AR226-0157
Pt-2

3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6053 in Rabbits

In-Vivo Study Reference Number: HWI#6329-136

Study Number: AMDT-010495.1
Test Substance: FC-99 (T-6053)

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-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/16/95
Completion Date

003764

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1.0 SUMMARY

Rabbit liver were analyzed by combustion for fluorine content at 48 hours after individual rabbits were dosed intravenously with a range of single doses of FC-99 (one rabbit/dose group). The fluorine was detected after a single dose of 20 ug/kg, but not after a 0.8, 4, 10 ug/kg dose at 48 hours postdose. Doses are calculated with respect to the C8 solids.

The perfluorooctanesulfonate salt form of FC-99 does not change the availability of total organic fluorine in whole liver after intravenous injection. The analysis of liver for total organic fluorine will provide a convenient marker to assess the dermal absorption of FC-99.

2.0 INTRODUCTION

This study was designed to provide information as to whether the perfluoro-octanesulfonate anion goes to the liver and other tissues after a dose of T-6053 (a 0.04% solution of FC-99 solids) is administered in an intravenous dose; and to ascertain the change in concentration with time after dose in serum and liver. FC-99 is a solution of perfluoroalkylsulfonates: perflourooctanesulfonate (about 20%), C4 - C7 perfluoroalkylsulfonates (about 5%), and water (75%). The sulfonates are as the diethanolamine salt.

It is known from studies done previously with rats, that the half-life of perfluoro-octanesulfonate anion is quite long (>1 month). It was shown in a previous study that the half-life of perfluorooctanesulfonate is >1 month in rabbits after an intravenous dose (HWI#6329-159). It is not expected that the half-life in rabbits would be different with just the salt form of the perfluorooctanesulfonate changed, as it is comparing FC-95 (potassium salt) with FC-99.

It was noted in the previous intravenous study with the perfluorooctanesulfonate that the serum levels lagged and even decreased after the injection and then returned to a maximum at 48 hours.

It was clear from the 28 day study (HWI#6329-159) that the perfluorooctane-sulfonate would provide a marker for dermal studies for which there was reasonable expectation that perfluorooctanesulfonate would be formed as a biotransformation product. It will therefore also be a useful marker for the assessment of the dermal absorption of FC-99.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

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

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

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

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

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

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

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

4.0 EXPERIMENTAL - Overview

Serum and tissues from animals dosed as described (HWI#6329-136) 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. The data are to provide evidence that perfluorooctanesulfonate and/or total organic fluorine in liver can be used to assess dermal absorption of FC-99.

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-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

6.0 DATA ANALYSIS

The data from combustion analysis of liver for total organic fluorine are attached. The liver at 48 hours post dose indicates that only the 250 mg/kg dose of T-6053 resulted in significant total organic fluorine in the liver (35 ug in total liver). This is a 0.04% solution of an aqueous solution in which the perfluorooctanesulfonate salt is about 20%. This corresponds to a dose in terms of perfluorooctanesulfonate salt as about 0.02 mg/kg. (The control value is estimated to be about 15 ug total in the whole liver, but the value is below the quantitation limit and is extrapolated from below the calibration of the instrument.) This result is as expected from a previous study in rabbits when perfluorooctanesulfonate as the potassium salt was injected and only doses above 0.6 mg/kg resulted in significant amounts of total organic fluorine in the liver. There is enough of the perfluorooctanesulfonate present to indicate that if appreciable amounts of FC-99 are absorbed in a dermal absorption study, it will be observed.

Other data was collected using electrospray mass spectrometry and Skalar segmented flow analyzer with ion selective electrode (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: The problem with this analysis is that there is too short a time period used for the study. In other studies, it has been observed that there is a lag in serum concentrations until 48 hours. There didn't appear to be any advantage in doing serums for this study, since the time period was too short and in view of the results from study HWI#6329-159.

7.0 CONCLUSION

The perfluorooctanesulfonate salt form (diethanolamine) of FC-99 does not change the availability of total organic fluorine in whole liver after intravenous injection. The analysis of liver for total organic fluorine will provide a convenient marker to assess the dermal absorption of FC-99. However a larger dose will be required.

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#6329-136 "Single-Dose Intravenous Pharmacokinetic Study of T-6053 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-010495.1

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-010495.1

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; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.1.1 Protocol and Final Report: HWI#6329-136 "Single-Dose Intravenous Pharmacokinetic Study of T-6053 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-6053 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

March 31, 1995

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-136

003771

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HWI 6329-136

QUALITY ASSURANCE STATEMENT

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

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/09/94	12/09/94	Protocol Review	12/09/94	01/10/95
12/27/94	12/27/94	Sample Collection	12/27/94	01/10/95
02/15/95	02/15/95	Data/Report Review	02/15/95	03/10/95
03/30/95	03/30/95	Report Rereview	03/30/95	04/10/95

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Cecilia M. Danner
Representative, Quality Assurance Unit

3/31/95
Date

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STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic
Study of T-6053 in Rabbits

Test Material	T-6053
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Experimental/In-life Start Date	December 27, 1994
Experimental/In-life Termination Date	December 29, 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-6053 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 10, 50, 125, or 250 mg of T-6053/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 in-life 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.

All five animals appeared normal throughout the study.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6053, 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-6053 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 was returned to the Sponsor after completion of all in-life 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 November 30, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

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

Animal Diet

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

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,572 to 2,836 g at initiation of treatment were placed into the following study groups:

Group	Treatment	Dose Level (mg T-6053/kg)	Dose Volume (mL/kg)	Number of Animals
1 (Control)	*	0	0.5	1
2	T-6053	10	0.5	1
3	T-6053	50	0.5	1
4	T-6053	125	0.5	1
5	T-6053	250	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. 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 seconds to 2.5 minutes 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°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the in-life 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 placed in a freezer set to maintain a temperature of -20°C ±10°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.

RESULTS

Body Weights

Individual body weights at initiation are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. All five animals appeared normal throughout the study.

Pathology

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

DISCUSSION

The level of systemic exposure of T-6053 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 10, 50, 125, and 250 mg/kg. All animals appeared normal throughout the study following administration of this material.

SIGNATURE

Steven R. Swenson FOR
Steven M. Glaza
Study Director
Acute Toxicology

3-31-95
Date

Table 1
Individual Body Weights (g)

Group	Dose Level (mg/kg)	Sex	Animal Number	Initial
1	0	Female	F52856	2,833
2	10	Female	F52857	2,802
3	50	Female	F52861	2,572
4	125	Female	F52862	2,746
5	250	Female	F52863	2,836

Table 2
Individual Clinical Signs

Group	Dose Level (mg/kg)	Sex	Animal Number	Observation	Hour 0.5	2	4	24	48
1	0	Female	F52856	Appeared normal	✓	✓	✓	✓	✓
2	10	Female	F52857	Appeared normal	✓	✓	✓	✓	✓
3	50	Female	F52861	Appeared normal	✓	✓	✓	✓	✓
4	125	Female	F52862	Appeared normal	✓	✓	✓	✓	✓
5	250	Female	F52863	Appeared normal	✓	✓	✓	✓	✓

✓ Condition existed.

APPENDIX A

Protocol Deviations
Protocol TP8084.PK

Protocol Deviations

Protocol	Actual Procedure
Page 8, 7. Experimental Design, C. Dosing Procedures, (1) Dosing Route. Intravenous injection into a marginal ear vein over approximately 30 to 60 seconds.	Animal Nos. F52856 (Group 1), F52861 (Group 3), and F52862 (Group 4), had a dosing duration of 157, 91, and 99 seconds, respectively.

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

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Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6053 in Rabbits

Date:

December 13, 1994

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-136

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STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6053 in Rabbits

HWI No.	6329-136
Test Material	T-6053
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	December 27, 1994
Experimental Termination Date	December 29, 1994
Draft Report Date	February 2, 1995

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

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

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

5. Test Material

A. Identification
T-6053

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 returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
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% ±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

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	10	1
3	50	1
4	125	1
5	250	1

a The dose volume will be 0.5 mL/kg of body weight.

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. 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°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

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

James D. Johnson or his alternate 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 (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section E. Termination, (3) Sample Collection, will be collected.

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

(3) 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°C ±10°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 (if applicable)
Gross pathology report (if applicable and requested by the Study Director)

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
Shipping 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 Toxicology Service Medical Department

12-15-94
Date

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

12-13-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12.13.94
Date

(6329-136.protdsk2)

9.1.2 Analytical protocol AMDT-010495.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6053 in Rabbits

In-Vivo Study Reference Number: HWI#6329-136

Study Number: AMDT-010495.1
Test Substance: FC-99 (T-6053)

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 Fluorochmicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

Author: James D. Johnson

Approved By:

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

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

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1.0 PURPOSE

To assess the level of systemic exposure when T-6053 is administered as a single intravenous injection to rabbits.

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.

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 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day dermal absorption study so all tissues will be retained.

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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#6329-136), 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. 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 Fluorochmicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Analyzer with Ion Selective Electrode

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 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include regression analysis of serum concentrations with time and averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as the Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

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

7.0 REFERENCES

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

8.0 ATTACHMENTS

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

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

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

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

8.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

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

003801

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

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

2.0 KEYWORDS

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

3.0 PRECAUTIONS

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

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

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

8.2 Calibration - Overview

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

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

8.3 Calibration - Procedure

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

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

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

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

8.4 Storage Conditions for Standards

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

9.0 PROCEDURES

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

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

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

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

9.4 Sample Calculations

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

10.0 VALIDATION

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

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

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

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

11.2 Analyzing the Data

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

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

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

Author: Rich Youngblom

Approved By:

James D. Johnson 10/3/95
Group Leader Date

Gale Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

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

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

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

1.2 APPLICABLE COMPOUNDS: Fluoride.

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

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

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

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

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

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

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

9.0 PROCEDURES

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

9.2 Calibration And Measurement, Using Orion 3E Software:

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

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

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

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

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

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

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

1.0 SCOPE

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

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

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

2.0 KEYWORDS

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

3.0 PRECAUTIONS

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

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

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

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

4.2 **Reagents**

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

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

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

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

8.2 Calibration

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

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

8.3 Storage Conditions for Standards

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

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

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

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

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

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

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

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

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

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

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

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

9.2 Manual Injection

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

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

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

9.3 **Using the Autosampler**

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

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

9.3.3 Set-up the sampler:

9.3.3.1 Push the sample button

9.3.3.2 Set sample loop size = 100 uL

9.3.3.3 Set inject/sample = 2

9.3.3.4 Set Cycle time = 0

9.3.3.5 Name the file: Livers

9.3.3.6 Identify the tray used

9.3.3.7 Add the samples to Queue by pressing "Enter"

9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 **Quality Control**

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

10.2 **Precision and Accuracy**

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

10.3 **Other Validation Parameters**

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

11.0 DATA ANALYSIS

11.1 Calculations

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

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

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

mg FC-95 anion in the total rabbit liver =

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

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

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

Method Identification Number: AMDT-M-8 **Adoption Date:** 10-5-95

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

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson
Group Leader 10/5/95
Date

Gale Von Bustenik
Quality Assurance 9-27-95
Date

Software: IBM MS Word, 6.0
Affected Documents: **AMDT-EP-26,** Operation and Maintenance of the Skalar Segmented Flow Analyzer

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1.0 SCOPE

1.1 This method is for the analysis for fluoride, thermally extracted from samples using the Dohrmann DX2000 (AMDT-M-1), and collected in TISAB for analysis with an Ion Selective Electrode (ISE). The analysis is performed using the Skalar Segmented Flow Analyzer with ISE.

1.2 Samples can be tissues, serum, biological material, or other materials extracted on the Dohrmann.

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps
4.1.2 Autopipets, oxford or equivalent with plastic tips
4.1.3 Polypropylene volumetric flasks, 100 mL
4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.
4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent
4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.
4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.
4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.
4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.
4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

Working Standard	mLs of Stock Standard	mLs of Intermediate Standard
0.015 ppm	-	0.15
0.03 ppm	-	0.3
0.06 ppm	-	0.6
0.09 ppm	-	0.9
0.12 ppm	-	1.2
0.15 ppm	-	1.5
0.3 ppm	0.3	-
0.6 ppm	0.6	-

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

5.1 Skalar Segmented Flow Auto Analyzer Sans^{Plus} System equipped with ISE

6.0 INTERFERENCES

6.1 High concentrations of alkalinity, chloride, phosphate, sulfate or iron can cause interferences.

7.0 SAMPLE HANDLING

7.1 Samples should be stored in polyethylene bottles. Samples should be analyzed within 30 days.

8.0 CALIBRATION AND STANDARDIZATION

8.1 **Preparation of Calibration Standards**

8.1.1 Prepare calibration standards as in section 4.3.

8.2 **Calibration**

8.2.1 The standards are analyzed at the beginning of the run.

8.3 **Storage Conditions for Standards**

8.3.1 Standards are stored in capped polypropylene volumetric flasks. New standards are prepared at a minimum of every six months, or as necessary.

9.0 PROCEDURE

9.1 **Start Up Procedure**

9.1.1 Clamp down the pumpdecks, air bars and sampler-pump tubing.

9.1.2 Put the fluoride electrodes in the electrode chamber.

9.1.3 Turn on the power of the sampler, pumps, offset potentiometer and heating bath.

9.1.4 Put the reagent-lines in the appropriate bottles.

9.1.5 Turn on the interface, computer, display and printer. **Make sure you turn on the interface before the computer.**

9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 **Starting a Run**

9.2.1 Create a sample table by selecting FILES, TABLE, and CREATE, type in the name of the file, and press ENTER.

9.2.2 Print the sample table, inserted in the system table by pushing ESC, PRINT, GROUP 1. This will print the entire run.

9.2.3 Dial the sampler settings to the appropriate number of samples, number of seconds for sample wash, and number of seconds for the sample.

9.2.4 Fill the sample tray with the standards, samples, washes and drifts. IW and FW/RUNOUT cups on the sampler do not need to be filled.

9.2.5 Set the baseline.

9.2.5.1 Select GRAPHICS, REAL TIME. If you cannot get real-time, you may be in the Data Handling Panel. Switch to the Analysis Panel by selecting CONTROL PANEL and pushing F7.

9.2.5.2 Use the small screwdriver for the offset potentiometer to set the base line. Adjust the baseline until it is approximately 3/4 inch from the bottom of the screen.

9.2.5.3 Check the highest standard and adjust the gain, if necessary, with the interface screw #3.

9.2.6 Go to CONTROL PANEL, and to analysis panel. Deselect the analysis that will not be run. (Select or deselect analysis by pressing ENTER.) Press Tab to return to the Analysis Panel.

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

9.2.9 Type your ID (initials), the sample table which you created under **9.2.1** (or press ENTER for choices), choose running with or without the system table and select START ANALYSIS.

9.2.10 After starting the software, start the sampler. Make sure that the sampler is set to the right number of samples and that the sample/wash/air times are OK.

9.2.11 Select GRAPHICS, REAL TIME to view the progress of the analysis.

9.3 Loading and Printing the Data-File

9.3.1 Go to CONTROL PANEL, press the spacebar to bring up the local menu and select LOAD. Select AUTOCALCULATION and enter the filename (or highlight the file to be printed and press ENTER).

9.3.2 To view the calibration curve, go to GRAPHICS, CALIBRATION CURVE.

9.3.3 To print the high level curve, push PRINT SCREEN.

9.3.4 To print the low level screen, push ESC to get out of graphics. Select SETTINGS. Change the max y value to approximately 900. Go to CAL CURVE and press ESC, and Enter. Press PRINT SCREEN.

9.3.5 Return to SETTINGS and change the max value back to 4095, go to EDIT, press ENTER and PRINT SCREEN to print sample peaks.

9.3.6 To print the results go to CONTROL PANEL, SPACEBAR, OUTPUT, OUTPUT. Select PRINTER for the Epson or PRN for the Laser.

9.4 Shutdown

9.4.1 Put all the reagent-lines in Milli-Q water.

9.4.2 Let the system rinse for approximately 30 minutes.

9.4.3 After the system has rinsed completely, turn off the sampler, pump and offset potentiometer. Turn off the heating bath on weekends. Leave liquid in the lines.

9.4.4 Take the electrode out and soak in 100 ppm F overnight.

9.4.5 Release the pump-decks, air bars and sampler pump-tubing.

9.4.6 Select FILES, press ALT F and select QUIT to exit the program.

9.4.7 On Friday, turn off the computer, display and interface for the weekend.

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard (mid to high concentration) every 10 samples. If a significant change in peak height occurs, 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-8.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-8.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

11.1.2 All calculations are done by the Skalar software. r^2 should be 0.995 or better.

11.2 Prepare spreadsheets to summarize data. Include sample volume, weights used etc.

11.3 Write the study number on the printouts, initial, date the printout, and bind together with all package documents and place in the study folder. Make a copy of the summary sheet and tape into the study notebook. Back up all data and spreadsheets onto study disk and backup disks.

11.4 Electronic Data

11.4.1 GLP studies: Electronic data is copied onto the Study floppy disk for each study, and also data is copied onto a floppy disk that is stored in the lab.

11.4.2 Other studies: All data is copied onto a floppy disk that is stored in the lab.

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

13.2 Skalar Methods, #335, Skalar Methods Manual

13.3 AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

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

Study Title: **Single-dose Intravenous Pharmacokinetic Study of T-6053 in Rabbits**

Study Number: AMDT-010495.1 Name of Auditor: Kari Rambo

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

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

Kari Rambo 10-19-95

QAU Auditor Date

003823

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

Thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

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

Summary of Combustion Data - Liver
AMDT-010495.1, HWI 6329-136
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group

	ug
Control Group	15.1
10 mg/kg dose (T6053)	12.7
50 mg/kg dose (T6053)	25.5
125 mg/kg dose (T6053)	17.7
250 mg/kg dose (T6053)	34.7

FC99 PK ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
liver spk 63-1	88%	1.08		0.1224			
liver spk 63-2	96%	0.970		0.1499			
liver spk 126-1	91%	2.06		0.1327			
liver spk 126-2	89%	2.16		0.1242			
liver spk 253-1	84%	4.16		0.122			
liver spk 253-2	83%	3.44		0.1462			
liver blank-1		0.314		0.1499			
F52856-1		0.250		0.136	77.8		
F52856-2		0.172	0.194	0.1263	77.8	15.1	0
F52856-3		0.160		0.1383	77.8		
F52857-1		0.150		0.141	77.4		
F52857-2		0.150	0.164	0.1428	77.4	12.7	10
F52857-3		0.192		0.1038	77.4		
F52861-1		0.186		0.1466	86.1		
F52861-2		0.311	0.296	0.1198	86.1	25.5	50
F52861-3		0.390		0.1366	86.1		
F52862-1		0.237		0.1456	79.5		
F52862-2		0.211	0.222	0.1053	79.5	17.7	125
F52862-3		0.219		0.136	79.5		
liver blk 1		0.412		0.105			
liver blk 2		0.359		0.1157			
liver spk 1	97%	1.24		0.1179			
liver spk 2	94%	0.857		0.1667			
F52863-1		0.503		0.1199	78.5		
F52863-2		0.426	0.441	0.1386	78.5	34.7	250
F52863-3		0.395		0.1232	78.5		
SPK 63-1	95%	0.940		0.1525			
SPK 63-2	96%	0.912		0.1593			
SPK 126-1	81%	1.76		0.1393			
SPK 126-2	82%	2.18		0.1136			

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

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.

A-1
Contains pages
A-1 through A-7
D. Christenson
11/22/95

Study:	Single-Dose Intravenous Pharmacokinetic
Protocol Number:	TP8084.PK
Test Material:	T-6063 in Rabbits (FC-99)
Matrix:	Liver
R Squared Value:	0.9816
Response Factor Amount:	1.00E-05
Analyst:	DLC
Date:	4/6/95
Method:	AMDT-M-4
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE File:	040695A

Group Dose	Sample #	Ion Count	Extracted wt g	Dilution factor	Concentration ug/g *	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: 0 mg/kg	F52856	20514	1.0361	1	0.1587	77.8299	0.012
Group 2: 10 mg /kg	F52857	8628	1.0019	1	0.0690	77.4061	0.005
Group 3: 50 mg/kg	F52861	7353	1.1609	1	0.0508	86.1239	0.004
Group 4: 125 mg/kg	F52862	27068	1.0462	1	0.2074	79.5049	0.016
Group 5: 250 mg/kg	F52863	29919	1.1937	1	0.2009	78.5453	0.016

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

A-2

Thurs 6 April 95

HWI# 6329- 131 (FC-135)

6329- 136 (FC-99)

- 138 (FC-129)

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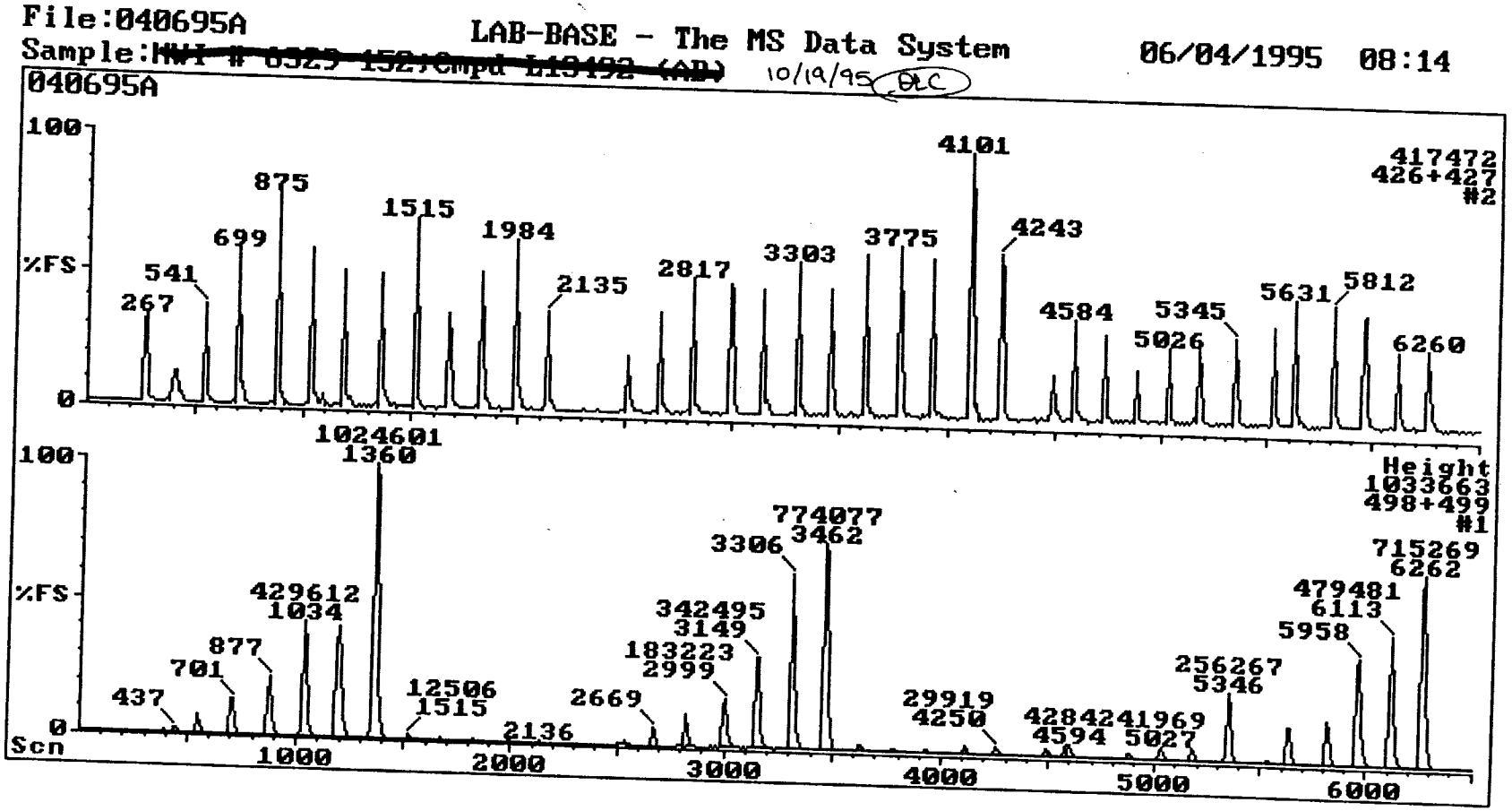
000063



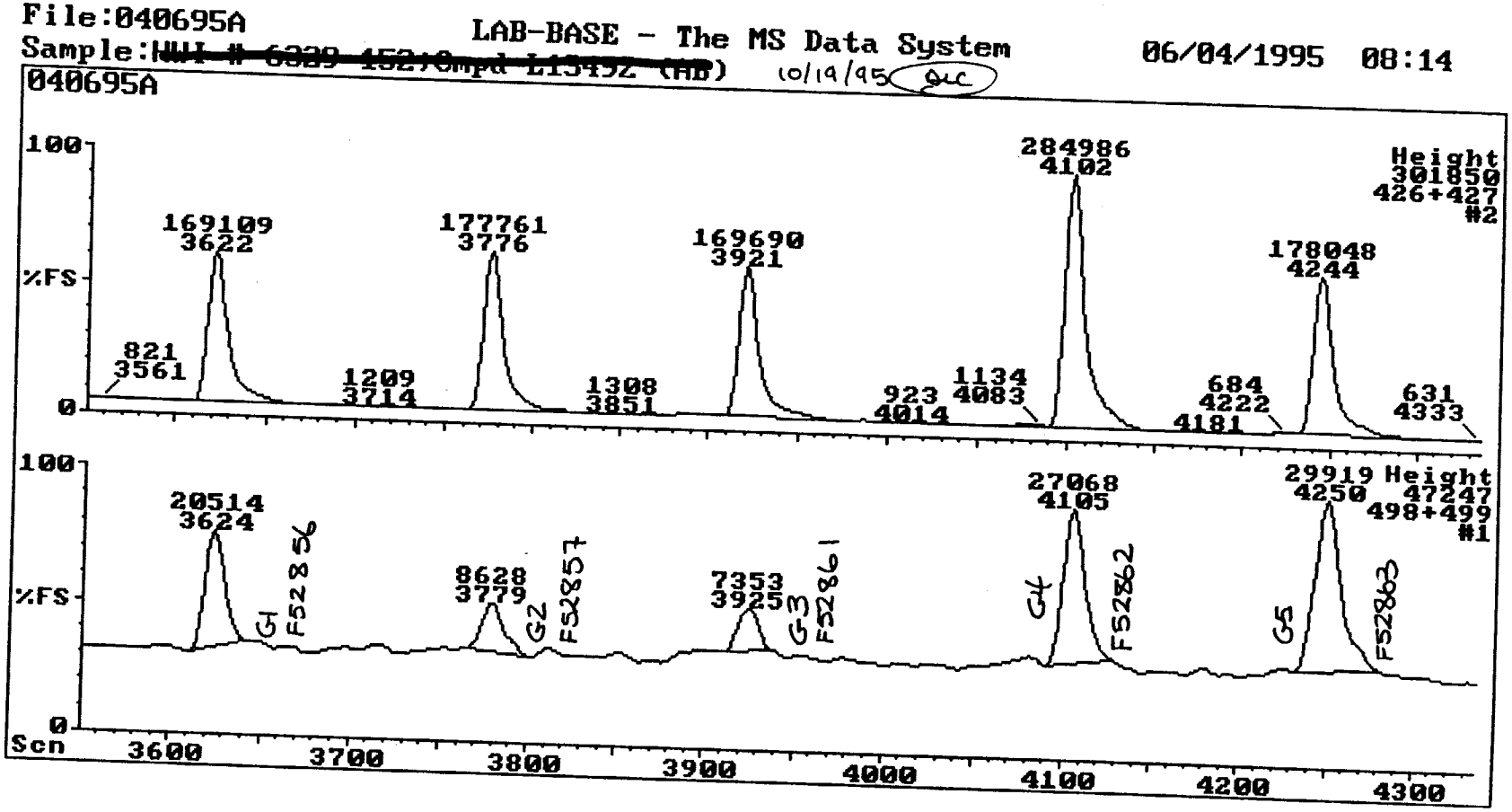
A-3

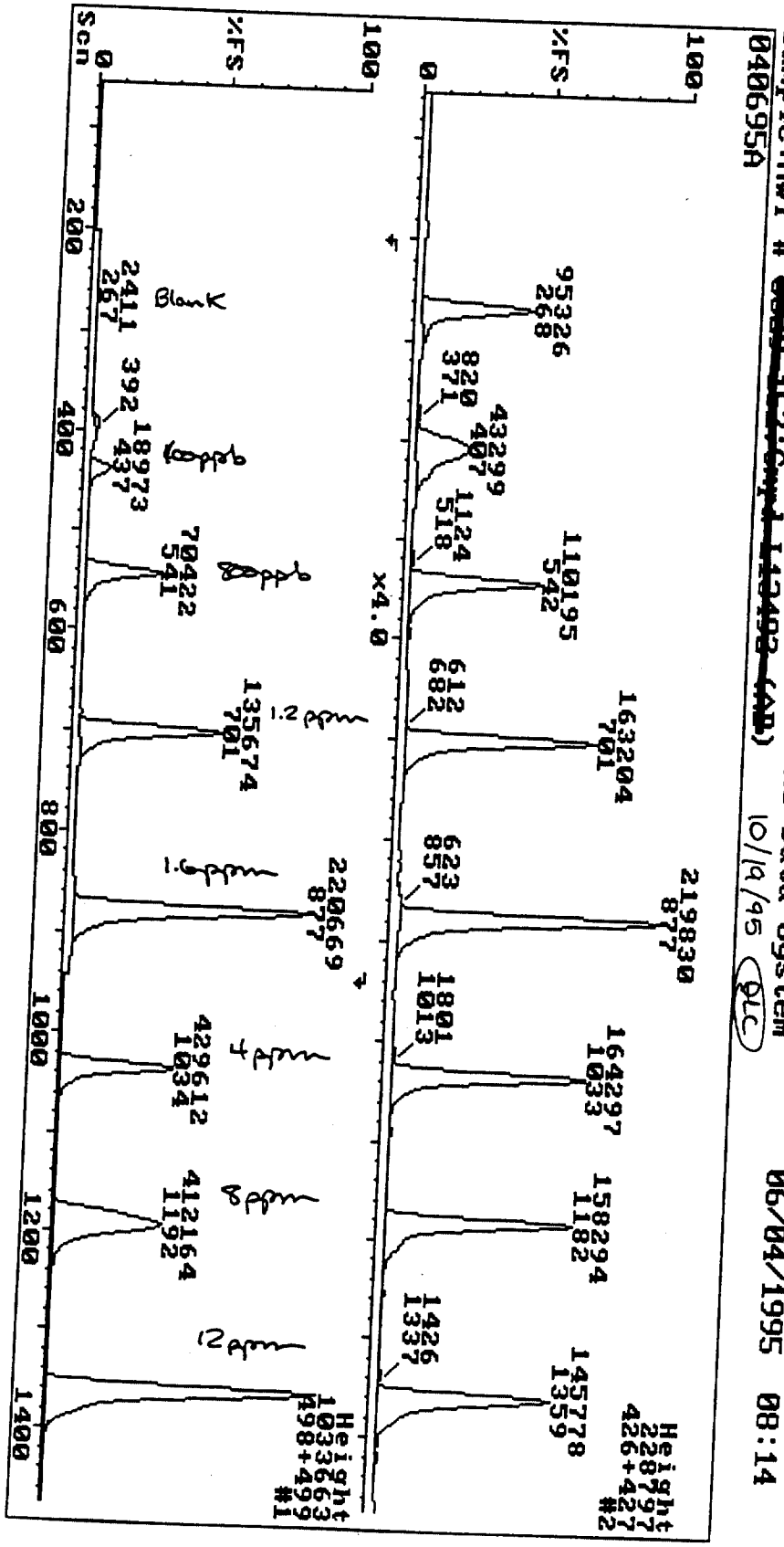
AWI #

6329-136 (FC-99)

C03833

000070

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Sample:HWI #
040695A
LAB-BASE - The MS Data System
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10/19/95
%FS
Scn
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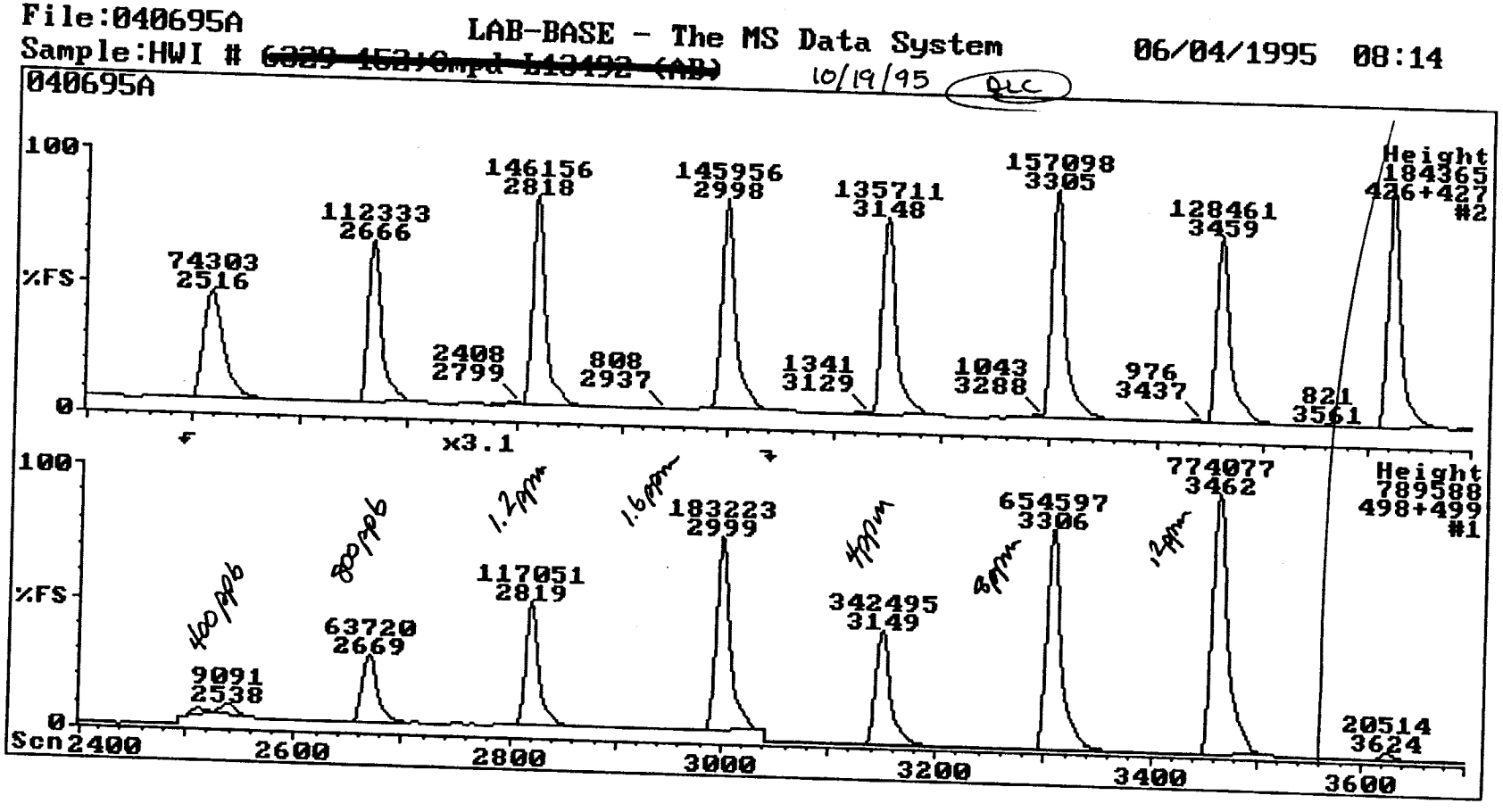
1.2 ppm
1.6 ppm
4 ppm
8 ppm
12 ppm
Height
1033663
498+499
#1
Height
228797
426+427
#2

A-5

G03835

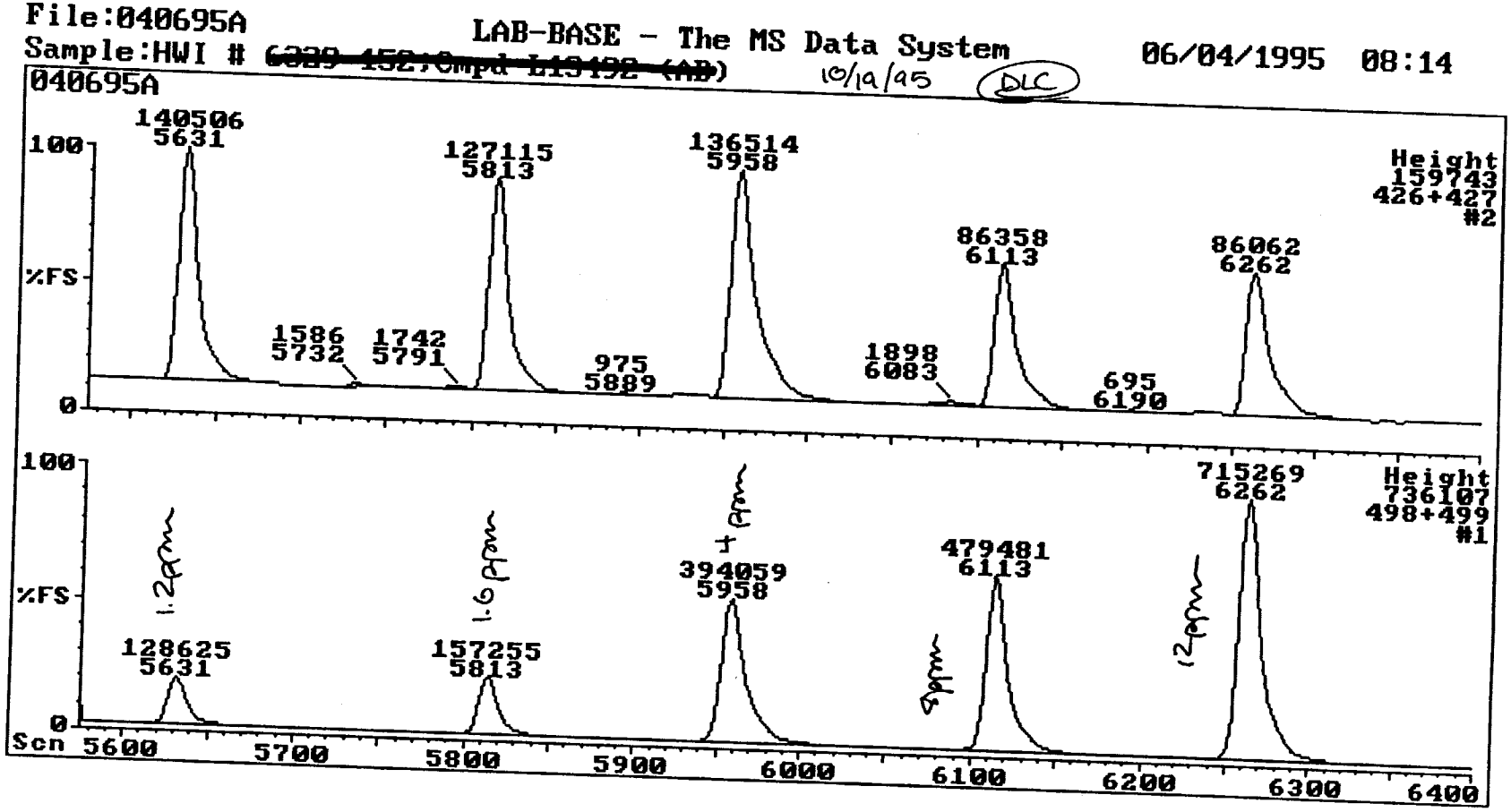
000072



A-6

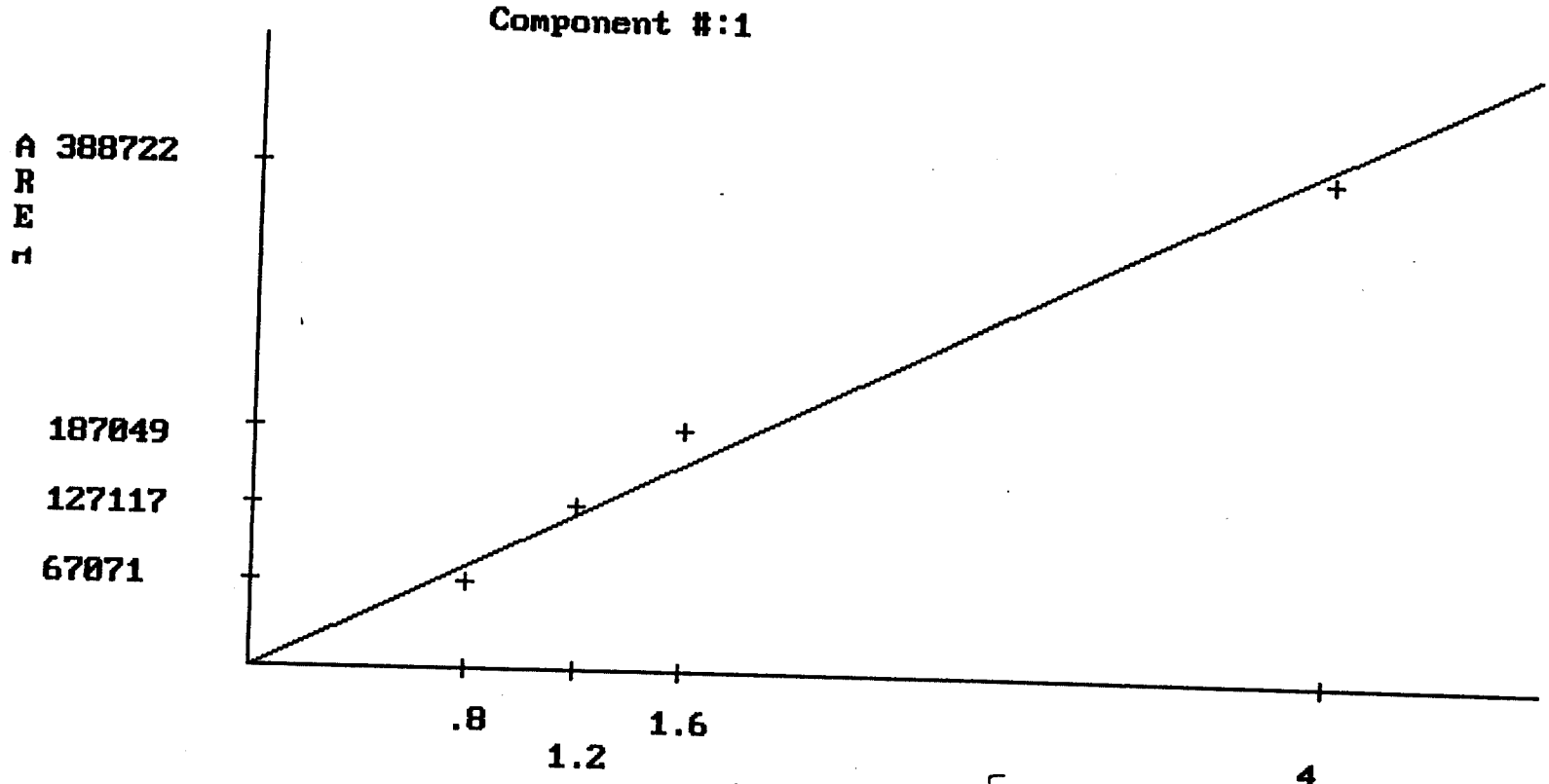
C03836

000073



040695A

Method C:DLCLIV
Sample
Operator
Run date 06-23-1995 09:19:00 version: 16
Printed on 06-23-1995 AT 09:19:13
Straight Line Fit forced through Origin.



from LABBASE FILE 040695A

Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.8000	67071
2	1.2000	127117
3	1.6000	187049
4	4.0000	388722

Y = SLOPE * X + INTERCEPT

Area = 9.9824E+04 * Amount + 0.0000E+00
Amount = 1.0018E-05 * Area + 0.0000E+00
R squared = 0.9816

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

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.

7/17/95 DDW

RE: 6329-136 LIVER SAMPLES
AMDT 02495.1
Date of Analysis: 4-6-95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using between 0.1 and 0.2 grams of the liver. The gas is collected in 1.0 mL of 1:1 TISAB/Milli-Q water then an additional 2 mL of 1:1 TISAB/Milli-Q is added to allow for sufficient volume for Skalar analysis. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Debra D Wright

C03839

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Pg 1 of 12

SUMMARY OF
6329-136 LIVERS
AMDT 02495.1

BEST COPY AVAILABLE

	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Average Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	Average Total F- per tissue (ug)
GROUP 1	F52856-1	ND	3.0	0.1360	ND	ND	77.8299	ND	ND
Dose Level : 0	F52856-2	0.03	3.0	0.1263	0.68		77.8299	53	
GROUP 2	F52857-1	0.08	3.0	0.1410	1.79		77.4061	139	
Dose Level : 10 mg/kg	F52857-2	0.11	3.0	0.1428	2.37	1.39	77.4061	184	108
	F52857-3	ND	3.0	0.1038	ND		77.4061	ND	
GROUP 3	F52861-1	ND	3.0	0.1466	ND		86.1239	ND	
Dose Level : 50 mg/kg	F52861-2	ND	3.0	0.1198	ND	ND	86.1239	ND	ND
	F52861-3	0.02	3.0	0.1366	0.49		86.1239	42	
GROUP 4	F52862-1	ND	3.0	0.1456	ND		79.5049	ND	
Dose Level : 125 mg/kg	F52862-2	ND	3.0	0.1053	ND	ND	79.5049	ND	ND
	F52862-3	ND	3.0	0.1360	ND		79.5049	ND	
GROUP 5	F52863-1	0.02	3.0	0.1199	0.62		78.5453	49	
Dose Level : 250 mg/kg	F52863-2	0.03	3.0	0.1386	0.55	0.56	78.5453	43	44
	F52863-3	0.02	3.0	0.1232	0.52		78.5453	41	

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003840

1995-07-10 10:23 OutPut of : 950406C1
Operator : DDW
Date of the Analysis : 1995-04-06 13:33
Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950406C1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol. (mL)	Qty Sample (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.49	99%										
2	Drift	1.50	1.51	101%										
3	Wash		0.00											
4	Standard 1	0.015	0.015	101%										
5	Standard 2	0.03	0.03	99%										
6	Standard 3	0.06	0.06	100%										
7	Standard 4	0.09	0.09	101%										
8	Standard 5	0.12	0.12	99%										
9	Standard 6	0.15	0.15	100%										
10	Standard 7	0.30	0.29	96%										
11	Standard 8	0.60	0.61	102%										
12	Standard 9	1.20	1.24	103%										
13	Standard 10	1.50	1.46	97%										
14	Drift	1.50	1.58	105%										
15	Wash		ND											
16	F52856-1		ND		3.0	0.1360	ND	77.8299	ND					
17	F52856-2		0.03		3.0	0.1263	0.68	77.8299	53					
18	BLANK		0.06		1.0	0.1499	0.39							
19	F52857-1		0.08		3.0	0.1410	1.79	77.4061	139					
20	F52857-2		0.11		3.0	0.1428	2.37	77.4061	184					
21	F52857-3		ND		3.0	0.1038	ND	77.4061	ND					
22	F52861-1		ND		3.0	0.1466	ND	86.1239	ND					
23	F52861-2		ND		3.0	0.1198	ND	86.1239	ND					
24	F52861-3		0.02		3.0	0.1366	0.49	86.1239	42					
25	F52862-1		ND		3.0	0.1456	ND	79.5049	ND					
26	Drift	1.50	1.56	104%										
27	Wash		ND											
28	F52862-2		ND		3.0	0.1053	ND	79.5049	ND					
29	F52862-3		ND		3.0	0.1360	ND	79.5049	ND					
30	LIVER BLK 1		ND		3.0	0.1050	ND							
31	LIVER BLK 2		ND		3.0	0.1157	ND							
32	LIVER SPK 1		0.05		3.0	0.1179	1.37			0.004	63.00	0.15	0.16	107%

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3

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI-TISAB final vol (mL)	Qty Sample (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
33	LIVER SPK 2		0.06		3.0	0.1667	1.07			0.004	63.00	0.15	0.18	117%
34	F52863-1		0.02		3.0	0.1199	0.62	78.5453	49					
35	F52863-2		0.03		3.0	0.1386	0.55	78.5453	43					
36	F52863-3		0.02		3.0	0.1232	0.52	78.5453	41					
37	SPK 63-1		0.06		3.0	0.1525	1.20							
38	Drift	1.50	1.56	104%						0.004	63.00	0.15	0.18	121%
39	Wash		ND											
40	SPK 63-2		Multi		3.0	0.1593	*			*	*			
41	SPK 126-1		Multi		3.0	0.1393	*			*	*			
42	SPK 126-2		Multi		3.0	0.1136	*			*	*			
43	Drift	1.50	1.54	103%										
44	Wash		ND											

* Samples # 40-42 had multiple peaks due to insufficient sample in cup. Disregard results for these three samples. DDW

1995-04-06 16:07 OutPut of : 950406C1

DDW 7/17/95
AMDT 0249.1
HWI 6329-17
Liners

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-04-06 13:33

Analysis File Name : C:\SKALAR\DATA\950406C1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00093
a0 = -1.24069

Fluoride L
Calibration order = 2

Correlation : r = 0.99928

Result = $a2 * x^2 + a1 * x + a0$

a2 = 0.00000
a1 = 0.00025
a0 = 0.00292

Sampler
Type : SA1000
Number : 1
Sample Time : 50 sec.
Wash Time : 120 sec.
Air Time : 1 sec.
Take up : Single
sPecial : None
needle Height : 70 mm.

Diluter
needle Height : 80 mm
dilution Factor : 10
dilution Volume : 2.5 ml.
Resample : 1
Dilution runs : 1

User file : . TXT
Reproces : No

003843

000080

5

1995-04-06 16:07 OutPut of : 950406C1

Fluoride 1.5

Path number	: 3
Signal type	: Debubbled
Decolor	: Yes
system Number	: 0
diLute	: No
Resample	: No
dil Threshold	: 4095
diG output	: 0
Window event	: Off

s1	sTandard	: Ignore
s2	sTandard	: Ignore
s3	sTandard	: Ignore
s4	sTandard	: Ignore
s5	sTandard	: Ignore
s6	sTandard	: 0.150
s7	sTandard	: 0.300
s8	sTandard	: 0.600
s9	sTandard	: 1.200
s10	sTandard	: 1.500

Order : Inverse Logarithm
Dimension : PPM

start Value	: 500 DU
trigger Limit	: 1800 Sec
Peak shape	: Pointed
stArt ignore	: 60 Sec
eNd ignore	: 120 Sec
Measure window	: 75 %
Filter	: No
Regeneration	: No
formUla	:
output	: ##.###

Fluoride L

Path number	: 0
Signal type	: Debubbled
Decolor	: No
system Number	: 0
diLute	: No
Resample	: No
dil Threshold	: 4095
diG output	: 0
Window event	: Off

003844

000081

6

1995-04-06 16:07 OutPut of : 950406C1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla     : c4:=c3
output      : #.####
```

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	Dil	F Cor.	Valu	Time
wt	iw	Initial Wash	1	1.000	3	0.057	1	0	280	65
					4	0.0029	0	0	0	0
1	t	Tracer	1	1.000	3	1.489	1	2284	2571	211
					4	1.0431	0	2284	0	0
2	d	Drift	1	1.000	3	1.508	1	2302	2595	387
					4	1.0550	0	2302	0	0
3	w	Wash	1	1.000	3	0.057	1	0	300	629
					4	0.0029	0	0	0	0
4	s1	Standard 1	1	1.000	3	0.064	1	48	348	735
					4	0.0152	0	48	0	0
5	s2	Standard 2	1	1.000	3	0.071	1	102	402	912
					4	0.0296	0	102	0	0
6	s3	Standard 3	1	1.000	3	0.089	1	211	512	1080
					4	0.0601	0	211	0	0
7	s4	Standard 4	1	1.000	3	0.109	1	313	616	1261
					4	0.0906	0	313	0	0
8	s5	Standard 5	1	1.000	3	0.129	1	404	708	1436
					4	0.1193	0	404	0	0
9	s6	Standard 6	1	1.000	3	0.154	1	497	804	1612
					4	0.1502	0	497	0	0
10	s7	Standard 7	1	1.000	3	0.287	1	863	1178	1786
					4	0.2868	0	863	0	0
11	s8	Standard 8	1	1.000	3	0.609	1	1387	1716	1962

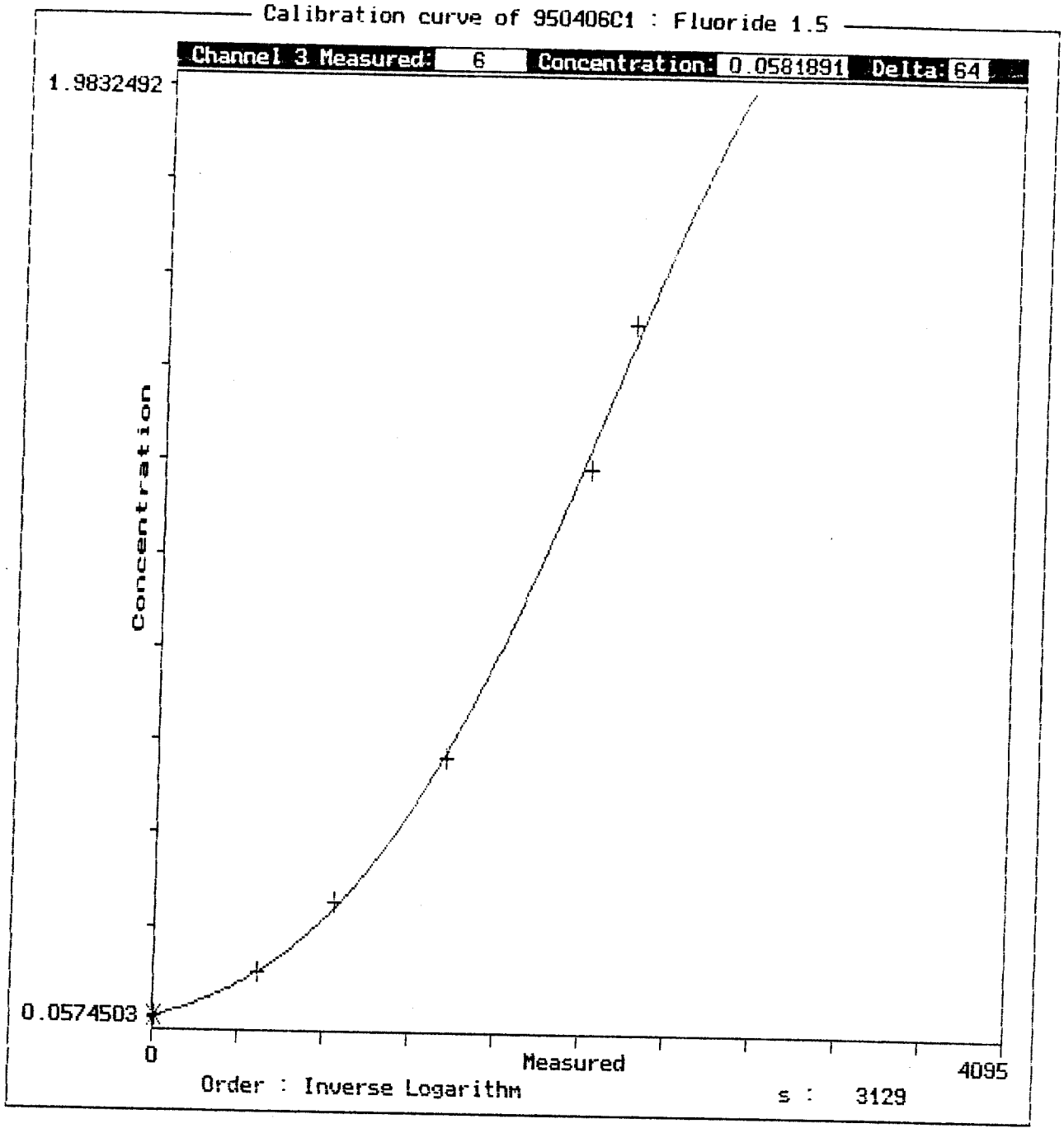
003845

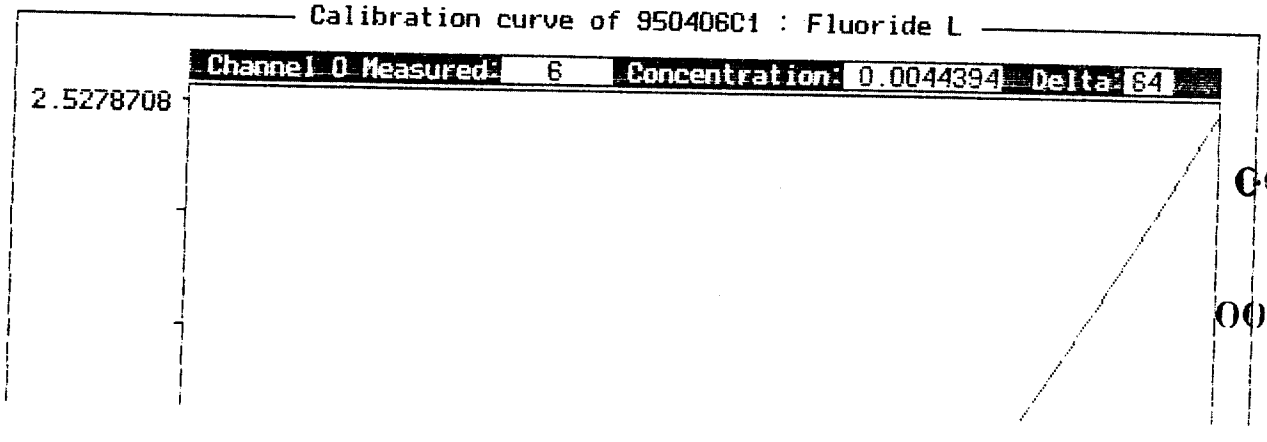
000082

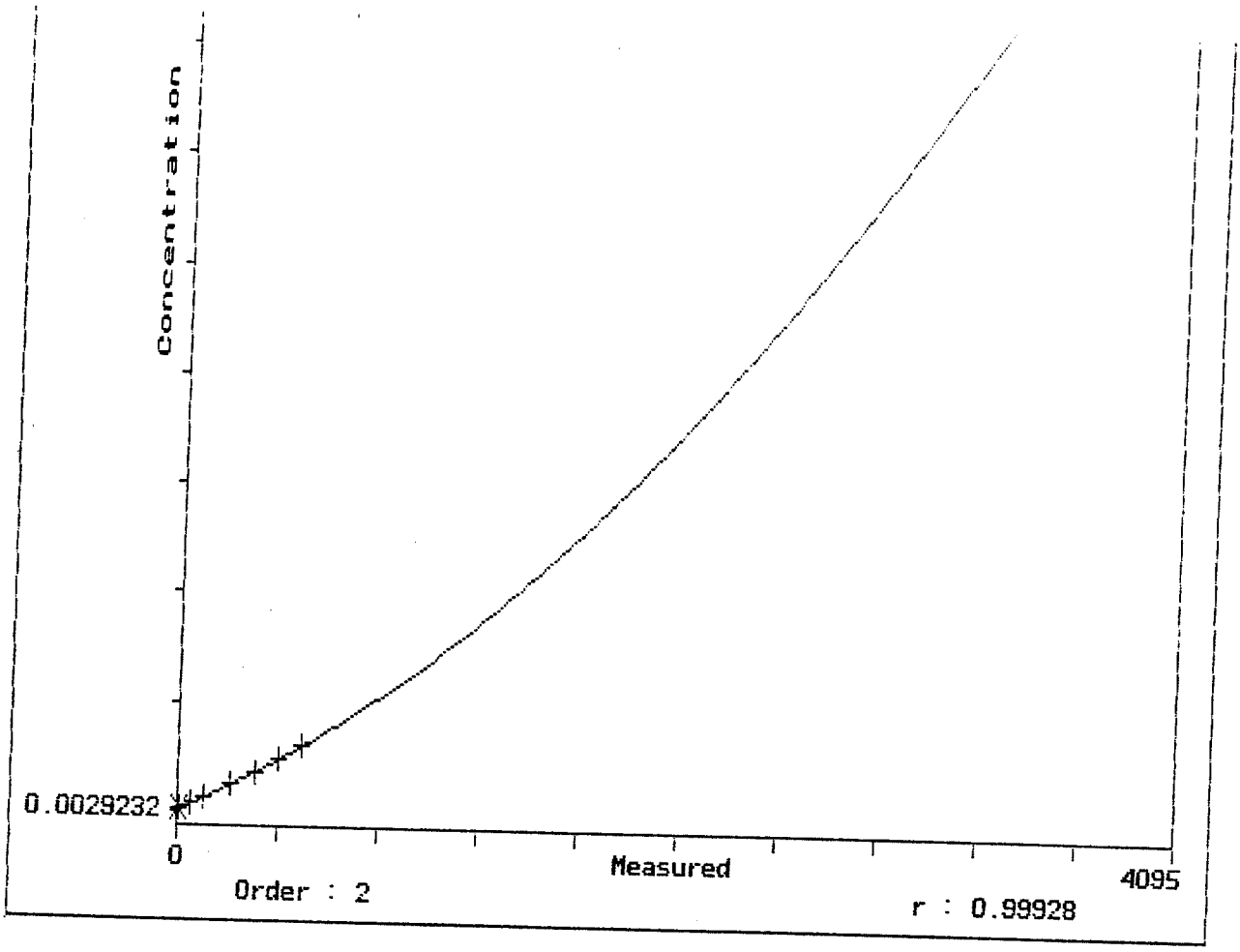
					4	0.5239	0	1387	0	0
12	s9	Standard 9	1	1.000	3	1.240	1	2053	2402	2136
					4	0.8957	0	2053	0	0
13	s10	Standard 10	1	1.000	3	1.459	1	2256	2616	2312
					4	1.0247	0	2256	0	0
14	d	Drift	1	1.000	3	1.582	1	2372	2668	2485
					4	1.1018	0	2372	0	0
15	w	Wash	1	1.000	3	0.057	1	0	296	2717
					4	0.0029	0	0	0	0
16	u	F52856-1	3	1.000	3	0.189	3	44	342	2834
					4	0.0142	0	44	0	0
17	u	F52856-2	3	1.000	3	0.212	3	98	400	3009
					4	0.0285	0	98	0	0
18	u	BLANK	1	1.000	3	0.088	1	206	512	3181
					4	0.0586	0	206	0	0
19	u	F52857-1	3	1.000	3	0.313	3	292	600	3360
					4	0.0841	0	292	0	0
20	u	F52857-2	3	1.000	3	0.373	3	384	696	3536
					4	0.1129	0	384	0	0
21	u	F52857-3	3	1.000	3	0.178	3	16	320	3676
					4	0.0070	0	16	0	0
22	u	F52861-1	3	1.000	3	0.178	3	15	320	3869
					4	0.0067	0	15	0	0
23	u	F52861-2	3	1.000	3	0.189	3	44	352	4053
					4	0.0142	0	44	0	0
24	u	F52861-3	3	1.000	3	0.201	3	74	384	4235
					4	0.0221	0	74	0	0
25	u	F52862-1	3	1.000	3	0.186	3	35	345	4411
					4	0.0119	0	35	0	0
26	d	Drift	1	1.000	3	1.555	1	2346	2657	4587
					4	1.0843	0	2346	0	0
27	w	Wash	1	1.000	3	0.057	1	0	312	4751
					4	0.0029	0	0	0	0
28	u	F52862-2	3	1.000	3	0.183	3	29	340	4934
					4	0.0103	0	29	0	0
29	u	F52862-3	3	1.000	3	0.183	3	27	336	5104
					4	0.0098	0	27	0	0
30	u	LIVER BLK 1	3	1.000	3	0.185	3	33	340	5286
					4	0.0113	0	33	0	0
31	u	LIVER BLK 2	3	1.000	3	0.181	3	24	329	5461
					4	0.0090	0	24	0	0
32	u	LIVER SPK 1	3	1.000	3	0.256	3	190	496	5637
					4	0.0540	0	190	0	0
33	u	LIVER SPK 2	3	1.000	3	0.265	3	208	512	5807

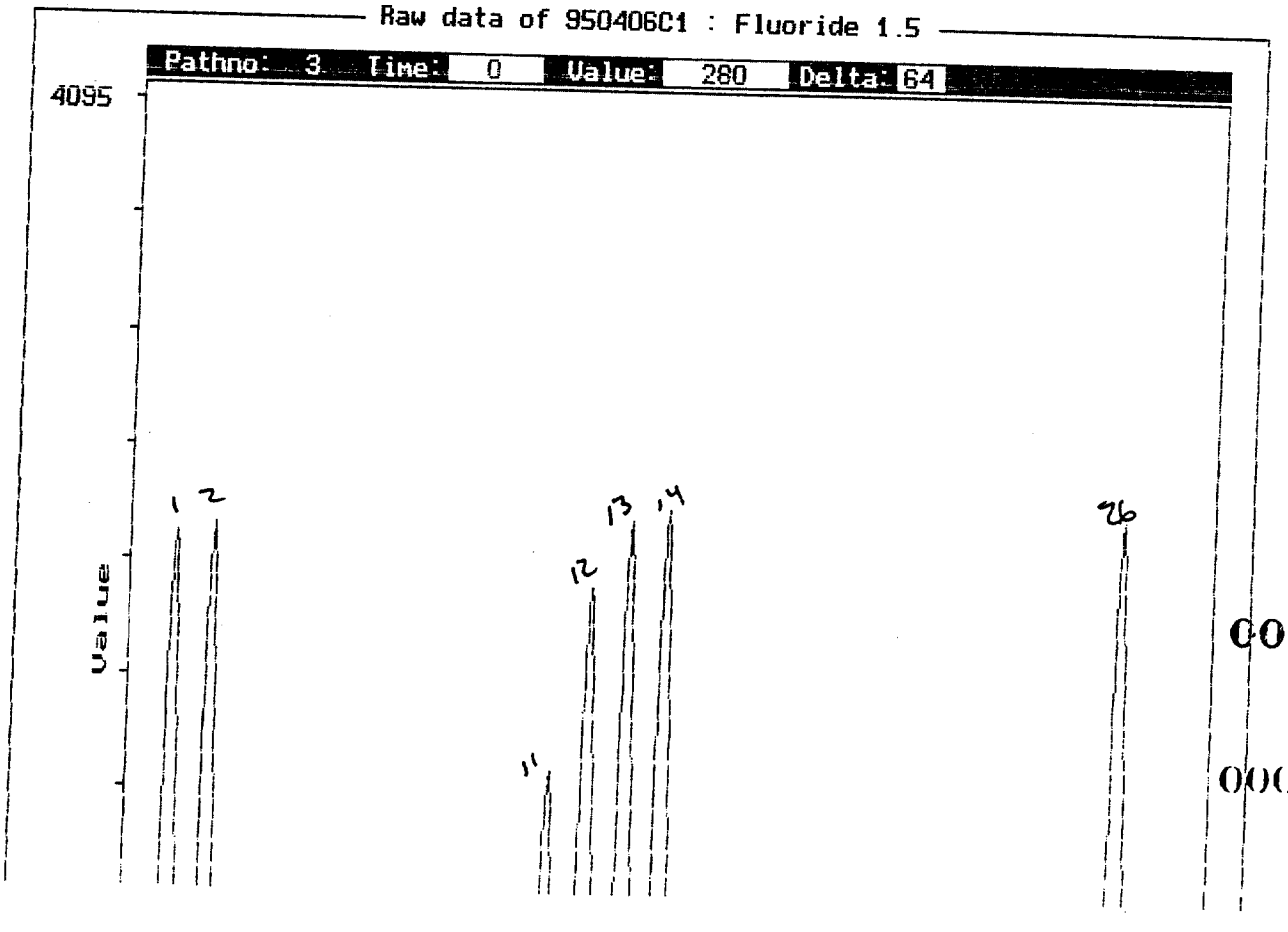
					4	0.0592	0		208	0	0
34	u	F52863-1	3	1.000	3	0.206	3		84	384	5985
					4	0.0247	0		84	0	0
35	u	F52863-2	3	1.000	3	0.207	3		86	384	6153
					4	0.0253	0		86	0	0
36	u	F52863-3	3	1.000	3	0.201	3		72	368	6336
					4	0.0215	0		72	0	0
37	u	SPK 63-1	3	1.000	3	0.269	3		215	512	6510
					4	0.0612	0		215	0	0
38	d	Drift	1	1.000	3	1.563	1		2354	2644	6688
					4	1.0896	0		2354	0	0
39	w	Wash	1	1.000	3	0.057	1		0	288	6861
					4	0.0029	0		0	0	0
40	u	SPK 63-2	3	1.000	3	Multi	3	M	1020	1360	7103
					4	0.3527	0		1020	0	0
41	u	SPK 126-1	3	1.000	3	Multi	3	M	1018	1388	7160
					4	0.3518	0		1018	0	0
42	u	SPK 126-2	3	1.000	3	Multi	3	M	992	1392	7344
					4	0.3406	0		992	0	0
43	d	Drift	1	1.000	3	1.544	1		2336	2752	7558
					4	1.0776	0		2336	0	0
44	w	Wash	1	1.000	3	0.057	1		0	448	7743
					4	0.0029	0		0	0	0
wt	rw	RunOut Wash	1	1.000	3	0.057	1		0	1405	8033
					4	0.0029	0		0	0	0

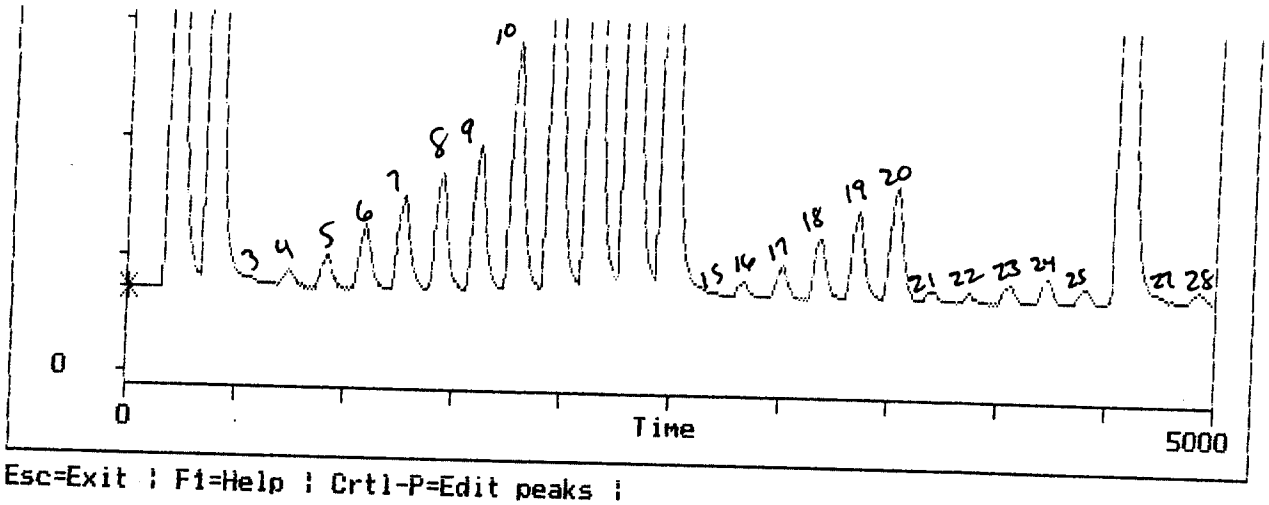
Multiple Peaks - Doc 4/6/95



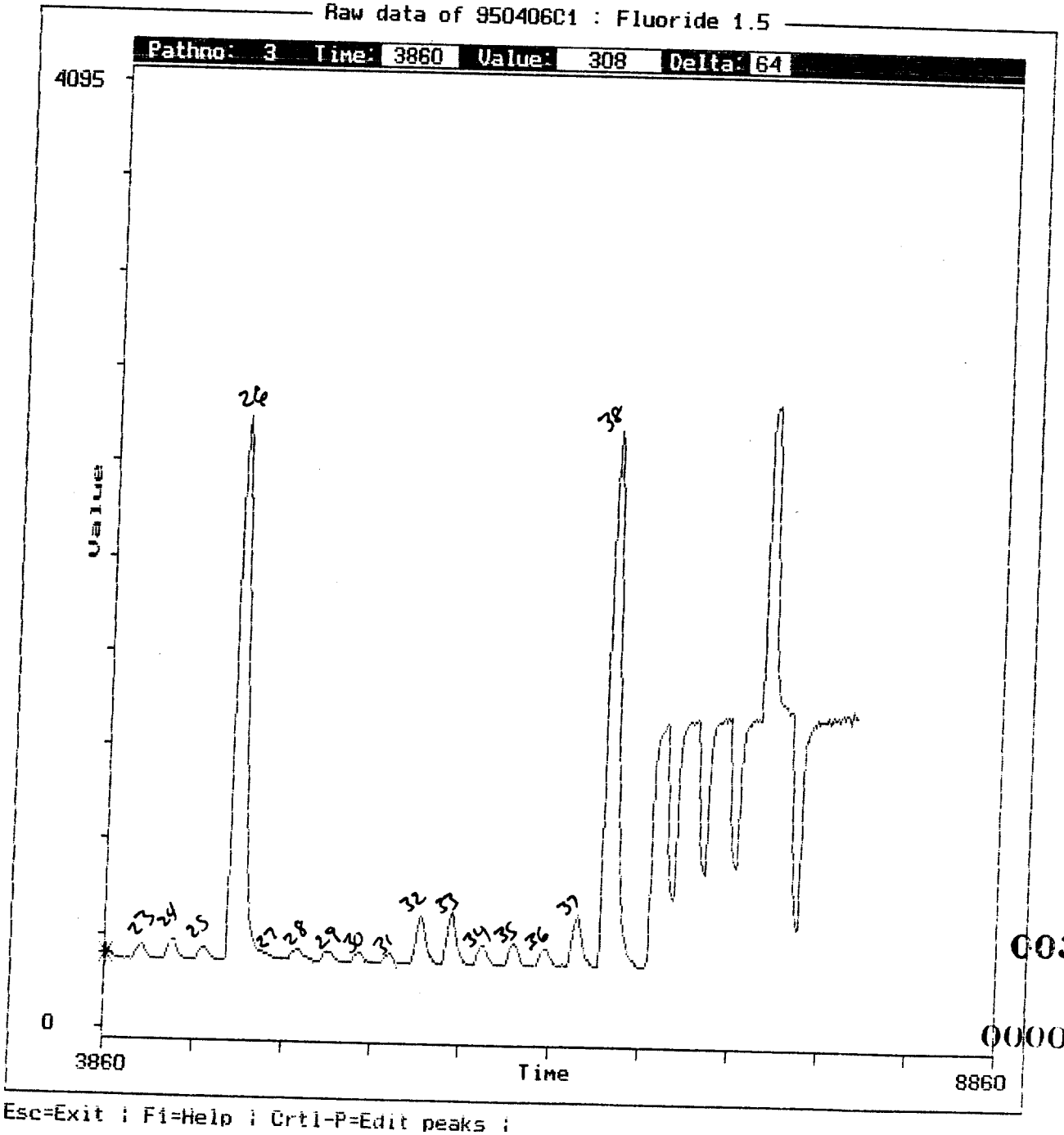








Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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