AR226-0225

3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6051 and T-6054 in Rabbits

In-Vivo Study Reference Number: HWI#6329-133

Study Number: AMDT-013195.1
Test Substance: FC-129 (T-6051 and T-6054)

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

Samples of liver from rabbits administered FC-129 (T-6054) or FC-129 treated fabric (T-6051) were analyzed at 28 days post dermal administration for total organic fluorine and perfluorooctanesulfonate. The results show that for the highest liquid formulation dose group (12.8 mg/kg) there is on the average about 0.2% of the dose in whole liver at 28 days.

Thus, dermal administration of FC-129 at higher levels results in some dermal absorption.

2.0 INTRODUCTION

Two studies were performed on FC-129. A pharmacokinetic study (HWI#6329-138) and this dermal absorption study (HWI #6329-133). The pharmacokinetic study showed that perfluorooctanesulfonate is a useful marker to assess the dermal absorption of FC-129. Liver, serum, and other tissues were available for analysis by combustion for total organic fluorine and electrospray mass spectrometry for analysis of specific molecules such as perfluorooctanesulfonate. By obtaining and then analyzing data from rabbits at 28 days post dermal dose, information for the assessment of the extent of dermal absorption of FC-129 is provided in this study.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

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

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

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

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

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

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

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

4.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-133), were available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests could be performed. The screening for fluoride in liver via combustion was the most likely analysis to present definitive data for absorption. Other available tests were electrospray mass spectroscopy and gas chromatography/mass spectrometry for metabolites. Liver samples were analyzed by both combustion and electrospray. Data were then analyzed 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 are attached. The level of total organic fluorine in whole liver for the control, 0.128, 1.28, and fabric doses are below the practical quantitation limit for this method. Just using meter readings and extrapolating from the standard curve, the values are on the order of 16 ug/whole liver. The 12.8 mg/kg dermal dose however, results in detectable amounts of organic fluoride in whole liver at 28 days

post dose. The values range from below the practical quantitation limit for one of the 6 rabbits (F52895) to 75 ug/whole liver for rabbit F52889. The average is 45 ug/whole liver with a standard deviation of 21 ug.

Electrospray mass spectrometry data are in agreement with the combustion data; there is very little perfluorooctanesulfonate in the groups other than the 12.8 mg/kg group. Small detectable amounts are observed in the 1.28 mg/kg and fabric groups; however, these are estimated to be on the order of 17 ug/whole liver or less. For the 12.8 mg/kg dose group, perfluorooctanesulfonate is detected in all rabbit liver samples at 28 days post dermal dose. The amounts are estimated to range from 25 to 85 ug/whole liver (mean of 48 ug/whole liver). The rabbit that showed 75 ug/whole liver of total organic fluorine for combustion analysis (F52889), had 56 ug/whole liver perfluorooctanesulfonate.

From the pharmacokinetic study on FC-129 (HWI#6329-138), it is known that a good portion of the intravenous dose will be biotransformed to perfluorooctanesulfonate. It is known that the half-life of perfluorooctanesulfonate in rabbits is >1 month. Thus, if FC-129 is dermally absorbed a portion of it will appear in liver at 28 days as perfluorooctanesulfonate.

Fifty ug perfluorooctanesulfonate/whole liver is 0.2% of the dose for the 12.8 mg/kg rabbits assuming a body weight of 2 kg and expressing the dose in potassium perfluorooctanesulfonate equivalents (FC-95). After an intravenous dose of 12.8 mg/kg a rabbit had 1.05% of the dose in liver at 48 hours. For comparison, 1.05% with a biological half-life of 30 days would be approximately 0.5% of the dose at day 28. Thus, estimated levels after an intravenous dose of 12.8 mg/kg would be 0.5% of the dose in whole liver and estimated levels after dermal administration of 12.8 mg/kg would be 0.2% of the dose in whole liver if the levels are compared at 28 days.

Other data was collected using 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 Have Affected the Quality of the Data: The problem with this analysis is that the extent of biotransformation of the rest of the fluorinated compounds in the liver at 48 hours in the pharmacokinetic study (HWI#6329-138) to perfluorooctanesulfonate is not known. There could be considerable biotransformation of the several percent of dose that fluorinated molecules other than perfluorooctanesulfonate represent. However, the 1.05% of the dose observed at 48 hours still indicates that perfluorooctanesulfonate is a sensitive marker to assess biotransformation of this compound. At 28 days, the value could

be somewhat higher than the above estimate of 0.5% of the dose due to delayed metabolism. If this were true, the value for dermal absorption has by definition (since it is measured at 28 days) a built in compensation for this delay and the value of 0.2% of the dose after dermal administration is being compared with a percentage of dose from the intravenous dose that is too low.

7.0 CONCLUSION

There is evidence of dermal absorption in rabbits of FC-129 after dermal administration of a 12.8 mg/kg dose.

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#6329-133: "Single-Dose Dermal Absorption/Toxicity Study of T-6054 and T-6051 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-013195.1

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-013195.1

9.10 Instrument Settings: See methods

9.11 Data: See attached.

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

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

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

9.1.1 Protocol and Final Report: HWI#6329-133: "Single-Dose Dermal Absorption/Toxicity Study of T-6054 and T-6051 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

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Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6054 and T-6051 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

June 16, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
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Laboratory Project Identification:

HWI 6329-133

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000276

HWI 6329-133

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
12/08/94	12/09/94	Protocol Review	12/09/94	01/10/95
12/28/94	12/28/94	Dose Administration	12/28/94	01/10/95
01/09/95	01/09/95	Protocol Amendment	01/09/95	02/10/95
01/30/95	01/30/95	Protocol Amendment	01/30/95	02/10/95
03/17/95	03/21/95	Data/Report Review	03/21/95	04/10/95
03/17/95	03/21/95	Data Review	03/21/95	04/10/95
06/14/95	06/15/95	Report Rereview	06/15/95	07/10/95
06/16/95	06/16/95	Report Rereview	06/16/95	07/10/95

Stacy Thuck for
Cecilia M. Danner
Representative, Quality Assurance Unit

6-16-95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity
Study of T-6054 and T-6051 in Rabbits

Test Materials	1. T-6054 2. T-6051
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Study Initiation Date	December 13, 1994
Experimental (In-life) Start Date	December 28, 1994
In-life End Date	January 25, 1995
Experimental Termination Date	June 16, 1995
Study Completion Date	June 16, 1995

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
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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-6054 and T-6051 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)	Sterile water	0[a]	3	3
2	T-6054	0.128	3	3
3	T-6054	1.28	3	3
4	T-6054	12.8	3	3
5	T-6051	[b]	3	3

a Administered at a dose volume of 2.0 mL/kg.
b Administered as a 10-cm x 10-cm section of test material (fabric).

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 in-life 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, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy and weighed (volume only determined for bile). The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6054 and T-6051 did not result in any test material-related changes in body weight gain or macroscopic findings at necropsy. All animals appeared clinically normal throughout the study with the exception of one female animal treated with T-6054 at 1.28 mg/kg that exhibited weakened hind limbs the last 22 days of study. This animal was also noted as having small feces on Day 8. These findings are considered to be due to an injury incurred during the sample collection procedures and are not considered to be test material-related. The control material and test material T-6051 did not produce any dermal irritation. No dermal irritation was observed as a result of T-6054 at a dose level of 0.128 or 1.28 mg/kg. T-6054 produced very slight dermal irritation in five animals at the 12.8 mg/kg dose level.

OBJECTIVE

The objective of this study was to assess the systemic toxicity/absorption and relative skin irritancy of test materials 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 mixtures prepared for the Groups 2, 3, and 4 animals for concentration, homogeneity/solubility, and stability was not conducted and the original test material usage log can not be located although a copy is retained in the study file. 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 materials were identified and described as follows:

Identification	Physical Description
T-6054	Amber liquid
T-6051	White plastic sheets

The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). Analysis of the test material mixtures prepared for the Groups 2, 3, and 4 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 materials were stored at room temperature. The control material was stored refrigerated. 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 phase according to HWI SOP. Any remaining control material is retained for other testing and will not be discarded after issuance of the final report.

Safety Precautions

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

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc., Kalamazoo, MI, on December 14, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

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

Animal Diet

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

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -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. F52890) was replaced in the study prior to treatment due to poor health. This animal was replaced with another female (No. F52877).

Study Design

Animals weighing from 2,157 to 2,508 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Sterile water	0[a]	3	3
2	T-6054	0.128	3	3
3	T-6054	1.28	3	3
4	T-6054	12.8	3	3
5	T-6051	[b]	3	3

a Administered at a dose volume of 2.0 mL/kg.
b Administered as a 10-cm x 10-cm section of test material (fabric).

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 test sites (intact skin) were 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 (sterile water for injection) was applied evenly to the test site at a rate of approximately 0.05 mL/cm^2.

Groups 2, 3, and 4. For the Groups 2, 3, and 4 animals (0.128, 1.28, 12.8 mg/kg, respectively), the test material (T-6054) was mixed with sterile water for injection to a concentration of 99, 990, and 9,920 mg/mL, respectively, and applied at a dose volume of 0.01 mL/kg. The mixtures were stored at room temperature until administered. An individual dose of the respective test material mixture was calculated for each animal based on its body weight on the day of treatment. For all three groups, the area of exposure was 4 cm^2 and the approximate rate of application was 0.006 mL/cm^2.

Group 5. The test material (T-6051) was applied to each animal's skin as a 10-cm x 10-cm section of material that was moistened with distilled water.

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. The only exception to this was the Day 8 erythema score for one female animal (No. F52889) in Group 4 was inadvertently not recorded.

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, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples were weighed (volume only determined for bile) and immediately placed on dry ice, then placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals were discarded.

Shipment of Blood, Bile, and Tissues

After experimental termination, the blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106), along with 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 exception of one female animal (No. F52900) treated with T-6054 at 1.28 mg/kg that exhibited weakened hind limbs during the last 22 days of study. This animal also had small feces on Day 8. These findings are considered to be due to an injury incurred during the sample collection procedures and are not considered to be test material-related.

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material and test material T-6051 produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6054 at a dose level of 0.128 or 1.28 mg/kg. T-6054 produced slight to moderate erythema reactions at Days 2 and 4 only in five animals at the 12.8 mg/kg dose level.

Pathology

Individual animal pathology comments are presented in Table 4. There were no lesions observed in any of the animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6054 and T-6051 were evaluated in male and female albino rabbits when administered as a single dermal application. Application of the these materials did not result in any test material-related effects on in-life clinical findings, body weight gain or macroscopic findings at necropsy. The control material and test material T-6051 did not produce any dermal irritation. No dermal irritation was observed with T-6054 applied at a dose level of 0.128 or 1.28 mg/kg. T-6054 produced very slight dermal irritation in five animals at the 12.8 mg/kg dose level.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

6-16-95
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 6329-133

PATHOLOGY REPORT

There were six rabbits (three males and three females) each from five dose levels euthanized and necropsied at the termination of the study. 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. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. After necropsy, the animals were discarded.

James Carter for Thomas Palmer

Thomas E. Palmer, PhD
Pathologist

6-16-95
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) - Sterile Water for Injection (0 mg/kg)							
F52885	2,292	2,483	2,853	F52967	2,204	2,368	2,863
F52873	2,204	2,321	2,745	F52878	2,331	2,475	2,978
F52898	2,169	2,366	2,745	F52883	2,274	2,499	2,985
Mean	2,222	2,390	2,781		2,270	2,447	2,942
Group 2 - T-6054 (0.128 mg/kg)							
F52887	2,272	2,235	2,621	F52901	2,281	2,340	2,839
F52893	2,204	2,260	2,603	F52876	2,270	2,358	2,842
F52897	2,338	2,423	2,913	F52882	2,127	2,323	2,814
Mean	2,271	2,306	2,712		2,226	2,340	2,832
Group 3 - T-6054 (1.28 mg/kg)							
F52965	2,248	2,369	2,966	F52884	2,351	2,508	2,928
F52891	2,077	2,383	2,757	F52900	2,210	2,460	2,588
F52892	2,261	2,333	2,614	F52968	2,166	2,289	2,822
Mean	2,195	2,362	2,779		2,242	2,419	2,779
Group 4 - T-6054 (12.8 mg/kg)							
F52886	2,286	2,417	2,793	F52889	2,292	2,357	2,853
F52880	2,161	2,219	2,525	F52894	2,160	2,489	2,837
F52899	2,353	2,393	2,741	F52895	2,219	2,462	2,791
Mean	2,267	2,343	2,686		2,224	2,436	2,827

Table 1 (Continued)
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 5 - T-6051 (10-cm x 10-cm Section)							
F52879	2,278	2,398	2,923	F52877[a]	2,021	2,157	2,782
F52963	2,173	2,258	2,622	F52888	2,191	2,273	2,752
F52874	2,048	2,265	2,495	F52966	2,264	2,322	2,792
Mean	2,166	2,307	2,680		2,159	2,251	2,775

a Animal No. F52890 was originally selected by the randomization program for use in the study but was replaced with No. F52877 due to poor health.

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 - 7	Day 8	Day 9 - 29
Group 1 (Control) - Sterile Water for Injection (0 mg/kg)						
Male	F52885	Appeared normal	✓	✓	✓	✓
	F52873	Appeared normal	✓	✓	✓	✓
	F52898	Appeared normal	✓	✓	✓	✓
Female	F52967	Appeared normal	✓	✓	✓	✓
	F52878	Appeared normal	✓	✓	✓	✓
	F52883	Appeared normal	✓	✓	✓	✓
Group 2 - T-6054 (0.128 mg/kg)						
Male	F52887	Appeared normal	✓	✓	✓	✓
	F52893	Appeared normal	✓	✓	✓	✓
	F52897	Appeared normal	✓	✓	✓	✓
Female	F52901	Appeared normal	✓	✓	✓	✓
	F52876	Appeared normal	✓	✓	✓	✓
	F52882	Appeared normal	✓	✓	✓	✓
Group 3 - T-6054 (1.28 mg/kg)						
Male	F52965	Appeared normal	✓	✓	✓	✓
	F52891	Appeared normal	✓	✓	✓	✓
	F52892	Appeared normal	✓	✓	✓	✓
Female	F52884	Appeared normal	✓	✓	✓	✓
	F52900	Appeared normal	✓	✓	-	-
		Weakened hind limbs	-	-	✓	✓
		Small feces	-	-	✓	-
	F52968	Appeared normal	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 - 7	Day 8	Day 9 - 29
		Group 4 - T-6054 (12.8 mg/kg)				
Male	F52886	Appeared normal	✓	✓	✓	✓
	F52880	Appeared normal	✓	✓	✓	✓
	F52899	Appeared normal	✓	✓	✓	✓
Female	F52889	Appeared normal	✓	✓	✓	✓
	F52894	Appeared normal	✓	✓	✓	✓
	F52895	Appeared normal	✓	✓	✓	✓
		Group 5 - T-6051 (10-cm x 10-cm Section)				
Male	F52879	Appeared normal	✓	✓	✓	✓
	F52963	Appeared normal	✓	✓	✓	✓
	F52874	Appeared normal	✓	✓	✓	✓
Female	F52877	Appeared normal	✓	✓	✓	✓
	F52888	Appeared normal	✓	✓	✓	✓
	F52966	Appeared normal	✓	✓	✓	✓

✓ Condition existed.

Table 3
Individual Dermal Irritation Scores

Group 1 (Control) - Sterile Water for Injection (0 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52885				Animal No. F52967			
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. F52873				Animal No. F52878			
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. F52898				Animal No. F52883			
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

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Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6054 (0.128 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52887				Animal No. F52901			
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. F52893				Animal No. F52876			
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. F52897				Animal No. F52882			
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-6054 (1.28 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52965				Animal No. F52884			
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. F52891				Animal No. F52900			
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. F52892				Animal No. F52968			
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

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Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6054 (12.8 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52886				Animal No. F52889			
Erythema	0	0	0	0	0	1	1	-
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. F52880				Animal No. F52894			
Erythema	0	1	1	0	0	1	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. F52899				Animal No. F52895			
Erythema	0	1	1	0	0	2	1	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

- Value not recorded.

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 5 - T-6051 (10-cm x 10-cm Section)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52879				Animal No. F52877			
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. F52963				Animal No. F52888			
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. F52874				Animal No. F52966			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
	Group 1 (Control) - Sterile Water for Injection (0 mg/kg)			
F52885	M	-	29	No visible lesions.
F52873	M	-	29	No visible lesions.
F52898	M	-	29	No visible lesions.
F52967	F	-	29	No visible lesions.
F52878	F	-	29	No visible lesions.
F52883	F	-	29	No visible lesions.
	Group 2 - T-6054 (0.128 mg/kg)			
F52887	M	-	29	No visible lesions.
F52893	M	-	29	No visible lesions.
F52897	M	-	29	No visible lesions.
F52901	F	-	29	No visible lesions.
F52876	F	-	29	No visible lesions.
F52882	F	-	29	No visible lesions.
	Group 3 - T-6054 (1.28 mg/kg)			
F52965	M	-	29	No visible lesions.
F52891	M	-	29	No visible lesions.
F52892	M	-	29	No visible lesions.
F52884	F	-	29	No visible lesions.
F52900	F	-	29	No visible lesions.
F52968	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 4 - T-6054 (12.8 mg/kg)		
F52886	M	-	29	No visible lesions.
F52880	M	-	29	No visible lesions.
F52899	M	-	29	No visible lesions.
F52889	F	-	29	No visible lesions.
F52894	F	-	29	No visible lesions.
F52895	F	-	29	No visible lesions.
		Group 5 - T-6051 (10-cm x 10-cm Section)		
F52879	M	-	29	No visible lesions.
F52963	M	-	29	No visible lesions.
F52874	M	-	29	No visible lesions.
F52877	F	-	29	No visible lesions.
F52888	F	-	29	No visible lesions.
F52966	F	-	29	No visible lesions.

- Not applicable.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
Group 1 (Control) - Sterile Water for Injection (0 mg/kg)					
Male	F52885	70.634	-	0.960	1.0
	F52873	75.513	16.417	0.866	1.6
	F52898	74.433	-	0.645	0.9
Female	F52967	62.517	-	0.777	2.1
	F52878	63.222	16.808	0.890	2.0
	F52883	75.418	-	0.618	2.5
Group 2 - T-6054 (0.128 mg/kg)					
Male	F52887	71.149	-	0.784	1.1
	F52893	64.537	13.509	0.836	0.6
	F52897	73.622	-	0.604	1.5
Female	F52901	71.908	14.610	0.640	1.7
	F52876	66.359	-	1.179	1.2
	F52882	77.959	-	1.337	1.6
Group 3 - T-6054 (1.28 mg/kg)					
Male	F52965	69.880	18.191	0.452	1.8
	F52891	65.695	-	0.525	1.1
	F52892	69.216	-	0.949	1.1
Female	F52884	65.907	-	0.590	1.2
	F52900	61.937	-	0.710	1.0
	F52968	67.347	12.513	0.866	1.3

- Not applicable.

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

Sex	Animal Number	Weight (g)			Bile Volume (mL)
		Liver	Kidneys	Dermal Application Site	
		Group 4 - T-6054 (12.8 mg/kg)			
Male	F52886	77.219	-	0.857	0.6
	F52880	59.985	12.254	0.634	0.8
	F52899	89.883	-	1.391	0.4
Female	F52889	69.288	-	0.953	0.5
	F52894	75.086	-	1.000	1.4
	F52895	59.174	15.603	0.635	0.9
		Group 5 - T-6051 (10-cm x 10-cm Section)			
Male	F52879	89.874	-	0.884	1.0
	F52963	71.814	-	1.535	0.6
	F52874	71.657	15.784	0.990	0.6
Female	F52877	71.284	-	1.154	0.6
	F52888	65.633	-	1.231	0.5
	F52966	68.719	15.855	0.898	1.5

- Not applicable.

APPENDIX A

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

Protocol Deviation

Protocol	Actual Procedure
Page 7, 7. Experimental Design, C. Observation of Animals, (2) Reading of Dermal Irritation, Second Sentence. Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8.	The Day 8 erythema score was inadvertently not recorded for one Group 4 female (No. F52889).

This deviation is not considered to have had an adverse effect on the outcome of the study.

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of
T-6054 and T-6051 in Rabbits

Date:

December 13, 1994

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-133

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

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of
T-6054 and T-6051 in Rabbits

HWI No.	6329-133
Test Materials	1. T-6054 2. T-6051
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	December 28, 1994
Experimental Termination Date	January 25, 1995
Draft Report Date	March 8, 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 test materials 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 Materials

A. Identification
1. T-6054
2. T-6051

B. Physical Description
1. (To be documented in the raw data)
2. (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 materials 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
15 males and 15 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	Number of Animals Females
1 (Control)	Distilled water	0*	3	3
2	T-6054	0.128	3	3
3	T-6054	1.28	3	3
4	T-6054	12.8	3	3
5	T-6051	**	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered as a 10.0-cm x 10.0-cm piece of test material (fabric)

(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 with an electric clipper. 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 respective doses for the animals in Groups 1, 2, 3, and 4 will be based on the animal's body weight just before administration and spread onto the area of exposure in a thin and uniform layer. The Group 1, 2, 3, and 4 materials will be applied undiluted. The Group 5 material will be applied as a 10.0-cm x 10.0-cm piece of the test material moistened with distilled water. The area of application (Groups 1-5) 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

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

12-15-94
Date

Steven M. Glaza

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

12-13-94
Date

Stacy Thiede

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12-13-94
Date

(6329-133.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-6054 and T-6051 in Rabbits

HWI 6329-133

Sponsor

3M Toxicology Service
Medical Department
3M Center, Bldg. 220-2E-02
P.O. Box 33220
St. Paul, MN 55133-3220

Contractor

Hazleton Wisconsin, Inc
3301 Kinsman Boulevard
Madison, WI 53704

Sponsor's Representative

John L. Butenhoff, PhD

Study Director

Steven M. Glaza

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective December 23, 1994

In order to obtain a measurable amount of test material (T-6054) for application in Groups 2, 3, and 4, the test material will be diluted with sterile water for injection and applied at a common dose volume of .01 mL/kg. Modify the following two sections of the protocol (protocol amendment items #1 and #2) to indicate these changes.

1. Page 6, 7. Experimental Design; B. Dose Administration; (1) Test Groups. Add the following shaded additions to this section:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0*	3	3
2	T-6054	0.128***	3	3
3	T-6054	1.28***	3	3
4	T-6054	12.8***	3	3
5	T-6051	**	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered as a 10.0-cm x 10.0-cm piece of test material (fabric)
*** To be administered at a dose volume of .01 mL/kg

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

Amendment No. 1

2. Page 6, 7. Experimental Design; B. Dose Administration; (3) Dose Administration. Delete the fourth sentence in this section and then add the following as the third and fourth sentences to this section.

> The control material (Group 1) will be applied undiluted. The dose for each animal in Groups 2, 3, and 4 will be diluted with sterile water for injection and applied at a dose volume of .01 mL/kg.

Effective December 28, 1994

Sterile water for injection will replace distilled water as the control material based on the fact that sterile water for injection will also be the vehicle in the test mixtures for Groups 2, 3, and 4 (see protocol amendment items #1 and #2). Modify the following three sections of the protocol to indicate this change.

3. Page 4, 6. Control Material; A. Identification. Replace *distilled water* with the following:

> Sterile Water for Injection

4. Page 4, 6. Control Material; D. Storage Conditions. Replace *Room temperature* with the following:

> Refrigerated

5. Page 6, 7. Experimental Design; B. Dose Administration; (1) Test Groups. Modify the table in this section with the following shaded change:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Sterile water	0*	3	3
2	T-6054	0.128***	3	3
3	T-6054	1.28***	3	3
4	T-6054	12.8***	3	3
5	T-6051	**	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered as a 10.0-cm x 10.0-cm piece of test material (fabric)
*** To be administered at a dose volume of .01 mL/kg

PROTOCOL APPROVAL

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

1-19-95
Date

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

1-12-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

1.12.95
Date

(6329-133.Aml.dsk2)

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study of
T-6054 and T-6051 in Rabbits

HWI 6329-133

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 portions of the protocol:

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.

1. Page 8, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the first paragraph of this section with the following underlined addition:

   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.

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

   The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.C.(4).(c).

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Amendment No. 2

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

Individual animal tissue weights and bile volumes

PROTOCOL AMENDMENT APPROVAL

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

2/15/95
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

(6329-133.Am2.dsk2)

9.1.2 Analytical protocol AMDT-013195.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6051 and T-6054 in Rabbits

In-Vivo Study Reference Number: HWI#6329-133

Study Number: AMDT-013195.1
Test Substance: FC-129 (T-6051 and T-6054)

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

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

1.0 PURPOSE

This study is designed to provide information as to whether FC-129 (T-6051 and T-6054) 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-129.

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 fluid from 24 test animals and 6 control animals. Tissues and fluids include liver, 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), $-20 \pm 10°C$ (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#6329-133), 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. To confirm the identity of fluorine-containing compounds present in liver (if any at 28 days) and serum at various intervals, electrospray mass spectrometry may be selected as one of the analytical techniques employed. Not all of the tissues and fluid samples will be analyzed. 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

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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.

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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.

000330

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

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

9.4 Sample Calculations

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

10.0 VALIDATION

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

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

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

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

11.2 Analyzing the Data

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

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

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

Author: Rich Youngblom

Approved By:

James D Johnson 10/3/95
Group Leader Date

Gale Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

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

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

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

1.2 APPLICABLE COMPOUNDS: Fluoride.

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

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

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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.

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9.2 Calibration And Measurement, Using Orion 3E Software:

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

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

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

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

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

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

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

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

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 10-31-95
Date

Quality Assurance 10-31-95
Date

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

1.0 SCOPE

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

2.0 KEYWORDS

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

3.0 PRECAUTIONS

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

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

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

4.2 Reagents

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

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

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

8.2 Prepare FC-95 Anion Standards

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

8.3 Prepare Beef Liver Homogenate to Use for Standards

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

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

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

8.4 Calculate the actual value of the standards:

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

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

8.5 **Calibration**

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

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

8.6 **Storage Conditions for Standards**

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

8.7 **Storage Conditions for Standards**

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

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

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

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

9.4 Record the liver weight in the study note book.

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

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

10.0 VALIDATION

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

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

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Worksheet AMDT-M-4

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

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

Liver Extraction Process:		Date & Initials
Pipet 1 mL of Liver Solution		
Pipet 100 uL of 12 ppm Internal Standard	Std. #	
Vortex 15 sec		
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #	
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer		
Pipet 5 mL of Ethyl Acetate		
Vortex 20-30 sec		
Shake 20 min		
Centrifuge 20-25 min		
Remove a 4 mL aliquot of organic layer		
Blow down to near dryness (<0.25 mL) with N_2		
Add 1 m: 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		

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3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

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

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

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 6/6/95
Date

Quality Assurance 6/6/95
Date

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

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.

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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 Von 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

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1.0 SCOPE

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

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

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps

4.1.2 Autopipets, oxford or equivalent with plastic tips

4.1.3 Polypropylene volumetric flasks, 100 mL

4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.

4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent

4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.

4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.

4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.

4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.

4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

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

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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-6051 and T-6054 in Rabbits**

Study Number: AMDT-013195.1 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
10/12/95	10/19/95	Final Report	10/19/95	10/19/95

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Kari Rambo 10-19-95
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-013195.1, HWI 6329-133
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	**16.2**	**±**	**5.5**
0.128 mg/kg dose (T6054)	**11.1**	**±**	**2.0**
1.28 mg/kg dose (T6054)	**17.1**	**±**	**2.6**
12.8 mg/kg dose (T6054)	**45.2**	**±**	**20.5**
Fabric Exposure (T6051)	**14.7**	**±**	**2.7**

***Calculated as the mean of triplicate samples from each of three male and three female rabbits.**

FC129 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
liver blank-3		0.230		0.140			
liver spike-4	83%	0.950		0.132			
liver spike-5	83%	1.06		0.118			
liver spike-6	102%	1.28		0.121			
liver spike-7	94%	1.00		0.142			
liver spike-8	84%	2.34		0.108			
liver spike-9	83%	2.49		0.101			
liver blank-4		0.328		0.116			
F52873-1		0.245		0.121	74.2		
F52873-2		0.334	0.303	0.107	74.2	22.4	0.0
F52873-3		0.329		0.104	74.2		
F52885-1		0.449		0.150	69.2		
F52885-2		0.222	0.326	0.114	69.2	22.5	0.0
F52885-3		0.306		0.108	69.2		
F52898-1		0.139		0.150	73.3		
F52898-2		0.131	0.124	0.148	73.3	9.11	0.0
F52898-3		0.103		0.146	73.3		
liver blank-1		0.228		0.152			
liver blank-2		0.298		0.134			
liver spike-1	91%	1.36		0.101			
liver spike-2	95%	0.995		0.145			
F52878-1		0.286		0.105	70.2		
F52878-2		0.230	0.231	0.150	70.2	16.2	0.0
F52878-3		0.176		0.151	70.2		
F52883-1		0.211		0.115	73.8		
F52883-2		0.242	0.211	0.107	73.8	15.6	0.0
F52883-3		0.179		0.135	73.8		
F52967-1		0.158		0.144	61.5		
F52967-2		0.194	0.184	0.116	61.5	11.3	0.0
F52967-3		0.200		0.108	61.5		
F52887-1		0.252		0.108	70.6		
F52887-2		0.189	0.205	0.128	70.6	14.5	0.128
liver blank-1		0.281		0.129			
liver spike-1	69%	0.968		0.108			
liver spike-2	85%	0.973		0.132			
liver spike-3	83%	0.875		0.143			
liver spike-4	109%	1.29		0.128			
liver spike-5	101%	1.11		0.138			
liver spike-6	101%	1.26		0.121			
F52887-3		0.173		0.148	70.6		
F52893-1		0.168		0.133	63.5		
F52893-2		0.147	0.163	0.135	63.5	10.4	0.128
F52893-3		0.175		0.135	63.5		

000361

FC129 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
F52897-1		0.156		0.134	72.4		
F52897-2		0.174	0.169	0.113	72.4	12.3	0.128
F52897-3		**0.179**		0.123	72.4		
F52876-1		**0.117**		0.149	65.2		
F52876-2		**0.155**	0.139	0.119	65.2	9.09	0.128
F52876-3		**0.146**		0.138	65.2		
F52882-1		0.112		0.146	76.1		
F52882-2		0.154	0.145	0.130	76.1	11.0	0.128
F52882-3		0.169		0.112	76.1		
F52901-1		0.126		0.140	70.5		
F52901-2		0.127	0.132	0.143	70.5	9.29	0.128
F52901-3		0.142		0.112	70.5		
Liver blk 1		0.331		0.112			
Liver blk 2		0.218		0.137			
Liver blk 1		1.22		0.146			
Liver blk 2		0.322		0.159			
Liver blk 3		0.280		0.139			
Liver spk-1	92%	1.10		0.127			
Liver spk-2	89%	0.869		0.156			
Liver spk-3	83%	0.864		0.145			
F52965-1		0.235		0.125	68.7		
F52965-2		0.205	0.219	0.163	68.7	15.0	1.28
F52965-3		0.216		0.151	68.7		
F52891-1		0.239		0.114	64.5		
F52891-2		0.197	0.225	0.144	64.5	14.5	1.28
F52891-3		0.239		0.128	64.5		
F52892-1		0.333		0.132	68.7		
F52892-2		0.270	0.292	0.134	68.7	20.1	1.28
F52892-3		0.273		0.102	68.7		
F52884-1		0.206		0.146	65.0		
F52884-2		0.255	0.231	0.101	65.0	15.0	1.28
F52884-3		0.230		0.135	65.0		
F52968-1		0.299		0.145	66.6		
F52968-2		0.261	0.267	0.116	66.6	17.8	1.28
F52968-3		0.242		0.121	66.6		
F52900-1		0.335		0.147	61.4		
F52900-2		0.390	0.328	0.115	61.4	20.1	1.28
F52900-3		0.259		0.115	61.4		
F52880-1		0.487		0.117	59.6		
F52880-2		0.548	0.538	0.142	59.6	32.1	12.8
F52880-3		0.579		0.138	59.6		
F52886-1		0.611		0.137	75.3		
F52886-2		0.728	0.610	0.114	75.3	46.0	12.8
F52886-3		0.490		0.113	75.3		
F52899-1		0.428		0.103	89.1		
F52899-2		0.366	0.378	0.115	89.1	33.7	12.8
F52899-3		0.340		0.126	89.1		

000362

FC129 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
liver blank 1		0.265		0.125			
liver blank 2		0.327		0.111			
liver blank 3		0.220		0.123			
liver spike-1	87%	0.921		0.143			
liver spike-2	0.7786	**1.05**		0.112			
liver spike-3	0.7852	**0.823**		0.144			
liver spike-4	**0.9656**	**1.06**		0.138			
liver spike-5	**0.8486**	**0.859**		0.149			
liver spike-6	82%	0.978		0.127			
F52889-1		0.812		0.114	68.7		
F52889-2		0.896	1.10	0.113	68.7	75.4	12.8
F52889-3		1.59		0.141	68.7		
F52894-1		0.835		0.127	74.0		
F52894-2		0.794	0.849	0.119	74.0	62.8	12.8
F52894-3		0.917		0.118	74.0		
F52895-1		0.307		0.151	58.1		
F52895-2		0.523	0.368	0.109	58.1	21.4	12.8
F52895-3		0.276		0.154	58.1		
F52874-1		0.331		0.144	69.7		
F52874-2		0.249	0.251	0.122	69.7	17.5	Fabric
F52874-3		0.173		0.145	69.7		
F52879-1		0.168		0.134	89.1		
F52879-2		0.194	0.182	0.105	89.1	16.3	Fabric
F52879-3		0.185		0.147	89.1		
F52963-1		0.216		0.130	70.2		
F52963-2		0.175	0.182	0.123	70.2	12.8	Fabric
F52963-3		0.154		0.135	70.2		
F52877-1		0.222		0.119	69.9		
F52877-2		0.190	0.191	0.118	69.9	13.3	Fabric
F52877-3		0.160		0.141	69.9		
F52888-1		0.158		0.144	64.2		
F52888-2		0.211	0.170	0.104	64.2	10.9	Fabric
F52888-3		0.142		0.139	64.2		
F52966-1		0.321		0.123	67.6		
F52966-2		0.277	0.255	0.131	67.6	17.3	Fabric
F52966-3		0.167		0.132	67.6		
Liver spike-7	60%	0.881		0.102			
Liver spike-8	71%	1.05		0.103			
Liver spike-9	75%	0.775		0.146			
Liver spike-10	98%	0.990		0.150			
Liver spike-11	103%	1.15		0.136			
Liver spike-12	101%	1.16		0.133			
Liver spike-13	78%	2.16		0.110			
Liver spike-14	107%	2.46		0.131			
Liver spike-15	102%	2.39		0.130			

000363

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

A-1
Contains pages
A-1 through A-
10-31-95
D. Christenson

Study: Single Dose Dermal Absorption
Protocol Number: TP3016.AB
Test Material: T-6054 & T-6051 in Rabbits (FC-129)
Matrix: Liver
R Squared Value: 0.9889
Response Factor Amount: 1.15E-05
Analyst: DLC
Date: 4/3/95
Method: AMDT-M-4
Instrument: Fisons VG 2000 Electrospray MS
LABBASE FILE 040395D

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g *	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: Sterile Water 2.0 mL/kg	F52883	N/D			N/D		N/D
	F52898	N/D			N/D		N/D
	F52885	N/D			N/D		N/D
	F52967	N/D			N/D		N/D
	F52873	N/D			N/D		N/D
	F52878	N/D			N/D		N/D
Group 2: 0.128 mg /kg	F52893	N/D			N/D		N/D
	F52887	N/D			N/D		N/D
	F52901	N/D			N/D		N/D
	F52876	N/D			N/D		N/D
Group 3: 1.28 mg/kg	F52892	11413	1.0139	1	0.1033	68.707	0.007
	F52891	14922	1.0615	1	0.1290	64.487	0.008
	F52965	8191	1.1217	1	0.0670	68.667	0.005
	F52884	12572	1.0881	1	0.1060	64.989	0.007
Group 4: 12.8 mg/kg	F52895	51584	1.117	1	0.4237	58.094	0.025
	F52899	58481	1.1894	1	0.4511	89.078	0.040
	F52894	127876	1.0236	1	1.1462	73.983	0.085
	F52880	58961	1.0931	1	0.4949	59.624	0.030
	F52886	84958	1.0915	1	0.7142	75.341	0.054
	F52889	93984	1.0615	1	0.8124	68.670	0.056
Group 5: Fabric	F52879	16734	1.2566	1	0.1222	89.125	0.011
	F52877	26249	1.0106	1	0.2383	69.913	0.017
	F52874	N/D			N/D		N/D
	F52963	9735	1.0772	1	0.0829	70.161	0.006
	F52888	N/D			N/D		N/D

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

HWI # 6329-133

Study:	Single Dose Dermal Absorption
Protocol Number:	TP3016.AB
Test Material:	T-6054 & T-6051 in Rabbits (FC-129)
Matrix:	Liver
R Squared Value:	0.9946
Response Factor Amount:	9.10E-06
Analyst:	DLC
Date:	4/4/95
Method:	AMDT-M-4
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE FILE	040495B

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g **	Total mass of liver g	Total amount of FC-95 per liver mg
Group 2: **0.128 mg /kg**	F52897	18736	1.1033	1	0.1236	72.433	0.009
	F52882	N/D	1.1025	1	N/D	76.060	N/D
Group 3: **1.28 mg/kg**	F52900	16432	1.3161	1	0.0909	61.356	0.006
	F52968	27181	1.2011	1	0.1648	66.642	0.011
Group 5: **Fabric ***	F52966	8521	1.1457	1	0.0542	67.632	0.004

*** Administered as a 10.0 cm x 10.0 cm piece of test fabric**

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

Replot

A-3

Method DLCLIV
Sample DLCLIV
Operator DLC

BEST COPY AVAILABLE

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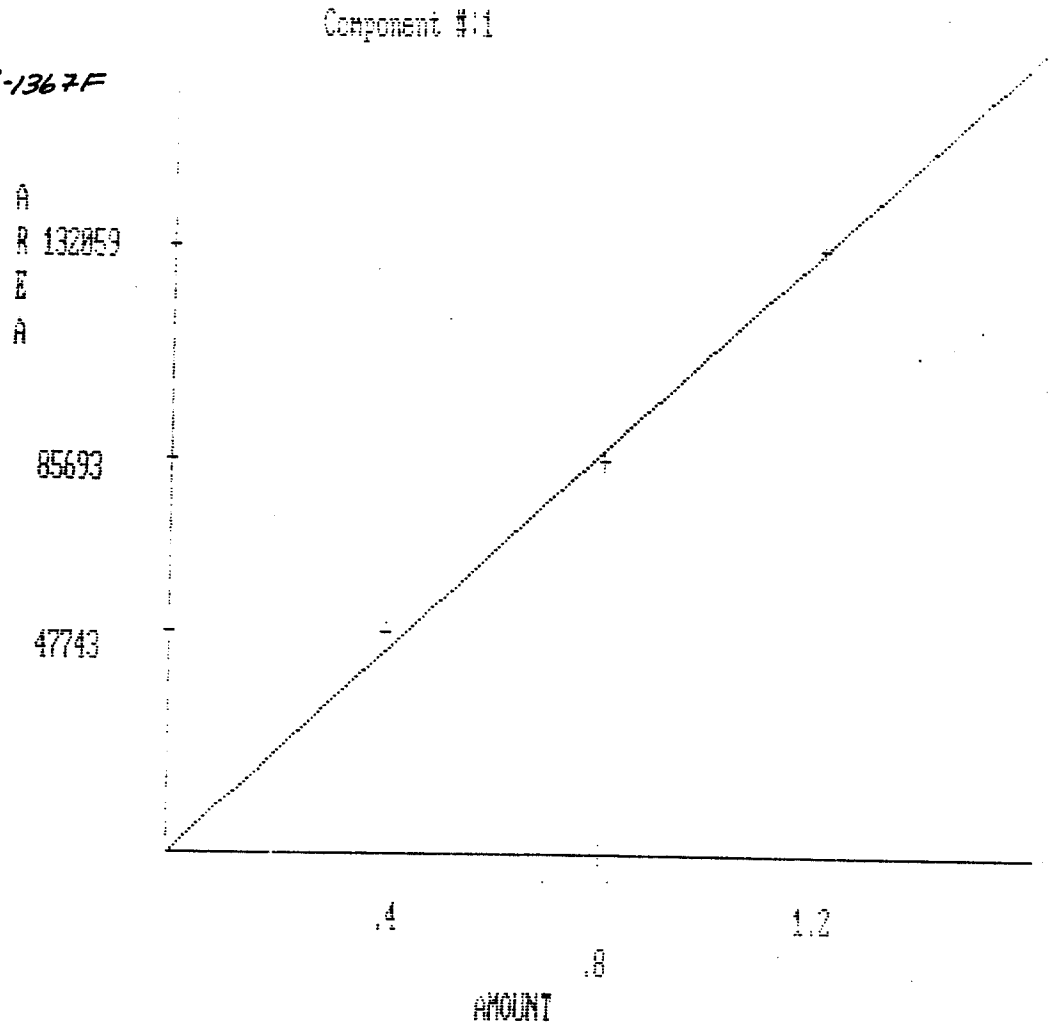
6329-143 FC-1367F
6329-133



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2	0.8000	85693
3	1.2000	132059

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Amount = 9.1012E-06 * Area + 0.0000E+00
R squared = 0.9946

FILE: 040395D

Keplot

A-4

HWL 6329-133

FC-129

Method DLCLIV
Sample DLCLIV
Operator DLC

BEST COPY AVAILABLE

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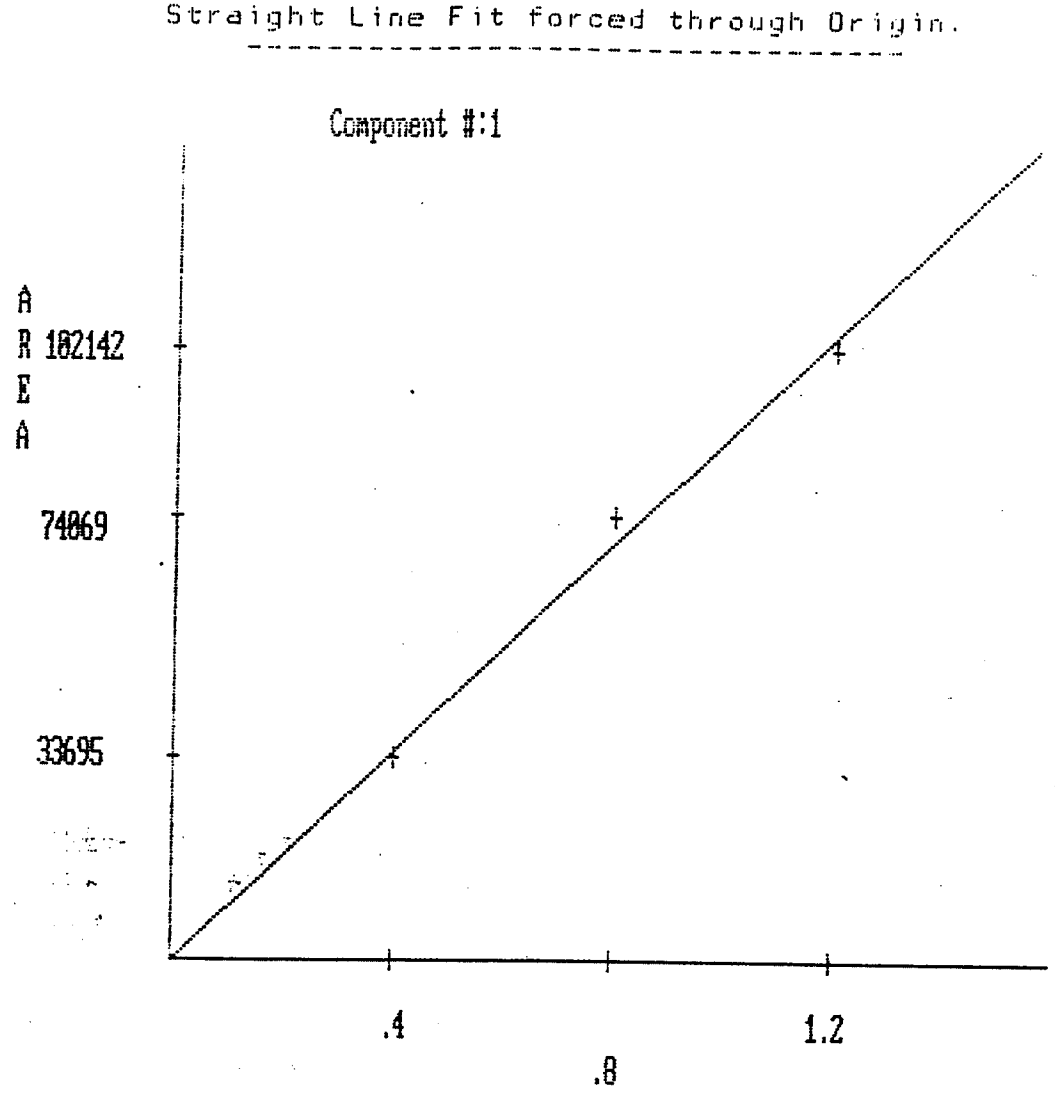
DLC Initial

6/22/95 Date

Exact Copy of Original



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Amount = 1.1469E-05 * Area + 0.0000E+00
R squared = 0.9889

HwI 6329-133

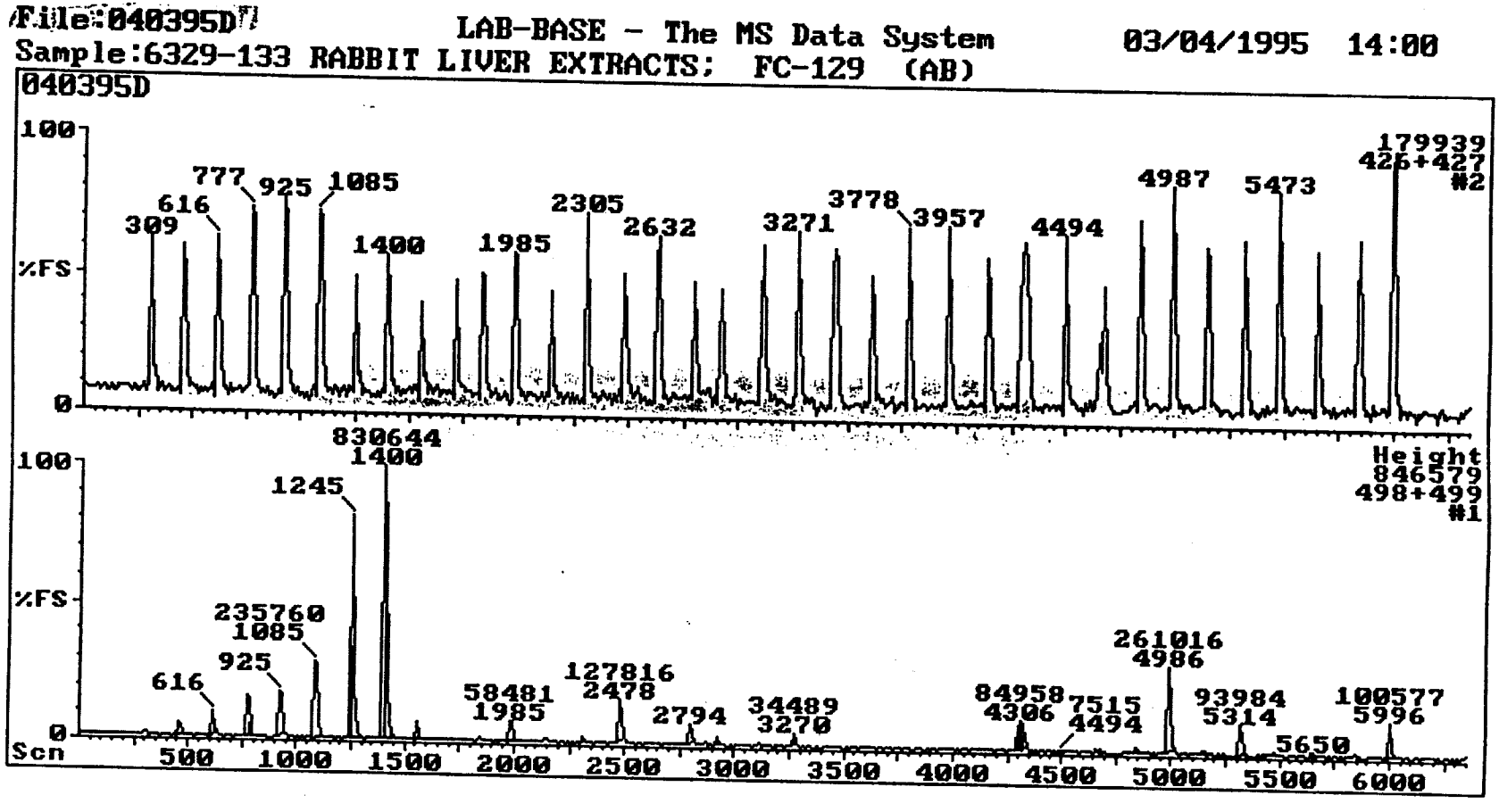
FC-129

A-6

Exact Copy of Original

DLC
Initial

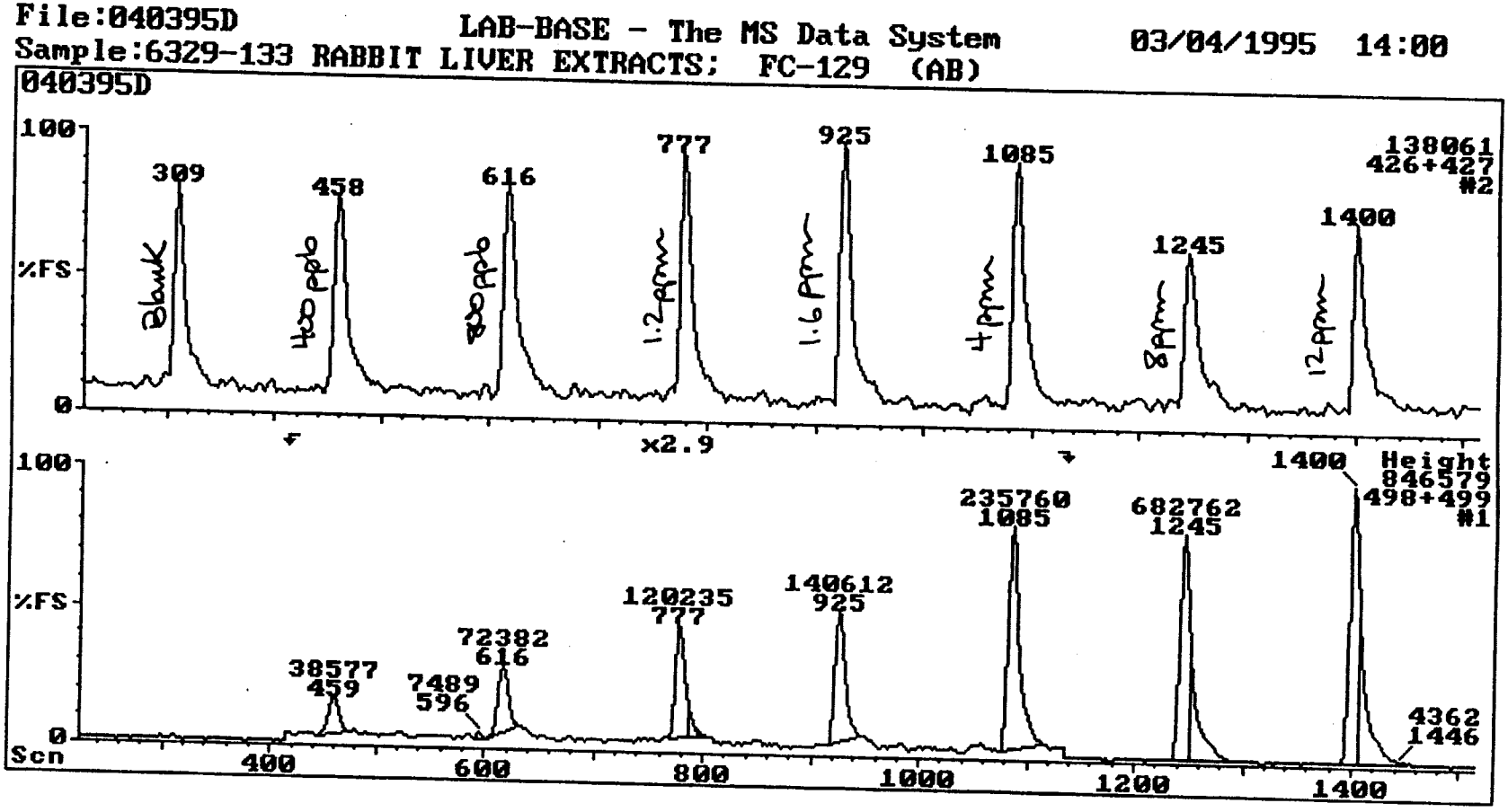
6/22/95
Date



000369

A-10 10-1 (82)
A-7

Beef liver extracts for std. curve



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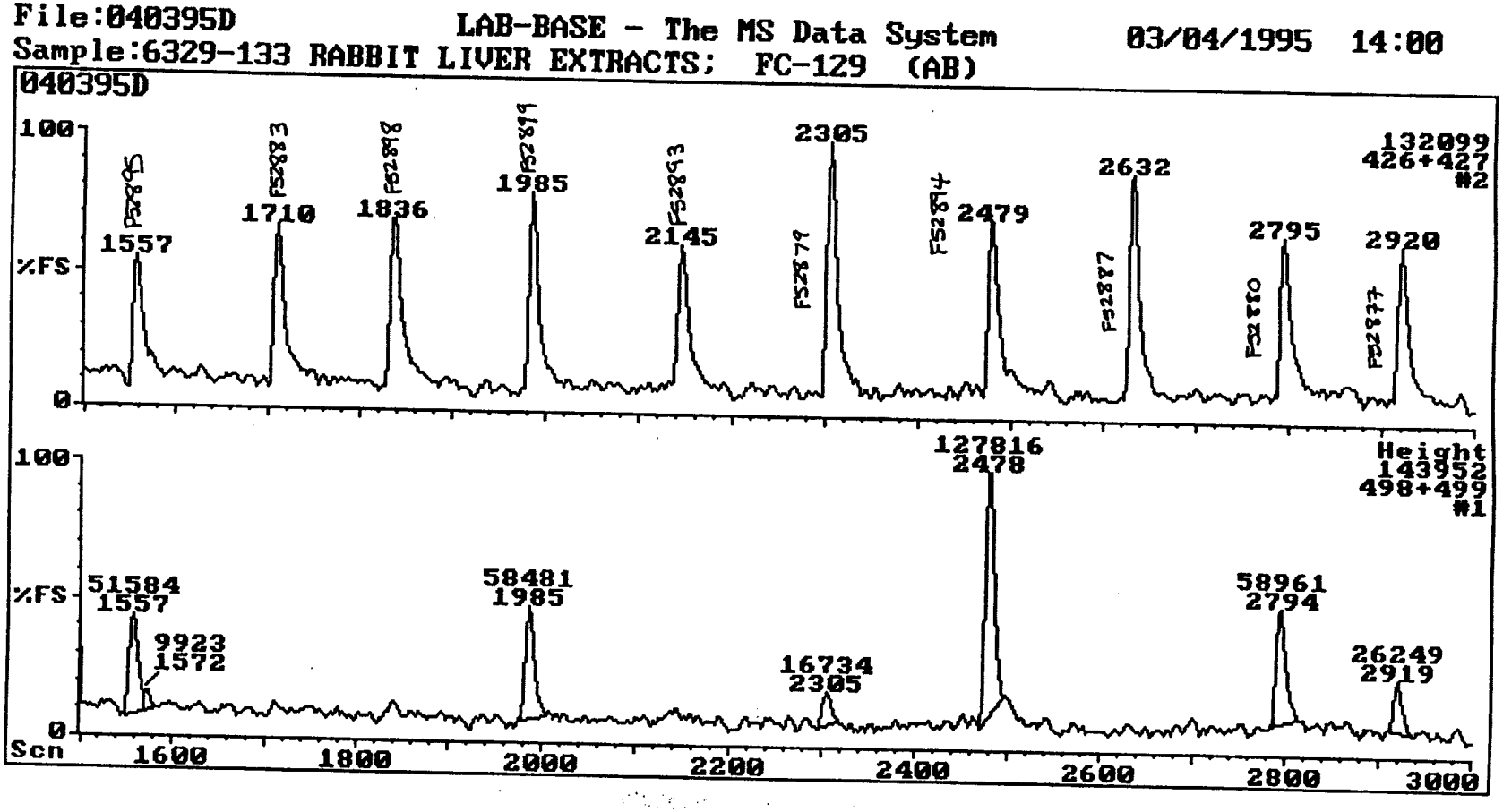
A-8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03/04/1995 14:00
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040395D
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%FS
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1836
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2145
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9923
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2800
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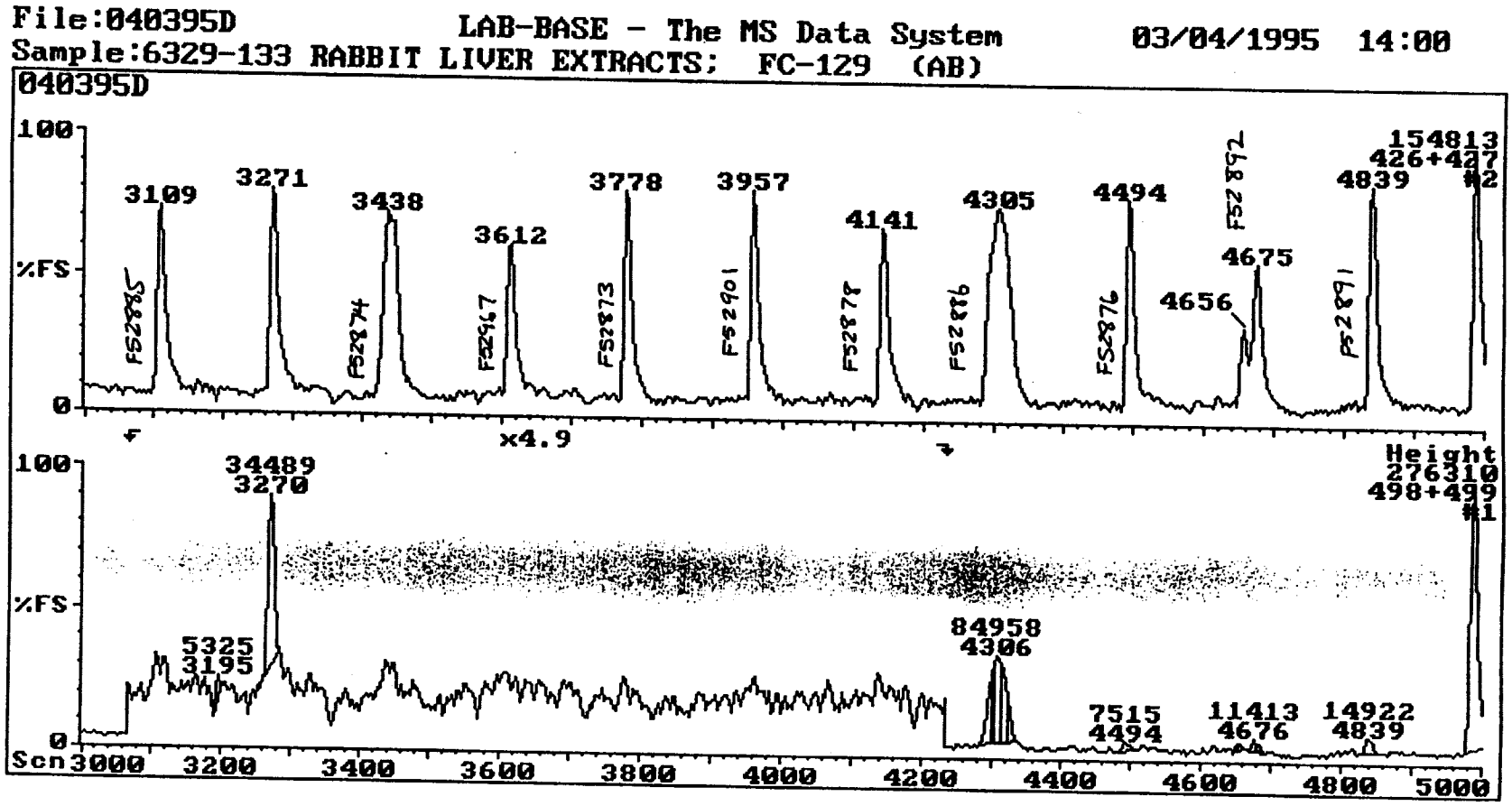
000371

A-9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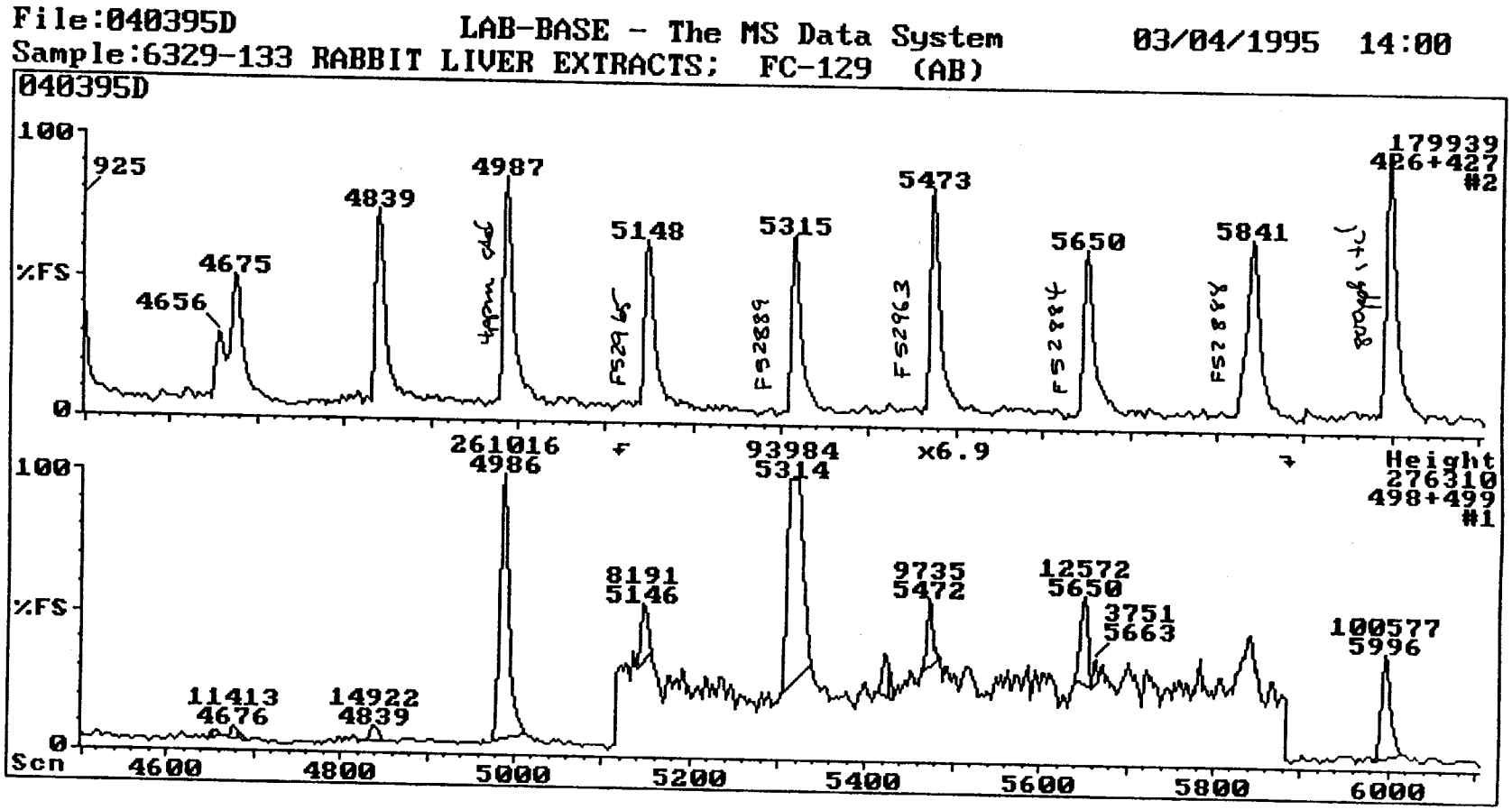
LAB-BASE - The MS Data System
03/04/1995 14:00
Sample:6329-133 RABBIT LIVER EXTRACTS; FC-129 (AB)
040395D
100
%FS
0
3109
3271
3438
3612
3778
3957
4141
4305
4494
4656
4675
4839
154813
426+427
#2
FS2885
FS2874
FS2917
FS2873
FS2901
FS2878
FS2876
FS2892
FS2891
x4.9
34489
3270
Height
276310
498+499
#1
5325
3195
84958
4306
7515
4494
11413
4676
14922
4839
Scn 3000 3200 3400 3600 3800 4000 4200 4400 4600 4800 5000

000372


File:040395D
LAB-BASE - The MS Data System
03/04/1995 14:00
Sample:6329-133 RABBIT LIVER EXTRACTS; FC-129 (AB)
040395D
100
%FS
0
925
4656
4675
4839
4987
5148
5315
5473
5650
5841
179939
426+427
#2
261016
4986
93984
5314
x6.9
Height
276310
498+499
#1
8191
5146
9735
5472
12572
5650
3751
5663
100577
5996
11413
4676
14922
4839
Scn
4600
4800
5000
5200
5400
5600
5800
6000

A-11

DLC

HWI 6329-133 & 6329-143

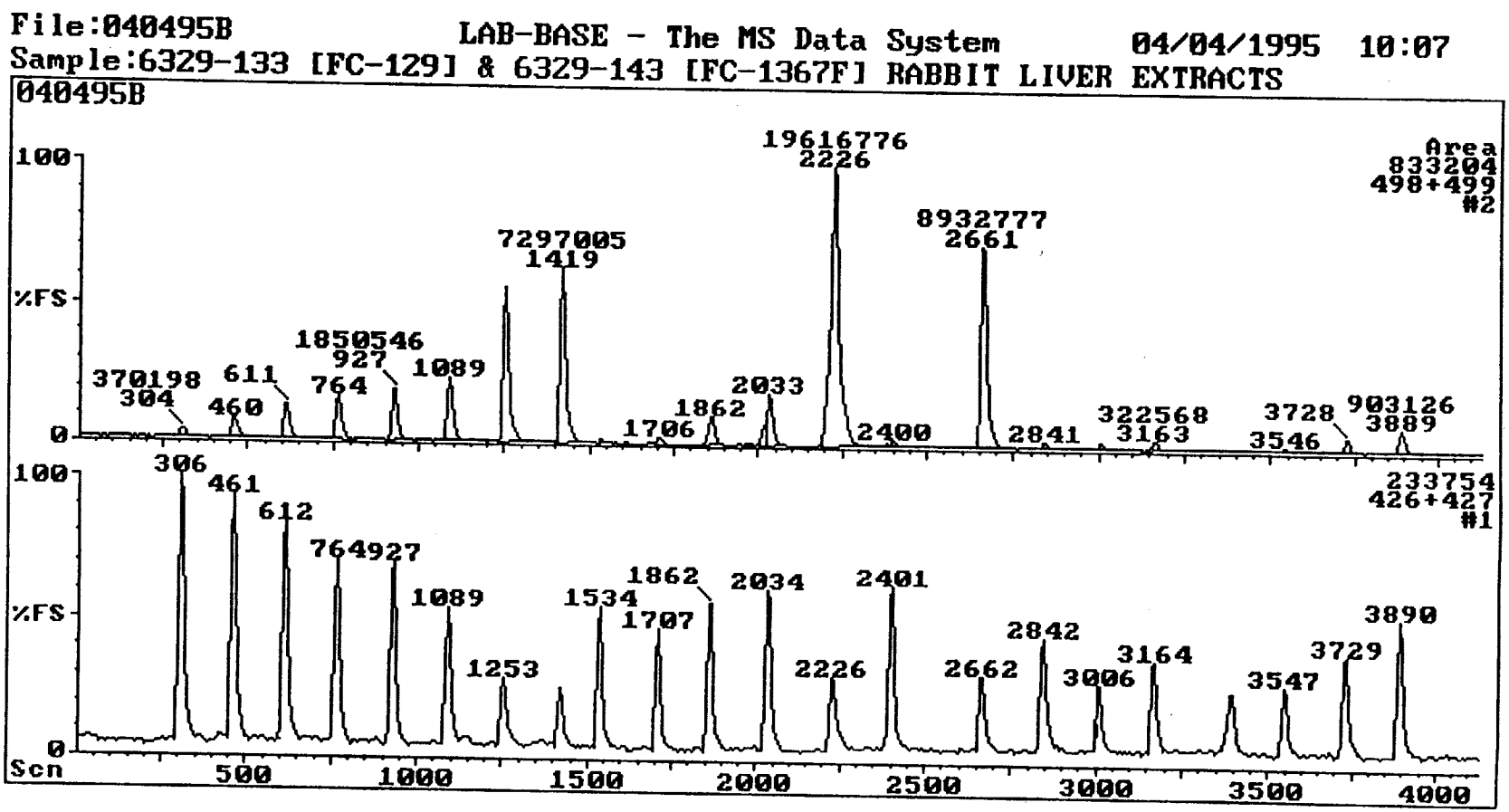
Copy of Original

DLC — Initial

6/2295 — Date

6329-133 Animals Analyzed

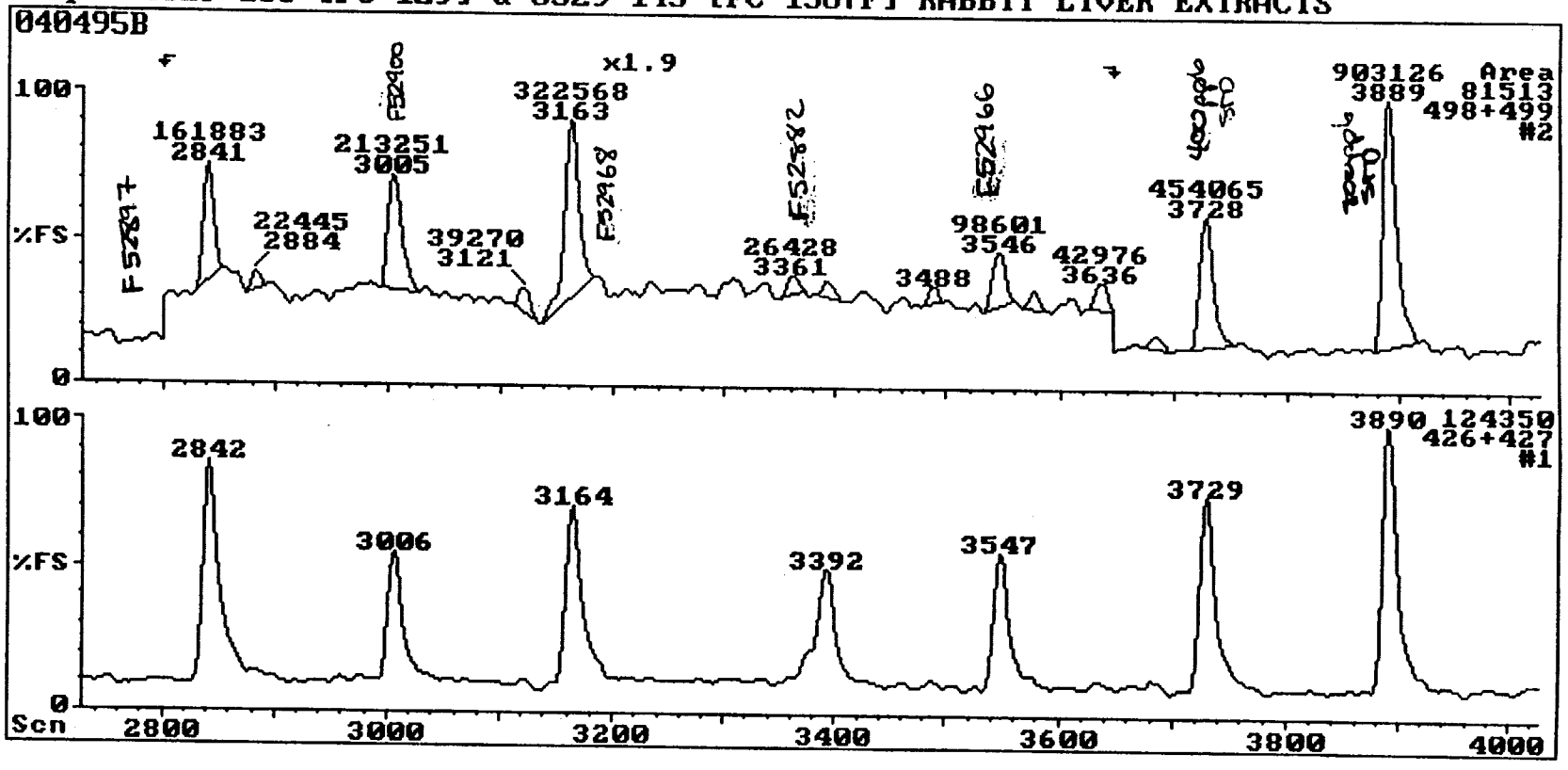
F52897
F52900
F52968
F52882
F52966



C003374

A-45
A-12
10-18-95
DLC

File:040495B LAB-BASE - The MS Data System 04/04/1995 10:07
Sample:6329-133 [FC-129] & 6329-143 [FC-1367F] RABBIT LIVER EXTRACTS



000375

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 section 6.0 and therefore is not discussed in detail.

DDW 7/18/95
Skalar Data

RE: 6329-133 LIVER SAMPLES
AMDT 13195.1
Date of Analysis: 3/28, 3/29 and 3/30/95
Analyst: DDW

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

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

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

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

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

Deborah D Wright

SUMMARY of 6329-133
LIVER SAMPLES
AMDT 013195.1

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	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Average Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	Average Total F- per tissue (ug)

** This is only a partial study. All samples were not saved from the Dohrmann analysis. DDW

	F52884-1	ND	3.0	0.1463	ND		64.9889	ND	
	F52884-2	ND	3.0	0.1011	ND	ND	64.9889	ND	ND
	F52884-3	ND	3.0	0.1345	ND		64.9889	ND	
	F52891-1	ND	3.0	0.1140	ND		64.4869	ND	
	F52891-2	ND	3.0	0.1443	ND	ND	64.4869	ND	ND
	F52891-3	ND	3.0	0.1284	ND		64.4869	ND	
GROUP 3	F52892-1	0.02	3.0	0.1322	0.43	0.20	68.7067	29	15
Dose Level : 1.28 mg/kg	F52892-2	ND	3.0	0.1342	ND		68.7067	ND	
	F52900-1	ND	3.0	0.1474	ND		61.3555	ND	
	F52900-2	ND	3.0	0.1153	ND	ND	61.3555	ND	ND
	F52900-3	ND	3.0	0.1150	ND		61.3555	ND	
	F52965-1	0.02	3.0	0.1246	0.40	0.36	68.6665	28	25
	F52965-3	0.02	3.0	0.1510	0.31		68.6665	21	
	F52968-1	ND	3.0	0.1448	ND		66.6415	ND	
	F52968-2	ND	3.0	0.1159	ND	ND	66.6415	ND	ND
	F52968-3	ND	3.0	0.1211	ND		66.6415	ND	

000378

Stolar Oct

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SUMMARY of 6329-133
LIVER SAMPLES
AMDT 013195.1

	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
	F52889-1	0.04	3.0	0.1139	1.17		68.6699	80	
	F52889-2	0.05	3.0	0.1125	1.34	1.56	68.6699	92	107
	F52889-3	0.10	3.0	0.1410	2.18		68.6699	149	
	F52894-1	0.05	3.0	0.1272	1.20		73.9834	89	
GROUP 4	F52894-2	0.06	3.0	0.1183	1.43	1.27	73.9834	105	94
Dose Level : 12.8 mg/kg	F52894-3	0.05	3.0	0.1185	1.17		73.9834	87	
	F52895-1	0.03	3.0	0.1513	0.62		58.0944	36	
	F52895-2	0.03	3.0	0.1091	0.87	0.65	58.0944	51	38
	F52895-3	0.02	3.0	0.1537	0.44		58.0944	26	
	F52874-1	0.02	3.0	0.1438	0.46		69.6893	32	
	F52874-2	0.02	3.0	0.1221	0.50	0.44	69.6893	35	31
	F52874-3	0.02	3.0	0.1454	0.35		69.6893	25	
GROUP 5	F52879-1	0.02	3.0	0.1338	0.34	0.17	89.1246	30	16
Dose Level : 10.0 cm x 10.0 cm fabric	F52879-2	ND	3.0	0.1050	ND		89.1246	ND	
	F52888-1	ND	3.0	0.1438	ND	ND	64.2262	ND	ND
	F52963-2	ND	3.0	0.1228	ND	ND	70.1610	ND	ND
	F52963-3	ND	3.0	0.1345	ND		70.1610	ND	

600379

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% 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	1.20	80%										
2	Drift	1.50	1.23	82%										
3	Wash		ND											
4	Std 1	0.015	0.016	104%										
5	Std 2	0.03	0.03	101%										
6	Std 3	0.06	0.06	97%										
7	Std 4	0.09	0.09	99%										
8	Std 5	0.12	0.13	104%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	98%										
11	Std 8	0.60	0.60	101%										
12	Std 9	1.20	1.23	102%										
13	Std 10	1.50	1.47	98%										
14	Drift	1.50	1.25	83%										
15	Wash		ND											
16	Blk-1		ND		3									
17	Blk-2		ND		3									
18	Spk-1		ND		3					0.004	63	0.15	ND	0%
19	Spk-2		ND		3					0.004	63	0.15	ND	0%
20	Spk-3		0.06		3					0.004	63	0.15	0.17	112%
21	F52900-1		ND		3	0.1474	ND	61.3555	ND					
22	F52900-2		ND		3	0.1153	ND	61.3555	ND					
23	F52900-3		ND		3	0.1150	ND	61.3555	ND					
24	F52968-1		ND		3	0.1448	ND	66.6415	ND					
25	F52968-2		ND		3	0.1159	ND	66.6415	ND					
26	Drift	1.50	1.26	84%										
27	Wash		ND				ND		ND					
28	F52968-3		ND		3	0.1211	ND	66.6415	ND					
29	F52884-1		ND		3	0.1463	ND	64.9889	ND					
30	F52884-2		ND		3	0.1011	ND	64.9889	ND					
31	F52884-3		ND		3	0.1345	ND	64.9889	ND					
32	F52892-2		ND		3	0.1342	ND	68.7067	ND					
33	F52548-2		ND		3		ND		ND					
34	F52888-1		ND		3	0.1438	ND	64.2262	ND					
35	F52963-2		ND		3	0.1228	ND	70.1610	ND					
36	F52963-3		ND		3	0.1345	ND	70.1610	ND					

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000380

AMBT 13195.1
HWI 6329-133
Guerro

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% 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
37	Blk		ND		3									
38	Drift	1.50	1.26	84%										
39	Wash		ND											

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G00381

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% 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	1.21	81%										
2	Drift	1.50	1.23	82%										
3	Wash		ND											
4	Std 1	0.015	0.016	108%										
5	Std 2	0.03	0.03	98%										
6	Std 3	0.06	0..0578											
7	Std 4	0.09	0.09	100%										
8	Std 5	0.12	0.12	104%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	98%										
11	Std 8	0.60	0.60	101%										
12	Std 9	1.20	1.23	102%										
13	Std 10	1.50	1.47	98%										
14	Drift	1.50	1.25	83%										
15	Wash		ND											
16	Blk-1		0.07		3.0									
17	Blk-2		0.02		3.0									
18	Blk-3		0.02		3.0									
19	Spk-1		0.05		3.0					0.004	63.00	0.15	0.15	100%
20	Spk-2		0.05		3.0					0.004	63.00	0.15	0.16	103%
21	Spk-3		0.05		3.0					0.004	63.00	0.15	0.15	96%
22	F52891-1		ND		3.0	0.1140	ND	64.4869	ND					
23	F52891-2		ND		3.0	0.1443	ND	64.4869	ND					
24	F52891-3		ND		3.0	0.1284	ND	64.4869	ND					
25	F52892-1		0.02		3.0	0.1322	0.43	68.7067	29.31					
26	Drift	1.50	1.25	83%										
27	Wash		ND											
28	F52965-1		0.02		3.0	0.1246	0.40	68.6665	27.78					
29	F52965-3		0.02		3.0	0.1510	0.31	68.6665	21.15					
30	Drift		1.26											
31	Wash		ND											

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000382

MST 13195.1
HWT 6329-13
Shimo

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% 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	1.25	83%										
2	Drift	1.50	1.28	85%										
3	Wash		0.02											
4	Std 1	0.015	0.018	123%										
5	Std 2	0.03	0.03	88%										
6	Std 3	0.06	0.06	100%										
7	Std 4	0.09	0.09	98%										
8	Std 5	0.12	0.12	104%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	96%										
11	Std 8	0.60	0.61	101%										
12	Std 9	1.20	1.24	104%										
13	Std 10	1.50	1.46	97%										
14	Drift	1.50	1.31	87%										
15	Wash		0.02											
16	Blk-1		0.02		3.0									
17	Blk-2		0.02		3.0									
18	Blk-3		ND		3.0									
19	Spk-1		0.06		3.0					0.004	63.00	0.15	0.17	113%
20	Spk-2		0.05		3.0					0.004	63.00	0.15	0.15	100%
21	Spk-3		0.05		3.0					0.004	63.00	0.15	0.16	107%
22	Spk-4		0.06		3.0					0.004	63.00	0.15	0.18	120%
23	Spk-5		0.06		3.0					0.004	63.00	0.15	0.19	124%
24	Spk-6		0.06		3.0					0.004	63.00	0.15	0.18	117%
25	F52874-1		0.02		3.0	0.1438	0.46	69.6893	32.13					
26	Drift	1.50	1.27	84%										
27	Wash		0.02											
28	F52874-2		0.02		3.0	0.1221	0.50	69.6893	34.93					
29	F52874-3		0.02		3.0	0.1454	0.35	69.6893	24.59					
30	F52879-1		0.02		3.0	0.1338	0.34	89.1246	30.17					
31	F52879-2		ND		3.0	0.1050	ND	89.1246	ND					
32	F52889-1		0.04		3.0	0.1139	1.17	68.6699	80.31					
33	F52889-2		0.05		3.0	0.1125	1.34	68.6699	91.74					
34	F52889-3		0.10		3.0	0.1410	2.18	68.6699	149.47					
35	F52894-1		0.05		3.0	0.1272	1.20	73.9834	88.64					
36	F52894-2		0.06		3.0	0.1183	1.43	73.9834	105.44					

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C00383

ARDT 12195.1
HWI 6329-133
Jimeno

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% 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
37	F52894-3		0.05		3.0	0.1185	1.17	73.9834	86.91					
38	Drift	1.50	1.28	85%										
39	Wash		0.02											
40	F52895-1		0.03		3.0	0.1513	0.62	58.0944	36.17					
41	F52895-2		0.03		3.0	0.1091	0.87	58.0944	50.64					
42	F52895-3		0.02		3.0	0.1537	0.44	58.0944	25.74					
43	Drift	1.50	1.31	87%										
44	Wash													

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C00384

DDW 7/15/95
AMDT 13195.1
HWL 6329-133
Lewis

1995-03-28 17:30 **OutPut of : 950328F1**

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-03-28 15:28

Analysis File Name : C:\SKALAR\DATA\950328F1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

```
          Ö x - c1 ¢      x  = corrected value of the sample
          º ááááá º      c1 = corrected value of the concentration 1
Result = 10â   s   ì      s  = Slope of the electrode
```

a2 = -0.00000
a1 = 0.00092
a0 = -1.24810

Fluoride L
Calibration order = 2

Correlation : r = 0.99716

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00022
a0 = 0.00604

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

1995-03-28 17:30 OutPut of : 950328F1

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	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
wt	iw	Initial Wash	1	1.000	3	0.056		0	219	65	
					4	0.0060		0	0	0	
1	t	Tracer	1	1.000	3	1.199		2065	2282	203	TV=1.2 ppm
					4	0.9756		2065	0	0	
2	d	Drift	1	1.000	3	1.230		2096	2312	380	TV=1.2 ppm
					4	0.9981		2096	0	0	
3	w	Wash	1	1.000	3	0.056		0	214	557	
					4	0.0060		0	0	0	
4	s1	Standard 1	1	1.000	3	0.062		43	258	732	
					4	0.0156		43	0	0	
5	s2	Standard 2	1	1.000	3	0.070		106	322	907	
					4	0.0303		106	0	0	
6	s3	Standard 3	1	1.000	3	0.088		214	432	1081	
					4	0.0579		214	0	0	
7	s4	Standard 4	1	1.000	3	0.109		325	544	1257	
					4	0.0893		325	0	0	
8	s5	Standard 5	1	1.000	3	0.135		440	661	1432	
					4	0.1250		440	0	0	
9	s6	Standard 6	1	1.000	3	0.152		506	728	1606	
					4	0.1469		506	0	0	
10	s7	Standard 7	1	1.000	3	0.293		895	1121	1781	
					4	0.2979		895	0	0	
11	s8	Standard 8	1	1.000	3	0.604		1410	1641	1956	
					4	0.5548		1410	0	0	
12	s9	Standard 9	1	1.000	3	1.229		2095	2332	2132	
					4	0.9974		2095	0	0	
13	s10	Standard 10	1	1.000	3	1.473		2335	2576	2305	
					4	1.1797		2335	0	0	
14	d	Drift	1	1.000	3	1.248		2113	2338	2482	TV=1.2 ppm
					4	1.0106		2113	0	0	
15	w	Wash	1	1.000	3	0.056		0	226	2661	
					4	0.0060		0	0	0	

Fluoride 1.5
Fluoride L

PPM
PPM

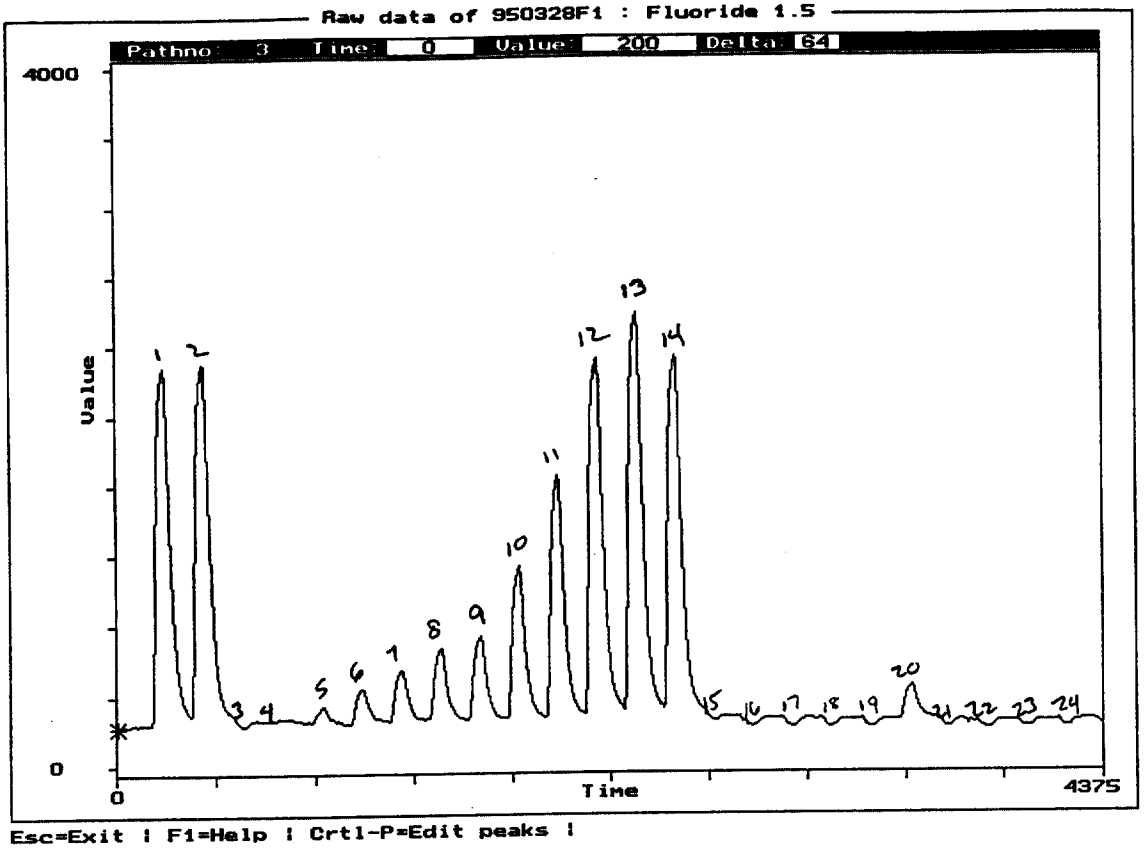
Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
16	u	BLK 1	1	1.000	3	too e	<	14	240	2779	
					4	0.0091		14	0	0	ND
17	u	BLK 2	1	1.000	3	Absen	A	-32	194	3007	
					4	#.####		-32	0	0	ND
18	u	SPK 1	1	1.000	3	Absen	A	-23	202	3182	
					4	0.0011		-23	0	0	ND
19	u	SPK 2	1	1.000	3	Absen	A	-33	192	3357	
					4	#.####		-33	0	0	ND
20	u	SPK 3	1	1.000	3	0.087		209	436	3533	
					4	0.0566		209	0	0	0.1698
21	u	F52900-1	1	1.000	3	too l	>	17	242	3765	
					4	0.0098		17	0	0	ND
22	u	F52900-2	1	1.000	3	too e	<	-1	224	3820	
					4	0.0058		-1	0	0	ND
23	u	F52900-3	1	1.000	3	Absen	A	-33	192	4057	
					4	#.####		-33	0	0	ND
24	u	F52968-1	1	1.000	3	0.058		12	236	4184	
					4	0.0087		12	0	0	ND
25	u	F52968-2	1	1.000	3	Absen	A	-32	192	4407	
					4	#.####		-32	0	0	ND
26	d	Drift	1	1.000	3	1.259		2124	2348	4581	TV=1.2 ppm
					4	1.0187		2124	0	0	
27	w	Wash	1	1.000	3	0.056		0	224	4751	
					4	0.0060		0	0	0	
28	u	F52968-3	1	1.000	3	too e	<	32	256	4871	
					4	0.0131		32	0	0	ND
29	u	F52884-1	1	1.000	3	0.061		33	256	5089	.0399
					4	0.0133		33	0	0	ND .030
30	u	F52884-2	1	1.000	3	0.061		34	256	5282	.0405
					4	0.0135		34	0	0	ND .02
31	u	F52884-3	1	1.000	3	0.061		34	256	5435	.0405
					4	0.0135		34	0	0	ND .03

Dehrman Samples
Skalar @ 3x (not .045) 0.1 (.0

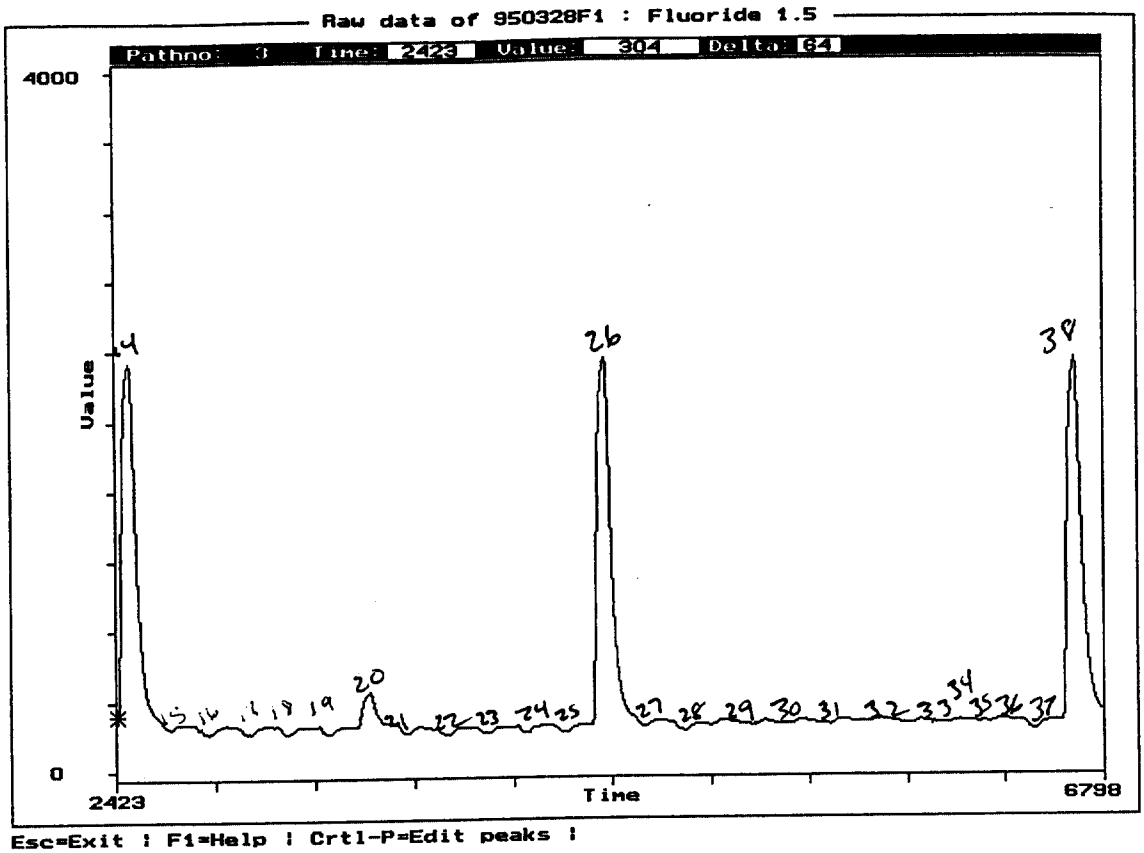
Fluoride 1.5
Fluoride L

PPM
PPM

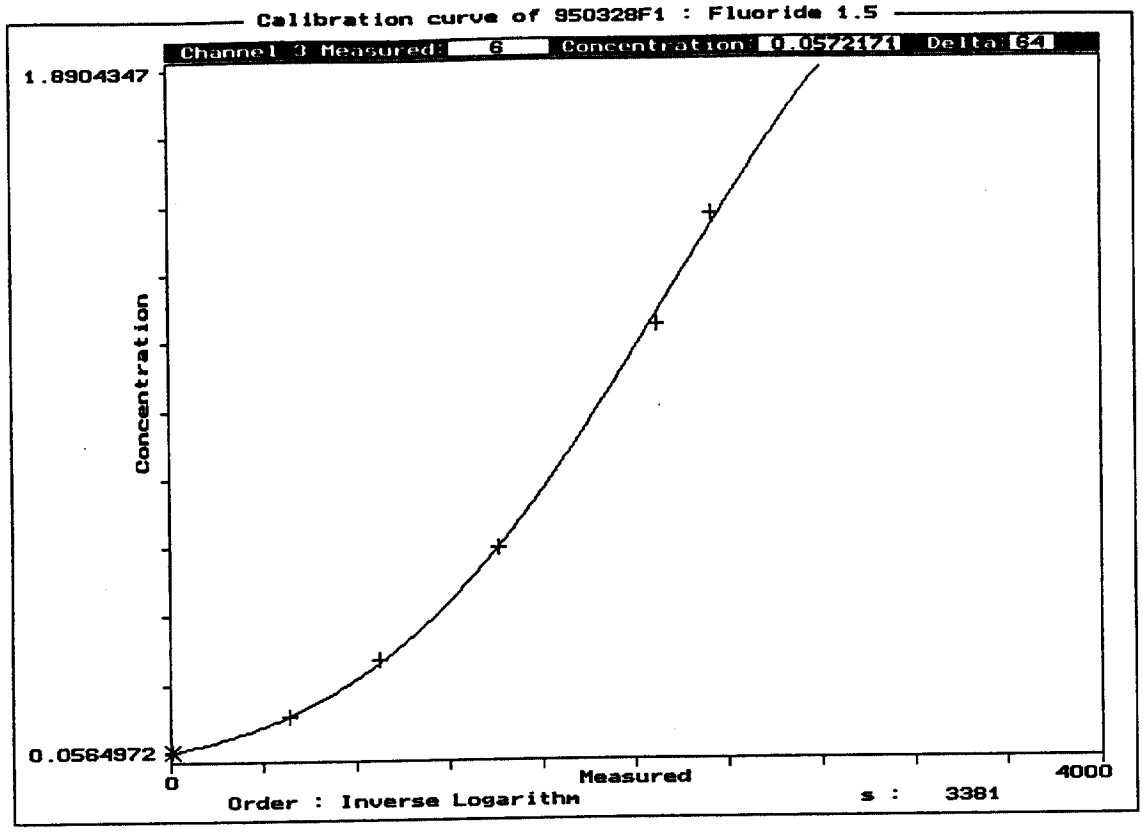
Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	Dohrman	
32	u	F52892-2	1	1.000	3	0.061		38	260	5635	ND	035
					4	0.0144		38	0	0	.0432	N
33	u	F52548-2	1	1.000	3	0.061		39	260	5805	ND	
					4	0.0147		39	0	0		
34	u	F52888-1	1	1.000	3	0.061		36	256	5983	.0420	.022
					4	0.0140		36	0	0	ND	ND
35	u	F52963-2	1	1.000	3	0.061		36	256	6159	ND	.021
					4	0.0140		36	0	0	.042	N
36	u	F52963-3	1	1.000	3	0.061		36	256	6333	ND	.020
					4	0.0140		36	0	0	.042	N
37	u	~~F52963-4~~ Blank TISAB	1	1.000	3	Absen	A	-23	196	6506	ND	
					4	0.0011		-23	0	0		
38	d	Drift	1	1.000	3	1.257		2122	2340	6684	TV=1.2ppm	
					4	1.0172		2122	0	0		
39	w	Wash	1	1.000	3	0.056		0	218	6858		
					4	0.0060		0	0	0		
wt	rw	RunOut Wash	1	1.000	3	0.056		0	0	7159		
					4	0.0060		0	0	0		

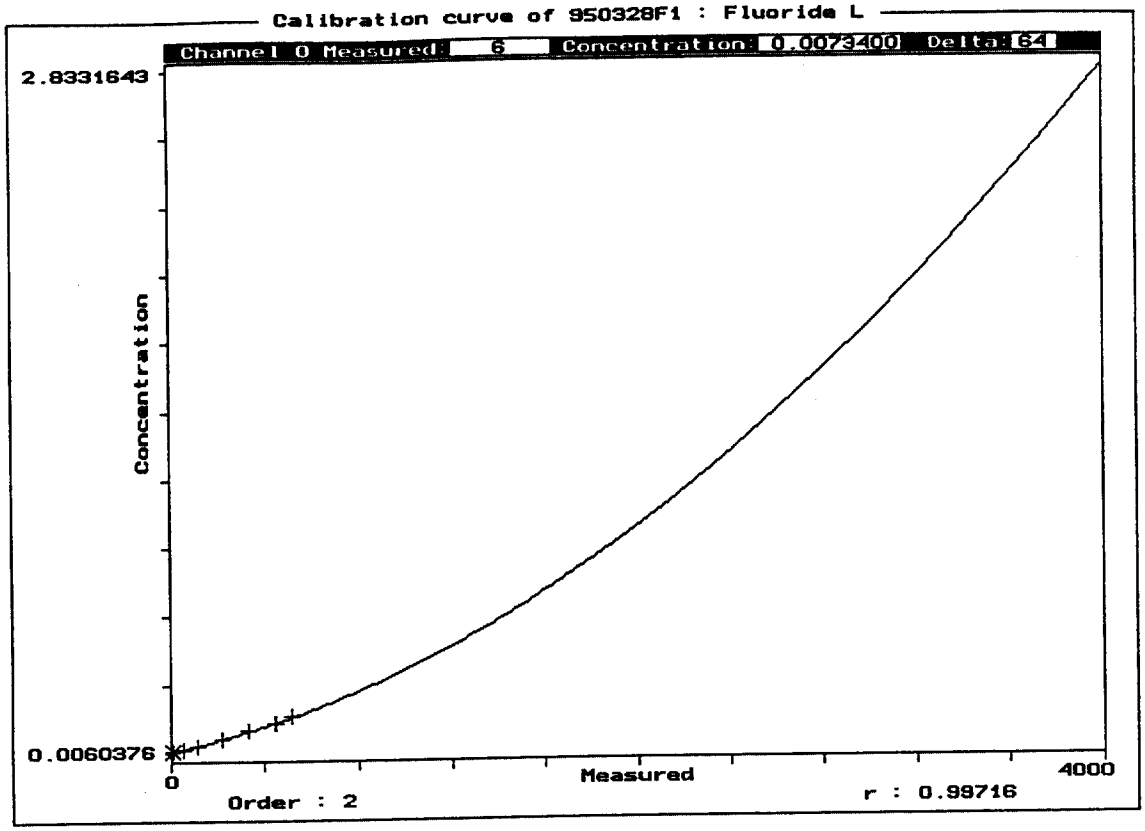


Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !



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


Calibration curve of 950328F1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0572171 Delta: 64
1.8904347
0.0564972
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 3381


Calibration curve of 950328F1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0073400
Delta: 64
2.8331643
0.0060376
Concentration
0
Measured
4000
Order : 2
r : 0.99716

DDW 7/18/95
AMDT 13195.1
HWI 6329-133
Lewis

1995-03-29 12:46 **OutPut of : 950329A1**

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-03-29 10:24

Analysis File Name : C:\SKALAR\DATA\950329A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

```
          Ö x - c1 ¢      x  = corrected value of the sample
          º ááááá º      c1 = corrected value of the concentration 1
Result = 10â   s    ì      s  = Slope of the electrode
```

a2 = -0.00000
a1 = 0.00087
a0 = -1.21638

Fluoride L
Calibration order = 2

Correlation : r = 0.99645

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00020
a0 = 0.00843

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

1995-03-29 12:46 OutPut of : 950329A1

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

1995-03-29 12:46 OutPut of : 950329A1

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

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
wt	iw	Initial Wash	1	1.000	3	0.061		0	216	65	
					4	0.0084		0	0	0	
1	t	Tracer	1	1.000	3	1.209		2068	2285	208	TV=1.2 ppm
					4	1.1307		2068	0	0	
2	d	Drift	1	1.000	3	1.234		2091	2308	382	
					4	1.1510		2091	0	0	
3	w	Wash	1	1.000	3	0.061		0	218	555	
					4	0.0084		0	0	0	
4	s1	Standard 1	1	1.000	3	0.065		37	258	732	
					4	0.0162		37	0	0	
5	s2	Standard 2	1	1.000	3	0.073		96	320	901	
					4	0.0294		96	0	0	
6	s3	Standard 3	1	1.000	3	0.091		208	436	1086	
					4	0.0578		208	0	0	
7	s4	Standard 4	1	1.000	3	0.112		320	552	1260	
					4	0.0902		320	0	0	
8	s5	Standard 5	1	1.000	3	0.136		425	660	1436	
					4	0.1244		425	0	0	
9	s6	Standard 6	1	1.000	3	0.152		489	728	1608	
					4	0.1470		489	0	0	
10	s7	Standard 7	1	1.000	3	0.293		893	1136	1784	
					4	0.3207		893	0	0	
11	s8	Standard 8	1	1.000	3	0.603		1415	1664	1957	
					4	0.6246		1415	0	0	
12	s9	Standard 9	1	1.000	3	1.228		2085	2342	2131	
					4	1.1457		2085	0	0	
13	s10	Standard 10	1	1.000	3	1.474		2308	2570	2309	
					4	1.3519		2308	0	0	
14	d	Drift	1	1.000	3	1.247		2103	2356	2483	TV=1.2 ppm
					4	1.1617		2103	0	0	
15	w	Wash	1	1.000	3	0.061		0	256	2663	
					4	0.0084		0	0	0	

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Fluoride 1.5
Fluoride L

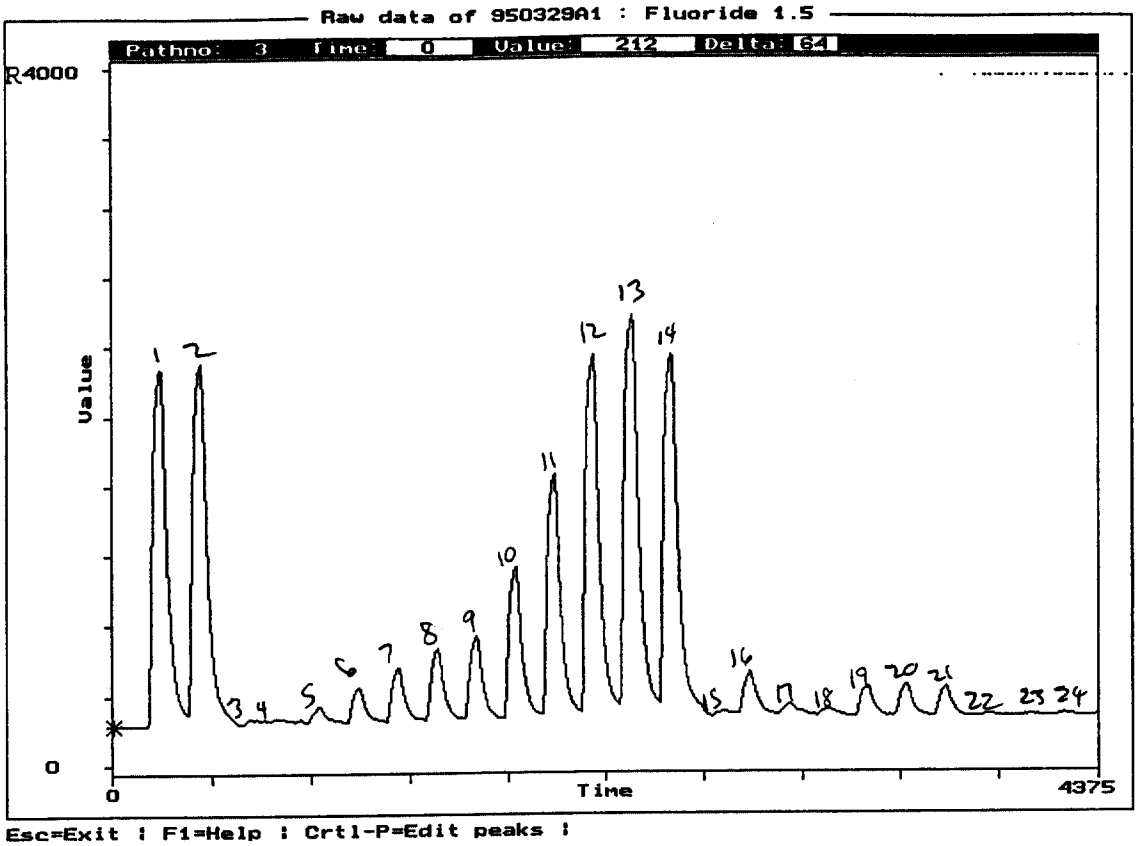
PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
16	u	BLK 1	1	1.000	3	0.100		255	512	2829	
					4	0.0709		255	0	0	+20% .2127
17	u	BLK 2	1	1.000	3	0.070		68	321	3010	
					4	0.0230		68	0	0	+35% .069
18	u	BLK 3	1	1.000	3	0.067		48	300	3185	
					4	0.0186		48	0	0	.0558
19	u	SPK 1	1	1.000	3	0.086		180	432	3357	
					4	0.0503		180	0	0	+8% .1509
20	u	SPK 2	1	1.000	3	0.087		186	436	3533	
					4	0.0519		186	0	0	+15% .1557
21	u	SPK 3	1	1.000	3	0.085		173	422	3711	
					4	0.0485		173	0	0	+9% .1455
22	u	F52891-1	1	1.000	3	Absen	A	13	260	3883	
					4	0.0111		13	0	0	.0333 ⇒ ND
23	u	F52891-2	1	1.000	3	Absen	A	13	258	4058	
					4	0.0111		13	0	0	.0333 ⇒ ND
24	u	F52891-3	1	1.000	3	0.064		28	272	4231	
					4	0.0142		28	0	0	.0426 ⇒ ND
25	u	F52892-1	1	1.000	3	0.067		49	292	4410	
					4	0.0188		49	0	0	.0564
26	d	Drift	1	1.000	3	1.252		2107	2348	4584	TV=1.2 ppm
					4	1.1653		2107	0	0	
27	w	Wash	1	1.000	3	0.061		0	240	4747	
					4	0.0084		0	0	0	
28	u	F52965-1	1	1.000	3	0.066		40	280	4938	
					4	0.0168		40	0	0	.0504
29	u	F52965-3	1	1.000	3	0.065		34	274	5110	
					4	0.0155		34	0	0	.0465
30	d	Drift	1	1.000	3	1.263		2118	2358	5284	TV=1.2 ppm
					4	1.1752		2118	0	0	
31	w	Wash	1	1.000	3	0.061		0	240	5455	
					4	0.0084		0	0	0	

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
wt	rw	RunOut Wash	1	1.000	3	0.061		0	256	5759
					4	0.0084		0	0	0


Raw data of 950329A1 : Fluoride 1.5
Pathno: 3 Time: 0 Value: 212 Delta: 64
R4000
Value
0
0
Time
4375
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !

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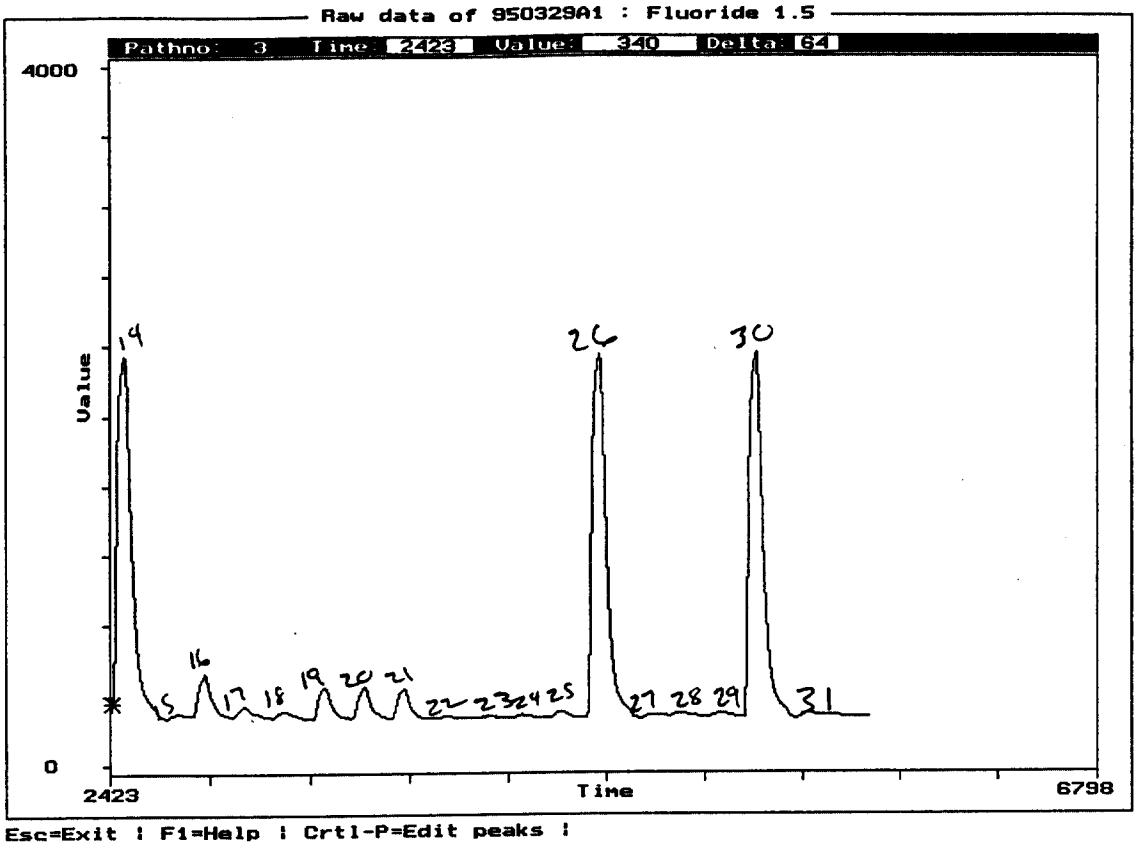
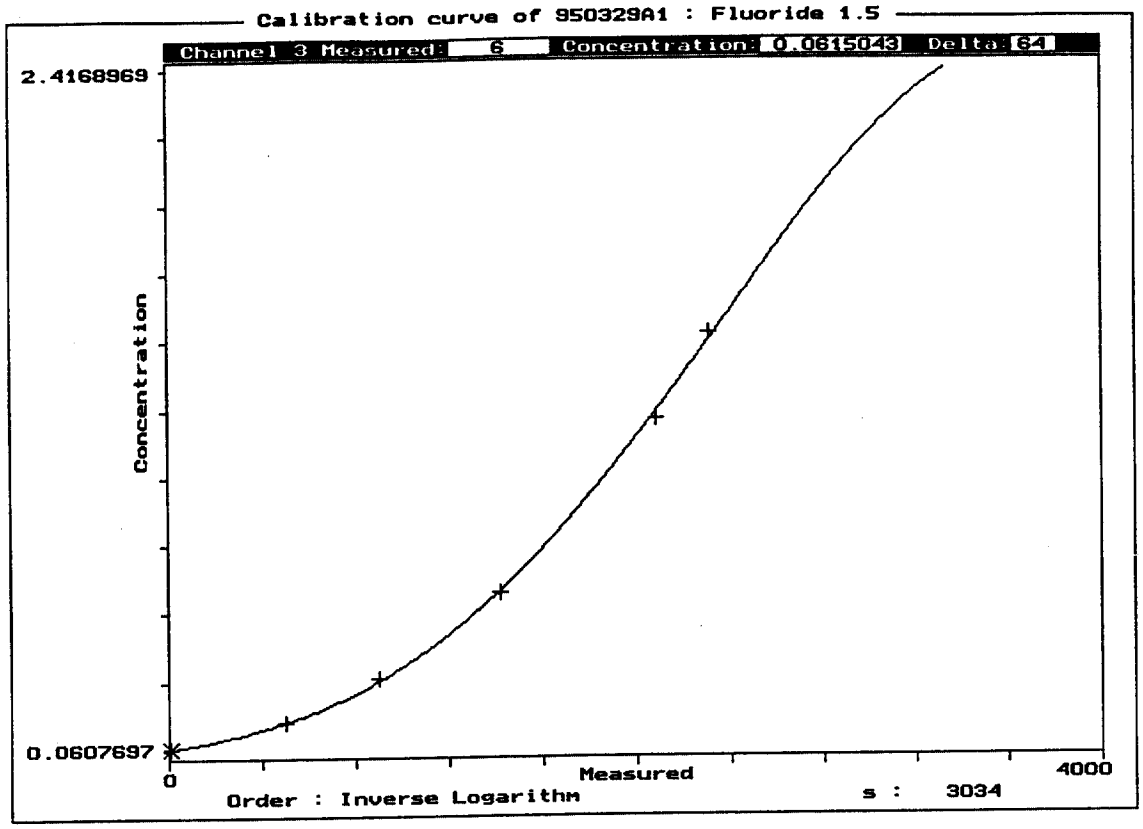
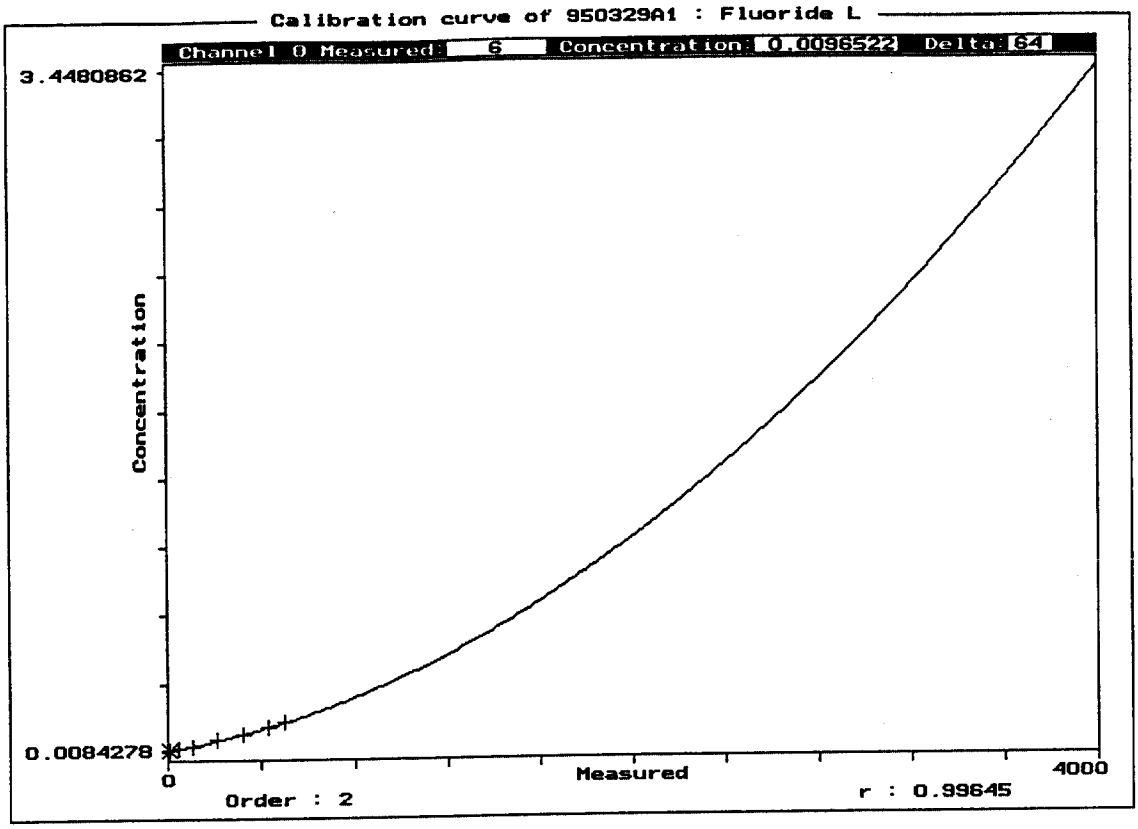



Calibration curve of 950329A1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0615043
Delta: 64
2.4168969
0.0607697
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 3034


Calibration curve of 950329A1 : Fluoride L
Channel 0 Measured: 6 Concentration: 0.0096522 Delta: 64
3.4480862
0.0084278
Concentration
0
4000
Measured
Order : 2
r : 0.99645

DDW 7/12/95
AMDT 13.9S.1
HWI 6329-133
Lucas

1995-03-30 10:59 **OutPut of : 950330A1**

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-03-30 08:43

Analysis File Name : C:\SKALAR\DATA\950330A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

Ö x - c1 ¢ x = corrected value of the sample
° ááááá ° c1 = corrected value of the concentration 1
Result = 10â s ì s = Slope of the electrode

a2 = -0.00000
a1 = 0.00088
a0 = -1.12348

Fluoride L
Calibration order = 2

Correlation : r = 0.99781

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00033
a0 = 0.01842

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

1995-03-30 10:59 OutPut of : 950330A1

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	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
wt	iw	Initial Wash	1	1.000	3	0.075		0	155	65
					4	0.0184		0	0	0
1	t	Tracer	1	1.000	3	1.248		2101	2274	211
					4	0.8362		2101	0	0
2	d	Drift	1	1.000	3	1.279		2139	2329	386
					4	0.8533		2139	0	0
3	w	Wash	1	1.000	3	0.075		0	208	569
					4	0.0184		0	0	0
4	s1	Standard 1	1	1.000	3	0.075		0	209	755
					4	0.0184		0	0	0
5	s2	Standard 2	1	1.000	3	0.079		24	234	906
					4	0.0264		24	0	0
6	s3	Standard 3	1	1.000	3	0.096		124	336	1084
					4	0.0599		124	0	0
7	s4	Standard 4	1	1.000	3	0.113		207	420	1262
					4	0.0882		207	0	0
8	s5	Standard 5	1	1.000	3	0.137		313	528	1435
					4	0.1248		313	0	0
9	s6	Standard 6	1	1.000	3	0.154		377	594	1612
					4	0.1472		377	0	0
10	s7	Standard 7	1	1.000	3	0.287		753	976	1785
					4	0.2835		753	0	0
11	s8	Standard 8	1	1.000	3	0.606		1308	1540	1962
					4	0.4990		1308	0	0
12	s9	Standard 9	1	1.000	3	1.242		2093	2338	2137
					4	0.8327		2093	0	0
13	s10	Standard 10	1	1.000	3	1.461		2383	2636	2312
					4	0.9645		2383	0	0
14	d	Drift	1	1.000	3	1.306		2173	2392	2487
					4	0.8686		2173	0	0
15	w	Wash	1	1.000	3	0.075		0	220	2729
					4	0.0184		0	0	0

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F Cor.	Valu	Time
16	u	blk 1	1	1.000	3	0.076	6	224	2822
					4	0.0204	6	0	0
17	u	blk 2	1	1.000	3	0.074	-7	208	3013
					4	0.0161	-7	0	0
18	u	blk 3	1	1.000	3	0.072	-21	192	3183
					4	0.0115	-21	0	0
19	u	spk 1	1	1.000	3	0.095	116	328	3363
					4	0.0572	116	0	0
20	u	spk 2	1	1.000	3	0.091	96	304	3536
					4	0.0505	96	0	0
21	u	spk 3	1	1.000	3	0.093	106	312	3706
					4	0.0539	106	0	0
22	u	spk 4	1	1.000	3	0.097	126	330	3888
					4	0.0606	126	0	0
23	u	spk 5	1	1.000	3	0.098	131	332	4063
					4	0.0623	131	0	0
24	u	spk 6	1	1.000	3	0.096	121	320	4235
					4	0.0589	121	0	0
25	u	F52874-1	1	1.000	3	0.077	11	208	4410
					4	0.0221	11	0	0
26	d	Drift	1	1.000	3	1.265	2122	2316	4587
					4	0.8456	2122	0	0
27	w	Wash	1	1.000	3	0.075	0	192	4766
					4	0.0184	0	0	0
28	u	F52874-2	1	1.000	3	0.076	6	196	4935
					4	0.0204	6	0	0
29	u	F52874-3	1	1.000	3	0.075	-4	184	5116
					4	0.0171	-4	0	0
30	u	F52879-1	1	1.000	3	0.074	-10	176	5274
					4	0.0151	-10	0	0
31	u	F52879-2	1	1.000	3	0.073	-16	169	5464
					4	0.0131	-16	0	0

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
32	u	F52889-1	1	1.000	3	0.088		78	261	5640
					4	0.0444		78	0	0
33	u	F52889-2	1	1.000	3	0.091		95	276	5812
					4	0.0501		95	0	0
34	u	F52889-3	1	1.000	3	0.122		248	426	5990
					4	0.1023		248	0	0
35	u	F52894-1	1	1.000	3	0.091		97	274	6164
					4	0.0508		97	0	0
36	u	F52894-2	1	1.000	3	0.094		113	288	6340
					4	0.0562		113	0	0
37	u	F52894-3	1	1.000	3	0.089		84	258	6514
					4	0.0464		84	0	0
38	d	Drift	1	1.000	3	1.276		2136	2308	6688
					4	0.8519		2136	0	0
39	w	Wash	1	1.000	3	0.075		0	170	6872
					4	0.0184		0	0	0
40	u	F52895-1	1	1.000	3	0.081		39	208	7034
					4	0.0314		39	0	0
41	u	F52895-2	1	1.000	3	0.082		40	208	7212
					4	0.0317		40	0	0
42	u	F52895-3	1	1.000	3	0.077		13	180	7388
					4	0.0227		13	0	0
43	d	Drift	1	1.000	3	1.310		2178	2344	7565
					4	0.8708		2178	0	0
44	w	Wash	1	1.000	3	0.075		0	165	7796
					4	0.0184		0	0	0
wt	rw	RunOut Wash	1	1.000	3	0.075		0	138	8040
					4	0.0184		0	0	0

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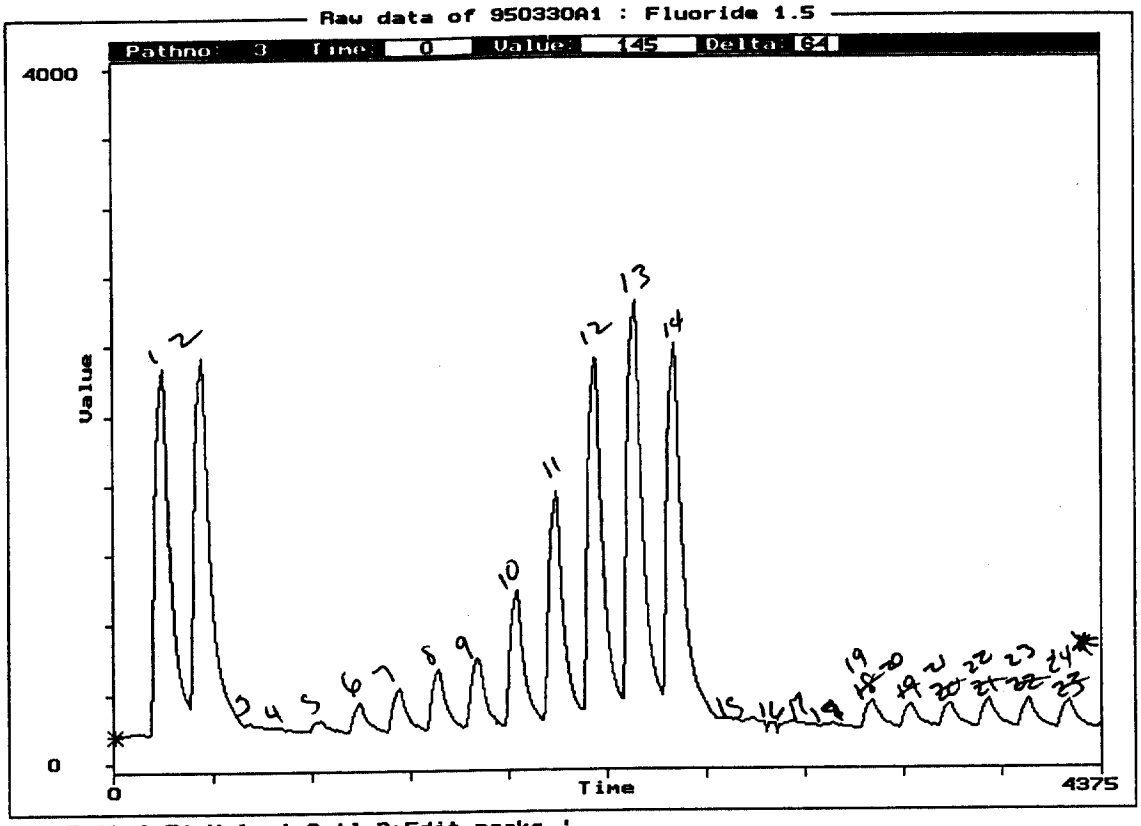

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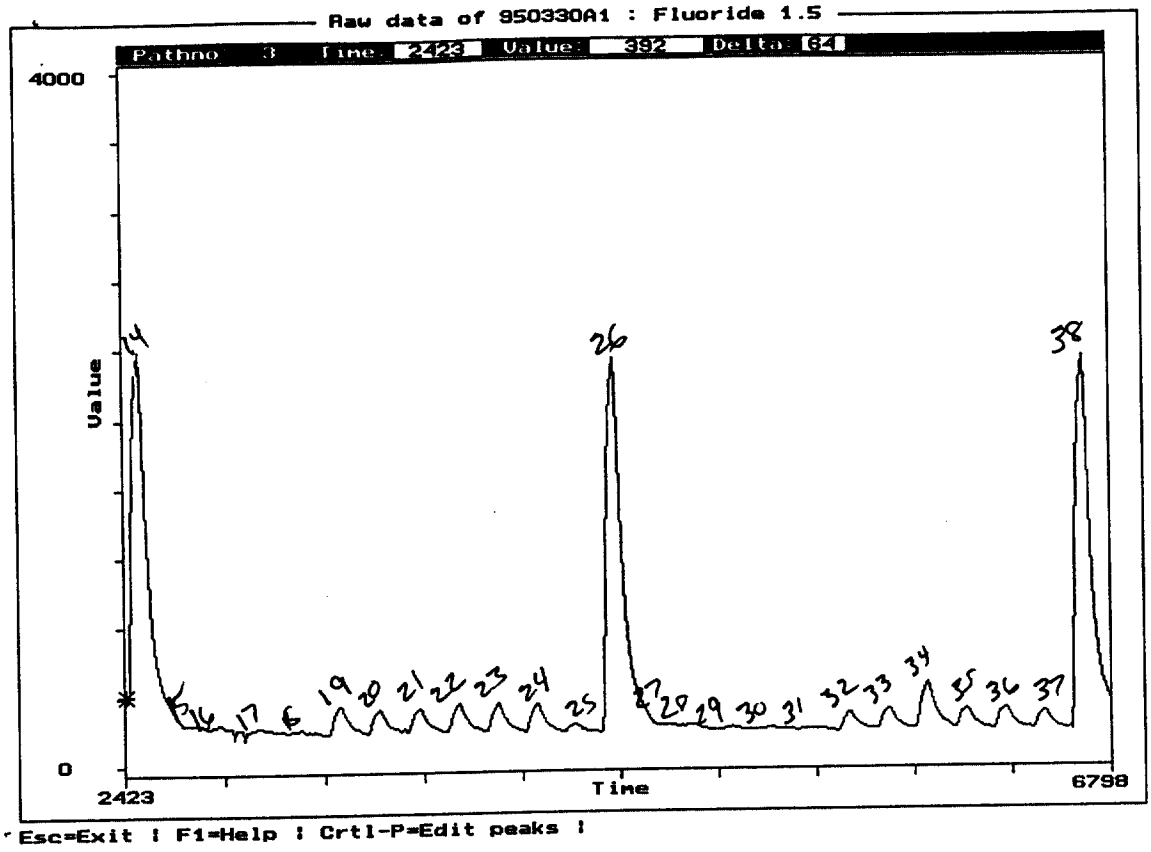
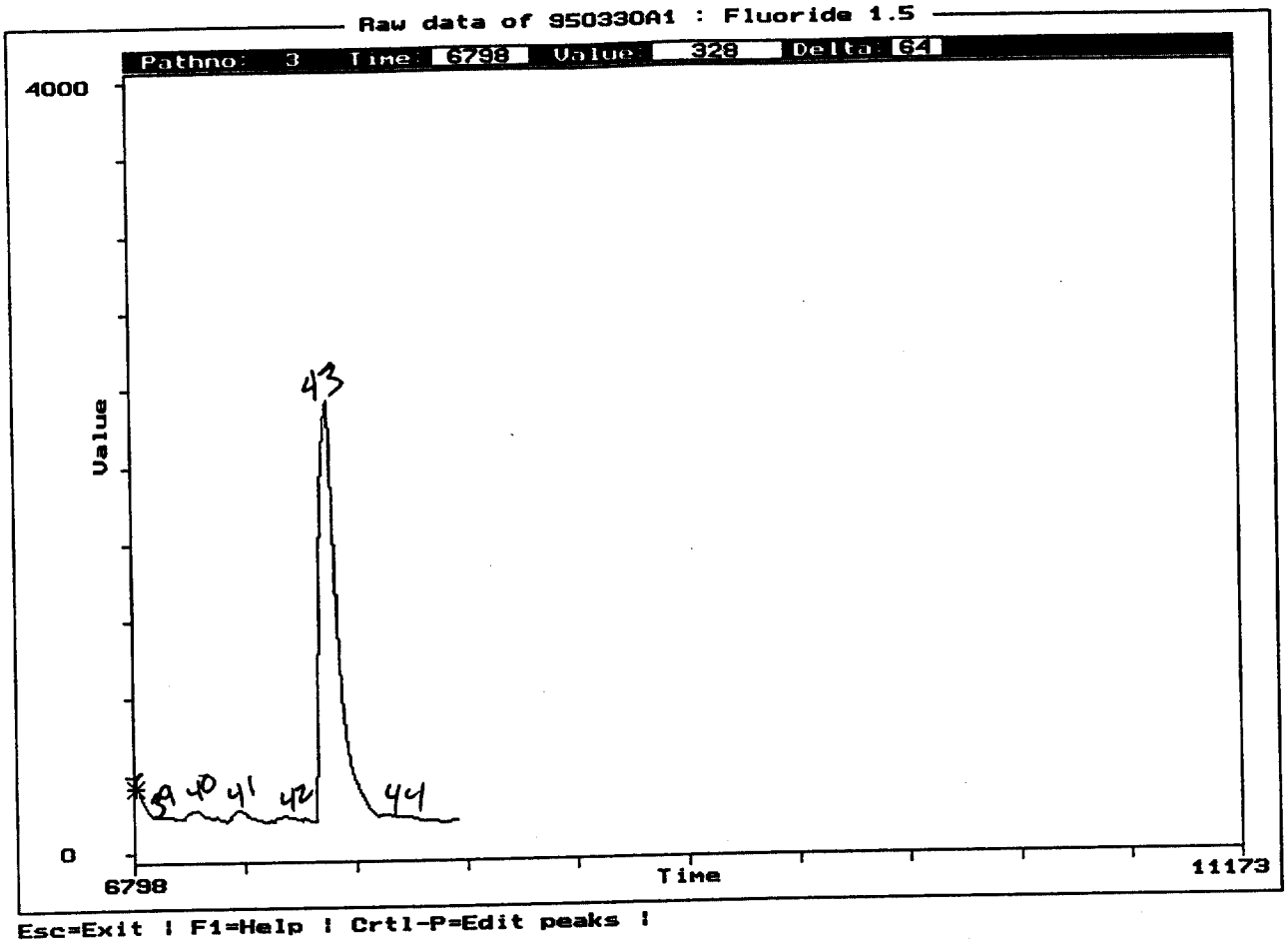
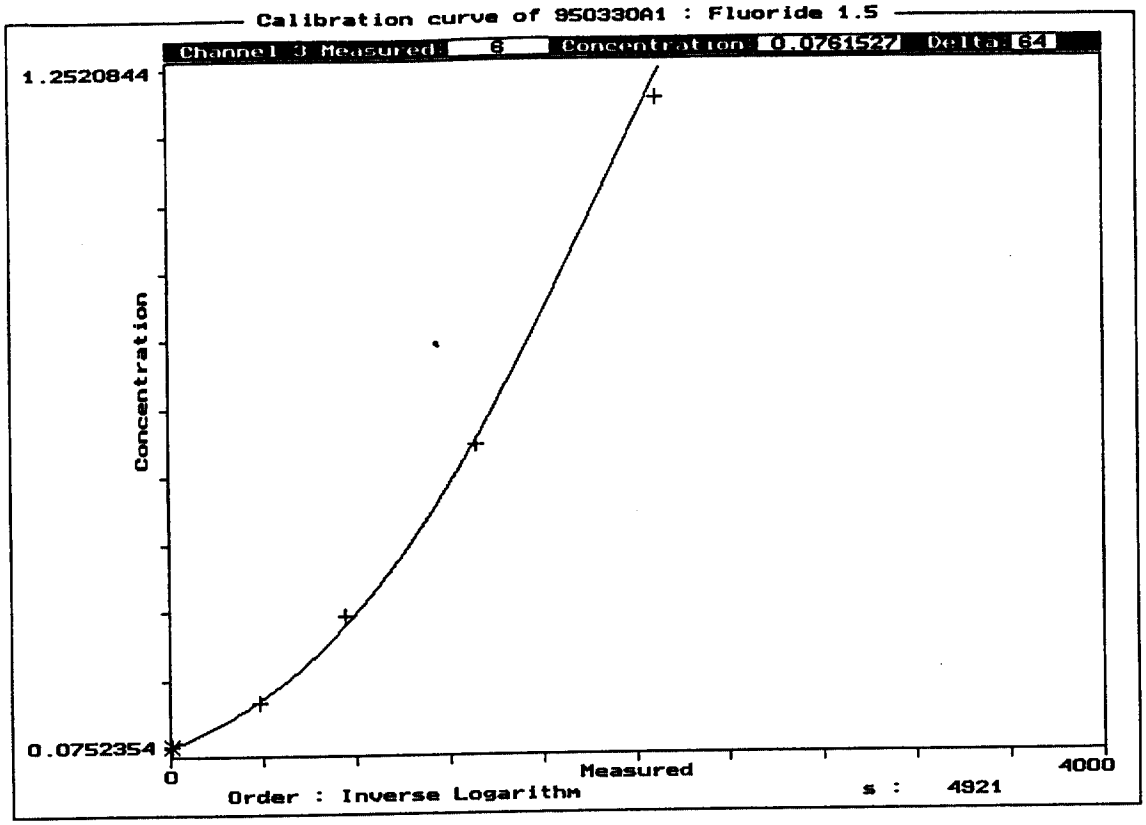
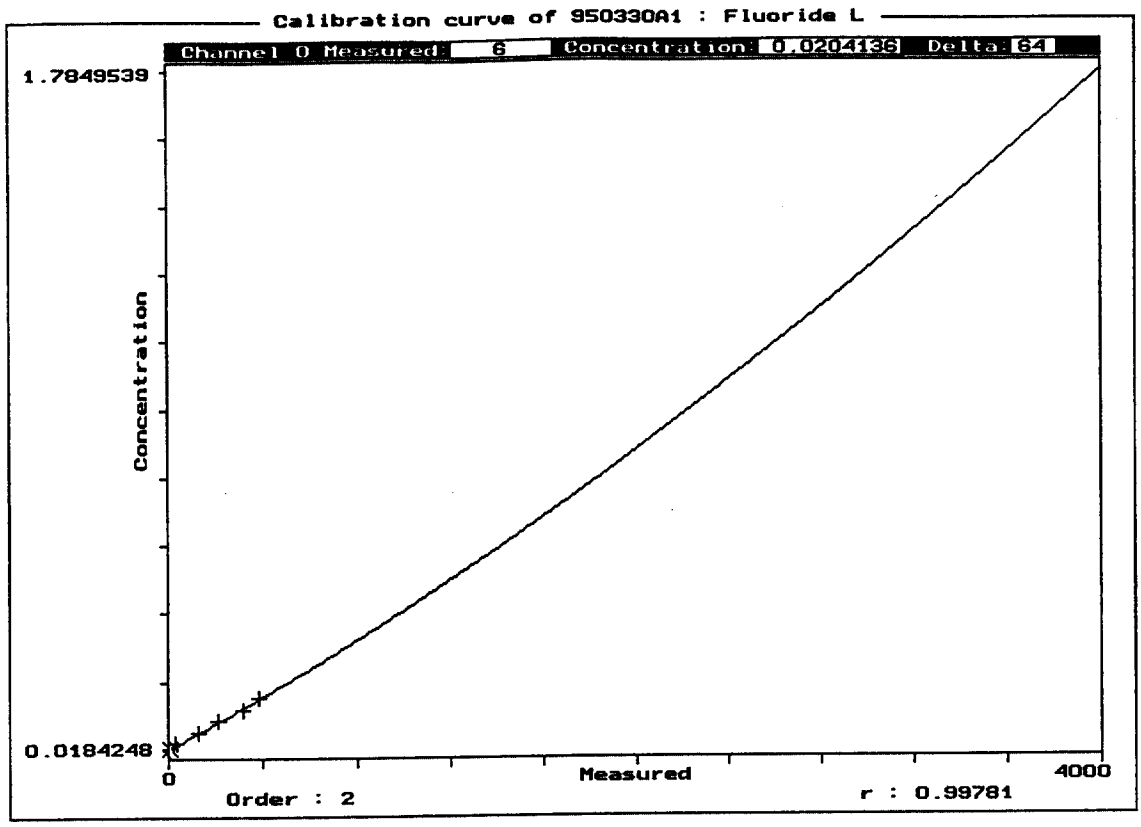



Raw data of 950330A1 : Fluoride 1.5
Pathno: 3 Time: 6798 Value: 328 Delta: 64
4000
Value
0
43
39
40
41
42
44
6798
Time
11173
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !


Calibration curve of 950330A1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0761527 Delta: 64
1.2520844
0.0752354
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 4921


Calibration curve of 950330A1 : Fluoride L
Channel 0 Measured: 6 Concentration: 0.0204136 Delta: 64
1.7849539
0.0184248
Concentration
0
Measured
4000
Order : 2
r : 0.99781