3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6053 in Rabbits

In-Vivo Study Reference Number: HWI#6329-137

Study Number: AMDT-022195.1
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

11/22/95
Completion Date

1.0 SUMMARY

After dermal application of FC-99 (T-6053), tissue and serum samples were collected (HWI#6329-137). The liver at 28 days was combusted and analyzed for total organic fluorine. The results of this analysis showed no differences between the controls and treated rabbits including the high dose which were rabbits treated with 250 mg/kg (T-6053). This formulation is a diethanolamine salt of perfluorooctanesulfonate. Twenty percent of these solids are the C8. T-6053 is a solution that is 0.04% of FC-99 solids. The dose for the high group was 20 ug/kg with respect to C8 perfluorooctanesulfonate. The dose was below detection limits of the method even if all of the samples were absorbed.

2.0 INTRODUCTION

This study was performed in order to provide data for the assessment of dermal absorption of FC-99. Data from other forms of perfluorooctanesulfonate (FC-95, the potassium salt) were available (HWI#6329-130, HWI#6329-159). A pharmacokinetic study (HWI#6329-136) had shown that perfluorooctanesulfonate would be a convenient marker for FC-99 if the doses administered were high enough.

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 then 28 days.

4.0 EXPERIMENTAL - Overview

Tissue and serum samples were available for analysis from HWI#6329-137. Liver samples collected at 28 days post dose were analyzed for total organic fluorine by combustion and selective ion electrode. The data were used to assess the extent of dermal absorption.

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 and subsequent combustion analysis for total organic fluorine of liver samples from rabbits dosed dermally with FC-99 is attached. The data from livers at 28 days post dose show no differences between dosing groups. Rabbits were dosed at 0, 10, 125, 250 mg/kg (0, and about 0.8, 10, and 20 ug/kg with respect to the C8 perfluorooctanesulfonate). The formulation of FC-99 is a diethanolamine salt of perfluorooctanesulfonate with about 25% solids. Twenty percent of these solids are the C8. T-6053 is a 0.04% solution of FC-99 solids. The dose for the high group was 20 ug/kg with respect to the perfluorooctanesulfonate. In previous studies, FC-95 has been shown to be persistent in rabbits after an intravenous dose (HWI#6329-159), not dermally absorbed at doses of 0.30 mg/kg (HWI#6329-130), and suitable as a marker to assess dermal absorption

(HWI#6329-159). In a pharmacokinetic study of FC-99 (HWI#6329-136), it was concluded that perfluorooctanesulfonate would be a useful marker for assessment of dermal absorption of FC-99. Since FC-99 contains perfluorooctanesulfonate the same as FC-95 but just a different salt form (FC-95 is the potassium salt), it is consistent that no dermal absorption appears to occur at these dose levels of T-6053. However, if all of the 20 ug/kg dose were absorbed, the method would not have been able to detect it. Since T-6053 is the same preparation of FC-99 used in the pharmacokinetic study, a direct comparison is appropriate.

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 circumstances that may affect this data analysis is that a longer term pharmacokinetics study on this salt form of perfluorooctanesulfonate was not performed. The dose was very low (below detection limits). However, it seems unlikely that there would be a substantial difference in the pharmacokinetics of two salt forms at 28 days especially with respect to deposition in liver.

7.0 CONCLUSION

There does not appear to be dermal absorption of FC-99 (T-6053) at dermal dose levels at 20 ug/kg. The dose was below the detection limit of the method.

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#6329-137 "Single-Dose Dermal Absorption/Toxicity Study of T-6053 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-022195.1

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-022195.1

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 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#6329-137 "Single-Dose Dermal Absorption/Toxicity Study of T-6053 in Rabbits" (Protocol type TP-3016.AB for dosing of animals, tissue collection, etc.)

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6053 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

July 11, 1995

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-137

000007

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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.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/21/94	12/21/94	Protocol Review	12/22/94	01/10/95
01/25/95	01/26/95	Protocol Amendment	01/26/95	02/10/95
01/30/95	01/30/95	Protocol Amendment	01/30/95	02/10/95
02/10/95	02/10/95	Necropsy	02/10/95	03/10/95
04/10/95	04/10/95	Protocol Amendment	04/10/95	05/10/95
04/20/95	04/20/95	Data/Report Review	04/20/95	05/10/95
04/20/95	04/20/95	Data Review	04/20/95	05/10/95
07/10/95	07/10/95	Report Rereview	07/10/95	08/10/95

Randy Lentz
Representative, Quality Assurance Unit

7.11.95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity
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 Locations	Initial Test: Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704 Replacement Animal: Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	December 30, 1994
Experimental (In-life) Start Date	January 13, 1995
In-life End Date	February 17, 1995
Experimental Termination Date	July 11, 1995
Study Completion Date	July 11, 1995

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

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Anatomical Pathologist

Jack Serfort/
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Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6053 when applied to the skin of rabbits.

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

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6053	10	3	3
3	T-6053	125	3	3
4	T-6053	250	3	3*

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

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

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks were conducted daily thereafter for 28 days. Body weights were determined on Day -9 for randomization purposes, before test or control material administration (Day 1), and at in-life termination (Day 29). The initial dermal irritation reading was made before test or control material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. Blood samples were collected from a marginal ear vein of the animals before experimental initiation (Day 1), approximately 24-hours postdose (Day 2), on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from each animal. All samples were centrifuged and separated into serum and cellular fractions. All animals were euthanized at termination of the in-life phase and necropsied. The whole liver, bile, a section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy and weighed (volume only determined for bile). The tissues from the female dosed at 250 mg/kg and sacrificed on Day 2 were collected but were not weighed. The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6053 did not result in any test material-related changes in body weight gain. All animals appeared clinically normal throughout the study with the exception of one Group 4 female animal that was sacrificed on Day 2 due to an injury (possible broken back). This animal was replaced in the study and the replacement animal appeared normal throughout the study. No dermal irritation was observed at the dermal scoring intervals as a result of the application of distilled water or T-6053 at any of the dose levels. There were no test material-related lesions observed at necropsy.

OBJECTIVE

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

REGULATORY COMPLIANCE

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

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6053 and described as a clear, colorless liquid. The control material was distilled water and was described as a clear, colorless liquid.

Purity and Stability

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

Storage and Retention

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

Safety Precautions

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

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc., Denver, Pennsylvania on December 28, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin. The replacement animal was received from HRP, Inc., Kalamazoo, Michigan on January 11, 1995 and was maintained at the Hazleton Wisconsin facility at 3301 Kinsman Boulevard, Madison, Wisconsin.

Housing

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

Animal Diet

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

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -9. The weight variation of the animals for each group of each sex selected for the study did not exceed ±2 standard deviations of the mean weight, and the mean body weights for each group of each sex were not statistically different at the 5% probability level. One female animal (No. F53454) was replaced after test material exposure due to a possible broken back. This animal was replaced with another female animal (No. F53504).

Study Design

Animals weighing from 2,311 to 2,609 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6053	10	3	3
3	T-6053	125	3	3
4	T-6053	250	3	3*

a Administered at a dose volume of 2.0 mL/kg.
* One animal sacrificed on Day 2 due to a possible broken back and replaced with another female animal. This animal weighed 2,352 g at initiation.

Justification for Species Selection

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

PROCEDURES

Preparation of Exposure Area

On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair. The clipped area made up approximately 20% of the total body surface area. The intact skin of the test sites was inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped on Days 8 and 29 to aid in visualizing the application sites.

Dose Administration

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

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

Groups 2, 3, and 4. For the Group-2 animals (10 mg/kg), the test material (T-6053) was mixed with distilled water to a concentration of 100 mg/mL and applied at a dose volume of 0.1 mL/kg. This mixture was stored at room temperature until administered. The test material was administered undiluted to the test sites of the Groups 3 and 4 animals (125 or 250 mg/kg, respectively) using the average bulk density of 0.975 g/mL to determine the dose volume for each dose level (0.13 and 0.26 mL/kg, respectively). An individual dose of the respective test material or test material mixture was calculated for each animal based on its body weight on the day of treatment. The area of exposure for the 10, 125, and 250 mg/kg dose levels was 4, 16, and 25 cm^2, respectively. The approximate rate of application ranged from 0.02 to 0.06 mL/cm^2.

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

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

Reason for Route of Administration

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

Observations of Animals

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks (morning and afternoon) were conducted daily thereafter for 28 days.

Body weights were determined for randomization purposes on Day -9, before test material administration (Day 1), and at in-life termination (Day 29).

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

Sample Collections

Blood samples (approximately 4 mL) were collected from a marginal ear vein of all animals before experimental initiation (Day 1). Subsequent collection of blood was conducted approximately 24-hours postdose (Day 2), and on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately

20 mL of blood was obtained from the posterior vena cava of each animal. All samples were centrifuged and separated into serum and cellular fractions. These samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

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

Shipment of Blood, Bile, and Tissues

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

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

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

RESULTS

Body Weights

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

Clinical Observations

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

- One Group 4 female (No. F53454) treated with T-6053 at 250 mg/kg appeared to have injured its back at the time of the 24-hour blood collection. This animal was sacrificed, necropsied, and replaced with No. F53504. The replacement animal appeared normal throughout the study.

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6053 at any of the dose levels.

Pathology

Individual animal pathology comments are presented in Table 4. Individual animal tissue weights and bile volumes are in Table 5. The necropsy of the Group 4 female (No. F53454), treated with T-6053 at 250 mg/kg and sacrificed on Day 2, revealed a fracture of the first lumbar vertebra. There were no lesions observed in any of the remaining animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6053 were evaluated in male and female albino rabbits when administered as a single dermal application. There were no test material-related changes in body weight gain or in-life clinical findings at any of the dose levels. No test material-related dermal irritation was observed during the study.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

7-11-95
Date

REFERENCE

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

HWI 6329-137

PATHOLOGY REPORT

There were six rabbits (three males and three females) each from four dose levels of 0, 10, 125, and 250 mg/kg of body weight euthanized and necropsied at the termination of the study. One female (Animal No. F53454) dosed at 250 mg/kg of body weight was sacrificed on Day 2 because of an apparent broken back. This animal was replaced with another female (Animal No. F53504). The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, there were no visible lesions in any of the animals sacrificed at study termination. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from each of these animals, and both kidneys from one male and one female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. The tissues from the female (dosed at 250 mg/kg) sacrificed on Day 2 were collected but were not weighed. After necropsy, the animals were discarded.

The animal sacrificed on Day 2 had a fracture of the first lumbar vertebra. This finding correlated with the clinical observation of an apparent broken back.

Thomas E. Palmer, PhD
Pathologist

7-11-95
Date

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Table 1
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -9	Day 1	Day 29	Animal Number	Randomization Day -9	Day 1	Day 29
Group 1 (Control) - Distilled Water (0 mg/kg)							
F53464	2,210	2,378	2,923	F53461	2,428	2,597	2,891
F53441	2,253	2,413	3,030	F53455	2,347	2,484	2,929
F53451	2,285	2,536	3,058	F53442	2,437	2,456	3,097
Mean	2,249	2,442	3,004		2,404	2,512	2,972
Group 2 - T-6053 (10 mg/kg)							
F53447	2,241	2,539	2,977	F53444	2,358	2,338	2,798
F53463	2,207	2,464	2,937	F53448	2,476	2,486	2,944
F53452	2,085	2,362	2,777	F53460	2,411	2,516	2,972
Mean	2,178	2,455	2,897		2,415	2,447	2,905
Group 3 - T-6053 (125 mg/kg)							
F53453	2,275	2,497	2,944	F53443	2,434	2,544	2,844
F53458	2,265	2,425	2,863	F53467	2,476	2,581	2,965
F53446	2,156	2,323	2,743	F53450	2,427	2,537	3,003
Mean	2,232	2,415	2,850		2,446	2,554	2,937
Group 4 - T-6053 (250 mg/kg)							
F53459	2,221	2,338	2,742	F53449	2,297	2,390	2,660
F53445	2,090	2,311	2,798	F53456	2,220	2,336	2,782
F53439	2,206	2,380	2,942	F53454[a]	2,424	2,609	2,627[b]
				F53504[c]	NA	2,352	2,939
Mean	2,172	2,343	2,827		2,314	2,359	2,794

NA Not available.
a Animal No. F53454 was originally selected by the randomization program for use in the study and was treated. This animal was sacrificed after completion of the exposure period due to a possible broken back and was replaced with No. F53504. The body weights for No. F53454 are not included in the Day 1 or Day 29 group means.
b Day 2 body weight.
c Replacement animal (not included in group assignment randomization).

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2	Day 3 through 29
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F53464	Appeared normal	✓	✓	✓
	F53441	Appeared normal	✓	✓	✓
	F53451	Appeared normal	✓	✓	✓
Female	F53461	Appeared normal	✓	✓	✓
	F53455	Appeared normal	✓	✓	✓
	F53442	Appeared normal	✓	✓	✓
		Group 2 - T-6053 (10 mg/kg)			
Male	F53447	Appeared normal	✓	✓	✓
	F53463	Appeared normal	✓	✓	✓
	F53452	Appeared normal	✓	✓	✓
Female	F53444	Appeared normal	✓	✓	✓
	F53448	Appeared normal	✓	✓	✓
	F53460	Appeared normal	✓	✓	✓

✓ Condition existed.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2	Day 3 through 29
		Group 3 - T-6053 (125 mg/kg)			
Male	F53453	Appeared normal	✓	✓	✓
	F53458	Appeared normal	✓	✓	✓
	F53446	Appeared normal	✓	✓	✓
Female	F53443	Appeared normal	✓	✓	✓
	F53467	Appeared normal	✓	✓	✓
	F53450	Appeared normal	✓	✓	✓
		Group 4 - T-6053 (250 mg/kg)			
Male	F53459	Appeared normal	✓	✓	✓
	F53445	Appeared normal	✓	✓	✓
	F53439	Appeared normal	✓	✓	✓
Female	F53449	Appeared normal	✓	✓	✓
	F53456	Appeared normal	✓	✓	✓
	F53454	Appeared normal	✓	-	
		Possible broken back (at time of 24-hour bleeding interval)	-	✓	
		Moribund sacrifice	-	✓	
	F53504[a]	Appeared normal	✓	✓	✓

✓ Condition existed.
- Condition not evident.
a Animal No. F53504 replaced Animal No. F53454.

HWI 6329-137

Table 3
Individual Dermal Irritation Scores

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

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53464				Animal No. F53461			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53441				Animal No. F53455			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53451				Animal No. F53442			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6053 (10 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53447				Animal No. F53444			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53463				Animal No. F53448			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53452				Animal No. F53460			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 3 - T-6053 (125 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53453				Animal No. F53443			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53458				Animal No. F53467			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53446				Animal No. F53450			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6053 (250 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F53459				Animal No. F53449			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53445				Animal No. F53456			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F53439				Animal No. F53454[a]			
Erythema	0	0	0	0	0	0	-	-
Edema	0	0	0	0	0	0	-	-
Atonia	0	0	0	0	0	0	-	-
Desquamation	0	0	0	0	0	0	-	-
Coriaceousness	0	0	0	0	0	0	-	-
Fissuring	0	0	0	0	0	0	-	-
					Animal No. F53504[b]			
Erythema					0	0	0	0
Edema					0	0	0	0
Atonia					0	0	0	0
Desquamation					0	0	0	0
Coriaceousness					0	0	0	0
Fissuring					0	0	0	0

a Animal replaced with No. F53504.
b Replacement animal for No. F53454.
- Not applicable.

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 1 (Control) - Distilled Water (0 mg/kg)		
F53464	M	-	29	No visible lesions.
F53441	M	-	29	No visible lesions.
F53451	M	-	29	No visible lesions.
F53461	F	-	29	No visible lesions.
F53455	F	-	29	No visible lesions.
F53442	F	-	29	No visible lesions.
		Group 2 - T-6053 (10 mg/kg)		
F53447	M	-	29	No visible lesions.
F53463	M	-	29	No visible lesions.
F53452	M	-	29	No visible lesions.
F53444	F	-	29	No visible lesions.
F53448	F	-	29	No visible lesions.
F53460	F	-	29	No visible lesions.

- Not applicable.

Table 4 (Continued)
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
			Group 3 - T-6053 (125 mg/kg)	
F53453	M	-	29	No visible lesions.
F53458	M	-	29	No visible lesions.
F53446	M	-	29	No visible lesions.
F53443	F	-	29	No visible lesions.
F53467	F	-	29	No visible lesions.
F53450	F	-	29	No visible lesions.
			Group 4 - T-6053 (250 mg/kg)	
F53459	M	-	29	No visible lesions.
F53445	M	-	29	No visible lesions.
F53439	M	-	29	No visible lesions.
F53449	F	-	29	No visible lesions.
F53456	F	-	29	No visible lesions.
F53454[a]	F	-	2	The first lumbar vertebra is fractured.
F53504[b]	F	-	29	No visible lesions.

- Not applicable.
a Animal replaced with No. F53504.
b Replacement animal for No. F53454.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F53464	87.407	-	0.468	0.3
	F53441	114.827	16.722	0.331	2.5
	F53451	89.123	-	0.394	0.3
Female	F53461	73.175	-	0.371	0.7
	F53455	94.647	16.740	0.512	1.0
	F53442	91.590	-	0.391	1.5
		Group 2 - T-6053 (10 mg/kg)			
Male	F53447	81.754	-	0.213	2[a]
	F53463	83.148	15.119	0.357	0.6
	F53452	82.687	-	0.535	0.3
Female	F53444	70.587	-	0.719	0.7
	F53448	74.702	-	0.361	0.7
	F53460	92.881	15.925	0.368	0.7

- Not applicable.
a Volume not measured to tenths of a mL.

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

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 3 - T-6053 (125 mg/kg)			
Male	F53453	96.249	-	0.612	1.5
	F53458	77.755	-	0.390	0.5
	F53446	83.574	17.429	0.321	0.7
Female	F53443	79.767	-	0.602	0.6
	F53467	78.684	15.937	0.467	0.7
	F53450	79.782	-	0.580	2[a]
		Group 4 - T-6053 (250 mg/kg)			
Male	F53459	79.255	15.567	0.372	0.4
	F53445	97.381	-	0.270	0.5
	F53439	83.548	-	0.349	0.5
Female	F53449	73.983	-	0.391	0.4
	F53456	77.804	14.412	0.572	1.0
	F53454[b]	NA	-	NA	NA
	F53504[c]	83.081	-	1.253	1.1

- Not applicable.

a Volume not measured to tenths of a mL.

b Moribund sacrifice on Day 2 (01/14/95) due to possible broken back. Tissues were collected, however, their corresponding weights or volumes were not taken since this requirement became effective on 01/24/95. Animal replaced with No. F53504.

c Replacement animal for No. F53454.

APPENDIX A

Protocol Deviations
Protocol TP3016.AB
Protocol Amendment No. 1
Protocol Amendment No. 2

Protocol Deviations

Protocol	Actual Procedure
Page 8, 7. Experimental Design, C. Observation of Animals, (4) Sample Collections, (c) Method of Collection, Second Sentence. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal sacrificed in a moribund condition or sacrificed at the time of necropsy (Day 29).	Blood was inadvertently not collected at the time of necropsy from the female animal (No. F53454) treated at 250 mg/kg that was sacrificed on Day 2 due to an apparent broken back.
Page 8, 7. Experimental Design, D. Pathology, (1) Unscheduled Sacrifices and Deaths, Second Sentence. 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.	The female animal (No. F53454) treated at 250 mg/kg was sacrificed on Day 2 due to an apparent broken back using an overexposure to carbon dioxide.
Page 9, 7. Experimental Design, D. Pathology, (3) Sample Collection, Second Sentence. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necropsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C.	The size of the dermal application site collected at necropsy was inadvertently not documented for the female animal (No. F53454) treated at 250 mg/kg that was sacrificed on Day 2 due to an apparent broken back. Also, the tissues collected from the animals in the initial test were placed on dry ice (during transportation) prior to being stored in a freezer.

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

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6053 in Rabbits

Date:

December 30, 1994

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-137

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

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of
T-6053 in Rabbits

HWI No.	6329-137
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	January 13, 1995
Experimental Termination Date	February 10, 1995
Draft Report Date	March 24, 1995

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

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

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

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

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

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

5. Test Material

A. Identification
T-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).

D. Storage
Room temperature

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

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

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

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Distilled water

B. Physical Description
Clear, colorless liquid

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

D. Storage Conditions
Room temperature

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

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

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

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

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.0 kg

(5) Number and Sex
12 males and 12 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

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

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

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

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

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

(f) Acclimation
At least 7 days

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

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

B. Dose Administration

(1) Test Groups

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0*	3	3
2	T-6053	10**	3	3
3	T-6053	125	3	3
4	T-6053	250	3	3

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

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

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

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

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

C. Observation of Animals

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

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

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

(4) Sample Collections

(a) Frequency
Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 8, 15, 22, and at experimental termination (Day 29)

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from the marginal ear vein of either ear on Days 1, 2, 4, 8, 15, and 22. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal sacrificed in a moribund condition or sacrificed at the time of necropsy (Day 29). The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain -20°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

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

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

D. Pathology

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

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

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

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

E. Statistical Analyses
No statistical analyses are required.

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

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

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

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

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

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

PROTOCOL APPROVAL

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

1-5-95
Date

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

12-30-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12/30/94
Date

(6329-137.protdsk2)

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema

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

Edema

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

Atonia

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

Desquamation

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

Coriaceousness

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

Fissuring

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

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study of
T-6053 in Rabbits

HWI 6329-137

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

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective January 13, 1995

1. Page 6, 7. Experimental Design; B. Dose Administration; (1) Test Groups. The test material mixture could not be prepared at the concentration needed to utilize a dose volume of .01 mL/kg. Modify the double asterisked footnote (**) in this section with the following underlined change:

   ** To be administered at a dose volume of .10 mL/kg

2. Page 6, 7. Experimental Design; B. Dose Administration; (3) Dose Administration. The test material mixture could not be prepared at the concentration needed to utilize a dose volume of .01 mL/kg. Modify the third sentence in this section with the following underlined change:

   The dose for each animal in Group 2 will be diluted with distilled water and applied at a dose volume of .10 mL/kg.

000047

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EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON, WI 53704

Effective January 17, 1995

3. Page 6, 7. Experimental Design; B. Dose Administration; (3) Dose Administration. Animal No. F53454 (Group 4 female) was sacrificed on Day 2 due to an injury (apparent broken back). Add the following as the second paragraph to this section:

> Due to the sacrifice on Day 2 of one Group 4 female (Animal No. F53454) because of an injury (apparent broken back), a replacement animal will be treated at the same dose level in the same manner as for the other animals in the study. The observations (clinical observations, reading of dermal irritation, body weights and sample collections) and the pathology of the animals (unscheduled sacrifices and deaths, scheduled sacrifice, and sample collection) will be conducted in the same manner as for the other animals in the study.

Effective January 24, 1995

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

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

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

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

> Individual animal tissue weights and bile volumes

PROTOCOL AMENDMENT APPROVAL

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

2/15/95
Date

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

2-6-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

2.7.95
Date

(6329-137.Am1.dsk2)

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study
of T-6053 in Rabbits

HWI 6329-137

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

Amendment No. 2

This amendment modifies the following portion of the protocol:

Effective January 20, 1995

1. Page 2, Study Location. The replacement animal was treated and maintained at the main facility of Hazleton Wisconsin, Inc., 3301 Kinsman Boulevard, instead of at Hazleton Wisconsin's Acute Studies Laboratory, 3802 Packers Avenue, since the replacement animal was received and acclimated in this facility. Replace this section with the following:

Study Location (Initial Test)	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Location (Replacement Animal)	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704

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

PROTOCOL AMENDMENT APPROVAL

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

5-23-95
Date

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

4-17-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

4.17.95
Date

(6329-137.Am2.dsk3)

9.1.2 Analytical protocol AMDT-022195.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6053 in Rabbits

In-Vivo Study Reference Number: HWI#6329-137

Study Number: AMDT-022195.1
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 Fluorochemicals 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 11/16/95
James D. Johnson Date
Study Director

John Butenhoff, PhD Date
Sponsor Representative

1.0 PURPOSE

This study is designed to provide information as to whether FC-99 (T-6053) is dermally absorbed. The analytical aspect of this study is to determine fluorine-containing compounds (biotransformation products) in the tissue and serum of rabbits at various times post dose dermal application of FC-99. The data are to be used to make an assessment of the extent of dermal absorption of FC-99.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

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

2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

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

2.3 Number of Test and Control Samples: Tissues and fluids from 18 test animals and 6 control animals. One animal was replaced on day 2 (animal F53454 in the 250 mg/kg dose group was replaced with animal F53504). Tissues and fluids include liver, kidney, serum, cellular fraction, dermal application site and bile. Analysis of these tissues will be at the discretion of the Study Director.

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

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

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

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

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

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

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-137), 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. When sufficient data has been collected to meet the objectives of the study in the opinion of the Study Director, analysis will cease.

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-8-0, Analysis of Fluoride Using the Skalar Segmented Flow 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 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 Fluorochemicals 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

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

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1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

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

1.2 APPLICABLE COMPOUNDS: Fluoride.

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

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

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

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

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

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

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

9.0 PROCEDURES

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

9.2 Calibration And Measurement, Using Orion 3E Software:

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

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

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

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

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

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 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-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

000065

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 uL
- 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 um.
- 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 100 mLs Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
- 4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
- 4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
- 4.2.4 Ethyl Acetate
- 4.2.5 Methanol
- 4.2.6 Milli-Q water

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-perfluoroctanesulphonic acid to be added to each liver sample.

8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluoroctanesulphonic acid. Record the actual weight.

8.1.3 Bring it up to volume with 100 mLs of methanol, this is the stock standard.

8.1.4 Dilute 3 mLs of the stock standard to 250 mLs final volume with Milli-Q water. Calculate the actual value of the standard.

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

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 and record the actual weight.

8.2.3 Bring up to volume with dilute acetonitrile in a 100 mL volumetric flask.

8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approx. 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 resuspend 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.

Approximate Conc. ug FC-95/ g liver	Working Standard (Approximate Conc.)	uL
Blank	-	-
400 ppb	500 ppb	100
800 ppb	500 ppb	200
1.2 ppm	500 ppb	300
1.6 ppm	500 ppb	400
4 ppm	1 ppm	500
8 ppm	5 ppm	200
12 ppm	5 ppm	300

8.4 Calculate the actual value of the standards:

uL of standard x mg/L x 1L/1000000 uL = mg FC-95

$$\frac{\text{mg FC-95}}{\text{137 mg liver*/1 mL solution}} \times \frac{4}{5}\text{(organic layer recovery factor)} = \text{mg/mL FC-95 equivalents in liver}$$

mg/mL x 1000 = mg/L = ppm FC-95 in liver

*Average weight of bovine liver in solution. 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.

8.6 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 spectometer 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.
9.15 Cap the sample and vortex 20 to 30 seconds.
9.16 Put them in the shaker for 20 min.
9.17 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.18 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.
9.19 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.20 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.21 Vortex 15 seconds.
9.22 Transfer the sample to a 3 mL syringe. Attach a 0.2 nm 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..
9.23 Cap and hold for analysis by electrospray mass spectroscopy.
9.24 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 Quality Control
10.2 Precision and Accuracy
10.3 Other Validation Parameters

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

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

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

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

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

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

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

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 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 10/5/95
Group Leader Date

Gale Nelson Burkett 9-27-95
Quality Assurance Date

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

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 Dermal Absorption/Toxicity Study of T-6053 in Rabbits**

Study Number: AMDT-022195.1 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
10/14/95	11/03/95	Final Report	11/03/95	11/03/95

Kari Rambo 11-3-95

QAU Auditor Date

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-022195.1, HWI 6329-137
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group*

	µg		Std. Dev.
Control Group	**20.2**	**±**	**7.9**
10 mg/kg dose (T6053)**	**18.5**	**±**	**8.7**
125 mg/kg dose (T6053)	**16.5**	**±**	**4.0**
250 mg/kg dose (T6053)	**17.2**	**±**	**6.6**

***Calculated as the mean of triplicate samples from each of three male and three female rabbits.**
****One outlier not included in mean.**

FC99 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 (µg)	Dosage (mg/kg)
SPK 63-1	95%	0.940		0.1525			
SPK 63-2	96%	0.912		0.1593			
SPK 126-1	81%	1.755		0.1393			
SPK 126-2	82%	2.180		0.1136			
LIVER BLK 1		0.373		0.1216			
LIVER BLK 2		0.265		0.1145			
F53442-1		0.365		0.1460			
F53442-2		0.231	0.262	0.1132	90.68	23.75	0
F53442-3		0.189		0.1596			
F53464-1		0.194		0.1240			
F53464-2		0.170	0.175	0.1447	86.57	15.17	0
F53464-3		0.162		0.1223			
F53455-1		0.210		0.1375			
F53455-2		0.145	0.178	0.1558	93.31	16.57	0
F53455-3		0.177		0.1126			
liver blank-1		0.436		0.1099			
liver blank-2		0.200		0.1376			
liver spike-1	93%	1.231		0.1140			
liver spike-2	90%	0.960		0.1420			
liver spike-3	90%	0.950		0.1440			
F53441-1		0.399		0.1388			
F53441-2		0.271	0.301	0.1435	114.12	34.35	0
F53441-3		0.232		0.1343			
F53451-1		0.211		0.1398			
F53451-2		0.235	0.209	0.1152	88.41	18.47	0
F53451-3		0.181		0.1413			
F53461-1		0.193		0.1552			
F53461-2		0.165	0.175	0.1277	72.71	12.73	0
F53461-3		0.168		0.1316			
F53460-1		0.182		0.1230			
F53460-2		0.202	0.188	0.1370	90.52	17.03	10
F53460-3		0.181		0.1265			
liver blank-1		0.604		0.1361			
liver blank-2		0.277		0.1326			
liver spike-1	90%	1.013		0.1342			
liver spike-2	63%	0.875		0.1087			
liver spike-3	97%	1.450		0.1011			
liver spike-4	95%	1.397		0.1028			
liver blank-3		0.265		0.1518			
F53447-1		0.233		0.1007			
F53447-2		0.152	0.185	0.1376	79.96	14.80	10
F53447-3		0.170		0.1161			
F53452-1		0.143		0.1295			
F53452-2		0.152	0.147	0.1159	80.42	11.85	10
F53452-3		0.146		0.1309			
LIVER BLK 1		0.202		0.1580			
LIVER BLK 2		0.131		0.1622			
LIVER SPK 1	92%	1.128		0.1240			
LIVER SPK 2	100%	1.074		0.1402			

SPIKE DATA FOR 63 AND 126 PPM
FC95 COPIED FROM 6329136L
ENDING QC
DDW 4-5-95

000091

FC99 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 (µg)	Dosage (mg/kg)
F53463-1		0.696		0.1285			
F53463-2*		1.869	0.433	0.0152	81.40	35.28	10
F53463-3		0.170		0.1283			
F53444-1		0.629		0.1190			
F53444-2		0.068	0.287	0.1399	69.27	19.85	10
F53444-3		0.163		0.1348			
F53448-1		0.188		0.1252			
F53448-2		0.146	0.172	0.1199	72.33	12.47	10
F53448-3		0.184		0.1155			
F53446-1		0.187		0.1169			
F53446-2		0.140	0.170	0.1332	82.78	14.07	125
F53446-3		0.183		0.1164			
F53453-1		0.246		0.1196			
F53453-2		0.145	0.225	0.1479	94.51	21.30	125
F53453-3		0.285		0.1513			
F53458-1		0.503		0.1133			
F53458-2		0.160	0.284	0.1332	76.79	21.84	125
F53458-3		0.190		0.1001			
F53443-1		0.175		0.1461			
F53443-2		0.175	0.181	0.1238	76.45	13.87	125
F53443-3		0.194		0.1082			
F53450-1		0.164		0.1303			
F53450-2		0.171	0.163	0.1270	76.89	12.53	125
F53450-3		0.153		0.1072			
F53467-1		0.222		0.1365			
F53467-2		0.203	0.201	0.1087	77.56	15.61	125
F53467-3		0.178		0.1004			
F53439-1		0.119		0.1366			
F53439-2		0.139	0.216	0.1419	81.71	17.63	250
F53439-3		0.390		0.1328			
F54445-1		0.200		0.1320			
F54445-2		0.151	0.198	0.1261	95.81	18.97	250
F54445-3		0.242		0.1324			
F54459-1		0.140		0.1479			
F54459-2		0.150	0.144	0.1273	78.24	11.26	250
F54459-3		0.142		0.1177			
Liver Blank-2		0.432		0.1255			
Liver Blank-3		0.148		0.1328			
Liver Spike-1	83%	1.096		0.1143			
Liver Spike-2	81%	1.149		0.1063			
Liver Spike-3	90%	0.932		0.1466			
Liver Spike-4	96%	1.306		0.1110			
Liver blank-4		0.277		0.1079			
F53449-1		0.248		0.1284			
F53449-2		0.173	0.194	0.1257	70.80	13.76	250
F53449-3		0.163		0.1495			

* Outlier not included in average.

000092

FC99 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 (µg)	Dosage (mg/kg)	
F53504-1		0.138		0.1274				
F53504-2		0.141	0.148	0.1368	82.31	12.22	250	Replacement for F54454
F53504-3		0.166		0.1116				
F53456-1		0.188		0.1017				
F53456-2		0.730	0.379	0.1398	77.10	29.25	250	
F53456-3		0.220		0.1258				
F53454-1		0.126		0.1304				
F53454-2		0.111	0.151	0.1414	93.04	14.02	250	Replaced on Day 2 by F53504
F53454-3		0.215		0.1245				
Liver Blk-1		0.146		0.1149				
Liver Blk-2		0.140		0.1029				
Liver Spike-1	94%			0.1074				
Liver Spike-2	108%			0.1378				
Liver Spike-3	104%			0.1052				
Liver Spike-4	94%			0.1200				

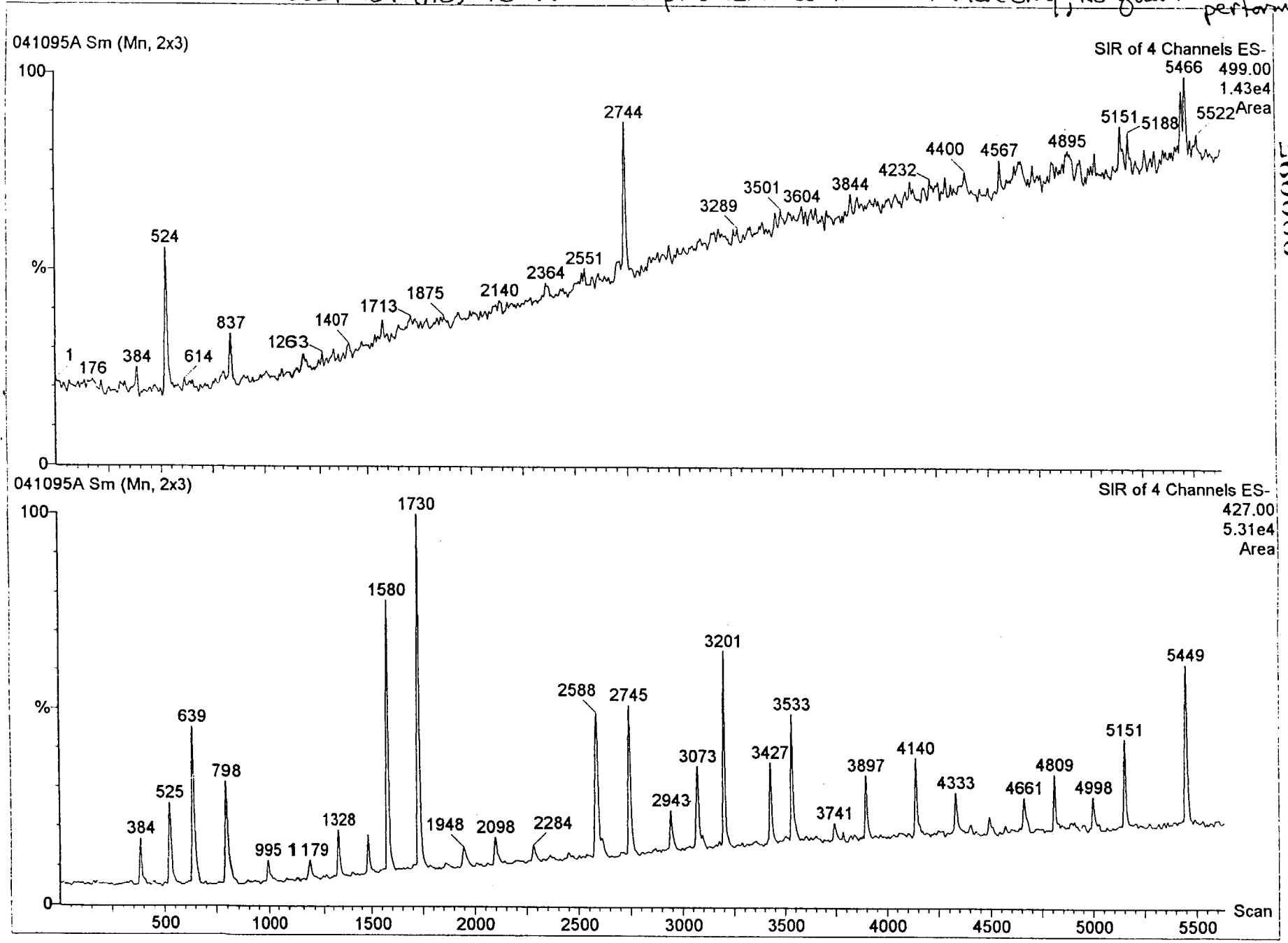
9.11.2 Raw data; analysis of liver extracts using electrospray mass spectrometry.

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.

D. Christensen 11/15/95

HWI # 6329-137 (AB) FC-99 Samples Screened for M⁻ 499 ion only, no quantitation performed.

A-1



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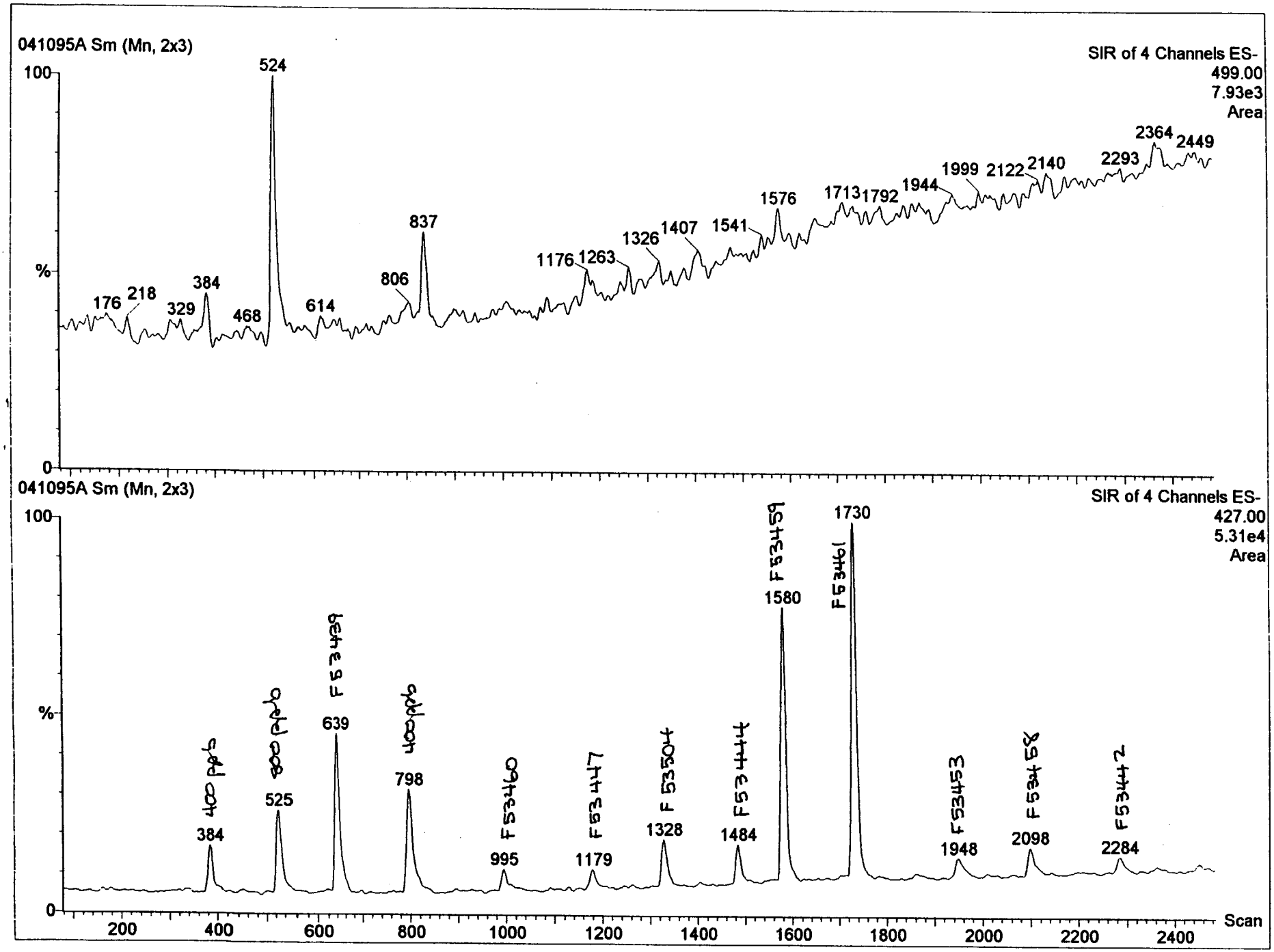
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384
468
614
806
1176
1263
1326
1407
1541
1576
1713
1792
1944
1999
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2140
2293
2364
2449
041095A Sm (Mn, 2x3)
SIR of 4 Channels ES-
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5.31e4
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100
%
0
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525
639
798
995
1179
1328
1484
1580
1730
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2200
2400
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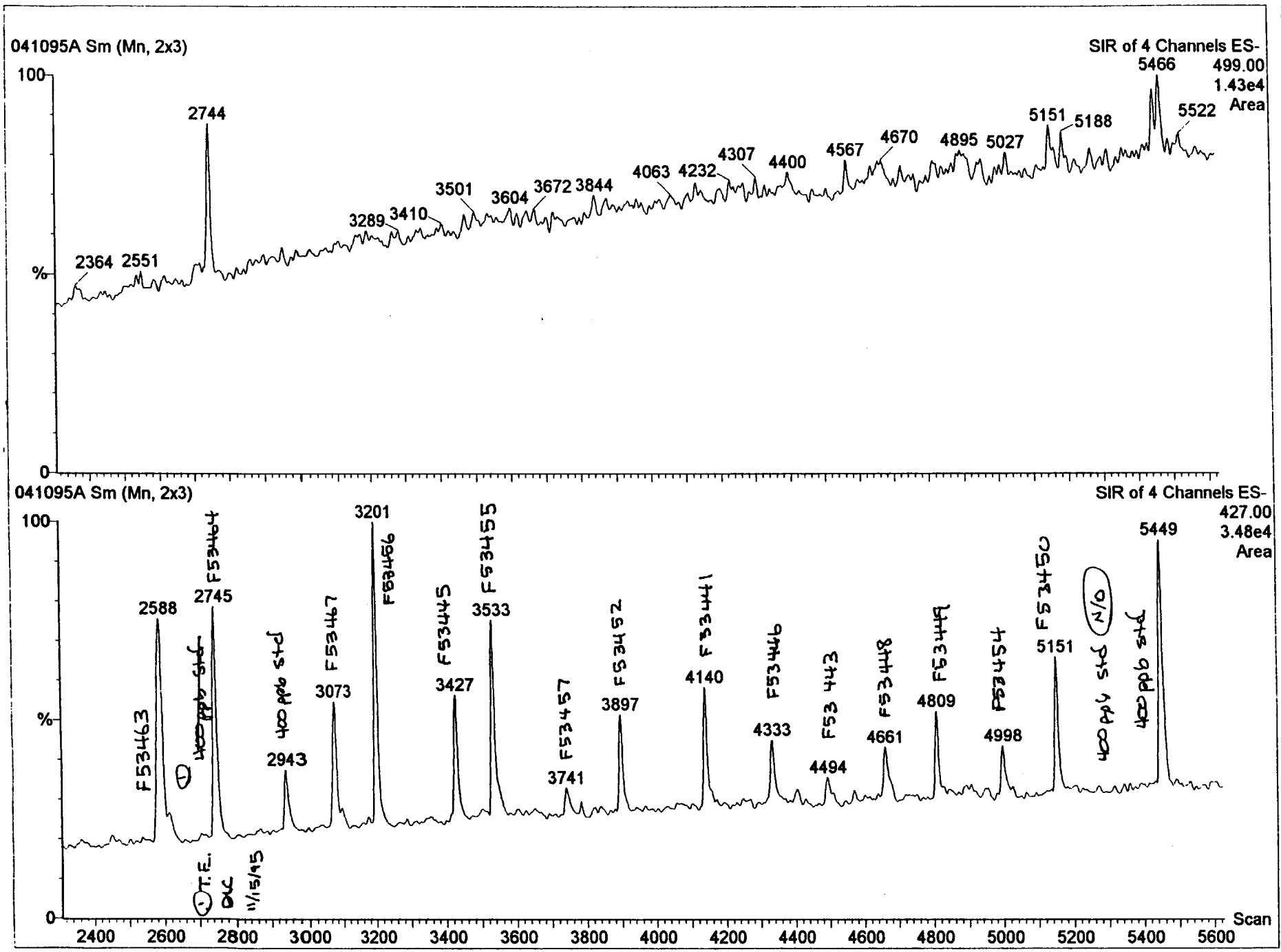
A-3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3604
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4063
4232
4307
4400
4567
4670
4895
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5151
5188
5466
5522
041095A Sm (Mn, 2x3)
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%
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F53463
2588
2745
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2943
F53467
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F53456
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F53455
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3741
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2800
3000
3200
3400
3600
3800
4000
4200
4400
4600
4800
5000
5200
5400
5600

000092

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.

DDW 4/20/95
Skalar Data

RE:6329-137 LIVER SAMPLES
AMDT 22195.2
Date of Analysis: 5-2-95
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 1 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

missed a page between 22 and 23 when numbering.
000093 DDW 11/22

Pg 1 of 34

SUMMARY of 6329-137
LIVER SAMPLES
AMDT 22195.2

	Sample ID	Skalar Result (ppm)	DI/TISAB final vol (mL)	Dry Samp (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)
	F53442-1	0.02	2.0	0.1460	0.33		90.6763	29.6	
	F53442-2	ND	2.0	0.1132	ND	ND	90.6763	ND	ND
	F53442-3	ND	2.0	0.1596	ND		90.6763	ND	
	F53464-1	ND	2.0	0.1240	ND		86.5739	ND	
	F53464-2	ND	2.0	0.1447	ND	ND	86.5739	ND	ND
	F53464-3	ND	2.0	0.1223	ND		86.5739	ND	
	F53455-1	ND	2.0	0.1375	ND		93.3090	ND	
	F53455-2	ND	2.0	0.1558	ND	ND	93.3090	ND	ND
GROUP 1	F53455-3	ND	2.0	0.1126	ND		93.3090	ND	
Dose Level : 0	F53441-1	0.03	2.0	0.1388	0.39		114.1164	45.1	
	F53441-2	0.02	2.0	0.1435	0.23	0.21	114.1164	26.1	23.7
	F53441-3	ND	2.0	0.1343	ND		114.1164	ND	
	F53451-1	ND	2.0	0.1398	ND		88.4088	ND	
	F53451-2	ND	2.0	0.1152	ND	ND	88.4088	ND	ND
	F53451-3	ND	2.0	0.1413	ND		88.4088	ND	
	F53461-1	ND	2.0	0.1552	ND		72.7104	ND	
	F53461-2	ND	2.0	0.1277	ND	ND	72.7104	ND	ND
	F53461-3	ND	2.0	0.1316	ND		72.7104	ND	
	F53460-1	ND	2.0	0.1230	ND		90.5154	ND	
	F53460-2	ND	2.0	0.1370	ND	ND	90.5154	ND	ND
	F53460-3	ND	2.0	0.1265	ND		90.5154	ND	
	F53447-1	ND	2.0	0.1007	ND		79.9589	ND	
	F53447-2	ND	2.0	0.1376	ND	ND	79.9589	ND	ND
	F53447-3	ND	2.0	0.1161	ND		79.9589	ND	
	F53452-1	ND	2.0	0.1295	ND		80.4220	ND	
	F53452-2	ND	2.0	0.1159	ND	ND	80.4220	ND	ND
GROUP 2	F53452-3	ND	2.0	0.1309	ND		80.4220	ND	
Dose Level : 10 mg/kg	F53463-1	0.06	2.0	0.1285	0.95		81.3971	77.7	
	F53463-2	ND	2.0	0.1516	ND	0.32	81.3971	ND	25.9
	F53463-3	ND	2.0	0.1283	ND		81.3971	ND	
	F53444-1	ND	2.0	0.1190	ND		69.2675	ND	
	F53444-2	ND	2.0	0.1399	ND	ND	69.2675	ND	ND
	F53444-3	ND	2.0	0.1348	ND		69.2675	ND	
	F53448-1	ND	2.0	0.1252	ND		72.3334	ND	
	F53448-2	ND	2.0	0.1199	ND	ND	72.3334	ND	ND
	F53448-3	ND	2.0	0.1155	ND		72.3334	ND	
	F53446-1	ND	2.0	0.1169	ND		82.7817	ND	
	F53446-2	ND	2.0	0.1332	ND	ND	82.7817	ND	ND
	F53446-3	0.02	2.0	0.1164	0.30		82.7817	24.5	
	F53453-1	0.02	2.0	0.1196	0.29		94.5134	27.2	
	F53453-2	0.02	2.0	0.1479	0.21	0.29	94.5134	20.2	27.4
	F53453-3	0.03	2.0	0.1513	0.37		94.5134	34.9	
	F53458-1	0.04	2.0	0.1133	0.75		76.7873	57.9	

SUMMARY of 6329-137
LIVER SAMPLES
AMDT 22195.2

	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Average Actual ppm F-	Total Tissue Wt. (grams)	Total F- per tissue (ug)	Average Total F- per tissue
GROUP 3	F53458-2	0.02	2.0	0.1332	0.26	0.34	76.7873	19.8	25.9
Dose Level : 125 mg/kg	F53458-3	ND	2.0	0.1001	ND		76.7873	ND	
	F53443-1	ND	2.0	0.1461	ND		76.4455	ND	
	F53443-2	ND	2.0	0.1238	ND	ND	76.4455	ND	ND
	F53443-3	ND	2.0	0.1082	ND		76.4455	ND	
	F53450-1	ND	2.0	0.1303	ND		76.8935	ND	
	F53450-2	ND	2.0	0.1270	ND	ND	76.8935	ND	ND
	F53450-3	ND	2.0	0.1072	ND		76.8935	ND	
	F53467-1	0.02	2.0	0.1365	0.23		77.5604	17.6	
	F53467-2	ND	2.0	0.1087	ND	ND	77.5604	ND	ND
	F53467-3	ND	2.0	0.1004	ND		77.5604	ND	
	F53439-1	ND	2.0	0.1366	ND		81.7065	ND	
	F53439-2	ND	2.0	0.1419	ND	ND	81.7065	ND	ND
	F53439-3	ND	2.0	0.1328	ND		81.7065	ND	
	F53445-1	ND	2.0	0.1320	ND		95.8121	ND	
	F53445-2	ND	2.0	0.1261	ND	ND	95.8121	ND	ND
	F53445-3	ND	2.0	0.1479	ND		95.8121	ND	
	F53459-1	ND	2.0	0.1479	ND		78.2415	ND	
	F53459-2	ND	2.0	0.1273	ND	ND	78.2415	ND	ND
	F53459-3	0.02	2.0	0.1177	0.39		78.2415	30.3	
GROUP 4	F53449-1	ND	2.0	0.1284	ND		70.7962	ND	
Dose Level : 250 mg/kg	F53449-2	ND	2.0	0.1257	ND	ND	70.7962	ND	ND
	F53449-3	ND	2.0	0.1495	ND		70.7962	ND	
	F53504-1	ND	2.0	0.1274	ND		82.3142	ND	
	F53504-2	ND	2.0	0.1368	ND	ND	82.3142	ND	ND
	F53504-3	ND	2.0	0.1116	ND		82.3142	ND	
	F53456-1	ND	2.0	0.1017	ND		77.1014	ND	
	F53456-2	0.06	2.0	0.1398	0.82	0.27	77.1014	62.9	21
	F53456-3	ND	2.0	0.1258	ND		77.1014	ND	
	F53454-1	ND	2.0	0.1304	ND		93.0439	ND	
	F53454-2	ND	2.0	0.1414	ND	ND	93.0439	ND	ND
	F53454-3	ND	2.0	0.1245	ND		93.0439	ND	

1995-06-13 08:39 OutPut of : 950502A1
Operator : DDW
Date of the Analysis : 1995-05-02 08:48
Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950502A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	Skalar % Recovery	DI:TISAB final vol (mL)	Qty Sampl (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	1.46	97%										
2	Drift	1.50	1.48	99%										
3	Wash													
4	Standard 1	0.015	0.01	97%										
5	Standard 2	0.03	0.03	108%										
6	Standard 3	0.06	0.06	95%										
7	Standard 4	0.09	0.09	101%										
8	Standard 5	0.12	0.12	101%										
9	Standard 6	0.15	0.15	103%										
10	Standard 7	0.30	0.29	95%										
11	Standard 8	0.60	0.61	102%										
12	Standard 9	1.20	1.23	103%										
13	Standard 10	1.50	1.47	98%										
14	Drift	1.50	1.51	100%										
15	Wash		0.00											
16	LIVER BLK 1		0.00		2.0	0.1216								
17	LIVER BLK 2		0.00		2.0	0.1145								
18	F53442-1		0.02		2.0	0.1460	0.33	90.6763	29.56					
19	F53442-2		0.00		2.0	0.1132	0.06	90.6763	5.13					
20	F53442-3		0.01		2.0	0.1596	0.12	90.6763	11.02					
21	F53464-1		0.00		2.0	0.1240	0.06	86.5739	5.17					
22	F53464-2		0.01		2.0	0.1447	0.13	86.5739	11.61					
23	F53464-3		0.00		2.0	0.1223	0.04	86.5739	3.82					
24	F53455-1		0.01		2.0	0.1375	0.10	93.3090	8.96					
25	F53455-2		0.01		2.0	0.1558	0.07	93.3090	6.71					
26	Drift	1.50	1.48	99%										
27	Wash		0.00											
28	F53455-3		0.01		2.0	0.1126	0.19	93.3090	18.07					
29	LIVER BLK 1		0.02		2.0	0.1099	0.41							
30	LIVER BLK 2		0.00		2.0	0.1376	0.05							
31	LIVER SPK 1		0.08		2.0	0.1140	1.40			0.004	63.00	0.15	0.16	105%

000102

Skalar Summary
6329-137 Livers

AHbT 22195.2

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	Skalar % 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
32	LIVER SPK 2		0.08		2.0	0.1420	1.09			0.004	63.00	0.15	0.16	103%
33	F53441-1		0.03		2.0	0.1388	0.39	114.1164	45.05					
34	F53441-2		0.02		2.0	0.1435	0.23	114.1164	26.08					
35	F53441-3		0.01		2.0	0.1343	0.19	114.1164	22.09					
36	F53451-1		0.01		2.0	0.1398	0.10	88.4088	8.85					
37	F53451-2		0.01		2.0	0.1152	0.14	88.4088	11.97					
38	Drift	1.50	1.50	100%	2.0									
39	Wash		0.00		2.0									
40	F53451-3		0.01		2.0	0.1413	0.15	88.4088	13.26					
41	F53461-1		0.01		2.0	0.1552	0.15	72.7104	10.68					
42	F53461-2		0.01		2.0	0.1277	0.10	72.7104	6.95					
43	F53461-3		0.01		2.0	0.1316	0.08	72.7104	5.64					
44	F53460-1		0.00		2.0	0.1230	0.07	90.5154	6.48					
45	F53460-2		0.01		2.0	0.1370	0.16	90.5154	14.67					
46	F53460-3		0.00		2.0	0.1265	0.05	90.5154	4.87					
47	F53447-1		0.00		2.0	0.1007	0.07	79.9589	5.88					
48	F53447-2		0.00		2.0	0.1376	0.07	79.9589	5.35					
49	F53447-3		0.01		2.0	0.1161	0.10	79.9589	7.99					
50	Drift	1.50	1.52	101%										
51	Wash		0.00											
52	F53452-1		0.01		2.0	0.1295	0.12	80.4220	9.69					
53	F53452-2		0.00		2.0	0.1159	0.05	80.4220	4.16					
54	F53452-3		0.00		2.0	0.1309	0.06	80.4220	4.79					
55	BLK 1		0.01		2.0	0.1580	0.10							
56	BLK 2		0.00		2.0	0.1622	0.03							
57	SPK 1		0.08		2.0	0.1240	1.35			0.004	63.00	0.15	0.17	110%
58	SPK 2		0.09		2.0	0.1402	1.27			0.004	63.00	0.15	0.18	117%
59	F53463-1		0.06		2.0	0.1285	0.95	81.3971	77.66					
60	F53463-2		0.01		2.0	0.1516	0.15	81.3971	12.46					
61	F53463-3		0.01		2.0	0.1283	0.09	81.3971	7.36					
62	Drift	1.50	1.52	101%										
63	Wash		0.00											
64	F53444-1		0.04		2.0	0.1190	0.61	69.2675	42.26					
65	F53444-2		0.00		2.0	0.1399	0.00	69.2675	0.00					
66	F53444-3		0.01		2.0	0.1348	0.11	69.2675	7.71					
67	F53448-1		0.00		2.0	0.1252	0.05	72.3334	3.70					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	Skalar % Recovery	DI TISAB final vol (mL)	Qty Sampl (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
68	F53448-2		0.00		2.0	0.1199	0.00	72.3334	0.00					
69	F53448-3		0.00		2.0	0.1155	0.07	72.3334	4.88					
70	Drift	1.50	1.53	102%										
71	Wash		0.00											
72	RunOut Wash		0.00											

1995-06-13 09:41 **OutPut of : 950502B1**
Operator : DDW
Date of the Analysis : 1995-05-02 12:41
Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950502B1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	Skalar % Recovery	DI TISAB final vol (mL)	Qty Sampl (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	1.49	99%										
3	Wash		0.00											
4	Standard 1	0.015	0.01	95%										
5	Standard 2	0.03	0.03	108%										
6	Standard 3	0.06	0.06	96%										
7	Standard 4	0.09	0.09	100%										
8	Standard 5	0.12	0.12	102%										
9	Standard 6	0.15	0.15	103%										
10	Standard 7	0.30	0.29	95%										
11	Standard 8	0.60	0.61	102%										
12	Standard 9	1.20	1.24	103%										
13	Standard 10	1.50	1.47	98%										
14	Drift	1.50	1.51	101%										
15	Wash		0.00											
16	F53446-1		0.01		2.0	0.1169	0.22	82.7817	18.41					
17	F53446-2		0.01		2.0	0.1332	0.15	82.7817	12.43					
18	F53446-3		0.02		2.0	0.1164	0.30	82.7817	24.46					
19	F53453-1		0.02		2.0	0.1196	0.29	94.5134	27.18					
20	F53453-2		0.02		2.0	0.1479	0.21	94.5134	20.19					
21	F53453-3		0.03		2.0	0.1513	0.37	94.5134	34.86					
22	F53458-1		0.04		2.0	0.1133	0.75	76.7873	57.88					
23	F53458-2		0.02		2.0	0.1332	0.26	76.7873	19.83					
24	F53458-3		0.01		2.0	0.1001	0.11	76.7873	8.59					
25	F53443-1		0.01		2.0	0.1461	0.16	76.4455	12.24					
26	Drift	1.50	1.48	98%										
27	Wash		0.00											
28	F53443-2		0.02		2.0	0.1238	0.37	76.4455	28.16					
29	F53443-3		0.01		2.0	0.1082	0.15	76.4455	11.16					
30	F53450-1		0.02		2.0	0.1303	0.33	76.8935	25.14					

000105

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	Skalar % Recovery	DI TISAB final vol (mL)	Qty Sampl (mls 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
31	F53450-2		0.01		2.0	0.1270	0.16	76.8935	12.11					
32	F53450-3		0.00		2.0	0.1072	0.09	76.8935	6.74					
33	F53467-1		0.02		2.0	0.1365	0.23	77.5604	17.61					
34	F53467-2		0.00		2.0	0.1087	0.08	77.5604	6.14					
35	F53467-3		0.00		2.0	0.1004	0.09	77.5604	6.95					
36	F53439-1		0.00		2.0	0.1366	0.06	81.7065	5.14					
37	F53439-2		0.01		2.0	0.1419	0.13	81.7065	10.59					
38	Drift	1.50	1.52	101%										
39	Wash		0.00											
40	F53439-3		0.03		2.0	0.1328	0.49	81.7065	40.11					
41	F53445-1		0.01		2.0	0.1320	0.21	95.8121	19.74					
42	F53445-2		0.00		2.0	0.1261	0.07	95.8121	6.23					
43	F53445-3		0.01		2.0	0.1479	0.13	95.8121	12.44					
44	F53459-1		0.01		2.0	0.1479	0.15	78.2415	11.53					
45	F53459-2		0.00		2.0	0.1273	0.06	78.2415	4.55					
46	F53459-3		0.02		2.0	0.1177	0.39	78.2415	30.31					
47	BLK 2		0.02		2.0	0.1255	0.34							
48	BLK 3		0.00		2.0	0.1328	0.00							
49	SPK 1		0.06		2.0	0.1143	1.12			0.004	63.00	0.15	0.13	85%
50	Drift	1.50	1.48	99%										
51	Wash		0.00											
52	SPK 2		0.06		2.0	0.1063	1.13			0.004	63.00	0.15	0.12	80%
53	SPK 3		0.07		2.0	0.1466	0.94			0.004	63.00	0.15	0.14	91%
54	SPK 4		0.08		2.0	0.1110	1.42			0.004	63.00	0.15	0.16	104%
55	LIVER BLK		0.00		2.0	0.1079	0.03							
56	F53449-1		0.01		2.0	0.1284	0.12	70.7962	8.27					
57	F53449-2		0.00		2.0	0.1257	0.00	70.7962	0.00					
58	F53449-3		0.00		2.0	0.1495	0.06	70.7962	4.45					
59	F53504-1		0.00		2.0	0.1274	0.00	82.3142	0.00					
60	F53504-2		0.00		2.0	0.1368	0.00	82.3142	0.00					
61	F53504-3		0.00		2.0	0.1116	0.00	82.3142	0.00					
62	Drift	1.50	1.46	97%										
63	Wash		0.00											
64	F53456-1		0.00		2.0	0.1017	0.01	77.1014	1.06					
65	F53456-2		0.06		2.0	0.1398	0.82	77.1014	62.87					
66	F53456-3		0.01		2.0	0.1258	0.12	77.1014	9.44					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	Skalar % Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Acrual 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
67	F53454-1		0.00		2.0	0.1304	0.00	93.0439	0.00					
68	F53454-2		0.00		2.0	0.1414	0.00	93.0439	0.00					
69	F53454-3		0.01		2.0	0.1245	0.08	93.0439	7.77					
70	BLK 1		0.00		2.0	0.1149	0.02							
71	BLK 2		0.00		2.0	0.1029	0.00							
72	SPK 1		0.08		2.0	0.1074	1.56			0.004	63.00	0.15	0.17	111%
73	SPK 2		0.10		2.0	0.1378	1.38			0.004	63.00	0.15	0.19	126%
74	Drift	1.50	1.49	100%										
75	Wash		0.00											
76	SPK 3		0.19		2.0	0.1052	3.52			0.004	126.00	0.30	0.37	122%
77	SPK 4		0.16		2.0	0.1200	2.73			0.004	126.00	0.30	0.33	108%
78	Drift	1.50	1.52	101%										
79	Wash		0.00											
wt	RunOut Wash		0.00											

DDW 6/20
AMDT 022195.
6329-137 Lw

1995-05-03 08:38 OutPut of : 950502A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-05-02 08:48

Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950502A1

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 = 0.00066
a0 = -1.20681

Fluoride L
Calibration order = 2

Correlation : r = 0.99926

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

a2 = 0.00000
a1 = 0.00024
a0 = -0.00641

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

000105

IC

1995-05-03 08:38 OutPut of : 950502A1

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    : ##.###
```

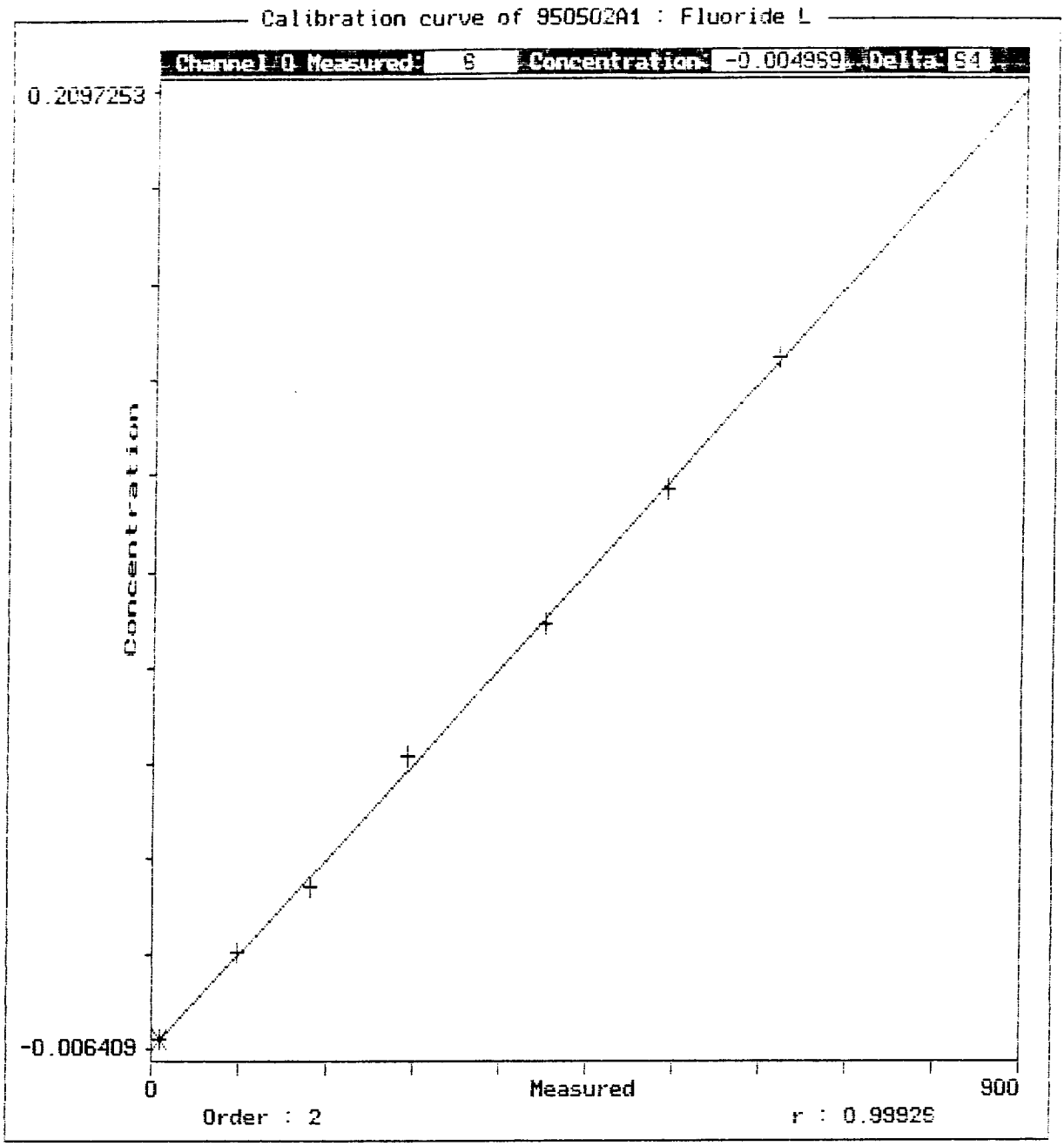
Fluoride L

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

```
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	Ch	Result	F	Time	Ch	Result	F	Time
wt	iw	Initial Wash	3	0.062		65	4	#.####		0
1	t	Tracer	3	1.458		211	4	0.7832		0
2	d	Drift	3	1.481		386	4	0.7926		0
3	w	Wash	3	0.062		626	4	#.####		0
4	s1	Standard 1	3	0.071		738	4	0.0145		0
5	s2	Standard 2	3	0.079		910	4	0.0325		0
6	s3	Standard 3	3	0.091		1084	4	0.0567		0
7	s4	Standard 4	3	0.112		1261	4	0.0908		0
8	s5	Standard 5	3	0.133		1437	4	0.1213		0
9	s6	Standard 6	3	0.154		1611	4	0.1492		0
10	s7	Standard 7	3	0.285		1787	4	0.2708		0
11	s8	Standard 8	3	0.611		1961	4	0.4552		0
12	s9	Standard 9	3	1.234		2135	4	0.6976		0
13	s10	Standard 10	3	1.465		2311	4	0.7863		0
14	d	Drift	3	1.505		2485	4	0.8025		0
15	w	Wash	3	0.062		2721	4	#.####		0
16	u	LIVER BLK 1	3	0.061	A	2835	4	#.####		0
17	u	LIVER BLK 2	3	0.060		3018	4	#.####		0
18	u	F53442-1	3	0.075		3187	4	0.0238		0
19	u	F53442-2	3	0.066		3360	4	0.0032		0
20	u	F53442-3	3	0.069		3536	4	0.0097		0
21	u	F53464-1	3	0.066		3708	4	0.0037		0
22	u	F53464-2	3	0.069		3879	4	0.0097		0
23	u	F53464-3	3	0.066		4063	4	0.0027		0
24	u	F53455-1	3	0.067		4237	4	0.0066		0
25	u	F53455-2	3	0.067		4411	4	0.0056		0
26	d	Drift	3	1.479		4587	4	0.7919		0
27	w	Wash	3	0.062		4812	4	#.####		0
28	u	F53455-3	3	0.069		4925	4	0.0109		0
29	u	LIVER BLK 1	3	0.074		5109	4	0.0224		0
30	u	LIVER BLK 2	3	0.066		5291	4	0.0034		0
31	u	LIVER SPK 1	3	0.105		5463	4	0.0798		0
32	u	LIVER SPK 2	3	0.103		5641	4	0.0776		0
33	u	F53441-1	3	0.077		5815	4	0.0274		0
34	u	F53441-2	3	0.072		5987	4	0.0164		0
35	u	F53441-3	3	0.070		6167	4	0.0130		0
36	u	F53451-1	3	0.068		6335	4	0.0070		0
37	u	F53451-2	3	0.068		6515	4	0.0078		0
38	d	Drift	3	1.502		6689	4	0.8013		0
39	w	Wash	3	0.062		6927	4	#.####		0
40	u	F53451-3	3	0.069		7029	4	0.0106		0
41	u	F53461-1	3	0.069		7214	4	0.0114		0
42	u	F53461-2	3	0.067		7386	4	0.0061		0
43	u	F53461-3	3	0.067		7563	4	0.0051		0
44	u	F53460-1	3	0.066		7740	4	0.0044		0
45	u	F53460-2	3	0.069		7908	4	0.0111		0
46	u	F53460-3	3	0.066		8088	4	0.0034		0
47	u	F53447-1	3	0.066		8262	4	0.0037		0
48	u	F53447-2	3	0.067		8440	4	0.0046		0
49	u	F53447-3	3	0.067		8616	4	0.0058		0
50	d	Drift	3	1.517		8790	4	0.8078		0
51	w	Wash	3	0.062		9029	4	#.####		0
52	u	F53452-1	3	0.068		9135	4	0.0078		0
53	u	F53452-2	3	0.066		9314	4	0.0030		0

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	F53452-3	3	0.066	9485	4	0.0039	0
55	u	BLK 1	3	0.068	9661	4	0.0078	0
56	u	BLK 2	3	0.066	9839	4	0.0025	0
57	u	SPK 1	3	0.107	10016	4	0.0834	0
58	u	SPK 2	3	0.110	10191	4	0.0887	0
59	u	F53463-1	3	0.094	10367	4	0.0613	0
60	u	F53463-2	3	0.070	10537	4	0.0116	0
61	u	F53463-3	3	0.067	10715	4	0.0058	0
62	d	Drift	3	1.519	10889	4	0.8085	0
63	w	Wash	3	0.062	11126	4	#.####	0
64	u	F53444-1	3	0.081	11239	4	0.0363	0
65	u	F53444-2	3	0.062	11425	4	#.####	0
66	u	F53444-3	3	0.068	11593	4	0.0075	0
67	u	F53448-1	3	0.066	11765	4	0.0032	0
68	u	F53448-2	3	0.064	11941	4	#.####	0
69	u	F53448-3	3	0.066	12118	4	0.0039	0
70	d	Drift	3	1.531	12291	4	0.8135	0
71	w	Wash	3	0.062	12529	4	#.####	0
wt	rw	RunOut Wash	3	0.062	12766	4	#.####	0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Channel 0 Measured: 0 Concentration: -0.004969 Delta: 94
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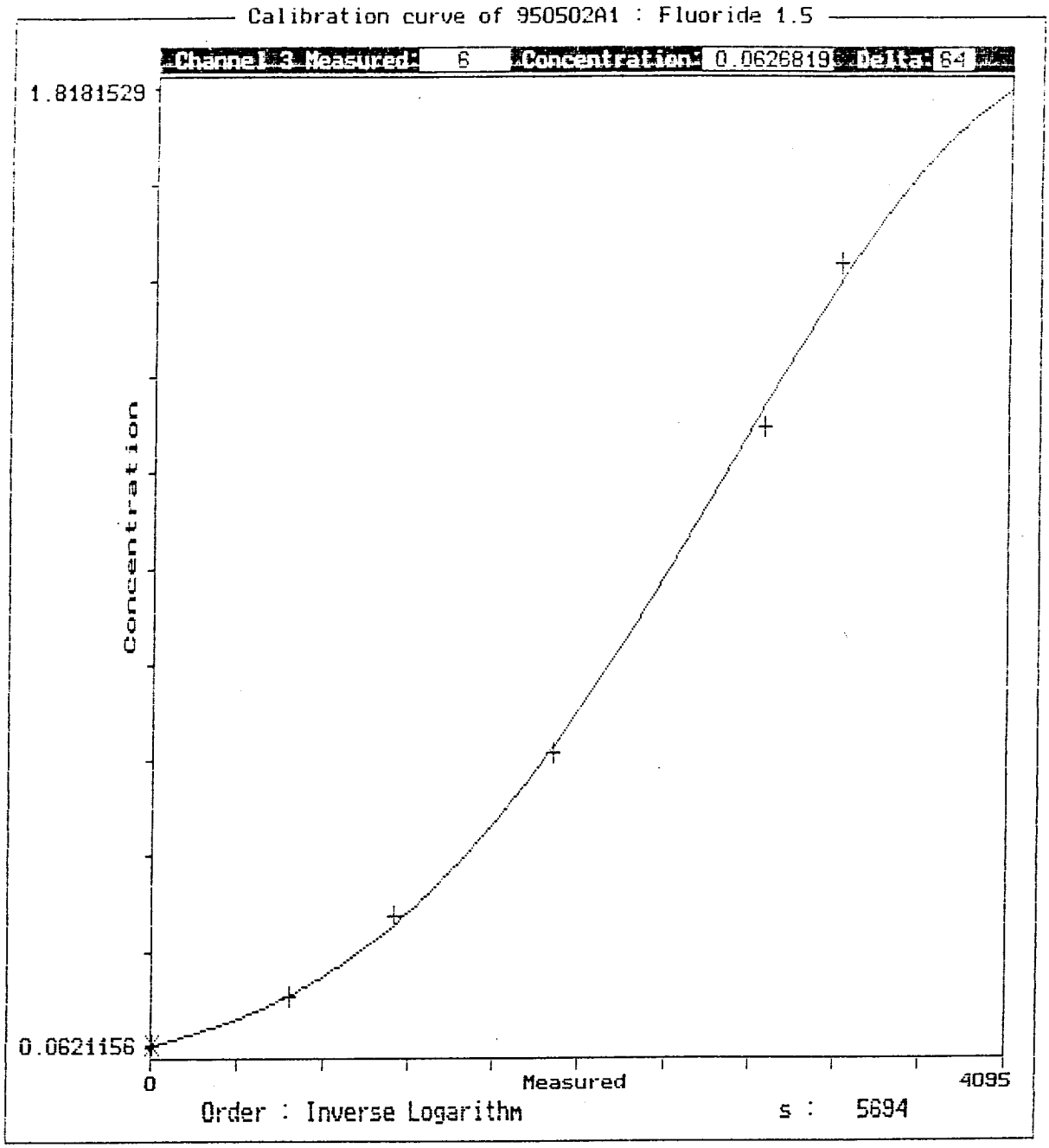
Concentration
0
Measured
900
Order : 2
r : 0.99929


Calibration curve of 950502A1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0626819 Delta: 64
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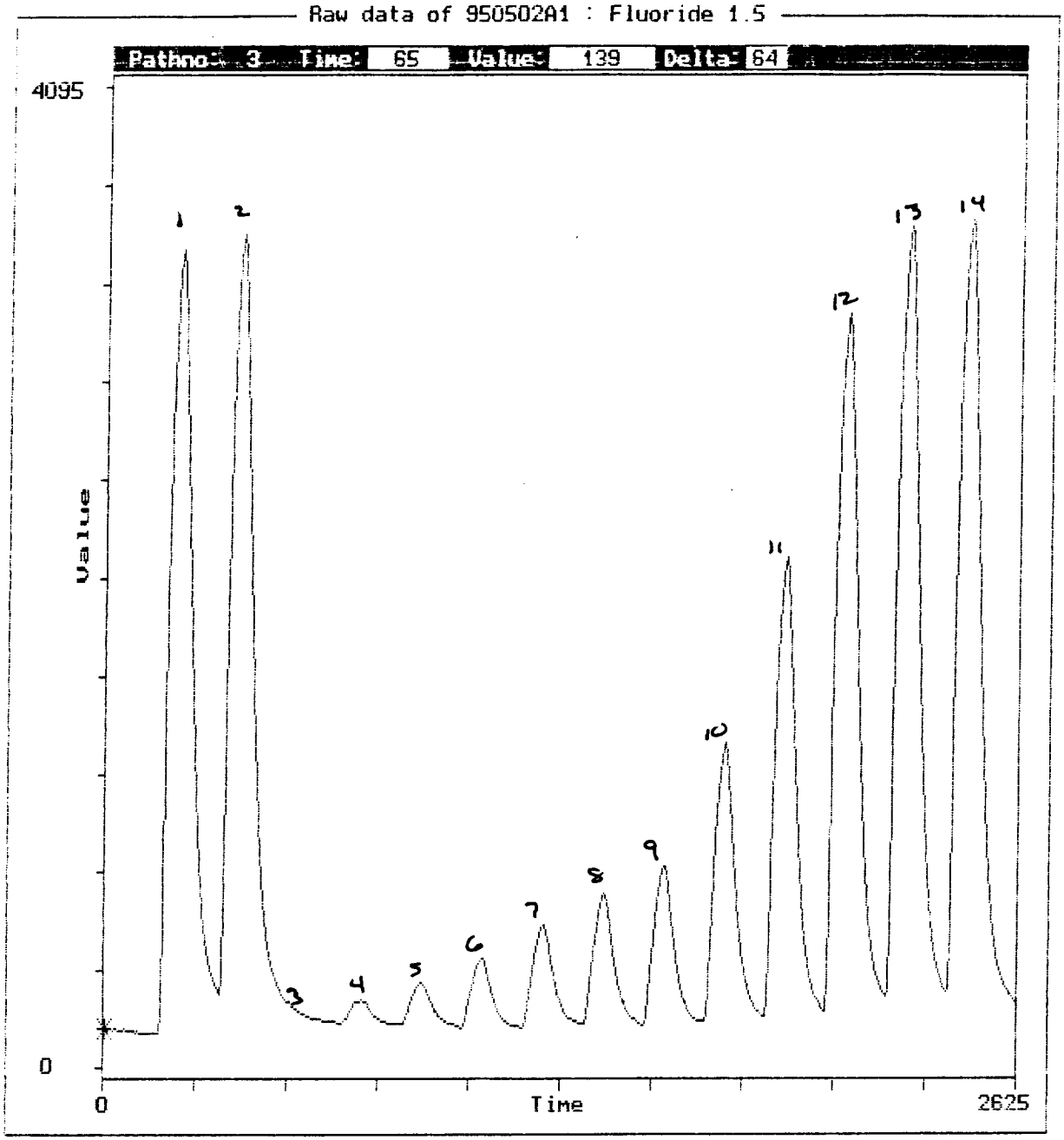
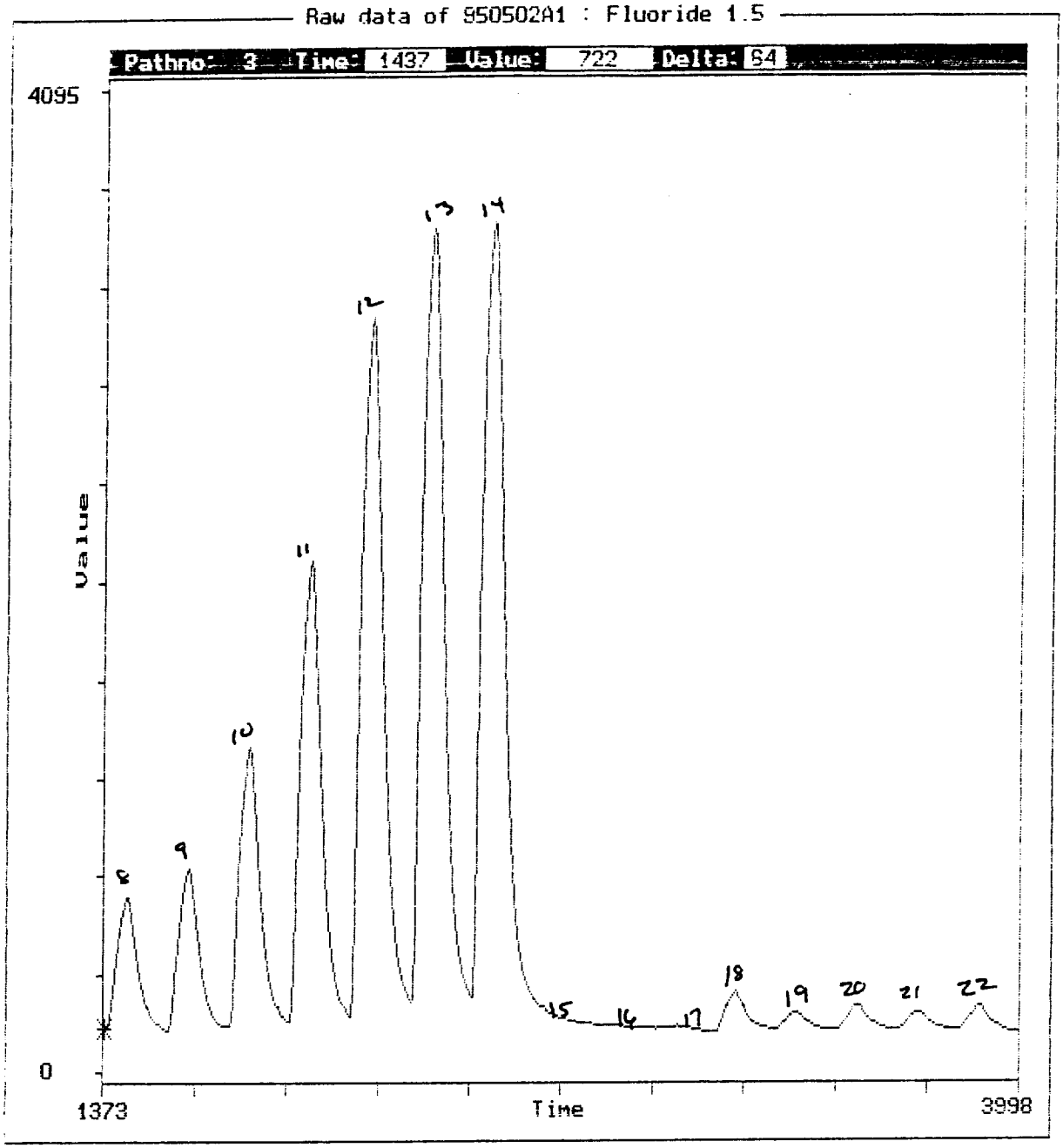
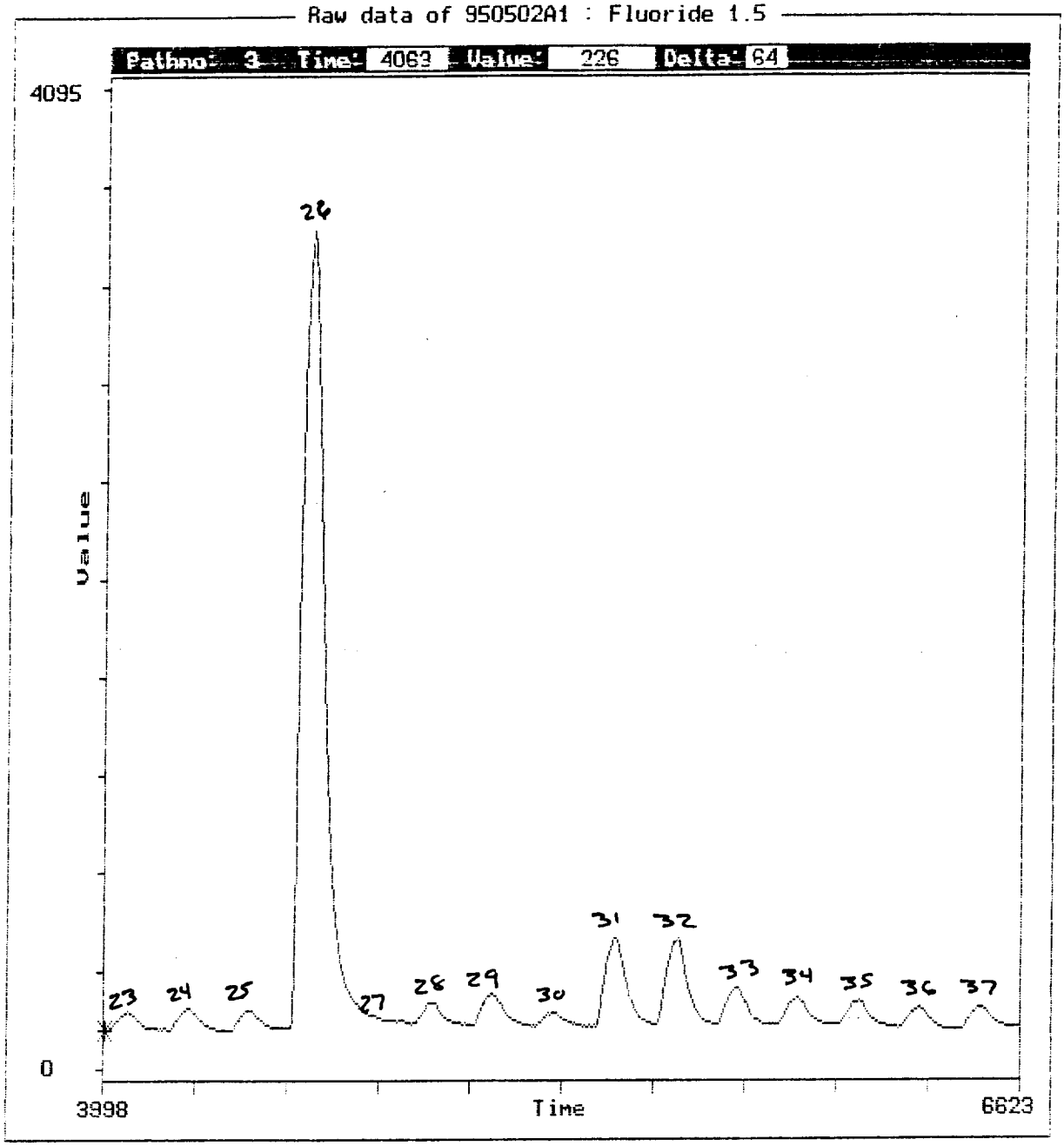
0.0621156
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 5694

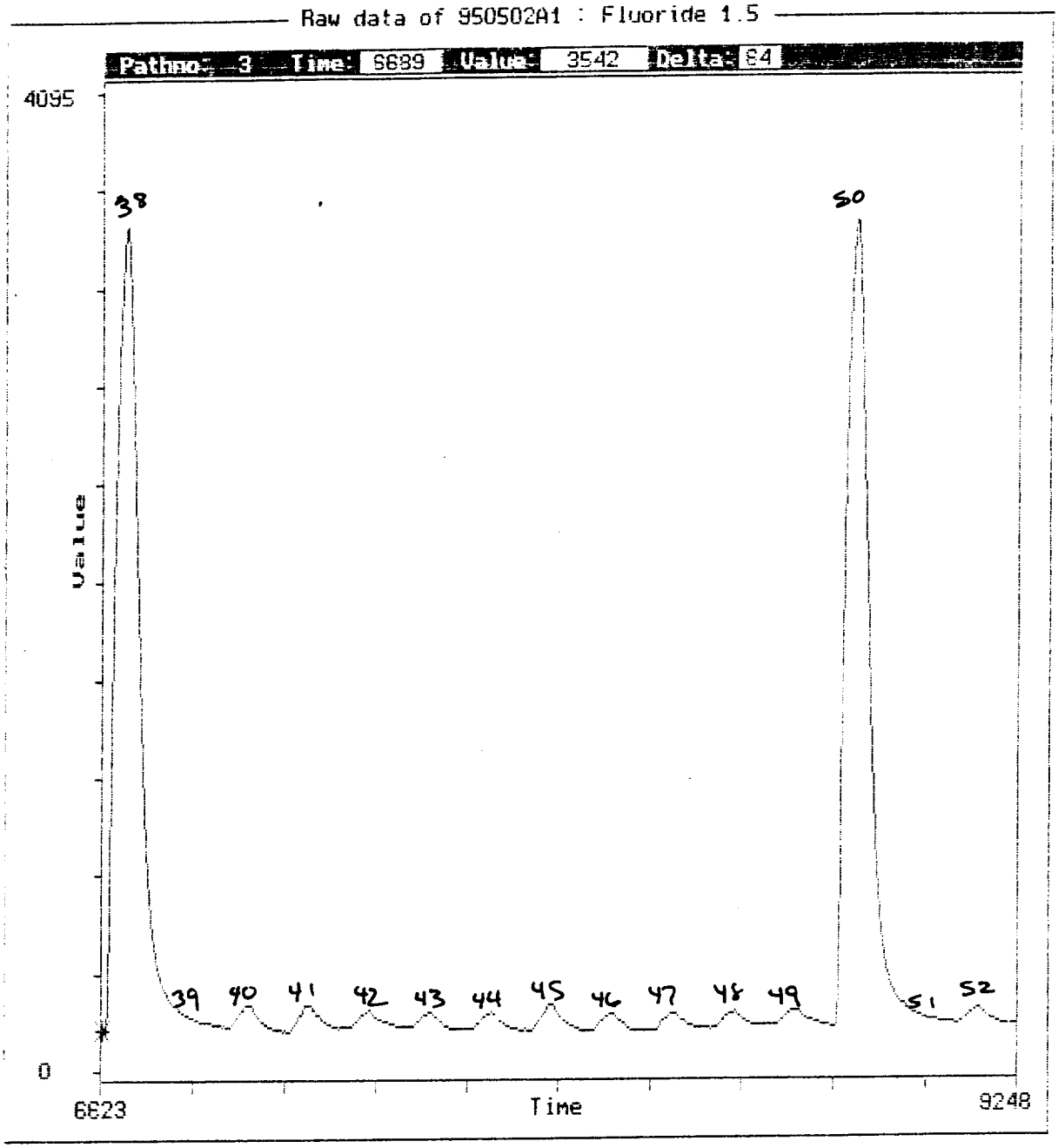


Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

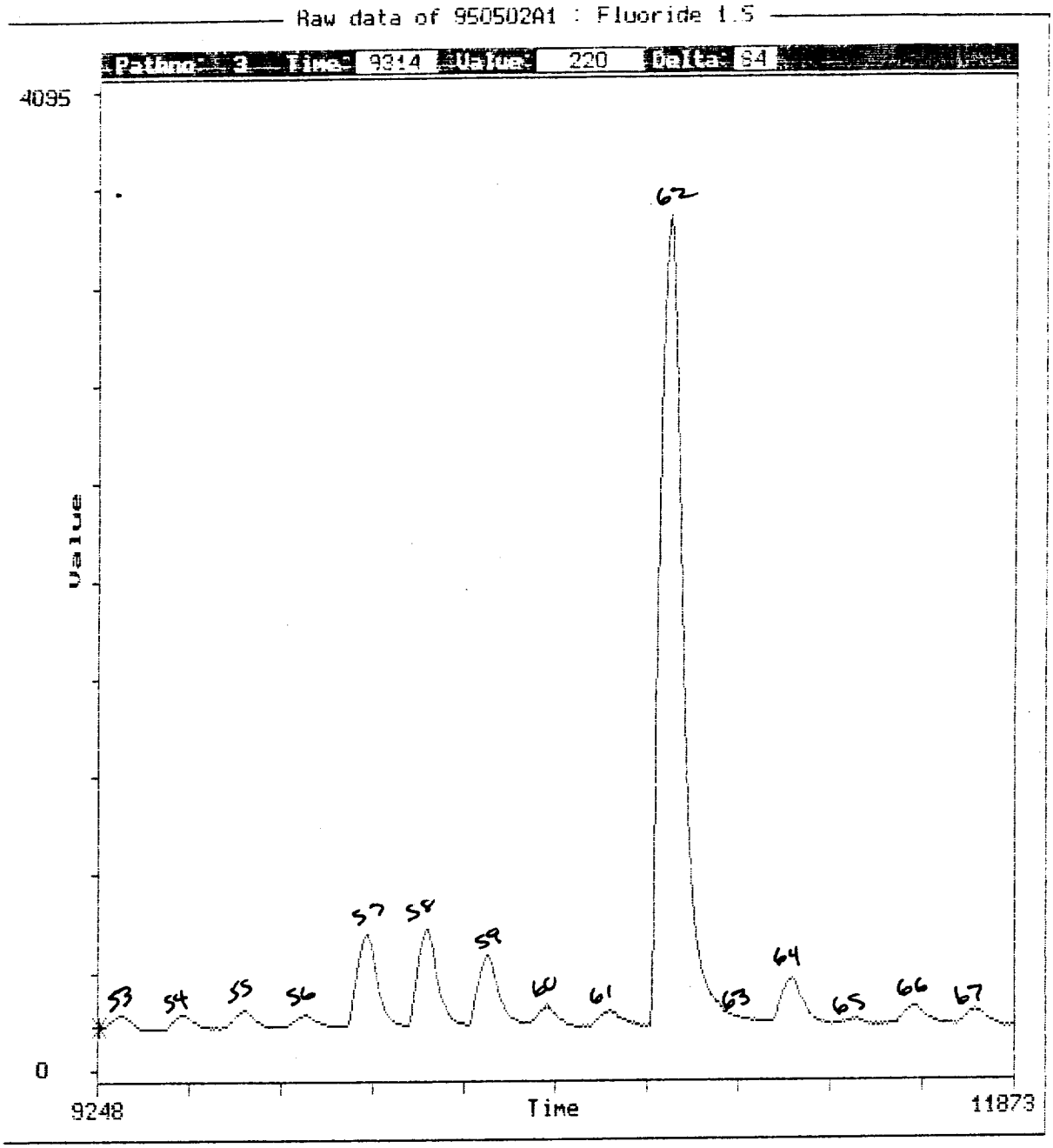

Raw data of 950502A1 : Fluoride 1.5
Pathno: 3 Time: 1437 Value: 722 Delta: 64
4095
0
Value
1373
Time
3998
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



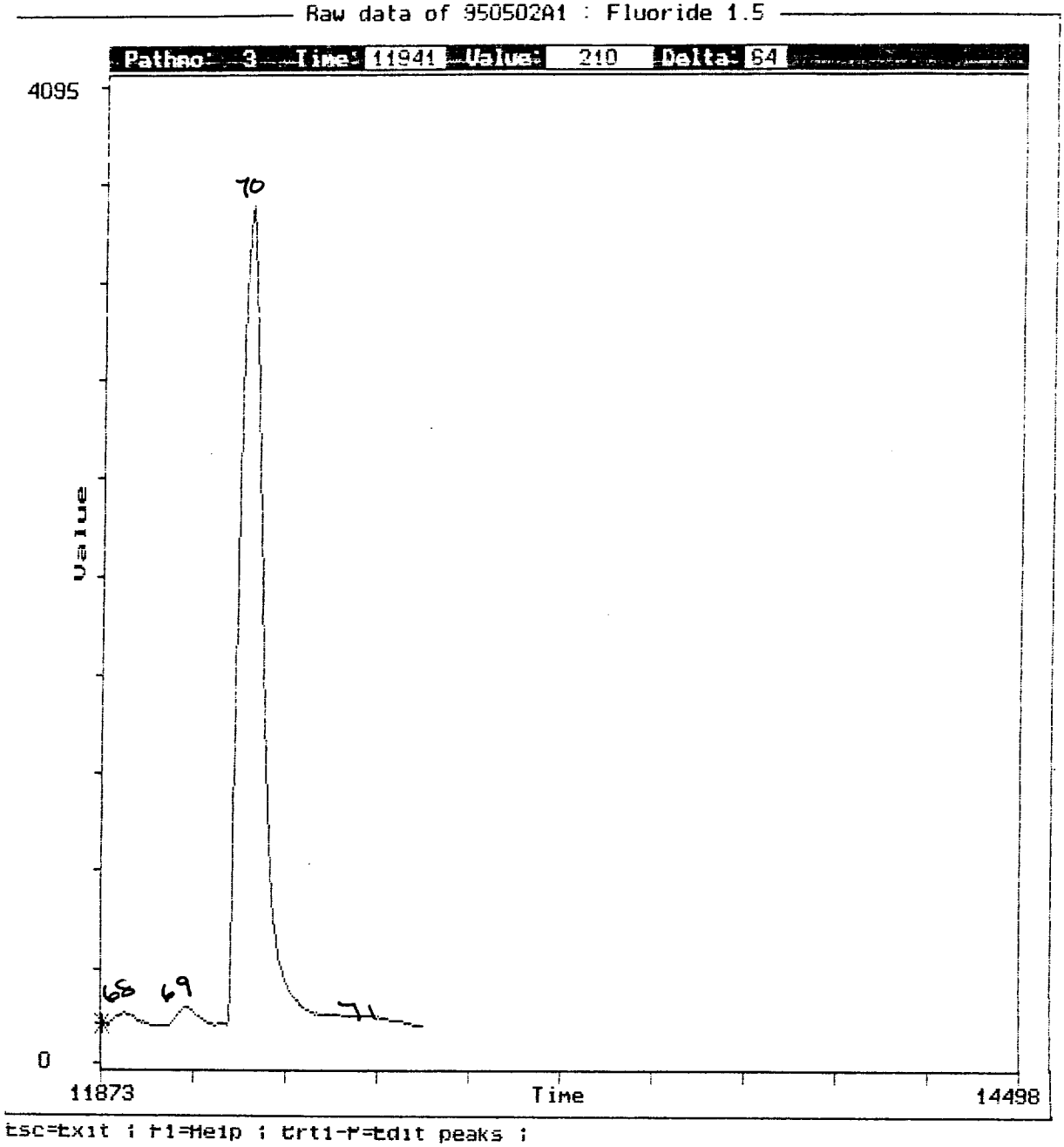
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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



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


Raw data of 950502A1 : Fluoride 1.5
Pathno: 3 Time: 11941 Value: 210 Delta: 64
4095
Value
0
11873
Time
14498
70
68
69
71
tsc=txit ; f1=Help ; trt1-f=tdit peaks ;

DDW 6/20/9
AMDT 022195
6329-137 Lu

1995-05-03 09:05 OutPut of : 950502B1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-05-02 12:41

Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950502B1

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 = 0.00068
a0 = -1.24604

Fluoride L
Calibration order = 2

Correlation : r = 0.99932

Result = a2 * x² + a1 * x + a0

a2 = 0.00000
a1 = 0.00021
a0 = -0.00305

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

000121

23

1995-05-03 09:05 OutPut of : 950502B1

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
```


Page missed

```
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	Ch	Result	F Time	Ch	Result	F Time
wt	iw	Initial Wash	3	0.057	65	4	#.####	0
1	t	Tracer	3	1.486	211	4	0.8506	0
2	d	Drift	3	1.491	387	4	0.8536	0
3	w	Wash	3	0.057	628	4	#.####	0
4	s1	Standard 1	3	0.064	737	4	0.0143	0
5	s2	Standard 2	3	0.073	913	4	0.0324	0
6	s3	Standard 3	3	0.087	1089	4	0.0576	0
7	s4	Standard 4	3	0.107	1264	4	0.0896	0
8	s5	Standard 5	3	0.131	1440	4	0.1221	0
9	s6	Standard 6	3	0.154	1613	4	0.1490	0
10	s7	Standard 7	3	0.286	1788	4	0.2673	0
11	s8	Standard 8	3	0.609	1964	4	0.4541	0
12	s9	Standard 9	3	1.235	2138	4	0.7300	0
13	s10	Standard 10	3	1.467	2312	4	0.8407	0
14	d	Drift	3	1.514	2488	4	0.8662	0
15	w	Wash	3	0.057	2723	4	#.####	0
16	u	F53446-1	3	0.064	2829	4	0.0130	0
17	u	F53446-2	3	0.062	3004	4	0.0100	0
18	u	F53446-3	3	0.066	3191	4	0.0172	0
19	u	F53453-1	3	0.066	3360	4	0.0172	0
20	u	F53453-2	3	0.065	3539	4	0.0158	0
21	u	F53453-3	3	0.071	3713	4	0.0279	0
22	u	F53458-1	3	0.079	3891	4	0.0427	0
23	u	F53458-2	3	0.066	4065	4	0.0172	0
24	u	F53458-3	3	0.060	4237	4	0.0056	0
25	u	F53443-1	3	0.063	4405	4	0.0117	0
26	d	Drift	3	1.476	4587	4	0.8457	0
27	w	Wash	3	0.057	4826	4	#.####	0
28	u	F53443-2	3	0.068	4941	4	0.0228	0
29	u	F53443-3	3	0.062	5113	4	0.0079	0
30	u	F53450-1	3	0.068	5289	4	0.0213	0
31	u	F53450-2	3	0.062	5463	4	0.0100	0
32	u	F53450-3	3	0.060	5636	4	0.0047	0
33	u	F53467-1	3	0.065	5817	4	0.0155	0
34	u	F53467-2	3	0.060	5987	4	0.0043	0
35	u	F53467-3	3	0.060	6162	4	0.0045	0
36	u	F53439-1	3	0.060	6337	4	0.0043	0
37	u	F53439-2	3	0.062	6516	4	0.0092	0
38	d	Drift	3	1.515	6688	4	0.8668	0
39	w	Wash	3	0.057	6927	4	#.####	0
40	u	F53439-3	3	0.073	7038	4	0.0326	0
41	u	F53445-1	3	0.064	7214	4	0.0136	0
42	u	F53445-2	3	0.060	7384	4	0.0041	0
43	u	F53445-3	3	0.062	7564	4	0.0096	0
44	u	F53459-1	3	0.063	7738	4	0.0109	0
45	u	F53459-2	3	0.060	7918	4	0.0037	0
46	u	F53459-3	3	0.068	8090	4	0.0228	0
47	u	BLK 2	3	0.068	8266	4	0.0215	0
48	u	BLK 3	3	0.056	8436	4	#.####	0
49	u	SPK 1	3	0.091	8616	4	0.0640	0
50	d	Drift	3	1.483	8790	4	0.8492	0
51	w	Wash	3	0.057	8930	4	#.####	0
52	u	SPK 2	3	0.089	9141	4	0.0603	0
53	u	SPK 3	3	0.094	9317	4	0.0688	0

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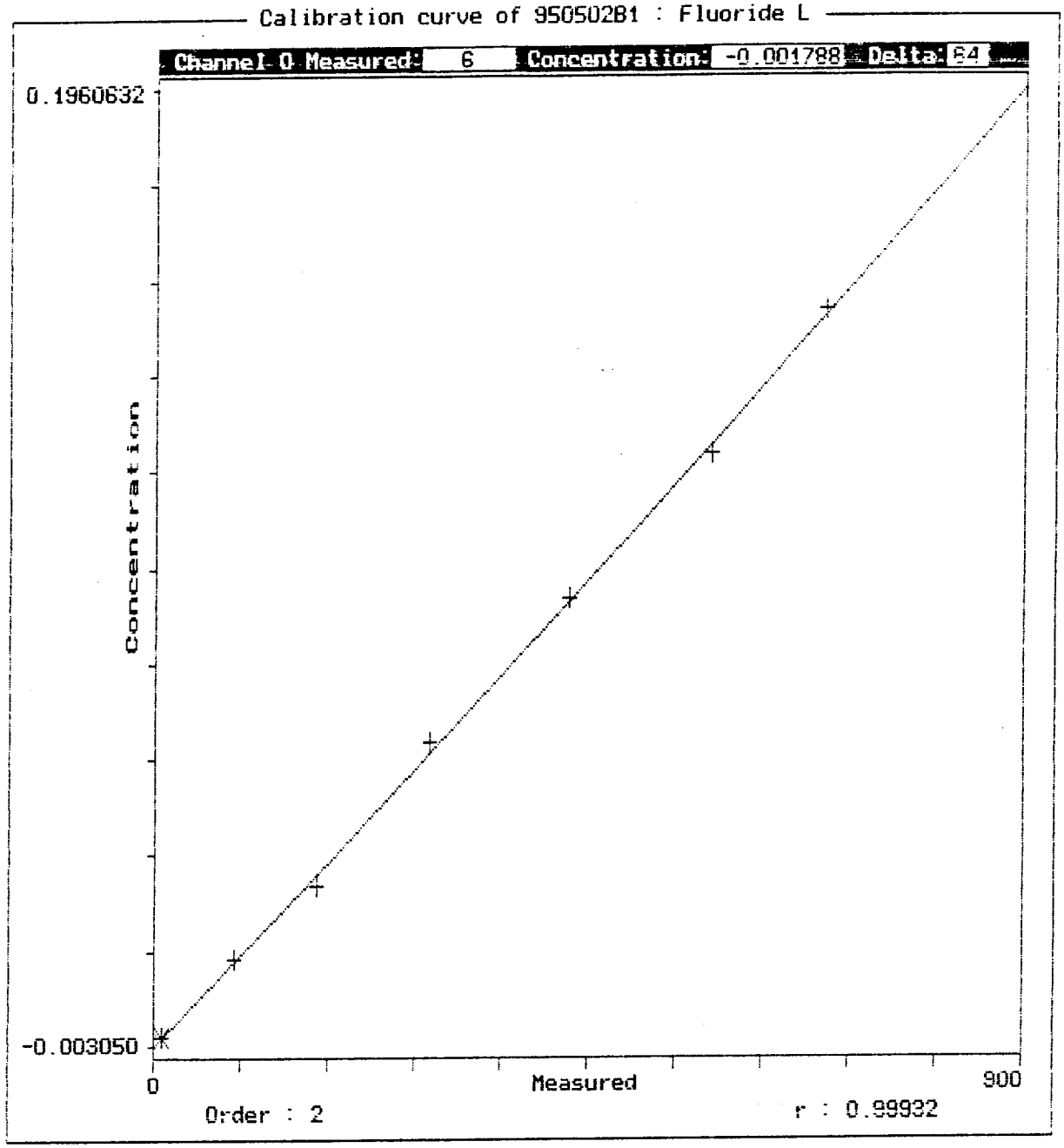
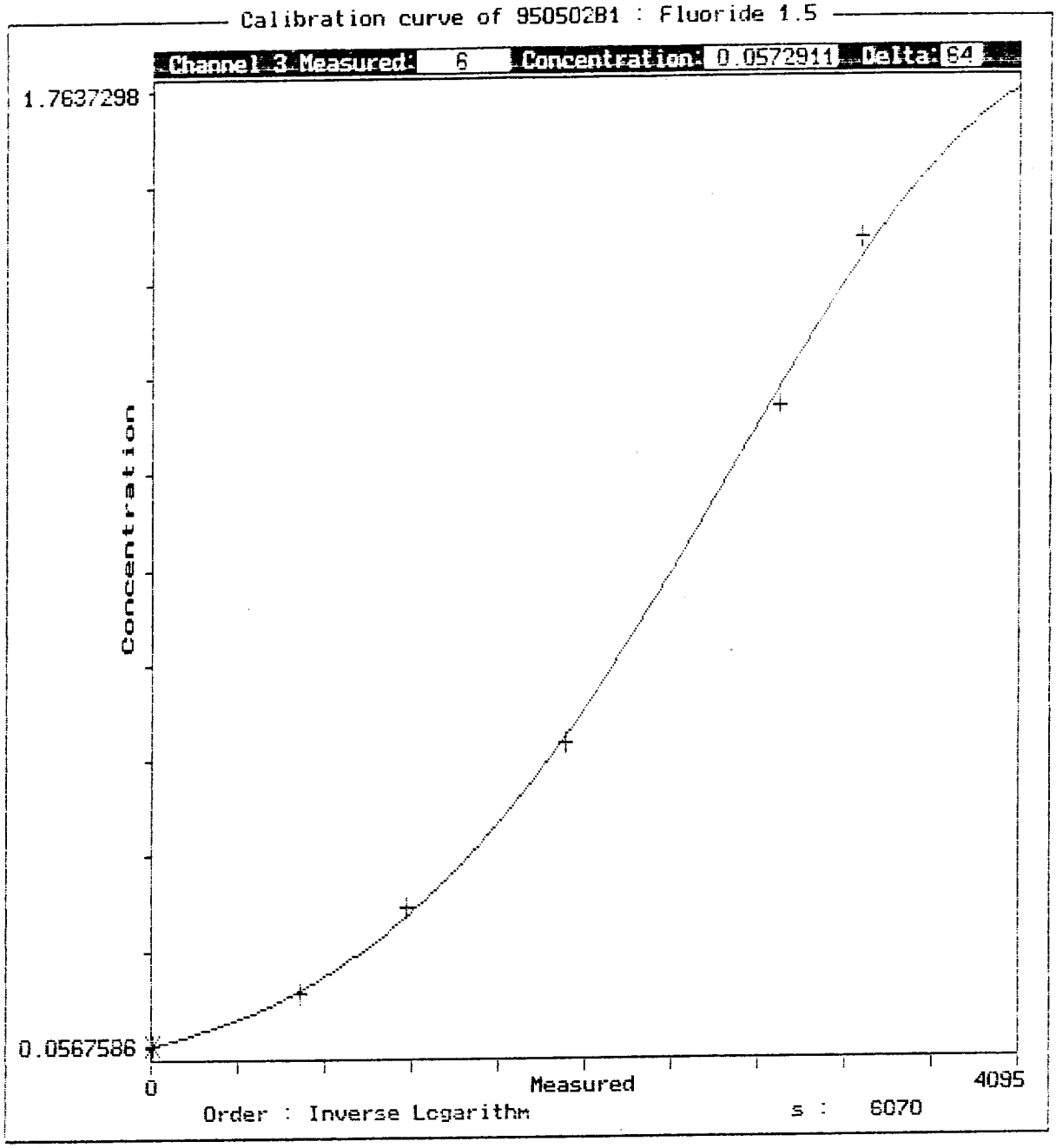
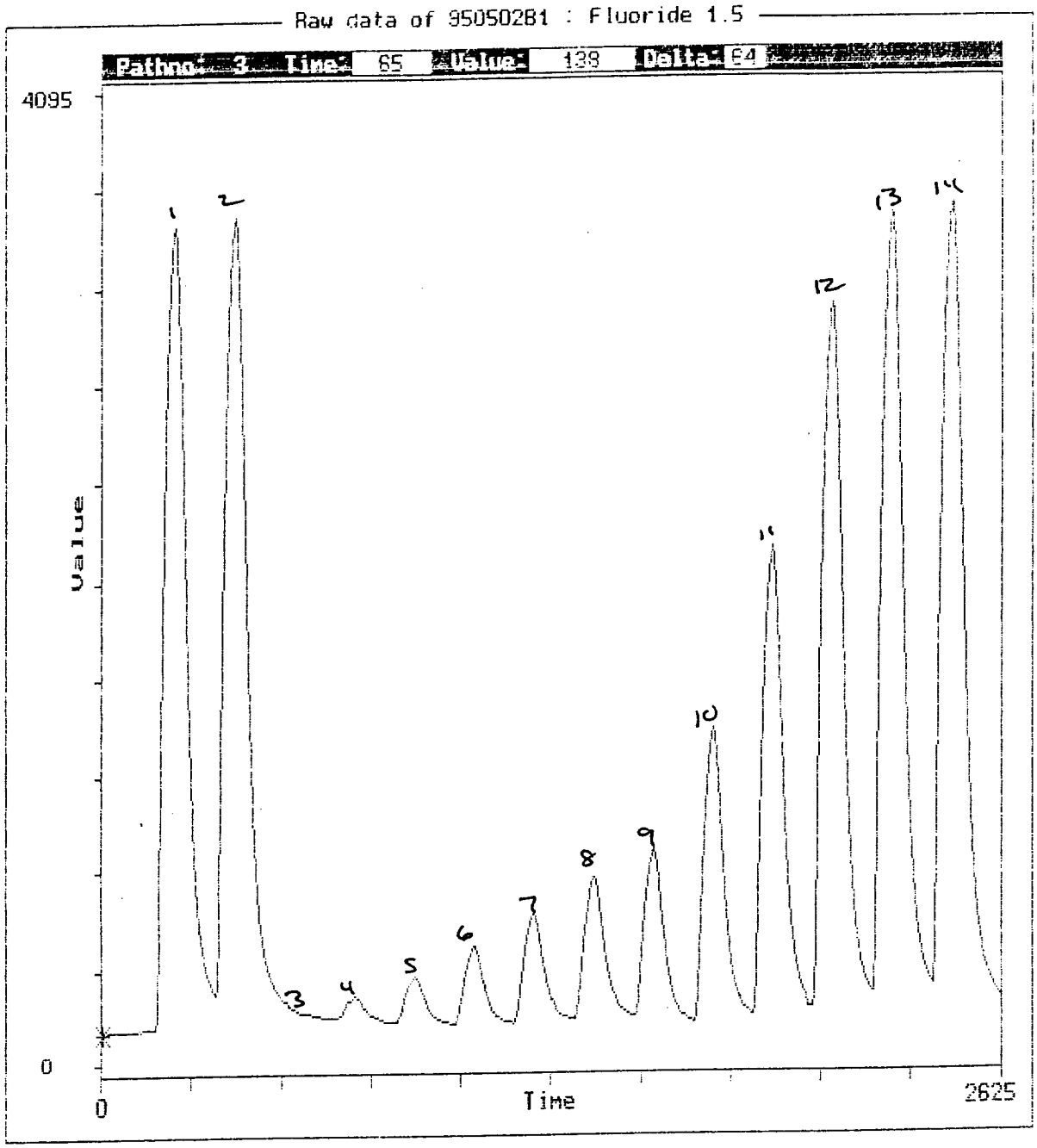
			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	SPK 4	3	0.100	9493	4	0.0786	0
55	u	LIVER BLK	3	0.059	9665	4	0.0018	0
56	u	F53449-1	3	0.061	9841	4	0.0075	0
57	u	F53449-2	3	0.056	10013	4	#.####	0
58	u	F53449-3	3	0.060	10189	4	0.0047	0
59	u	F53504-1	3	0.056	10361	4	#.####	0
60	u	F53504-2	3	0.054	10539	4	#.####	0
61	u	F53504-3	3	0.057	10711	4	#.####	0
62	d	Drift	3	1.459	10889	4	0.8366	0
63	w	Wash	3	0.057	11044	4	#.####	0
64	u	F53456-1	3	0.058	11237	4	0.0007	0
65	u	F53456-2	3	0.087	11413	4	0.0570	0
66	u	F53456-3	3	0.061	11593	4	0.0077	0
67	u	F53454-1	3	0.057	11763	4	#.####	0
68	u	F53454-2	3	0.057	11933	4	#.####	0
69	u	F53454-3	3	0.060	12108	4	0.0052	0
70	u	BLK 1	3	0.058	12288	4	0.0009	0
71	u	BLK 2	3	0.057	12463	4	#.####	0
72	u	SPK 1	3	0.103	12641	4	0.0839	0
73	u	SPK 2	3	0.111	12817	4	0.0954	0
74	d	Drift	3	1.494	12991	4	0.8550	0
75	w	Wash	3	0.057	13222	4	#.####	0
76	u	SPK 3	3	0.185	13345	4	0.1815	0
77	u	SPK 4	3	0.164	13517	4	0.1599	0
78	d	Drift	3	1.518	13691	4	0.8686	0
79	w	Wash	3	0.057	13890	4	#.####	0
wt	rw	RunOut Wash	3	0.057	14166	4	#.####	0

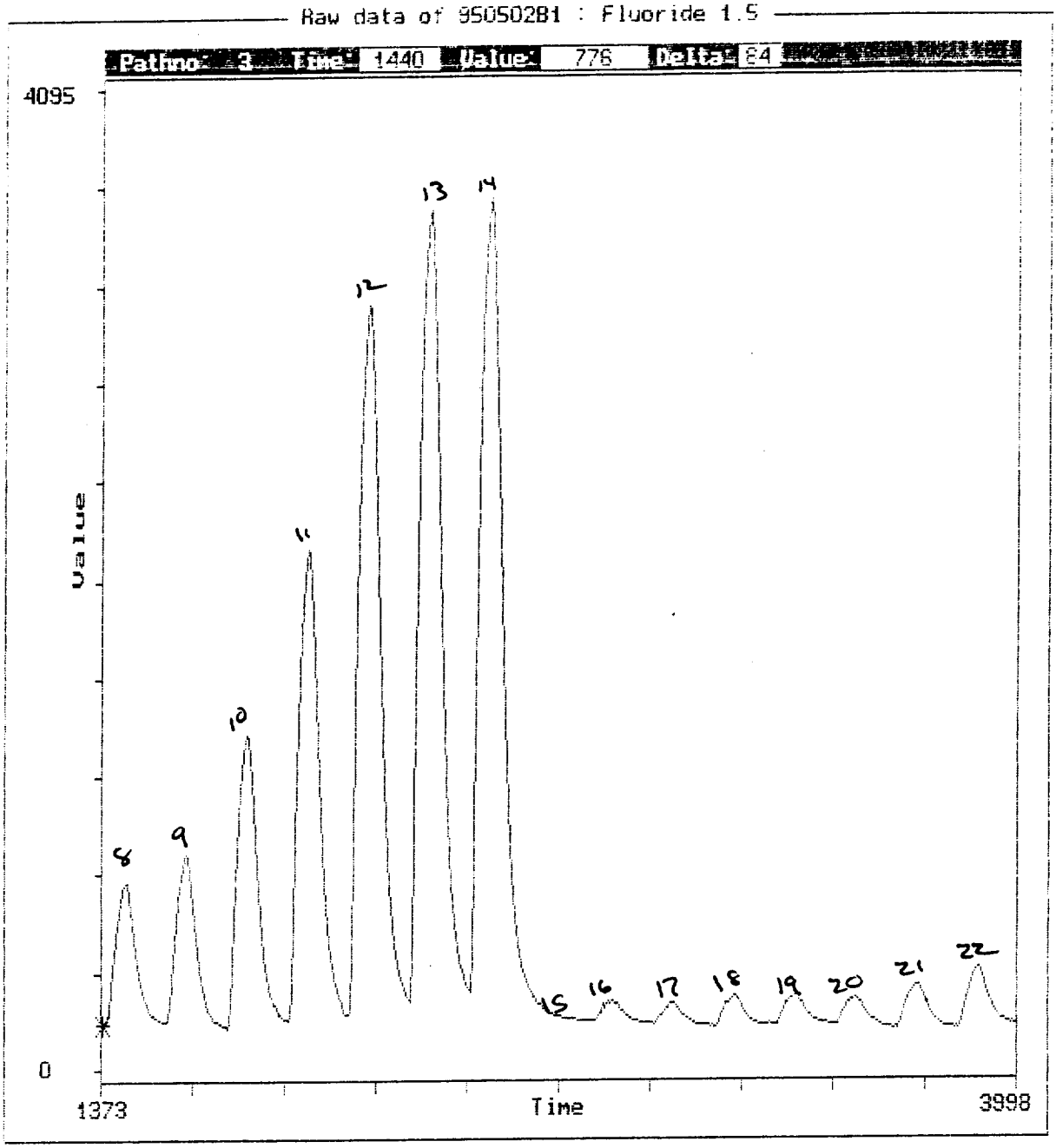
use high range result DDW 9/3/95


Calibration curve of 950502B1 : Fluoride L
Channel 0 Measured: 6
Concentration: -0.001788
Delta: 84
0.1960632
-0.003050
Concentration
0
Measured
900
Order : 2
r : 0.99932


Calibration curve of 950502B1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0572911 Delta: 64
1.7637298
Concentration
0.0567586
0
Measured
4095
Order : Inverse Logarithm
s : 6070



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



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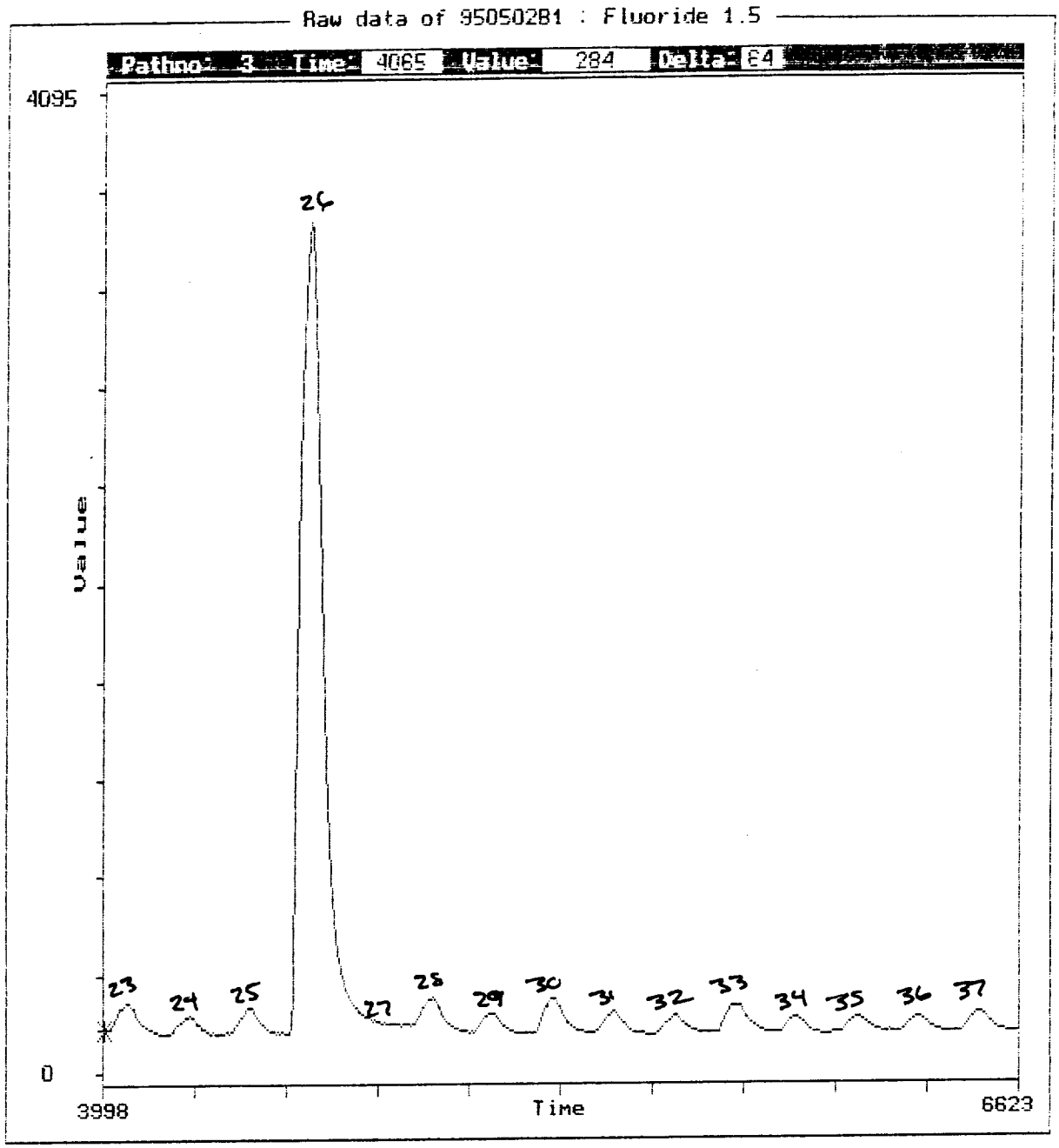
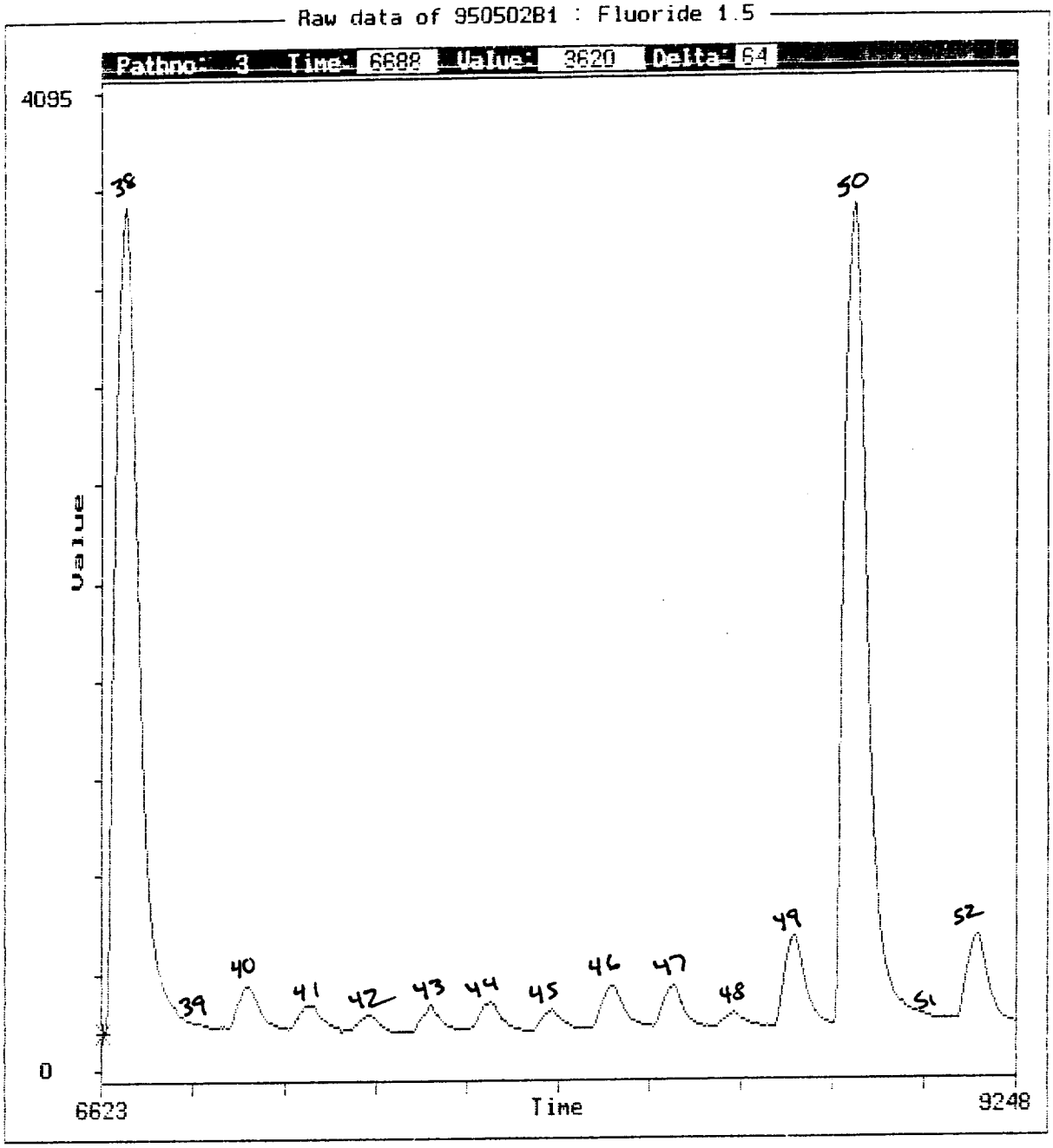
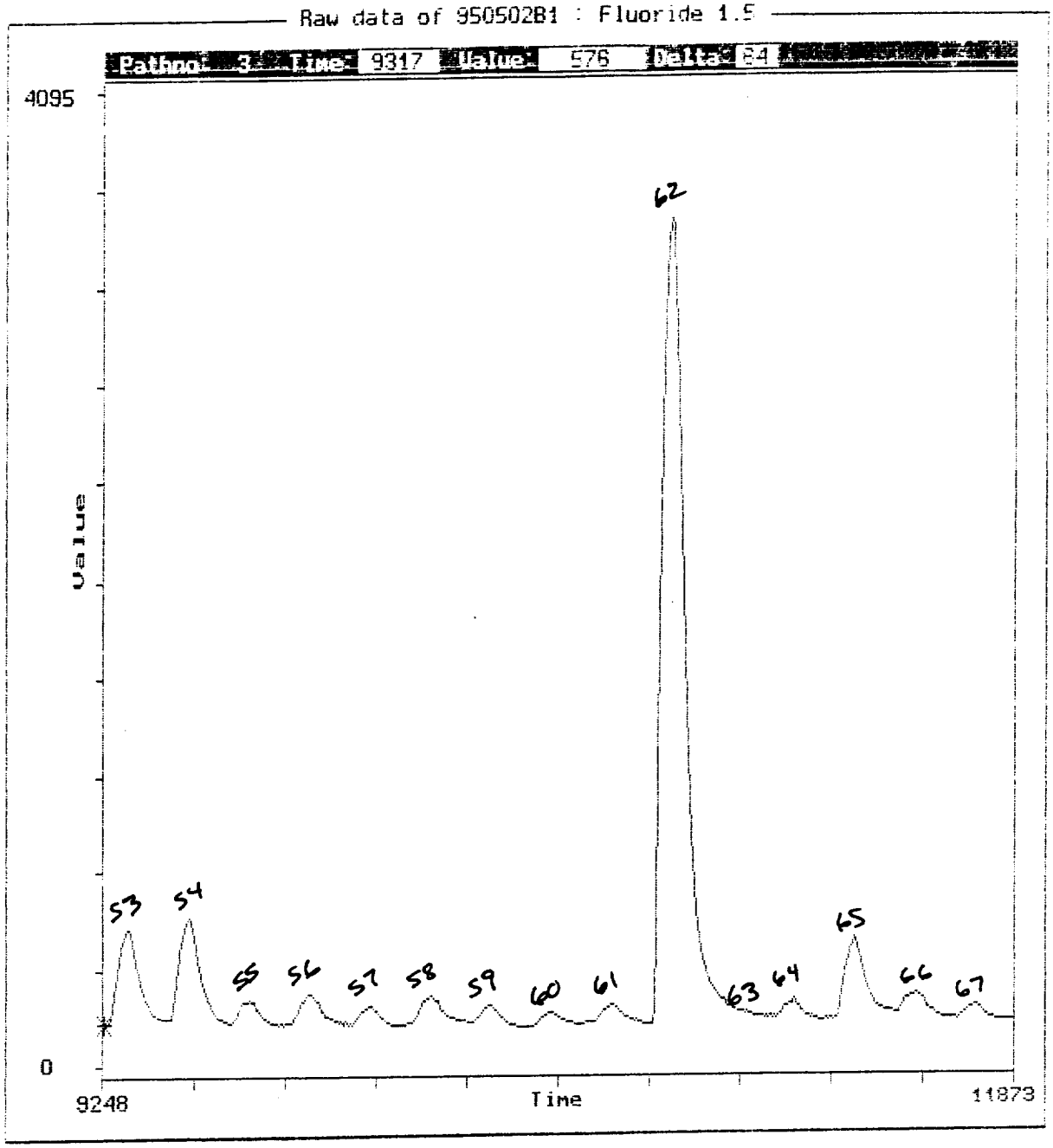


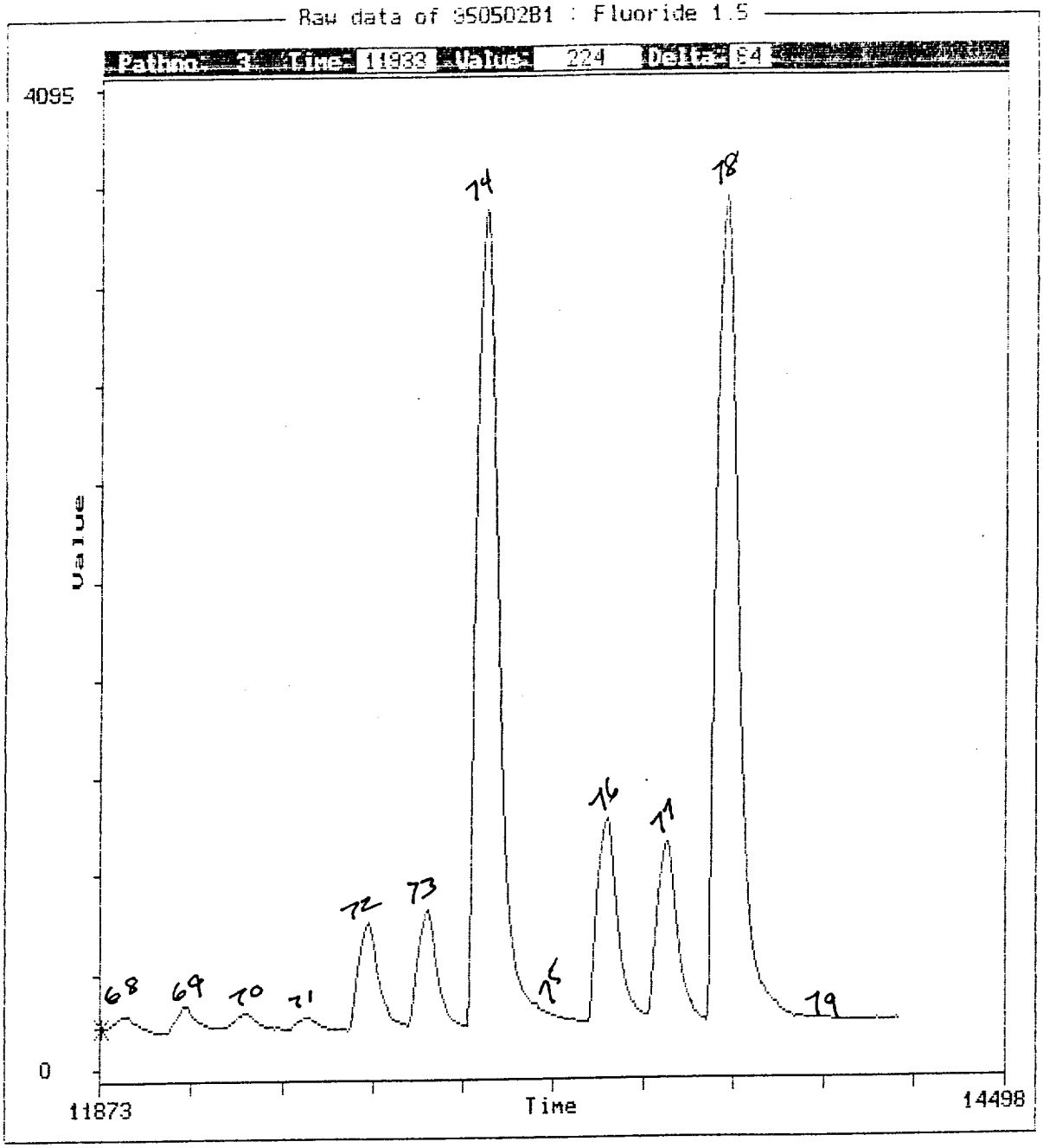
Esc=Exit ¦ F1=Help ¦ Ctrl-P=Edit peaks ¦



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



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



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