVER SPK 126-1	84%	2.192		0.1159			
LIVER SPK 126-2	83%	1.616		0.1554			
LIVER SPK 126-3	86%	2.353		0.1110			
LIVER SPK 253-1	82%	3.580		0.1399			
LIVER SPK 253-2	80%	3.966		0.1228			
LIVER SPK 253-3	86%	4.195		0.1242			
LIVER SPK 506-1	69%	6.493		0.1292			
LIVER SPK 506-2	68%	6.338		0.1297			
LIVER SPK 506-3	69%	7.100		0.1175			
LIVER SPK 1012-1	63%	12.884		0.1186			
LIVER SPK 1012-2	75%	14.244		0.1277			
LIVER SPK 1012-3	64%	13.635		0.1139			
BLANK LIVER-1		0.432		0.1144			
BLANK LIVER-2		0.303		0.1223			
BLANK LIVER-3		0.219		0.1402			
F52652-1		0.332		0.1329			
F52652-2		0.242	0.273	0.1202	85.62	23.37	0.0
F52652-3		0.245		0.1013			
F52653-1		0.319		0.1385			
F52653-2		0.217	0.254	0.1118	91.68	23.28	5.0
F52653-3		0.226		0.1155			
F52654-1		0.174		0.1464			
F52654-2		0.206	0.204	0.1173	78.65	16.01	10.0
F52654-3		0.231		0.1168			
F52665-1		0.480		0.1411			
F52665-2		0.384	0.434	0.1502	82.72	35.87	100.0
F52666-1		0.437		0.1325			
F52666-1		0.973		0.1415			
F52666-2		1.043	0.988	0.1146	93.11	92.03	500.0
F52666-3		0.949		0.1161			
LIVER SPK-A	66%	0.791		0.1269			
LIVER SPK-B	67%	0.895		0.1126			
LIVER SPK-C	73%			0.1290			
LIVER SPK-D	74%			0.1397			
LIVER SPK-E	76%			0.1457			
LIVER SPK-F	74%			0.1351			
LIVER BLNK-1				0.1232			

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

Study: Single-Dose Intravenous Pharmacokinetic
 Protocol Number: TP8084.PK
 Test Material: T-6049 in Rabbits (FC-95)
 Matrix: Liver
 R Squared Value: 0.9717
 Response Factor Amount: 1.07E-05
 Analyst: DLC
 Date: 3/28/95
 Method:
 Instrument: Fisons VG 2000 Electrospray MS
 LABBASE File: 032895C

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	F52652	N.D.	0.9979	1	N.D.	85.619	N.D.
Group 2: 5 mg /kg	F52653	N.D.	1.061	1	N.D.	91.683	N.D.
Group 3: 10 mg /kg	F52654	6056	1.1129	1	0.0466	78.656	0.004
Group 4: 100 mg/kg	F52665	39065	1.0209	1	0.3274	82.718	0.027
Group 5: 500 mg/kg	F52666	71977	1.1125	1	0.5536	93.111	0.052

* 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 than 171 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.

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HWI # 6329-124 PX 1801

6329-129 FC 95

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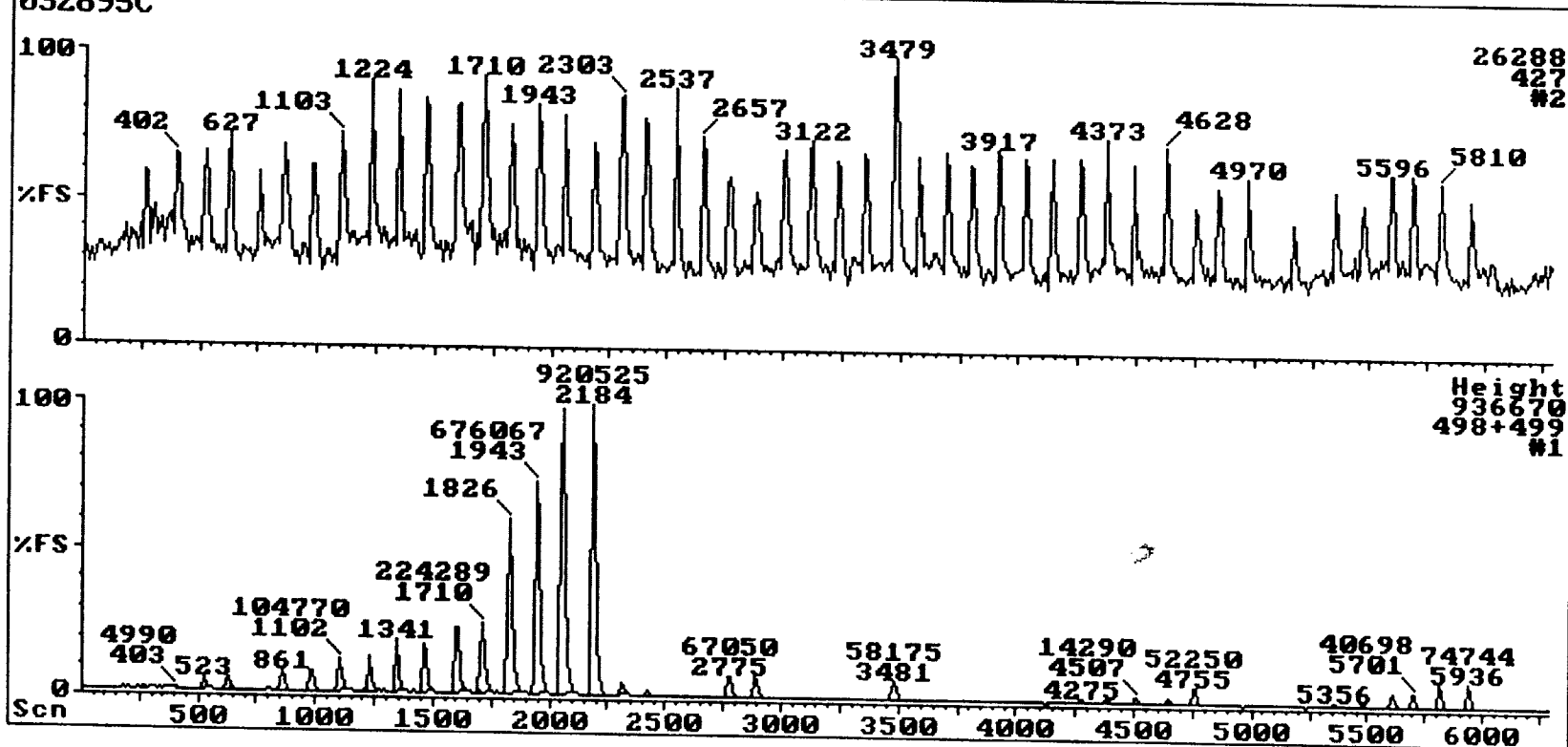
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Sample: ~~rat liver from absorption studies~~ 11/10/95 OLC

032895C



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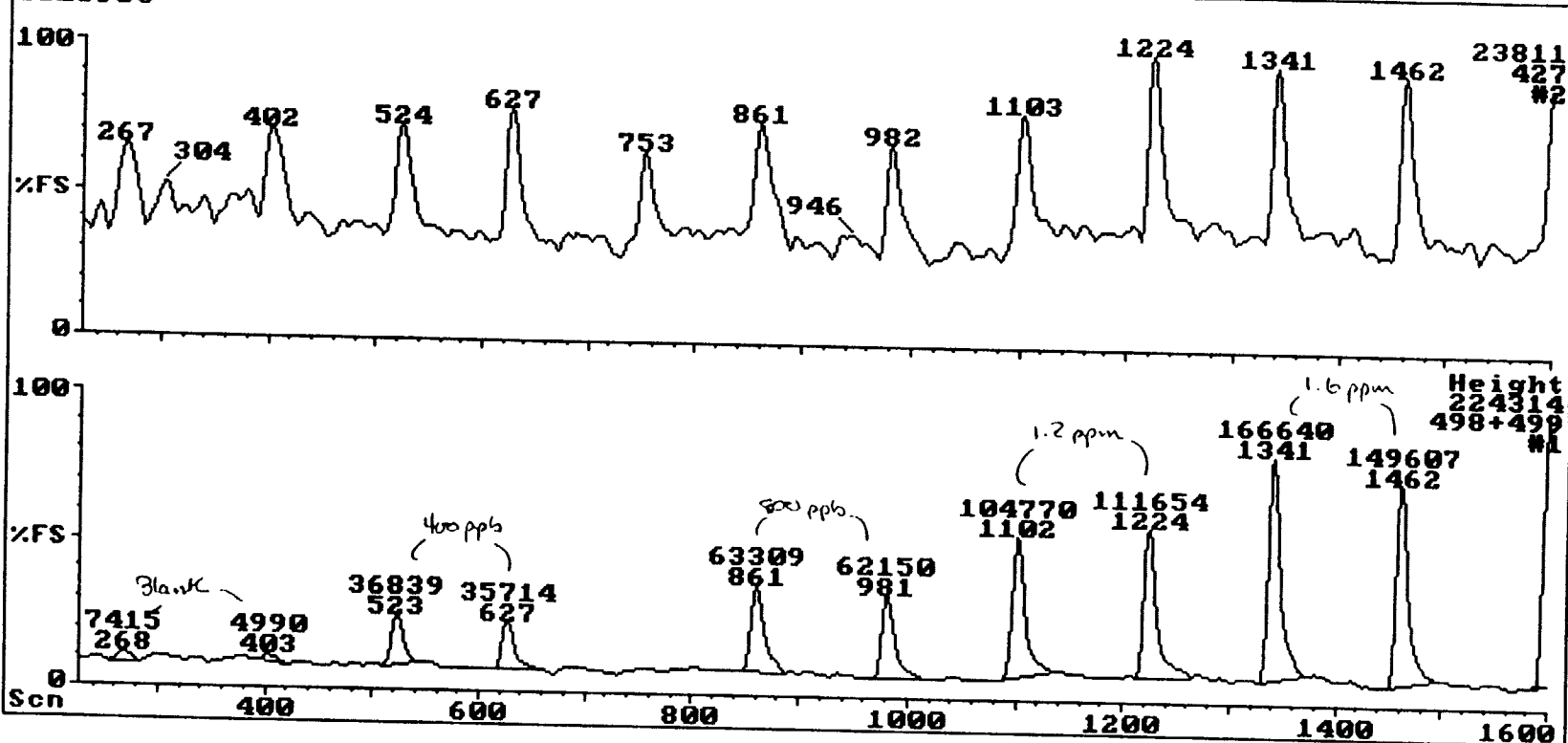
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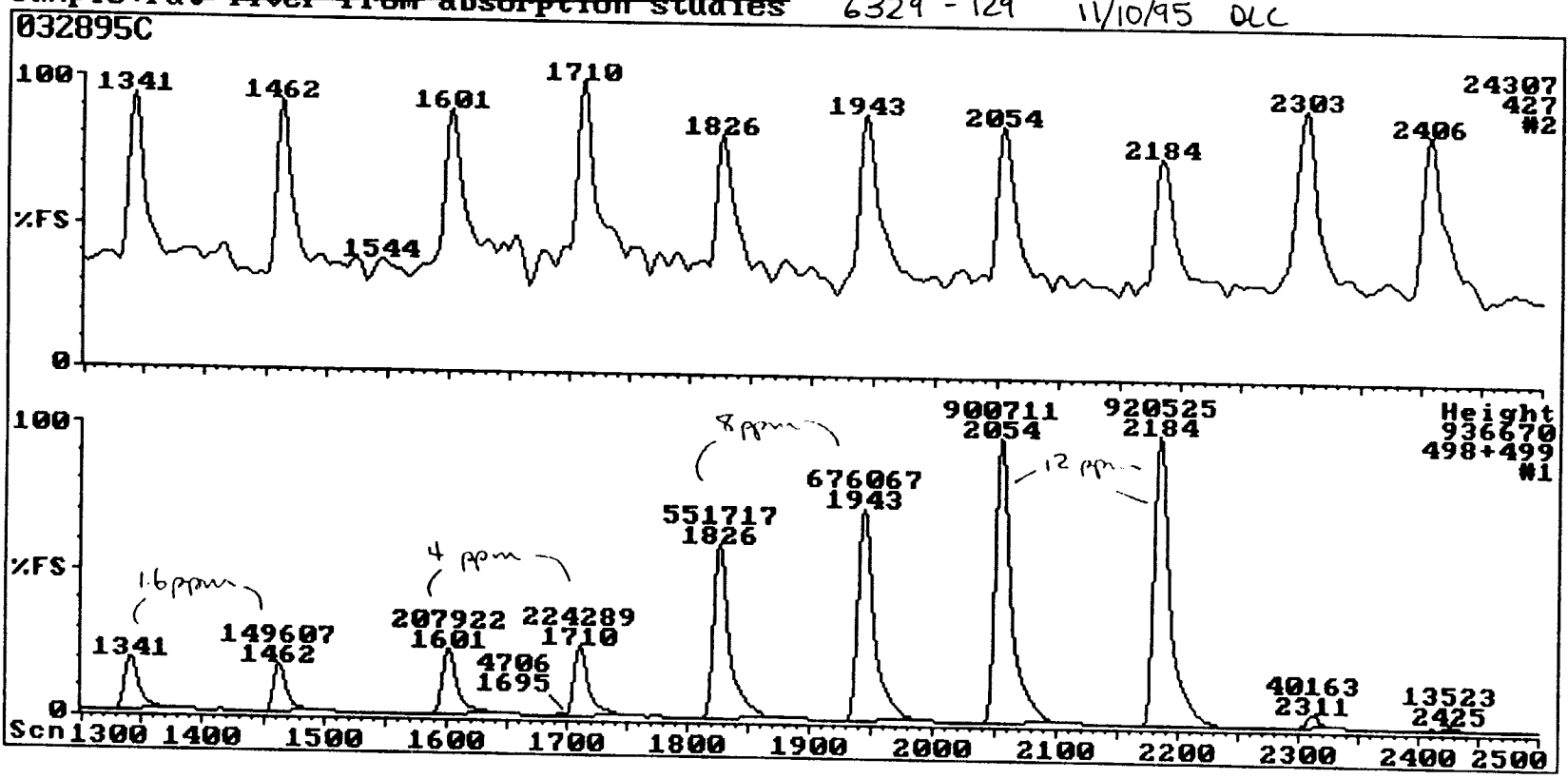
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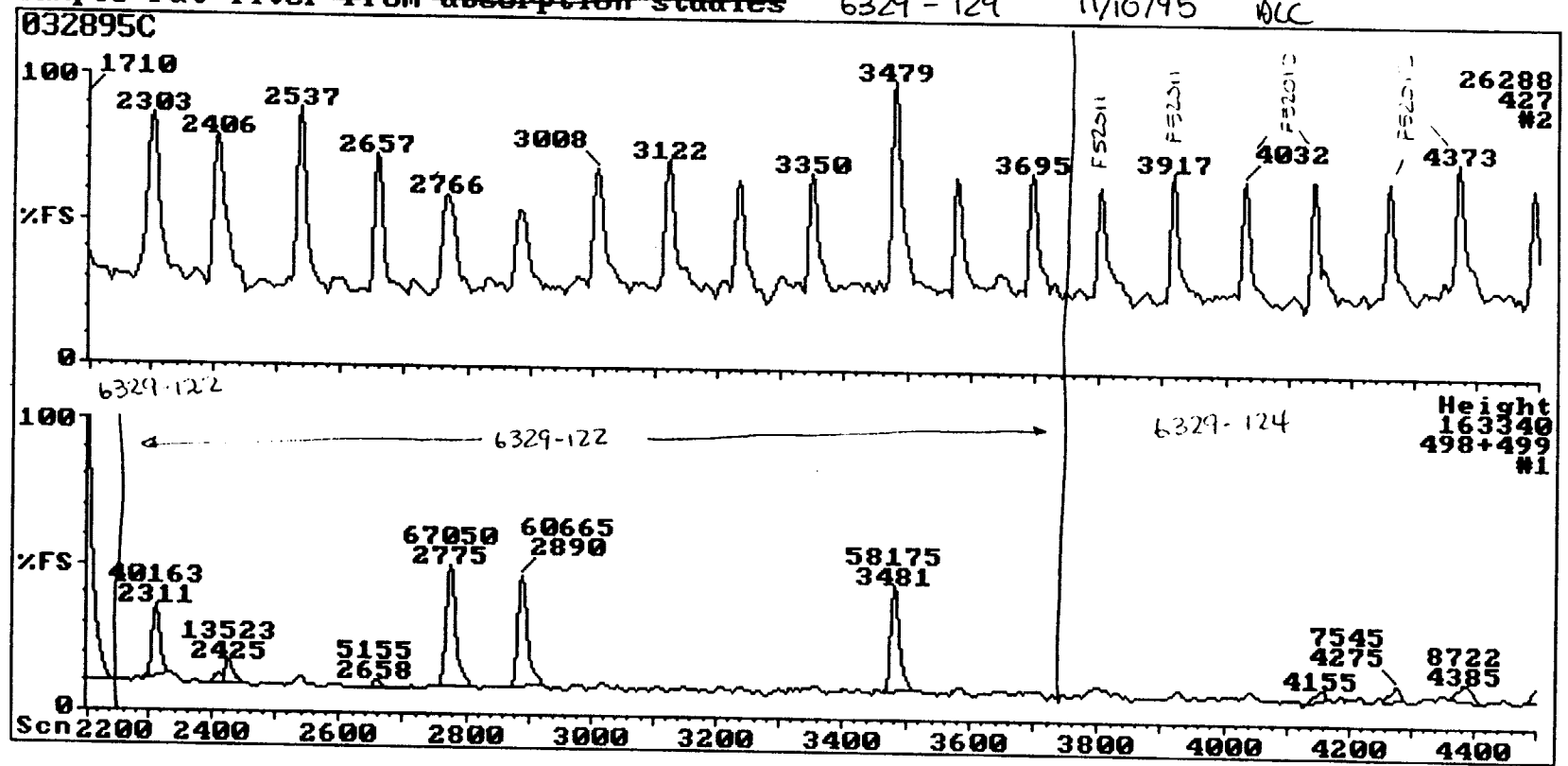


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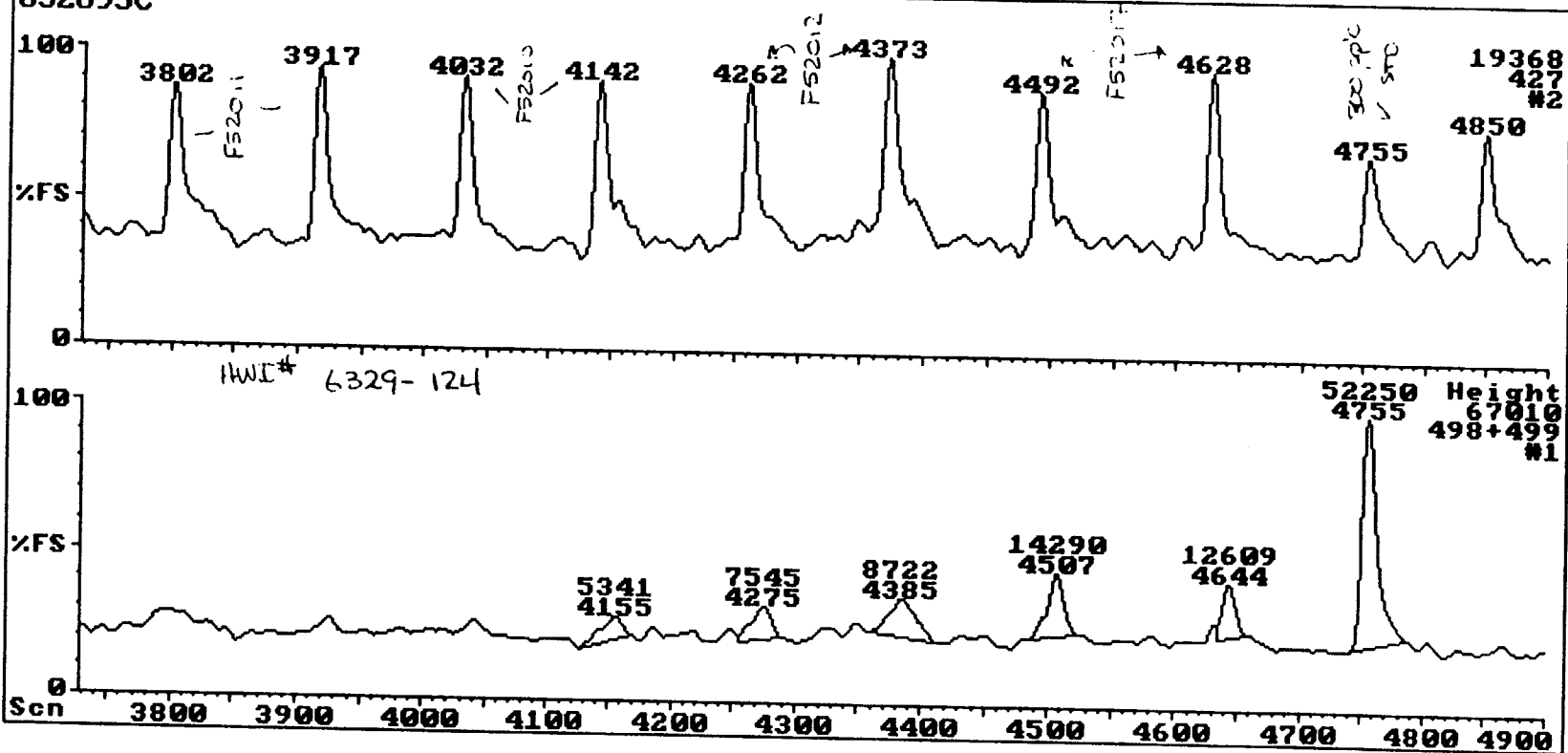
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11/10/95 DLG

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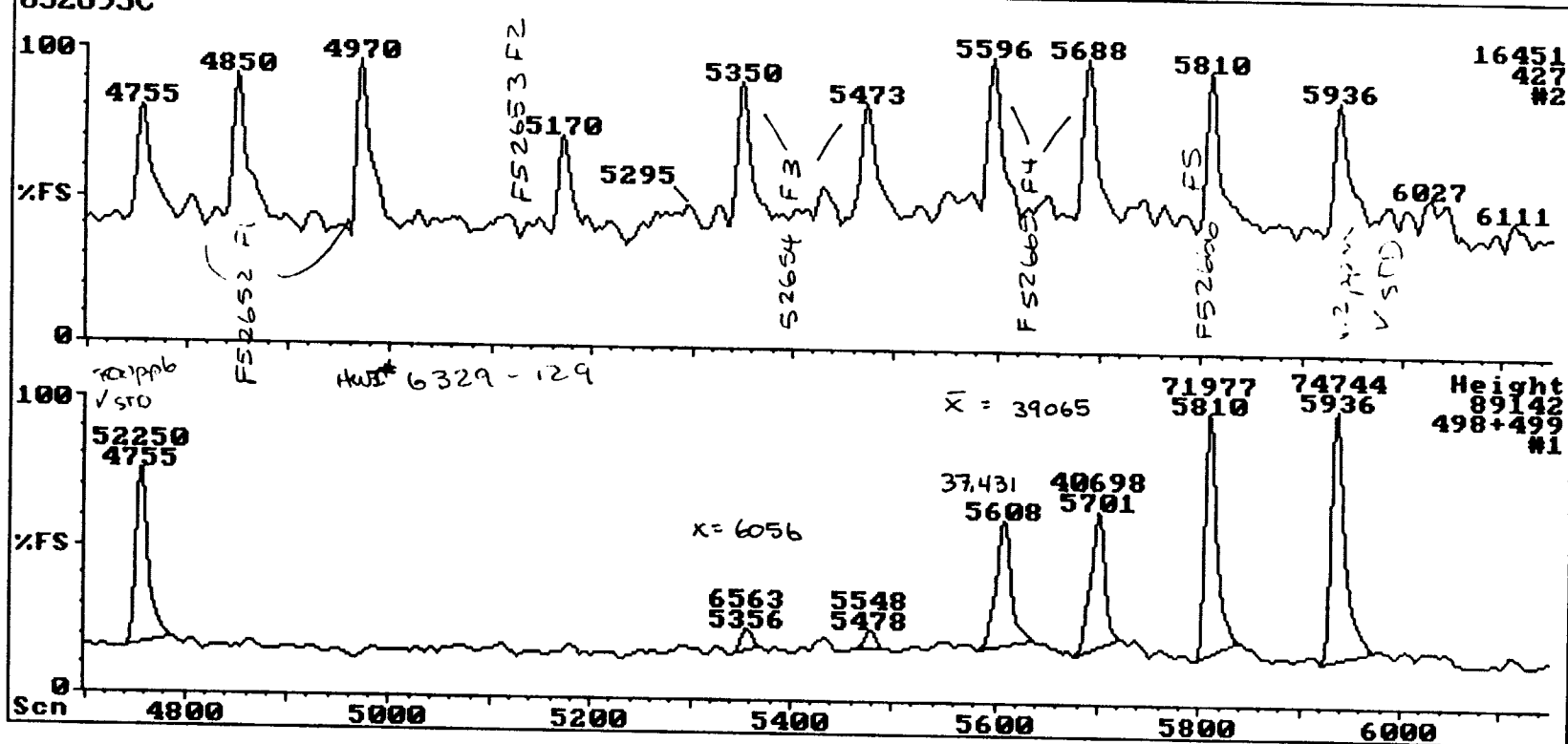
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Sample:rat liver from absorption studies

6329-129

11/10/95 OK

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9.11.3 Summary and raw data; ppm F⁻ in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

Summary of Combustion Data - Liver
AMDT-112294.1, HWI 6329-129

Concentration of Fluoride in Serum
Mean per Dose Group per Sampling Interval*

		Average ppm F- in serum (W/W)
500 mg/kg dosed rabbit	48 HR	0.633
	24 HR	0.707
	12 HR	0.789
	8 HR	0.620
	6 HR	0.595
	4 HR	0.614
	2 HR	0.705
100 mg/kg dosed rabbit	48 HR	0.341
	24 HR	0.289
	12 HR	0.395
	8 HR	0.293
	6 HR	0.246
	4 HR	0.265
	2 HR	0.255

***2-24 hour calculated as the mean of duplicate samples, 48 hour analyzed in triplicate.**

FC95 PK		Actual	Average	serum	Dosage
ID	% rcvry	ppm F- in serum (W/W)	ppm F- in serum (W/W)	burned (mL)	
Serum blk 1		0.931		0.1	
Serum blk 2		0.590		0.1	
Serum blk 3		0.391		0.1	
Serum blk 4		0.321		0.1	
Serum SPK 1	69%	2.10		0.1	
Serum SPK 2	184%	5.58		0.1	
Serum SPK 3	79%	2.40		0.1	
Serum SPK 4	78%	2.36		0.1	
Serum SPK 5	103%	3.13		0.1	
Serum SPK 6	98%	2.99		0.1	
SERUM BLANK-1		0.511		0.1	
SERUM BLANK-2		0.375		0.1	
F52666-F5-48 HR - 1		0.689		0.1	
F52666-F5-48 HR - 2		0.582	0.633	0.1	500
F52666-F5-48 HR - 3		0.628		0.1	
F52666-F5-24 HR - 1		0.735		0.1	
F52666-F5-24 HR - 2		0.680	0.707	0.1	500
F52666-F5-12 HR - 1		0.835		0.1	
F52666-F5-12 HR - 2		0.743	0.789	0.1	500
F52666-F5-8 HR - 1		0.636		0.1	
F52666-F5-8HR - 2		0.605	0.620	0.1	500
F52666-F5-6HR - 1		0.617		0.1	
F52666-F5-6HR - 2		0.573	0.595	0.1	500
F52666-F5-4HR - 1		0.580		0.1	
F52666-F5-4HR - 2		0.649	0.614	0.1	500
F52666-F5-2HR - 1		0.755		0.1	
F52666-F5-2HR - 2		0.655	0.705	0.1	500
SERUM BLANK 1		0.575		0.1	
SERUM BLANK 2		0.314		0.1	
F52665-F4-48 HR-1		0.333		0.1	
F52665-F4-48 HR-2		0.350	0.341	0.1	100
F52665-F4-48 HR-3		0.339		0.1	
F52665-F4-24HR-1		0.303		0.1	
F52665-F4-24HR-2		0.276	0.289	0.1	100
F52665-F4-12HR-1		0.464		0.1	
F52665-F4-12HR-2		0.326	0.395	0.1	100
F52665-F4-8HR-1		0.288		0.1	
F52665-F4-8HR-2		0.299	0.293	0.1	100
F52665-F4-6HR-1		0.242		0.1	
F52665-F4-6HR-2		0.249	0.246	0.1	100
F52665-F4-4HR-1		0.272		0.1	
F52665-F4-4HR-2		0.257	0.265	0.1	100
F52665-F4-2HR-1		0.254		0.1	
F52665-F4-2HR-2		0.256	0.255	0.1	100